Laura Dean

Blood Groups and Red Cell Antigens

Last Updated: 2.00

Blood Groups and Red Cell Antigens is a guide to the differences in our blood types that complicate blood transfusions and pregnancy. It accompanies the dbRBC, a new NCBI resource that contains clinical and DNA data about human red blood cells.

Author

Laura Dean

National Center for Biotechnology Information (NCBI), National Library of Medicine, National Institutes of Health, Bethesda, MD 20892-6510

Table of Contents

Preface

People come in all different shapes and sizes. There are also differences you can't see, such as their types of blood.

Blood type is determined by markers (antigens) that are scattered across the surface of red blood cells (RBCs). These antigens take a variety of different forms: they may be sugars that project above the cell surface, or they may be large proteins that form an important part of the RBC membrane. The presence and absence of these antigens make the blood from different people, different. And there are many types of blood because there are hundreds of antigens.

Rarely does a person's blood type matter in everyday life, even if their blood type is uncommon or rare. Blood type only becomes significant when the blood from two people mix, e.g., during a blood transfusion. If a person recieves RBCs that have antigens different to their own, his or her immune system may attack and destroy the transfused cells.

Therefore, before a blood transfusion, a person's blood type is determined. Serologically, this is done by testing for antibodies which are formed against antigens that are missing from the person's own RBCs. In other words, a person's blood type is determined by the antigens they lack. A complementary test can be used to determine a person's blood type by the antigens he or she produces, and the answer to which antigens are produced lies in the DNA. A person's DNA directly encodes the protein blood group antigens and indirectly encodes the sugar antigens by encoding the enzymes that produce them. By combining serological tests with a person's genotype, we can define a person's blood type more accurately and quickly

The database of red blood cells (dbRBC) , is a new resource from the NCBI. It contains information about blood, including clinical data about different blood groups and stores DNA sequences which encode different blood group antigens.

About the Author

Laura Dean, M.D.

Dr. Laura Dean is a Visiting Research Fellow at the National Center for Biotechnology Information (NCBI), which is part of the National Library of Medicine (NLM) at the National Institutes of Health (NIH). Laura graduated as a medical doctor from the University of Cambridge in 2000. Since that time she has completed a medical and surgical rotation in the UK and written two textbooks ("The Genetic Landscape of Diabetes" and "Blood Groups and Red Cell Antigens") and over 20 online articles for the NCBI.

Acknowledgments

I would like to thank the following individuals who were invaluable in the review process:

1. Blood and the cells it contains

The average human adult has more than 5 liters (6 quarts) of blood in his or her body. Blood carries oxygen and nutrients to living cells and takes away their waste products. It also delivers immune cells to fight infections and contains platelets that can form a plug in a damaged blood vessel to prevent blood loss.

Through the circulatory system, blood adapts to the body's needs. When you are exercising, your heart pumps harder and faster to provide more blood and hence oxygen to your muscles. During an infection, the blood delivers more immune cells to the site of infection, where they accumulate to ward off harmful invaders.

All of these functions make blood a precious fluid. Each year in the USA, 30 million units of blood components are transfused to patients who need them. Blood is deemed so precious that is also called "red gold" because the cells and proteins it contains can be sold for more than the cost of the same weight in gold.

This chapter introduces the components of blood.

Blood contains cells, proteins, and sugars
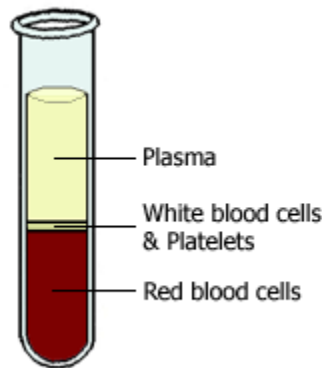
If a test tube of blood is left to stand for half an hour, the blood separates into three layers as the denser components sink to the bottom of the tube and fluid remains at the top.

The straw-colored fluid that forms the top layer is called plasma and forms about 60% of blood. The middle white layer is composed of white blood cells (WBCs) and platelets, and the bottom red layer is the red blood cells (RBCs). These bottom two layers of cells form about 40% of the blood.

Plasma is mainly water, but it also contains many important substances such as proteins (albumin, clotting factors, antibodies, enzymes, and hormones), sugars (glucose), and fat particles.

All of the cells found in the blood come from bone marrow. They begin their life as stem cells, and they mature into three main types of cells— RBCs, WBCs, and platelets. In turn, there are three types of WBC—lymphocytes, monocytes, and granulocytes—and three main types of granulocytes (neutrophils, eosinophils, and basophils). See them in action in "Meet the blood cells".

See a figure of all the cellular elements of blood in Janeway & Traver's Immunobiology

A sample of blood can be further separated into its individual components by spinning the sample in a centrifuge. The force of the spinning causes denser elements to sink, and further processing enables the isolation of a particular protein or the isolation of a particular type of blood cell. With the use of this method, antibodies and clotting factors can be harvested from the plasma to treat immune deficiencies and bleeding disorders, respectively. Likewise, RBCs can be harvested for blood transfusion.

Meet the blood cells.

Click on the cells to find out more about them.

Download file

To view "Meet the blood cells", you will need to have Flash installed on your computer.

Red blood cells transport oxygen

Every second, 2-3 million RBCs are produced in the bone marrow and released into the circulation. Also known as erythrocytes, RBCs are the most common type of cell found in the blood, with each cubic millimeter of blood containing 4-6 million cells. With a diameter of only 6 μm, RBCs are small enough to squeeze through the smallest blood vessels. They circulate around the body for up to 120 days, at which point the old or damaged RBCs are removed from the circulation by specialized cells (macrophages) in the spleen and liver.

In humans, as in all mammals, the mature RBC lacks a nucleus. This allows the cell more room to store hemoglobin, the oxygen-binding protein, enabling the RBC to transport more oxygen. RBCs are also biconcave in shape; this shape increases their surface area for the diffusion of oxygen across their surfaces. In non-mammalian vertebrates such as birds and fish, mature RBCs do have a nucleus.

See an electron micrograph of red blood cells in Albert's
Molecular Biology of the Cell

If a patient has a low level of hemoglobin, a condition called anemia, they may appear pale because hemoglobin gives RBCs, and hence blood, their red color. They may also tire easily and feel short of breath because of the essential role of hemoglobin in transporting oxygen from the lungs to wherever it is needed around the body.

White blood cells are part of the immune response

WBCs come in many different shapes and sizes. Some cells have nuclei with multiple lobes, whereas others contain one large, round nucleus. Some contain packets of granules in their cytoplasm and so are known as granulocytes.

See an electron micrograph of white blood cells in Albert's
Molecular Biology of the Cell

Despite their differences in appearance, all of the various types of WBCs have a role in the immune response. They circulate in the blood until they receive a signal that a part of the body is damaged. Signals include interleukin 1 (IL-1), a molecule secreted by macrophages that contributes to the fever of infections, and histamine, which is released by circulating basophils and tissue mast cells, and contributes to allergic reactions.

In response to these signals, the WBCs leave the blood vessel by squeezing through holes in the blood vessel wall. They migrate to the source of the signal and help begin the healing process.

Individuals who have low levels of WBCs may have more and worse infections. Depending upon which WBCs are missing, the patient is at risk for different types of infection. For example, macrophages are especially good at swallowing bacteria, and a deficiency in macrophages leads to recurrent bacterial infections. In contrast, T cells are particularly skilled in fighting viral infections, and a loss of their function results in an increased susceptibility to viral infections.

Neutrophils digest bacteria
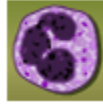
Neutrophils are also known as polymorphonuclear cells because they contain a nucleus whose shape (morph) is irregular and contains many (poly) lobes. They also belong to a group of WBCs known as granulocytes because their cytoplasm is dotted with granules that contain enzymes that helps them digest pathogens.

See a neutrophil phagocytose a bacterium in Albert's
Molecular Biology of the Cell

Monocytes become macrophages

Monocytes are young WBCs that circulate in the blood. They develop into macrophages after they have left the blood and migrated into tissue. There they provide an immediate defense because they can engulf (phagocytose) and digest pathogens before other types of WBCs reach the area.

In the liver, tissue macrophages are called Kupffer cells, and they specialize in removing harmful agents from blood that has left the gut. Alveolar macrophages are in the lungs and remove harmful agents that may have been inhaled. Macrophages in the spleen remove old or damaged red blood cells and platelets from the circulation.

See a macrophage phagocytose a red blood cell in Albert's
Molecular Biology of the Cell

Macrophages are also "antigen-presenting cells", presenting the foreign proteins (antigens) to other immune cells, triggering an immune response.

Lymphocytes consist of B cells and T cells

Lymphocytes are round cells that contain a single, large round nucleus. There are two main classes of cells, the B cells that mature in the bone marrow, and the T cells that mature in the thymus gland.

See an electron micrograph of a B cell and T cell in Albert's
Molecular Biology of the Cell

Once activated, the B cells and T cells trigger different types of immune response. The activated B cells, also known as plasma cells, produce highly specific antibodies that bind to the agent that triggered the immune response. T cells, called helper T cells, secrete chemicals that recruit other immune cells and help coordinate their attack. Another group, called cytotoxic T cells, attacks virally infected cells.

Platelets help blood to clot

Platelets are irregularly shaped fragments of cells that circulate in the blood until they are either activated to form a blood clot or are removed by the spleen. Thrombocytopenia is a condition of low levels of platelets and carries an increased risk of bleeding. Conversely, a high level of platelets (thrombocythemia) carries an increased risk of forming inappropriate blood clots. These could deprive essential organs such as the heart and brain, of their blood supply, causing heart attacks and strokes, respectively.

A diagram of a platelet in Albert's
Molecular Biology of the Cell

As with all the cells in the blood, platelets originate from stem cells in the bone marrow. The stem cells develop into platelet precursors (called megakaryocytes) that "shed" platelets into the bloodstream. There, platelets circulate for about 9 days. If they encounter damaged blood vessel walls during this time, they stick to the damaged area and are activated to form a blood clot. This plugs the hole. Otherwise, at the end of their life span they are removed from the circulation by the spleen. In a diverse number of diseases where the spleen is overactive, e.g. rheumatoid arthritis and leukemia, the spleen removes too many platelets, leading to increased bleeding.

Your complete blood count

A complete blood count (CBC) is a simple blood test that is commonly ordered as part of a routine medical assessment. As the name suggests, it is a count of the different types of cells found in the blood. The test can diagnose and monitor many different diseases, such as anemia, infection, inflammatory diseases, and malignancy. Table 1 gives an example of CBC values, but note that the reference ranges and the units used may differ, depending upon the laboratory that carried out the test.

Table 1. Complete blood count.

Blood component	Abbreviation used	Reference range	SI Reference range
White blood cells	WBC	4500-11,000/mm^3	4.5-11.0 x 10^9/L
Red blood cells*	RBC	Male: 4.3-5.9 million/mm^3 Female: 3.5-5.5 million/mm^3	Male: 4.3-5.9 x 10^{12}/L Female: 3.5-5.5 x 10^{12}/L
Hemoglobin*	HGB	Male: 13.5-17.5 g/dL Female: 12.0-16.0 g/dL	Male: 2.09-2.71 mmol/L Female: 1.86-2.48 mmol/L
Hematocrit*	HT	Male: 41%-53% Female: 36%-46%	Male: 0.41-0.53 Female: 0.36-0.46

Blood component	Abbreviation used	Reference range	SI Reference range
Mean corpuscular volume	MCV	80-100 μm^3	80-100 fl
Mean corpuscular hemoglobin	MCH	25.4-34.6 pg/cell	0.39-0.54 fmol/cell
Mean corpuscular hemoglobin concentration	MCHC	31%-36% Hb/cell	4.81-5.58 mmol Hb/L
Platelets	Platelets	150,000-400,000/mm^3	150-400 x 10^9/L

*Values differ depending upon altitude.

Red blood cell count detects anemia

A CBC measures the following features of RBCs:

- the total amount of hemoglobin (Hb) in the blood
- the number of RBCs (RBCs)
- the average size of a RBC (MCV)
- the amount of space RBCs take up in the blood (hematocrit)

The CBC also includes information about RBCs that is calculated from the other measurements, e.g., the amount (MCH) and concentration (MCHC) of hemoglobin in RBCs.

The number of RBCs and the amount of hemoglobin in the blood are lower in women than in men. This is because of the menstrual loss of blood each month. Below a certain level of hemoglobin, a patient is said to be anemic, suggesting a clinically significant drop in oxygen carrying capacity. Anemia is not a diagnosis but a symptom of an underlying disease that has to be investigated.

A clue to the cause of anemia is the average size of RBC (mean corpuscular volume, MCV). Causes of a high MCV include a deficiency of B_{12} or folate vitamins in the diet. B_{12} is found in red meat therefore, a deficiency of B_{12} is especially common in vegetarians and vegans. Conversely, folate is plentiful in fresh leafy green vegetables, therefore, a deficiency of folate is common in the elderly, who may have a poor diet.

Low MCV anemia is common and may be a result of hereditary blood disorders, such as thalassemia, but is most often caused by a deficiency of iron. For example, women of reproductive age may lose too much iron through heavy menstrual bleeding and are prone to this form of anemia, known as iron-deficiency anemia.

Hematocrit is the percentage of RBCs in relation to the total volume of blood

The hematocrit measures the fraction of the blood that is made up of RBCs. It reflects the combination of the total number of RBCs, and the volume that they occupy.

One of the changes seen in pregnancy is a drop in hematocrit. This occurs because although the production of RBCs does not change greatly, the plasma volume increases, i.e., the RBCs are "diluted". Alternatively, a low hematocrit can reflect a drop in RBC production by the bone marrow. This may be attributable to bone marrow disease (damage by toxins or cancer) or due to a decrease in erythropoietin, a hormone secreted by the kidney that stimulates RBC production. Decreased RBCs may also be the result of a reduced life span of the RBCs (e.g., chronic bleeding).

A high hematocrit value may truly reflect an increase in the fraction of RBCs (e.g., increased erythropoietin attributable to a tumor of RBCs called polycythemia rubra vera), or it may reflect a drop in the plasma component of the blood (e.g., fluid loss in burn victims).

The number of WBCs increases in infection and tumors

The WBC count is a count of the number of WBCs found in one cubic millimeter of blood.

An increased number of WBCs is most commonly caused by infections, such as a urinary tract infection or pneumonia. It may also be caused by WBC tumors, such as leukemia.

A decreased number of WBCs is caused by the bone marrow failing to produce WBCs or by an increased removal of WBCs from the circulation by a diseased liver or an overactive spleen. Bone marrow failure may be caused by toxins or by the normal bone marrow cells being replaced by tumor cells.

The WBC differential part of the CBC breaks down the WBCs into five different types: neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Finding out the count of each type of WBC gives more information about the underlying problem. For example, in the early stages of an infection, most of the increase in WBCs is attributable to the increase in neutrophils. As the infection continues, lymphocytes increase. Worm infections can trigger an increase in eosinophils, whereas allergic conditions, such as hay fever, trigger an increase in basophils.

The number of platelets indicates whether bleeding or clotting is likely

Normally, one cubic millimeter of blood contains between 150,000 and 400,000 platelets. If the number drops below this range, uncontrolled bleeding becomes a risk, whereas a rise above the upper limit of this range indicates a risk of uncontrolled blood clotting.

Hemoglobin binds oxygen

Hemoglobin is the oxygen-carrying protein that is found within all RBCs. It picks up oxygen where it is abundant (the lungs) and drops off oxygen where it is needed around the body. Hemoglobin is also the pigment that gives RBCs their red color.

Heme groups and globins

As its name suggests, hemoglobin is composed of "heme" groups (iron-containing rings) and "globins" (proteins). In fact, hemoglobin is composed of four globin proteins—two alpha chains and two beta chains—each with a heme group. The heme group contains one iron atom, and this can bind one molecule of oxygen. Because each molecule of hemoglobin contains four globins, it can carry up to four molecules of oxygen.

See hemoglobin structure in Albert's
Molecular Biology of the Cell

Hemoglobin transports oxygen

In the lungs, a hemoglobin molecule is surrounded by a high concentration of oxygen, therefore, it binds oxygen. In active tissues, the oxygen concentration is lower, so hemoglobin releases its oxygen.

This behavior is much more effective because the hemoglobin––oxygen binding is "co-operative". This means that the binding of one molecule of oxygen makes it easier for the binding of subsequent oxygen molecules. Likewise, the unbinding of oxygen makes it easier for other oxygen molecules to be released. This means that the response of hemoglobin to the oxygen needs of active tissues is much quicker.

Aside from the oxygen saturation of hemoglobin, other factors that influence how readily hemoglobin binds oxygen include plasma pH, plasma bicarbonate levels, and the pressure of oxygen in the air (high altitudes in particular).

The molecule 2,3-disphosphoglycerate (2,3-DPG) binds to hemoglobin and lowers its affinity for oxygen, thus promoting oxygen release. In individuals who have become acclimatized to living at high altitudes, the level of 2,3-DPG in the blood increases, allowing the delivery of more oxygen to tissues under low oxygen tension.

Fetal hemoglobin

Fetal hemoglobin differs from adult hemoglobin in that it contains two gamma chains instead of two beta chains. Fetal hemoglobin binds oxygen with a much greater affinity than adult hemoglobin; this is an advantage in the womb because it allows fetal blood to extract oxygen from maternal blood, despite its low concentration of oxygen.

Normally, all fetal hemoglobin is replaced by adult hemoglobin by the time of birth.

Breaking down hemoglobin

Old or damaged RBCs are removed from the circulation by macrophages in the spleen and liver, and the hemoglobin they contain is broken down into heme and globin. The globin protein may be recycled, or broken down further to its constituent amino acids, which may be recycled or metabolized. The heme contains precious iron that is conserved and reused in the synthesis of new hemoglobin molecules.

During its metabolism, heme is converted to bilirubin, a yellow pigment that can discolor the skin and sclera of the eye if it accumulates in the blood, a condition known as jaundice. Instead, the plasma protein albumin binds to bilirubin and carries it to the liver, where it is secreted in bile and also contributes to the color of feces.

Jaundice is one of the complications of an incompatible blood transfusion. This occurs when the recipient's immune system attacks the donor RBCs as being foreign. The rate of RBC destruction and subsequent bilirubin production can exceed the capacity of the liver to metabolize the bilirubin produced.

Hemoglobinopathies

Hemoglobinopathies form a group of inherited diseases that are caused by mutations in the globin chains of hemoglobin. Sickle cell anemia is the most common of these and is attributable to a mutation that changes one of the amino acids in the hemoglobin beta chain, producing hemoglobin that is "fragile". When the oxygen concentration is low, RBCs tend to become distorted and "sickle" shaped. These deformed cells can block small blood vessels and damage the organs they are supplying. This can be very painful, and if not treated, a sickle cell crisis can be fatal.

Sickle cell anemia in Genes and Disease

Another inherited anemia that particularly affects individuals of Mediterranean descent is thalassemia. A fault in the production of either alpha or beta globin chains causes a range of symptoms, depending on how many copies of the alpha and beta genes are affected. Some individuals may be carriers of the disease and have no symptoms, whereas if all copies of the genes are lost, the disease is fatal.

Thalassemia in Genes and Disease

The porphyrias are a group of inherited disorders in which the synthesis of heme is disrupted. Depending upon the stage at which the disruption occurs, there are a range of neurological and gastrointestinal side effects. King George III of England ("the madness of King George") was one of the most famous individuals who suffered from porphyria.

Porphyria in Genes and Disease

Resources

Karl Landsteiner, Nobel Laureate from Nobelprize.org

Red Gold: the epic story of blood from Public Broadcasting Service (PBS)

2. Blood group antigens are surface markers on the red blood cell membrane

Before the 1900s, it was thought that all blood was the same, a misunderstanding that led to frequently fatal transfusions of animal blood into humans and hazardous transfusions of blood between people. Human blood is not the same—people belong to different blood groups, depending upon the surface markers found on the red blood cell.

The cells that make up the body's tissues and organs are covered with surface markers, or antigens. Red blood cells are no different. This chapter will describe the types of red blood cell antigen and explain why they are so important in medicine today.

Antigens stimulate an immune response

An antigen is any substance to which the immune system can respond. For example, components of the bacterial cell wall can trigger severe and immediate attacks by neutrophils.

If the immune system encounters an antigen that is not found on the body's own cells, it will launch an attack against that antigen. Conversely, antigens that are found on the body's own cells are known as "self-antigens", and the immune system does not normally attack these.

The membrane of each red blood cell contains millions of antigens that are ignored by the immune system. However, when patients receive blood transfusions, their immune systems will attack any donor red blood cells that contain antigens that differ from their self-antigens. Therefore, ensuring that the antigens of transfused red blood cells match those of the patient's red blood cells is essential for a safe blood transfusion.

Red blood cell antigens can be sugars or proteins

Blood group antigens are either sugars or proteins, and they are attached to various components in the red blood cell membrane.

For example, the antigens of the ABO blood group are sugars. They are produced by a series of reactions in which enzymes catalyze the transfer of sugar units. A person's DNA determines the type of enzymes they have, and, therefore, the type of sugar antigens that end up on their red blood cells.

In contrast, the antigens of the Rh blood group are proteins. A person's DNA holds the information for producing the protein antigens. The RhD gene encodes the D antigen, which is a large protein on the red blood cell membrane. Some people have a version of the gene that does not produce D antigen, and therefore the RhD protein is absent from their red blood cells.

The figure below shows the red blood cell membrane and some of the blood group antigens attached to it. Aside from the sugar (glycan or carbohydrate) antigens, the red blood cell membrane contains three types of protein that carry blood group antigens: single-pass proteins, multi-pass proteins, and glycosylphosphatidylinositol (GPI)-linked proteins. Click on the blood groups to find out more about the antigens that define it.
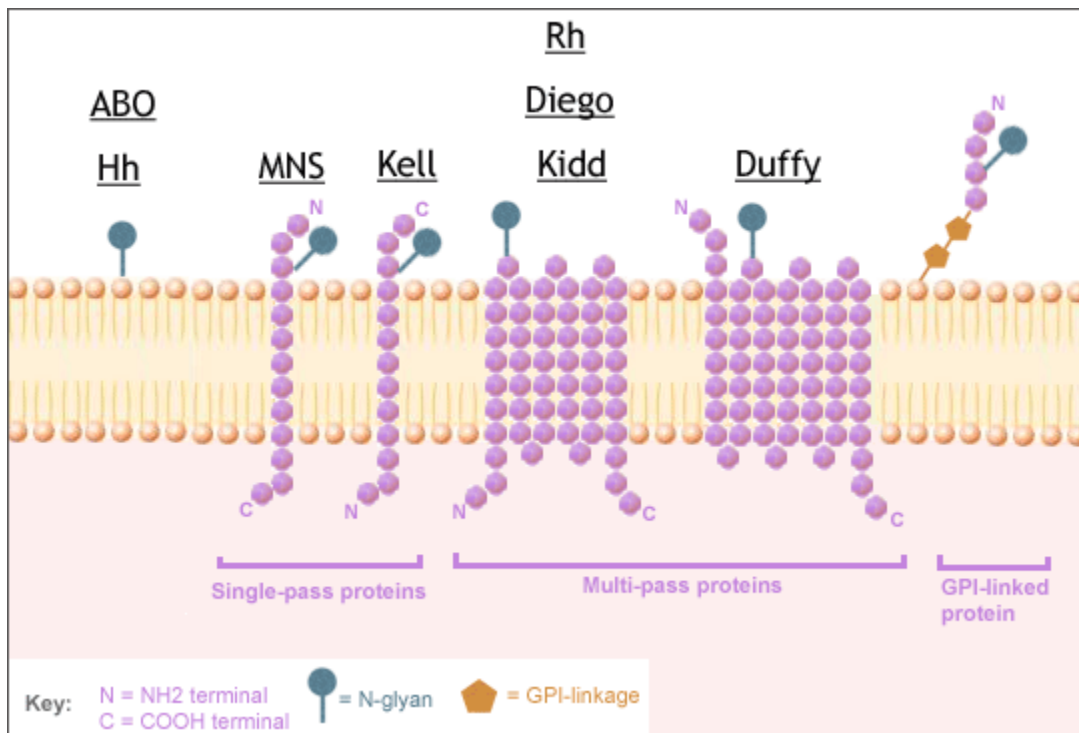
Red blood cell antigens determine your blood group

The antigens expressed on the red blood cell determine an individual's blood group. The main two blood groups are called ABO (with blood types A, B, AB, and O) and Rh (with Rh D-positive or Rh D-negative blood types).

The functions of many of the blood group antigens are not known, and if they are missing from the red blood cell membrane, there is no ill effect. This suggests that if the blood group antigens used to have a function, e.g., one particular blood group antigen made red blood cells more resistant to invasion from a parasite, it is no longer relevant today.

But the presence or absence of red blood cell antigens becomes extremely important when blood from different people mixes, e.g., when a patient receives a blood transfusion from a blood bank. This also happens when a mother becomes pregnant because during labor, a small amount of fetal blood enters her circulation. In these circumstances, exposure to the foreign antigens on the red blood cells can trigger immune reactions.

It is not possible to completely remove the danger of adverse reactions when blood from two people mix, but the danger can be minimized. Before a blood transfusion takes place, the blood to be donated must be "typed and cross matched" with the patient's blood to ensure immune compatibility (see Chapter 3). In pregnancy, the risk of the mother's immune system attacking the foreign antigens present on her fetus' red blood cells is prevented by giving the mother antibodies to cover fetal red blood cell antigens and removing them from the mother's circulation before her immune cells find them (see Chapter 4).

Blood groups differ around the world

The distribution of the four ABO blood types, A, B, AB, and O, varies in populations throughout the world. It is determined by the frequency of the three alleles of the ABO gene in different populations. Blood type O is the most common worldwide, followed by group A. Group B is less common, and group AB is the least common.

The frequencies of ABO and Rh type in the United States were recently examined by collecting data from blood donors over a 10 year period (1). The charts below summarize the findings for blood type and race:

Distribution of ABO phenotypes by Race or Ethnicity
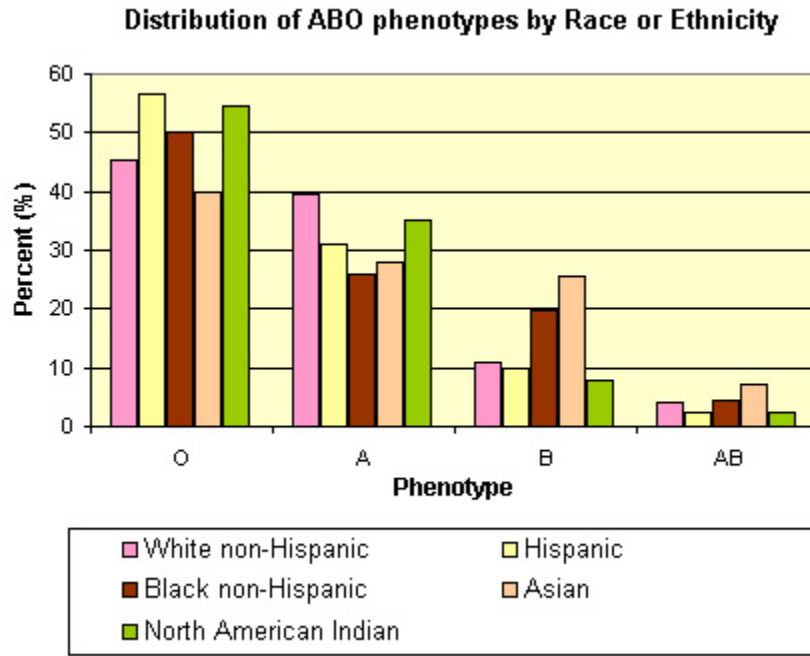
The highest percentage of type O (57%) was found in Hispanic donors (a group that includes donors of Mexican, Puerto Rican, and Cuban descent). The next highest percentage of type O was found in North American Indian (55%) and black (50%) donors.

Distribution of RhD phenotype by race or ethnicity
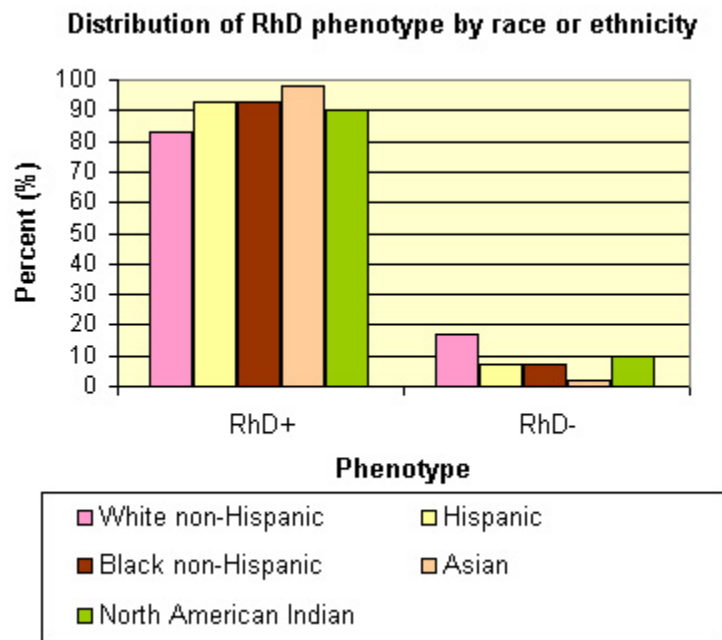
In all donors, the Rh D-positive (RhD+) blood type was more common than the Rh D-negative (RhD-) blood type. The highest percentage of RhD- was found in white donors (17.3%).

Blood type O: the Americas

People with blood type O are said to be "universal donors" because their blood is compatible with all ABO blood types. It is also the most common blood type in populations around the world, including the USA (1) and

Western Europe (2, 3). Among indigenous populations of Central and South America, the frequency of O blood type is extremely high, approaching 100%. It is also high among Australian aborigines.

Blood Type A: Central and Eastern Europe

Type A is common in Central and Eastern Europe. In countries such as Austria, Denmark, Norway, and Switzerland, about 45-50% of the population have this blood type, whereas about 40% of Poles and Ukrainians do so.

The highest frequencies are found in small, unrelated populations. For example, about 80% of the Blackfoot Indians of Montana have blood type A.

Blood type B: Asia

Blood type B is relatively common in Chinese and Indians, being present in up to 25% of the population. It is less common in European countries and Americans of European origin, being found in about 10% of these populations.

Blood type AB is the least common

Blood type AB individuals are known as "universal receivers" because they can receive blood from any ABO type.

It is also the rarest of the blood groups. It is most common in Japan, regions of China, and in Koreans, being present in about 10% of these populations.

The classification of blood cell antigens

Traditionally, newly discovered red blood cell antigens were named alphabetically (e.g. ABO, MNS, P) or were named for the first person who produced antibody against them (e.g. Duffy, Diego). In 1980, The International Society of Blood Transfusion (ISBT) Working Party on Terminology for Red Cell Surface antigens was formed to create a standard for blood group terminology. Under this terminology, each blood group antigen has a number, and it belongs to a blood group system, a collection, or a series (4).

Blood groups

A blood group system contains antigens controlled by a single gene (or by multiple closely linked loci), and the system is genetically distinct. At the time of writing, there are 22 blood group systems, including the ABO, Rh, and Kell blood groups which contain antigens that can provoke the most severe transfusion reactions.

Each blood group antigen is assigned a six-digit number by the ISBT. The first three digits represent the blood group (e.g., ABO is 001, Rh is 004), and the last three identify the antigen in the order it was discovered. For example, for ABO, the A antigen was the first to be discovered and has the number 001.001 whereas the B antigen was next and is designated 001.002.

Collections

A collection contains antigens that are related in some way, e.g., by genetics or biochemistry, but they do not meet the criteria to form a blood group. Once a collection of antigens can be proven to be genetically distinct, they are given the status of a blood group. At the time of writing, there are six collections of antigens.

Series

Red cell antigens that do not fit into a blood group or a collection are sorted into two series: if they are rare (frequency of less than 1%), they are placed in the 700 series, if they are common (frequency greater than 90%), they are placed in the 901 series. At the time of writing, there are 22 antigens in the 700 series and 11 antigens in the 901 series.

References

1. Garratty G, Glynn SA, McEntire R. ABO and Rh(D) phenotype frequencies of different racial/ethnic groups in the United States. Transfusion. 2004;44:703–6. PubMed PMID: 15104651.
2. Bloodbook.Com, Racial & Ethnic Distribution of ABO Blood Types Cited 15th March, 2005.
3. Reid ME and Lomas-Francis C. The Blood Group Antigen Facts Book. Second ed. 2004, New York: Elsevier Academic Press.
4. Daniels GL, Fletcher A, Garratty G, Henry S, Jorgensen J, Judd WJ, Levene C, Lomas-Francis C, Moulds JJ, Moulds JM, Moulds M, Overbeeke M, Reid ME, Rouger P, Scott M, Sistonen P, Smart E, Tani Y, Wendel S, Zelinski T. Blood group terminology 2004: from the International Society of Blood Transfusion committee on terminology for red cell surface antigens. Vox Sang. 2004;87:304–16. PubMed PMID: 15585029.

3. Blood transfusions and the immune system

The immune system never rests—its cells constantly patrol the circulation. Without the immune system, the body would be overwhelmed with infections. With it, blood transfusions must be performed with great care.

If incompatible blood is given in a transfusion, the donor cells are treated as if they were foreign invaders, and the patient's immune system attacks them accordingly. Not only is the blood transfusion rendered useless, but a potentially massive activation of the immune system and clotting system can cause shock, kidney failure, circulatory collapse, and death.

This chapter discusses the causes of transfusion reactions and how the hazards of blood transfusions are minimized.

How to launch an immune response against transfused red blood cells

Many of the adverse effects of blood transfusions are mediated by the recipient's immune system. In general, the formation of this and other immune responses occur in three stages:

- the immune system detects foreign material (antigen)
- the immune system processes the antigen
- the immune system mounts a response to remove the antigen from the body

The immune response varies tremendously, depending on the individual (the health of his or her immune system and genetic factors) and the antigen (how common it is and how "provocative" it is to the immune system).

Antigen detection

The red blood cells (RBCs) from one person may enter into the circulation of another person in two different ways, either by a blood transfusion or by pregnancy. The RBCs will appear foreign if they contain antigens that are not found on the patient's own RBCs.

Antigen processing

When the macrophage encounters an antigen, it engulfs it, digests it, and then presents the antigenic fragments on its cell surface together with MHCII (Major Histocompatibility Complex II).

A T helper cell binds to the antigen/MHCII on the macrophage, and the two cells interact. The macrophage secretes cytokines to stimulate the T cell, which in turn secretes cytokines to stimulate the growth and production of more T cells.

The T helper cell, now activated, leaves to activate a third type of cell, the B cell. Existing B cells are stimulated by the T cell to grow, divide, and produce genetically identical daughter cells. Some of the daughter cells become plasma cells that produce antibodies that are specific for the antigen that stimulated their production. The amount and type of antibody produced results from the interaction of T helper cells (which stimulate antibody production) and T suppressor cells (which inhibit antibody production). Other daughter cells remain as B cells in the circulation for many years. They serve as "memory cells", remembering the encounter with the antigen that stimulated their production.

Read a summary of antigen presentation to T cells in Janeway & Traver's Immunobiology

Immune response

If this is the first time the antigen has been encountered, a primary immune response is mounted. Usually there is a delay of several days, then IgM antibody is produced, followed by a switch to IgG antibody production. The initial IgM molecules bind the antigen weakly, but the subsequent IgG molecules are much better targeted. IgG continues to be produced long after the encounter with the antigen, providing long-lasting immunity.

If the immune system has encountered the antigen before, it will already be armed with primed B cells (memory cells) that accelerate the production of larger amounts of IgG (rather than IgM). This is called the secondary immune response. It is faster, more specific, and the production of the specific antibody may remain high for years. B cells may also undergo changes to further improve how the antibodies they produce bind to the antigen.

There are two main arms of immune response: humoral (using antibodies) and cellular (using immune cells). Severe immune-mediated transfusion reactions usually involve the humoral arm. In the case of a foreign red blood cell antigen, the patient's pre-existing antibodies bind to the antigen, coating the donor RBCs.

Some types of antibody may activate the complement cascade, a series of enzyme-driven reactions involving protein fragments. The cascade ends with the formation of a "membrane attack complex", a large molecule that punches a hole in the cell membrane. Other antibodies simply bind to the donor RBCs and cause them to clump together (agglutinate). The agglutinated cells may survive or may be prematurely removed from the circulation by the macrophages.

Otherwise, the fate of the incompatible RBCs largely rests in the hands of macrophages in the liver or the spleen. They remove the antibody-coated cells from the circulation and phagocytose them. Phagocytosis is aided by the macrophages having a receptor that binds to the antibodies and another receptor that binds to complement fragments. Therefore, incompatible RBCs are rapidly destroyed after antibody binding. In addition, this antibody response may cause dangerous hemolytic transfusion reactions as described below.

"Blood type and cross match"

To avoid a transfusion reaction, donated blood must be compatible with the blood of the patient who is receiving the transfusion. More specifically, the donated RBCs must lack the same ABO and Rh D antigens that the patient's RBCs lack. For example, a patient with blood group A can receive blood from a donor with blood group A (which lacks the B antigen) or blood group O (which lacks all ABO blood group antigens). However, they cannot receive blood from a donor with blood group B or AB (which both have the B antigen).

Before a blood transfusion, two blood tests known as a "type and cross match" are done. First, the recipient's blood type is determined, i.e., their ABO type and Rh D status. In theory, once the recipient's blood type is known, a transfusion of compatible blood can be given. However, in practice, donor blood may still be incompatible because it contains other antigens that are not routinely typed but may still cause a problem if the recipient's serum contains antibodies that will target them. Therefore, a "cross match" is done to ensure that the donor RBCs actually do match against the recipient's serum.

To perform a cross match, a small amount of the recipient's serum is mixed with a small amount of the donor RBCs. The mixture is then examined under a microscope. If the proposed transfusion is incompatible, the donor RBCs are agglutinated by antibodies in the recipient's serum.

Play the blood typing game at:
NobelPrize.Org

Transfusion reactions: Immune-mediated

Immune-mediated transfusion reactions occur when incompatible blood products are transfused into a patient's circulation, triggering a response from the patient's immune system. The destruction of incompatible RBCs is called a hemolytic transfusion reaction, which may occur immediately (acute) or after a period of days (delayed). The destruction of incompatible donor white blood cells (WBCs) causes a febrile non-hemolytic transfusion reaction (FNHTR), and the destruction of incompatible donor platelets causes post-transfusion purpura (PTP).

The symptoms produced by these transfusion reactions are often similar, beginning with chills, fever, shaking, and aching. Some transfusion reactions are mild and resolve by themselves (e.g., FNHTR) whereas others can develop into a life-threatening reaction (e.g., acute hemolytic transfusion reaction).

The risks are minimized by using blood products only when necessary and, even then, using a specific blood component rather than whole blood. Also, all WBCs are now removed from donated blood; leukodepletion reduces the risk of certain infections as well as the risk of fever due to white blood cell incompatibility.

Hemolytic transfusion reaction: Red blood cell incompatibility

Hemolytic transfusion reactions (HTRs) are reactions in which donor RBCs are destroyed by antibodies in the recipient's circulation. They occur when antigen-positive donor RBCs are transfused into a patient who has preformed antibodies to that antigen. The donor RBCs may be destroyed immediately (a potentially serious reaction) or may have a shortened or even normal survival time (milder reactions).

Red blood cell incompatibility may also occur when the patient's RBC antigens are attacked by antibodies from the donor's plasma. This tends to be a minor problem because of the small amount of antibody present in the donated plasma, which is further diluted on transfusion into the recipient's circulation.

Acute hemolytic transfusion reaction

Acute hemolytic transfusion reactions occur within 24 hours of the transfusion and often occur during the transfusion. Ominously, the patient may report a "feeling of impending doom". They may also complain of a burning sensation at the site of the infusion, together with chills, fever, and pain in the back and flanks.

The severity of the reaction depends upon: (1) how much incompatible antigen was transfused—how much blood was given and the number of antigens per red blood cell; (2) the nature of the antigen - its size and location on the red blood cell membrane; and (3) the nature of the recipient's antibodies - the type (IgG or IgM) and subtype (IgG3) of antibody, the amount present in the circulation at the time of the transfusion, its avidity for binding to the antigen, and its ability to activate complement.

Intravascular hemolysis

The most severe reactions involve an intravascular hemolysis; the donor RBCs are destroyed by the recipient's antibodies while they are still inside blood vessels. Such reactions involve antibodies that strongly activate complement, which in turn lyses the donor RBCs. Hemoglobin is released into the plasma and excreted in urine (hemoglobinuria), turning the urine a dark brown color. Bilirubin, a metabolite of hemoglobin usually secreted into bile by the liver, instead accumulates in the blood causing jaundice. Massive activation of complement can cause shock, as can the large amounts of tissue factor released by RBC debris that triggers an uncontrollable clotting cascade (disseminated intravascular coagulation).

The most common cause of an acute intravascular hemolytic transfusion reaction is ABO incompatibility. The ABO blood group antigens are densely expressed on the RBC surface, and most people have adequate amounts of preformed antibodies that can not only bind to the RBCs but can also activate complement. Although routine

typing and cross matching should prevent incompatible ABO blood group antigens from triggering this type of reaction, human error occasionally leads to the "wrong blood" being given during a transfusion.

Apart from anti-A and anti-B, other antibodies capable of intravascular hemolysis of transfused RBCs include anti-H produced in people with the Bombay blood group (see the H blood group), anti-Jk^a (see the Kidd blood group), and anti-P, P1, Pk (see the P blood group system).

Extravascular hemolysis

In extravascular hemolytic reactions, the donor RBCs are removed from the circulation by macrophages in the spleen and liver. The macrophages destroy the red blood cells inside these organs.

The donor RBCs may still be coated with the recipient's antibodies, but these antibodies do not trigger an immediate intravascular hemolysis. Instead, their presence (specifically, the Fc component of the antibody) is recognized by IgG-Fc receptors of macrophages, which aids the phagocytosis of the cells. Antibodies directed at antigens of the Rh blood group mediate this type of RBC removal.

Other types of antibody that bind to the donor RBCs may bind the complement component C3b without activating the entire cascade. This further aids the phagocytosis by macrophages that have C3b receptors. Such antibodies include those directed against antigens of the ABO, Duffy, and Kidd blood groups.

Because the extravascular destruction of RBCs is slower and more controlled than intravascular hemolysis, very little free hemoglobin is released into the circulation or excreted in the urine. The liver can keep up with the increased production of bilirubin, and jaundice rarely occurs. Therefore, the main symptoms of this type of reaction are fever and chills.

Delayed hemolytic transfusion reaction

Delayed hemolytic transfusion reactions may occur as soon as 1 day or as late as 14 days after a blood transfusion. The donor RBCs are destroyed by the recipient's antibodies, but the hemolysis is "delayed" because the antibodies are only present in low amounts initially.

The recipient's antibodies were formed during a previous sensitization (primary stimulation) with a particular antigen. However, by the time a cross match is done, the level of antibody in the recipient's plasma is too low to cause agglutination, making this type of reaction difficult to prevent. Likewise, during the blood transfusion the level of antibody is too low to cause an acute transfusion reaction.

However, during the blood transfusion, as the patient re-encounters the antigen, his or her immune system is stimulated to rapidly produce more antibodies (secondary stimulation). Over the following days, the recipient's antibodies bind to the donor RBCs, which are subsequently removed from the circulation by macrophages (extravascular hemolysis).

The clinical outcome depends upon the rate at which the patient can produce antibodies and hence destroy the donor RBCs. Usually, this type of reaction is much less severe than acute hemolytic reactions.

This type of transfusion reaction is associated with antibodies that target the Kidd and Rh antigens.

Febrile non-hemolytic transfusion reaction (FNHTR): White blood cell incompatibility

The most common transfusion reaction is a fever without signs of hemolysis. This is called a febrile non-hemolytic transfusion reaction (FNHTR). Most cases are mild—the patients may describe feeling hot and cold, their temperatures rise by at least 1°C, and they may have rigors. Only when other potentially severe causes of transfusion reactions have been excluded may FNHRT be diagnosed.

The cause is thought to be the patient's preformed antibodies attacking transfused WBCs, binding to their HLA antigens. Another factor might be that during the storage of blood units, WBCs release cytokines that may provoke a fever when the unit of blood is transfused into a patient.

The risk of FNHRT is reduced by removing WBCs from blood units prior to storage—a process known as leukodepletion. In addition, patients who receive multiple transfusions may be given an anti-pyretic before the transfusion to lessen fever symptoms.

Post transfusion purpura (PTP): Platelet incompatibility

Post transfusion purpura (PTP) is defined as a thrombocytopenia (low number of platelets) that occurs 5 to 10 days after a platelet transfusion. Patients are at risk of bleeding, and bleeding into the skin causes a purplish discoloration of the skin known as purpura.

PTP is caused by the recipient having a platelet-specific antibody that reacts with the donor platelets. The recipient's own platelets are also attacked. The platelet antigen HPA-1a appears to be most frequently targeted.

PTP is more common in women because pregnancy increases the likelihood of forming the platelet-specific antibody. It may also have formed after an earlier platelet transfusion. Treatment includes the use of intravenous immunoglobulin to neutralize the antibodies or to remove them from the plasma by plasmapheresis.

Allergic reactions: IgE anti-allergen antibodies

Some patients can have an allergic reaction after their blood transfusions—they report feeling itchy and break out into hives (urticaria). This is more common in patients who have a history of allergic conditions such as hay fever.

This type of allergic reaction happens when existing IgE antibody binds to its antigen and triggers the release of histamine from the patient's mast cells and basophils. In an allergic reaction to a blood transfusion, either the transfused blood contains IgE that binds to antigen from the recipient's blood, or the antibody is the recipient's own and binds to antigen in the transfused blood.

Fortunately, symptoms are usually mild and can be controlled by stopping the transfusion and giving antihistamines.

Anaphylaxis: IgA anti-plasma protein antibodies

Anaphylaxis is a life-threatening allergic reaction that can occur after only a few milliliters of blood have been transfused. The patient reports difficulty breathing and may be wheezing and coughing. There may also be nausea and vomiting in the absence of a fever. Other signs include low blood pressure, loss of consciousness, respiratory arrest, and circulatory shock. Urgent treatment is essential and includes giving epinephrine.

Usually the antigen that triggers the anaphylaxis is not known. In the case of patients with IgA deficiency, it is thought that the presence of IgA in the donor's plasma is the trigger. IgA-deficient patients have a mild immunodeficiency that may not have been diagnosed. Because they lack IgA, their immune systems can be sensitized to it. Although this type of transfusion reaction is rare in these patients, special precautions are taken to reduce their risk of exposure to IgA in blood products.

Transfusion associated lung injury (TRALI): Donor anti-leukocyte antibodies attack

Transfusion associated lung injury (TRALI) is a rare and occasionally fatal transfusion reaction characterized by a sudden onset of shortness of breath.

The underlying mechanism is not fully understood, but it is thought to involve the transfusion of donor plasma that contains antibodies that attack the recipient's WBCs. These donor antibodies bind to, and cause the aggregation of, the recipient's WBCs in the blood vessels that supply the lungs. The white cells release inflammatory mediators that increase the permeability of the lung capillaries, causing fluid to accumulate in the tissue of the lungs, a condition known as pulmonary edema for which supportive treatment is given.

Transfusion associated graft-versus-host disease (TA-GVHD): Donor T cells attack

Transfusion associated graft-versus-host disease (TA-GVHD) arises when transfused blood cells (the graft) attack the patient's own cells (the host). It is more common in immunocompromised patients whose immune systems fail to eliminate the transfused cells. Instead, the surviving donor T cells attack cells that bear HLA antigens.

This type of reaction becomes apparent about one week after the transfusion. Signs include a fever, characteristic skin lesions, and diarrhea. Blood tests reveal signs of bone marrow failure and liver malfunction.

To prevent TA-GVHD, special precautions are taken with high-risk patients. They only receive blood products that have been irradiated. This prevents all donor cells, including the T cells, from being able to divide and attack the host. In cases where TA-GVHD does develop, the outcome is grave. The patient usually dies several weeks after the blood transfusion.

Transfusion reactions: Non-immune

Not all of the problems that can arise during a blood transfusion are attributable to the immune system. Some are mechanical, especially in patients who need multiple blood transfusions. For example, blood that is not sufficiently warmed before transfusion can cause hypothermia. Also, the volume of blood that needs to be transfused may be too great for the patient's cardiovascular system, especially in elderly patients or patients with varying degrees of heart failure. In such cases, transfusion can cause volume overload and respiratory difficulty.

Metabolic disturbances can also occur, older or damaged RBCs release potassium, and transfusing such blood may cause hyperkalemia (an increased level of potassium) in the patient, putting them at risk of heart arrhythmias. In large amounts, citrate, a blood preservative that prevents clotting, can lower the level of calcium in the plasma (hypocalcemia), leading to muscle tremors and heart arrhythmias.

Finally, the risk of blood transfusions transmitting infectious diseases has been greatly reduced, but a small risk still remains. A virus can be passed on from the donor who is unaware that he or she has an infection. Infection may also occur after the blood has been donated; bacteria can contaminate blood products while they are being stored.

To minimize the risk of infection, blood donors are now screened, and people who are at risk of infectious diseases are excluded from donating blood. In addition, all donated blood is tested for infectious agents. Currently in the USA, blood is tested for HIV, hepatitis B virus, hepatitis C virus, syphilis, and HTLV types I and II, which are linked to leukemia. Since 2003, blood has also been screened for West Nile virus (WNV).

4. Hemolytic disease of the newborn

Hemolytic disease of the newborn (HDN) used to be a major cause of fetal loss and death among newborn babies. The first description of HDN is thought to be in 1609 by a French midwife who delivered twins—one baby was swollen and died soon after birth, the other baby developed jaundice and died several days later. For the next 300 years, many similar cases were described in which newborns failed to survive.

It was not until the 1950s that the underlying cause of HDN was clarified; namely, the newborn's red blood cells (RBCs) are being attacked by antibodies from the mother. The attack begins while the baby is still in the womb and is caused by an incompatibility between the mother's and baby's blood.

By the 1960s, trials in the United States and the United Kingdom tested the use of therapeutic antibodies that could remove the antibodies that cause HDN from the mother's circulation. The trials showed that giving therapeutic antibodies to women during their pregnancy largely prevented HDN from developing (1). By the 1970s, routine antenatal care included screening of all expectant mothers to find those whose pregnancy may be at risk of HDN, and giving preventative treatment accordingly. This has led to a dramatic decrease in the incidence of HDN, particularly severe cases that were responsible for stillbirth and neonatal death.

This chapter will discuss the causes of HDN and how the disease can be treated or minimized, if not prevented entirely.

Maternal antibodies cross the placenta and attack fetal red blood cells

During pregnancy, some of the mother's antibodies are transported across the placenta and enter the fetal circulation. This is necessary because by the time of birth, newborns have only a primitive immune system, and the continuing presence of maternal antibodies helps ensure that they survive while their immune system matures. A downside to this protection is that by targeting fetal RBCs, maternal antibodies can also cause HDN.

A major cause of HDN is an incompatibility of the Rh blood group between the mother and fetus. Most commonly, hemolytic disease is triggered by the D antigen, although other Rh antigens, such as c, C, E, and e, can also cause problems.

Pregnancies at risk of HND are those in which an Rh D-negative mother becomes pregnant with an RhD-positive child (the child having inherited the D antigen from the father). The mother's immune response to the fetal D antigen is to form antibodies against it (anti-D). These antibodies are usually of the IgG type, the type that is transported across the placenta and hence delivered to the fetal circulation.

HDN can also be caused by an incompatibility of the ABO blood group. It arises when a mother with blood type O becomes pregnant with a fetus with a different blood type (type A, B, or AB). The mother's serum contains naturally occurring anti-A and anti-B, which tend to be of the IgG class and can therefore cross the placenta and hemolyse fetal RBCs.

HDN due to ABO incompatibility is usually less severe than Rh incompatibility. One reason is that fetal RBCs express less of the ABO blood group antigens compared with adult levels. In addition, in contrast to the Rh antigens, the ABO blood group antigens are expressed by a variety of fetal (and adult) tissues, reducing the chances of anti-A and anti-B binding their target antigens on the fetal RBCs.

Less common causes of HDN include antibodies directed against antigens of the Kell blood group (e.g., anti-K and anti-k), Kidd blood group (e.g., anti-Jka and anti-Jkb), Duffy blood group (e.g., anti-Fya), and MNS and s blood group antibodies. To date, antibodies directed against the P and Lewis blood groups have not been associated with HDN.

Sensitization occurs during the first pregnancy

Sensitization to an antigen occurs when the immune system encounters an antigen for the first time and mounts an immune response. In the case of HDN caused by Rh incompatibility, an Rh D-negative mother may first encounter the D antigen while being pregnant with an Rh D-positive child, or by receiving a blood transfusion of Rh D-positive blood. Once a mother has been sensitized to the D antigen, her serum will contain anti-D. The direct Coombs test (see below) confirms the presence of anti-D and hence that the mother has been sensitized.

Only a small amount of fetal blood need enter the mother's circulation for sensitization to occur. Typically, this occurs during the delivery of the first-born Rh D-positive child. Fetal-maternal hemorrhage is common during labor and is increased during a prolonged or complicated labor, which in turn increases the risk of sensitization. Sensitization can also occur earlier in the pregnancy, for example during a prenatal bleed or a miscarriage. It may also occur during medical procedures, such as a termination of pregnancy or chorionic villus sampling.

The risk of sensitization to the Rh D antigen is decreased if the fetus is ABO incompatible. This is because any fetal cells that leak into the maternal circulation are rapidly destroyed by potent maternal anti-A and/or anti-B, reducing the likelihood of maternal exposure to the D antigen.

HDN occurs in subsequent pregnancies

Initially, the maternal anti-D that is formed at the time of sensitization is of the IgM type, which can not cross the placenta. In subsequent pregnancies, a repeat encounter with the Rh D antigen stimulates the rapid production of type IgG anti-D, which can be transported across the placenta and enter the fetal circulation. Once in the fetal circulation, anti-D attaches to the Rh D antigens found on the fetal RBCs, marking them to be destroyed.

The rate of hemolysis determines whether the nature of HDN is mild, moderate, or severe. In mild cases, the small increase in the rate of hemolysis is tolerated by the fetus. At birth and during the newborn period, symptoms include a mild anemia and jaundice, both of which may resolve without treatment.

In cases where there is a greater increase in the rate of hemolysis, the level of bilirubin may still remain low during the pregnancy because of the ability of the placenta to remove bilirubin from the fetal circulation. However, after birth the neonate's immature liver is unable to metabolize the increased amount of bilirubin that instead accumulates in his or her blood. Within 24 hours of birth, the level of bilirubin may rise dramatically. If levels continue to rise, bilirubin may enter the brain to cause kernicterus, a potentially fatal condition that leaves permanent neurological damage in the babies that survive.

An even greater rapid and prolonged destruction of RBCs leads to severe anemia in the fetus. The liver, spleen, and other organs increase their production of RBCs to compensate for their loss. The drive to produce RBCs causes the liver and spleen to increase in size (hepatosplenomegaly), and liver dysfunction can occur. Immature RBCs (erythroblasts) spill into the circulation, giving rise to the alternative name of this disease, erythroblastosis fetalis. A complication of severe HDN is hydrops fetalis, in which the fetal tissues become swollen (edematous). This condition is usually fatal, either *in utero* or soon after birth.

The Coombs test detects Rh incompatibility between mother and fetus

To detect HDN, the presence of maternal anti-Rh IgG must be identified. *In vivo*, these antibodies destroy Rh D-positive fetal RBCs, but *in vitro*, they do not lyse cells or even cause agglutination, making them difficult to identify. Therefore, the Coombs test is used. This test uses antibodies that bind to anti-D antibodies. The test is

named for Robin Coombs, who first developed the technique of using antibodies that are targeted against other antibodies.

Direct Coombs test: diagnoses HDN

The direct Coombs test detects maternal anti-D antibodies that have already bound to fetal RBCs.

First, a sample of fetal RBCs is washed to remove any unbound antibody (Ig). When the test antibodies (anti-Ig) are added, they agglutinate any fetal RBCs to which maternal antibodies are already bound.

This is called the direct Coombs test because the anti-Ig binds "directly" to the maternal anti-D Ig that coats fetal RBCs in HDN.

Indirect Coombs test: used in the prevention of HDN

The indirect Coombs test finds anti-D antibodies in the mother's serum. If these were to come into contact with fetal RBCs they would hemolyse them and hence cause HDN. By finding maternal anti-D before fetal RBCs have been attacked, treatment can be given to prevent or limit the severity of HDN.

For this test, the mother's serum is incubated with Rh D-positive RBCs. If any anti-D is present in the mother's serum, they will bind to the cells. The cells are then washed to remove all free antibodies. When anti-Ig antibodies are added, they will agglutinate any RBCs to which maternal antibodies are bound.

This is called the indirect Coombs test because the anti-Ig finds "indirect" evidence of harmful maternal antibodies, requiring the addition of fetal RBCs to show the capacity of maternal anti-D to bind to fetal RBCs.

See a diagram of the Direct and Indirect Coombs tests in Janeway & Travers Immunobiology

Preventing HDN

Determine Rh status of the mother

As part of routine prenatal or antenatal care, the blood type of the mother (ABO and Rh) is determined by a blood test. A test for the presence of atypical antibodies in the mother's serum is also performed. At present, Rh D incompatibility is the only cause of HDN for which screening is routine.

In the United States, the frequency of Rh D-negative status varies from about 17% in Caucasians to about 7% in Hispanics and Blacks. The frequency is much lower in people of Asian descent (including people from China, India, and Japan), averaging about 2% (2).

If the mother is not sensitized, reduce the risk of future sensitization

To find out whether a pregnant Rh D-negative mother has been sensitized to the Rh D antigen, an indirect Coombs test is done (see above). If anti-D is not found in the mother's serum, it is likely that she has not been sensitized to the Rh D antigen.

The risk of future sensitization can be greatly reduced by giving all unsensitized mothers anti-D Ig, which "mops up" any fetal RBCs that may have leaked into the maternal circulation, reducing the risk of first-time exposure to the D antigen.

Usually, Rh D-negative mothers receive on injection of anti-D Ig at about 28 weeks gestation, which is about the time when fetal RBCs start to express the D antigen, and mothers receive another dose at about 34 weeks, a few weeks before labor begins during which the risk of fetomaternal hemorrhage is high. A final dose of anti-D Ig is

given after the baby has been delivered. In addition, anti-D Ig is given to cover other events during the pregnancy that may lead to sensitization, e.g., antepartum bleeds and pre-eclampsia.

This prophylaxis regime against Rh D sensitization is effective. However, currently, there is no routine prophylaxis for HDN caused by incompatibility of other blood group antigens.

If the mother is sensitized, determine whether the fetus is at risk and monitor accordingly

Once the presence of maternal anti-D has been confirmed, the next step is to determine whether the fetal RBCs are a target, i.e., confirm the Rh status of the fetus. If the father is homozygous for the D allele (D/D), the fetus will be D positive. If however the father is heterozygous (D/d), there is a 50:50 chance that the fetus is D positive, and the only way to know the blood type for sure is to test a sample of fetal cells taken from the amniotic fluid or umbilical cord.

If the fetus is Rh D-positive, the pregnancy is carefully monitored for signs of HDN. Monitoring includes regular ultrasound scans of the fetus and monitoring of the amount of anti-D in the mother's serum. Active hemolysis is indicated by a rise in anti-D. If a fetal blood test confirms fetal anemia, depending upon its severity, a blood transfusion can be done *in utero* to replace the lysed fetal RBCs.

Blood transfusions may also be needed to correct anemia in the newborn period. During this period there may also be a sharp rise in the level of bilirubin in the neonate, which can be lowered by phototherapy and exchange transfusions.

References

1. Urbaniak SJ, Greiss MA. RhD haemolytic disease of the fetus and the newborn. Blood Rev. 2000;14:44–61. PubMed PMID: 10805260.
2. Garratty G, Glynn SA, McEntire R. ABO and Rh(D) phenotype frequencies of different racial/ethnic groups in the United States. Transfusion. 2004;44:703–6. PubMed PMID: 15104651.

5. The ABO blood group

The discovery of the ABO blood group, over 100 years ago, caused great excitement. Until then, all blood had been assumed to be the same, and the often tragic consequences of blood transfusions were not understood. As our understanding of the ABO group grew, not only did the world of blood transfusion become a great deal safer, but scientists could now study one of the first human characteristics proven to be inherited. A person's ABO blood type was used by lawyers in paternity suits, by police in forensic science, and by anthropologists in the study of different populations.

The ABO blood group antigens remain of prime importance in transfusion medicine—they are the most immunogenic of all the blood group antigens. The most common cause of death from a blood transfusion is a clerical error in which an incompatible type of ABO blood is transfused. The ABO blood group antigens also appear to have been important throughout our evolution because the frequencies of different ABO blood types vary among different populations, suggesting that a particular blood type conferred a selection advantage (e.g., resistance against an infectious disease.)

However, despite their obvious clinical importance, the physiological functions of ABO blood group antigens remain a mystery. People with the common blood type O express neither the A nor B antigen, and they are perfectly healthy. Numerous associations have been made between particular ABO phenotypes and an increased susceptibility to disease. For example, the ABO phenotype has been linked with stomach ulcers (more common in group O individuals) and gastric cancer (more common in group A individuals). Another observation is that individuals with blood type O tend to have lower levels of the von Willebrand Factor (vWF), which is a protein involved in blood clotting.

At a glance

Antigens of the ABO blood group.

Number of antigens	**4: A, B, AB, and A1**
Antigen specificity	**Carbohydrate** The sequence of oligosaccharides determines whether the antigen is A, B, or A1.
Antigen-carrying molecules	**Glycoproteins and glycolipids of unknown function** 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 A/B polymorphism arises from several SNPs in the ABO gene, which result in A and B transferases that differ by four amino acids. The O allele encodes an inactive glycosyltransferase that leaves the ABO antigen precursor (the H antigen) unmodified.
Frequency of ABO blood group antigens	**A:** 43% Caucasians, 27% Blacks, 28% Asians **B:** 9% Caucasians, 20% Blacks, 27% Asians **A1:** 34% Caucasians, 19% Blacks, 27% Asians Note: Does not include AB blood groups (1).
Frequency of ABO phenotypes	**Blood group O is the most common phenotype in most populations.** Caucasians: group O, 44%; A_1, 33%; A_2, 10%; B, 9%; A1B, 3%; A2B, 1% Blacks: group O, 49%; A_1, 19%; A_2, 8%; B, 20%; A1B, 3%; A2B, 1% Asians: group O, 43%; A_1, 27%; A_2, rare; B, 25%; A1B, 5%; A2B, rare Note: Blood group A is divided into two main phenotypes, A_1 and A_2 (1).

Antibodies produced against ABO blood group antigens.

Antibody type	**IgG and IgM** Naturally occurring. 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.

Background information

History

At the beginning of the 20th century an Austrian scientist, Karl Landsteiner, noted that the RBCs of some individuals were agglutinated by the serum from other individuals. He made a note of the patterns of agglutination and showed that blood could be divided into groups. This marked the discovery of the first blood group system, ABO, and earned Landsteiner a Nobel Prize.

Landsteiner explained that the reactions between the RBCs and serum were related to the presence of markers (antigens) on the RBCs and antibodies in the serum. Agglutination occurred when the RBC antigens were bound by the antibodies in the serum. He called the antigens A and B, and depending upon which antigen the RBC expressed, blood either belonged to blood group A or blood group B. A third blood group contained RBCs that reacted as if they lacked the properties of A and B, and this group was later called "O" after the German word "Ohne", which means "without". The following year the fourth blood group, AB, was added to the ABO blood group system. These RBCs expressed both A and B antigens.

In 1910, scientists proved that the RBCs antigens were inherited, and that the A and B antigens were inherited codominantly over O. There was initially some confusion over how a person's blood type was determined, but the puzzle was solved in 1924 by Bernstein's "three allele model".

The ABO blood group antigens are encoded by one genetic locus, the ABO locus, which has three alternative (allelic) forms—A, B, and O. A child receives one of the three alleles from each parent, giving rise to six possible genotypes and four possible blood types (phenotypes).

ABO genotype in the offspring		ABO alleles inherited from the mother		
		A	B	O
ABO alleles inherited from the father	A	A	AB	A
	B	AB	B	B
	O	A	B	O

Nomenclature

- Number of ABO blood group antigens: 4
- ISBT symbol: ABO
- ISBT number: 001
- Gene symbol: ABO
- Gene name: ABO blood group (A transferase, α1,3-N-acetylgalactosaminyltransferase; B transferase, α1,3-galactosyltransferase)

Basic biochemistry

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. A_1 red cells express about 5 times more A antigen than A_2 red cells, but both types of red cell react with anti-A, and as far as transfusion purposes are concerned, the A_1 and A_2 blood groups are interchangeable.

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 (2).

The immune system forms antibodies against whichever ABO blood group antigens are *not* found on the individual's RBCs. Thus, a group A individual will have anti-B antibodies and a group B individual will have 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. Blood group AB is the least common, and these individuals will have neither anti-A nor anti-B in their serum.

Blood group	Antigen(s) present on the red blood cells	Antibodies present in the serum	Genotype(s)
A	A antigen	Anti-B	AA or AO
B	B antigen	Anti-A	BB or BO
AB	A antigen and B antigen	None	AB
O	None	Anti-A and Anti-B	OO

ABO antibodies in the serum are formed naturally. Their production is stimulated when the immune system encounters the "missing" ABO blood group antigens in foods or in micro-organisms. This happens at an early age because sugars that are identical to, or very similar to, the ABO blood group antigens are found throughout nature.

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).

See the structures of the A, B, and O antigens in Stryer's Biochemistry

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. These antigens are incorporated into one of four types of oligosaccharide chain, type 2 being the most common in the antigen-carrying molecules in RBC membranes. Some of the other enzymes involved in the earlier stages of ABO antigen synthesis are also involved in producing antigens of the Hh blood group and the Lewis blood group.

Expression

Although the ABO blood group antigens are regarded as RBC antigens, they are actually expressed on a wide variety of human tissues and are present on most epithelial and endothelial cells.

Each human RBC expresses about 2 million ABO blood group antigens. Other blood cells, such as T cells, B cells, and platelets, have ABO blood group antigens that have been adsorbed from the plasma. In individuals who are "secretors", a soluble form of the ABO blood group antigens is found in saliva and in all bodily fluids except for the cerebrospinal fluid.

A number of illnesses may alter a person's ABO phenotype. Patients can "acquire" the B antigen during a necrotizing infection during which bacteria release an enzyme into the circulation that converts the A1 antigen into a B-like antigen (3). During this time, patients should not receive blood products that contain the B antigen because their sera will still contain anti-B. Once the underlying infection is treated, the patients' blood groups return to normal.

Illness can also cause patients to "lose" ABO blood group antigens. Any disease that increases the body's demand for RBCs may weaken the expression of ABO blood group antigens, e.g., thalassemia. In addition, ABO blood group antigens can be altered by hematological cancers that can modify the sugar chains that bear the ABO blood group antigens, lending to the use of the A and B antigens as tumor markers for acute leukemia, myeloproliferative disorders, and myelodysplasia.

Function of the A and B antigens

The functions of the ABO blood group antigens are not known. Individuals who lack the A and B antigens are healthy, suggesting that any function the antigens have is not important, at least not in modern times.

Diseases associated with ABO blood group antigens

No diseases are known to result from the lack of expression of ABO blood group antigens, but the susceptibility to a number of diseases has been linked with a person's ABO phenotype. Such correlations remain controversial and include the observation that gastric cancer appears to be more common in group A individuals (4), whereas gastric and duodenal ulcers occur more often in group O individuals (5).

A clear correlation has been established between the ABO phenotype and the level of two proteins involved in blood clotting; factor VII (FVIII) and von Willebrand factor (vWF) (6). Blood group O individuals have about 25% less FVIII and vWF in their plasma. It is well established that low levels of FVIII and vWF are a cause of excess bleeding, and therefore it may also be the case that increased levels make clotting more likely, increasing the risk of both arterial (ischemic heart disease) and venous (thromboembolic disease) problems. Indeed, non-group O individuals have been shown to be at an increased risk of both arterial and venous disease (6).

Clinical significance of ABO antibodies

ABO antibodies are of major clinical significance for two reasons: they are naturally occurring and are found universally, and, they are highly reactive.

Transfusion reactions

The routine practice of blood typing and cross matching blood products should prevent adverse transfusion reactions caused by ABO antibodies. However, clerical error can result in "the wrong blood" being transfused into a patient, an error which can result in the death of the patient (7, 8).

If a recipient who has blood group O is transfused with non-group O RBCs, the naturally occurring anti-A and anti-B in the recipient's serum binds to their corresponding antigens on the transfused RBCs. These antibodies fix complement and cause rapid intravascular hemolysis, triggering an acute hemolytic transfusion reaction that can cause disseminated intravascular coagulation, shock, acute renal failure, and death.

Anti-A1 is a less significant cause of transfusion reactions and does not appear to fix complement.

Hemolytic disease of the newborn

Most cases of hemolytic disease of the newborn (HDN) that arise from an ABO incompatibility require no treatment. Cases of severe hemolysis that require exchange transfusions are less common, and fetal hydrops is rare (9).

HDN caused by ABO antibodies occurs almost exclusively in infants of blood group A or B who are born to group O mothers (10). This is because the anti-A and anti-B formed in group O individuals tend to be of the IgG type (and therefore can cross the placenta), whereas the anti-A and anti-B found in the serum of group B and A

individuals, respectively, tends to be of the IgM type. Although uncommon, cases of HDN have been reported in infants born to mothers with blood group A2 (11) and blood group B (12).

HDN tends to be relatively mild in nature mainly because fetal RBCs don't express adult levels of A and B antigens. However, the strength of fetal ABO blood group antigens can vary, and therefore the degree of hemolysis and hence the severity of HDN can be unpredictable (13). Early studies suggested that the race of a neonate was a risk factor for developing ABO HDN (14). However, later studies showed that the prevalence of disease that required treatment did not differ significantly among Asian, Black, Hispanic, and Caucasian infants (15).

Molecular information

Gene

The ABO locus encodes specific glycosyltransferases that synthesize A and B antigens on RBCs. For A/B antigen synthesis to occur, a precursor called the H antigen must be present. In RBCs, the enzyme that synthesizes the H antigen is encoded by the H locus (FUT1). In saliva and other bodily secretions, the enzyme that synthesizes the H antigen is encoded by the Se locus (FUT2).

The ABO locus

The ABO locus is located on chromosome 9 at 9q34.1-q34.2. It contains 7 exons that span more than 18 kb of genomic DNA. Exon 7 is the largest and contains most of the coding sequence. Exon 6 contains the deletion that is found in most O alleles and results in a loss of enzymatic activity.

The A and B alleles differ from each other by seven nucleotide substitutions, four of which translate into different amino acids in the gene product (R176G, G235S, L266M, G268A). The residues at positions 266 and 268 determine the A or B specificity of the glycosyltransferase they encode (16).

The O allele differs from the A allele by deletion of guanine at position 261. The deletion causes a frameshift and results in translation of an almost entirely different protein that lacks enzymatic activity (16).

There are many variant ABO alleles that encode a number of variant ABO phenotypes, but they do not encode specific antigens other than the A and B antigens. For example, weak A subgroups, such as A_3, A_x, and A_{el}, express the A antigen, and weak B subgroups, such as B_3 and B_x, express the B antigen (2).

> View the sequences of variant ABO alleles at the
> dbRBC Sequence Alignment Viewer

The H locus (FUT1)

The H locus is located on chromosome 19 at 19q13.3. It contains three exons that span more than 5 kb of genomic DNA, and it encodes a fucosyltransferase that produces the H antigen on RBCs.

Individuals who are homozygous for null alleles at this locus (h/h) do not produce H antigen, and because the H antigen is an essential precursor to the ABO blood group antigens, they cannot produce A and B antigens. Therefore, their serum contains anti-A and anti-B, in addition to potent anti-H. This rare phenotype of H-deficient RBCs is called the "Bombay phenotype" (O_h) after the city in which it was first discovered. Individuals with the Bombay phenotype are healthy, but if they ever needed a blood transfusion, the antibodies in their serum would place them at a high risk of having an acute hemolytic transfusion reaction. This can be avoided by using only blood products from a donor who also has the Bombay phenotype (usually a relative).

Read more about the Hh blood group in Chapter 6.

The Se locus (FUT2)

The Se locus is located on chromosome 19 at 19q13.3. It contains two exons that span about 25 kb of genomic DNA.

The Se locus encodes a specific fucosyltransferase that is expressed in the epithelia of secretory tissues, such as salivary glands, the gastrointestinal tract, and the respiratory tract. The enzyme it encodes catalyzes the production of H antigen in bodily secretions.

"Secretors" have at least one copy of the Se gene that encodes a functional enzyme—their genotype is Se/Se or Se/se. They secrete H antigen which, depending on their ABO genotype, is then processed into A and/or B antigens.

Non-secretors are homozygous for null alleles at this locus (se/se). They are unable to produce a soluble form of H antigen and hence do not produce A and B antigens.

References

1. Reid ME and Lomas-Francis C. The Blood Group Antigen Facts Book. Second ed. 2004, New York: Elsevier Academic Press.
2. Daniels G. Human Blood Groups, Second ed. 2002, Blackwell Science.
3. Stayboldt C, Rearden A, Lane TA. B antigen acquired by normal A1 red cells exposed to a patient's serum. Transfusion. 1987;27:41–4. PubMed PMID: 3810822.
4. Reid ME, Bird GW. Associations between human red cell blood group antigens and disease. Transfus Med Rev. 1990;4:47–55. PubMed PMID: 2134616.
5. O'Donnell J, Laffan M A. The relationship between ABO histo-blood group, factor VIII and von Willebrand factor. Transfus Med. 2001;11(4):343–51. PubMed PMID: 11532189.
6. Fuchs CS, Mayer RJ. Gastric carcinoma. N Engl J Med. 1995;333:32–41. PubMed PMID: 7776992.
7. Sazama K. Reports of 355 transfusion-associated deaths: 1976 through 1985. Transfusion. 1990;30:583–90. PubMed PMID: 2402771.
8. Williamson LM, Lowe S, Love EM, Cohen H, Soldan K, McClelland DB, Skacel P, Barbara JA. Serious hazards of transfusion (SHOT) initiative: analysis of the first two annual reports. BMJ. 1999;319:16–9. PubMed PMID: 10390452.
9. Gilja BK, Shah VP. Hydrops fetalis due to ABO incompatibility. Clin Pediatr (Phila). 1988;27:210–2. PubMed PMID: 3349731.
10. Ozolek JA, Watchko JF, Mimouni F. Prevalence and lack of clinical significance of blood group incompatibility in mothers with blood type A or B. J Pediatr. 1994;125:87–91. PubMed PMID: 8021795.
11. Jeon H, Calhoun B, Pothiawala M, Herschel M, Baron BW. Significant ABO hemolytic disease of the newborn in a group B infant with a group A2 mother. Immunohematol. 2000;16:105–8. PubMed PMID: 15373613.
12. Haque KM, Rahman M. An unusual case of ABO-haemolytic disease of the newborn. Bangladesh Med Res Counc Bull. 2000;26:61–4. PubMed PMID: 11508073.
13. Grundbacher FJ. The etiology of ABO hemolytic disease of the newborn. Transfusion. 1980;20:563–8. PubMed PMID: 7191589.
14. Bucher KA, Patterson AM, Jr, Elston RC, Jones CA, Kirkman HN, Jr. Racial difference in incidence of ABO hemolytic disease. Am J Public Health. 1976;66:854–8. PubMed PMID: 961953.
15. Toy PT, Reid ME, Papenfus L, Yeap HH, Black D. Prevalence of ABO maternal-infant incompatibility in Asians, Blacks, Hispanics and Caucasians. Vox Sang. 1988;54:181–3. PubMed PMID: 3369141.
16. Yamamoto F, Clausen H, White T, Marken J, Hakomori S. Molecular genetic basis of the histo-blood group ABO system. Nature. 1990;345:229–33. PubMed PMID: 2333095.

6. The Hh blood group

The Hh blood group contains one antigen, the H antigen, which is found on virtually all RBCs and is the building block for the production of the antigens within the ABO blood group.

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. There is no ill effect with being H deficient, but if a blood transfusion is ever needed, people with this blood type can receive blood only from other donors who are also H deficient. (A transfusion of "normal" group O blood can trigger a severe transfusion reaction.)

Because the H antigen is the precursor of the ABO blood group antigens, if it is not produced, the ABO blood group antigens are also not produced. This can be misleading in paternity cases, a fact that has been exploited in soap opera story lines!

In the show "General Hospital", the father of Monica's child was in doubt. Monica had blood type A (genotype AO) and her child had blood type O (genotype OO). Because the child must inherit an O allele from the father, the father could have the genotype AO, BO, or OO. In other words, the child's father could have blood group A or B or O, which rules out Monica's husband Alan (type AB) and implicates Rick (type O).
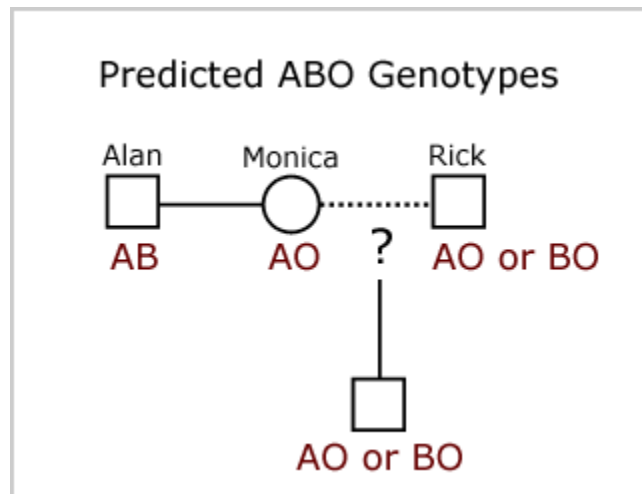
However, Alan is the father! This is possible because both he and Monica are carriers of incomplete H deficiency (H/h). Their h/h child is unable to produce any ABO blood group antigens and so despite inheriting the A or B allele from Alan, the child's RBC's lack the A and B antigens as in blood type O.

At a glance

Antigens of the Hh blood group.

Number of antigens	1: the H antigen
Antigen specificity	**Carbohydrate** The specificity of the H antigen is determined by the sequence of oligosaccharides. More specifically, the minimum requirement for H antigenicity is the terminal disaccharide fucose-galactose, where the fucose has an alpha-(1-2)- linkage.
Antigen-carrying molecules	**Glycoproteins and glycolipids of unknown function** The H antigen is 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 FUT1 gene indirectly encodes the H antigen expressed on RBCs.** FUT1 encodes a fucosyltransferase that catalyzes the final step in the synthesis of the H antigen. The FUT2 gene indirectly encodes a soluble form of the H antigen, which is found in bodily secretions.
Frequency of the H antigen	**Present on 99.9% of RBCs in all populations** H deficiency is rare: it is found in 1 of 8,000 in Taiwan, 1 of 10,000 in India, and 1 per million in Europe (1).
Frequency of the H phenotype	**Blood group O: 45% in Caucasians, 49% in Blacks, 43% in Asians, and 55% in Mexicans** The frequency of the H antigen is equivalent to the frequency of blood group O in which the H antigen remains unaltered (1).

Antibodies produced against the H antigen.

Anti-H type	**IgM is more common than IgG** Anti-H is naturally occurring in people with H antigen deficiency.
Anti-H reactivity	**Capable of hemolysis** Anti-H can activate the complement cascade which lyses RBCs while they are still in the circulation (intravascular hemolysis).
Transfusion reaction	**Yes—can cause an acute hemolytic transfusion reaction**
Hemolytic disease of the newborn	**Possible** HDN could arise in mothers with the Bombay phenotype (Oh, h/h)

Background information

History

In Bombay, India, an individual was discovered to have an interesting blood type that reacted to other blood types in a way that had not been seen before. Serum from this individual contained antibodies that reacted with all RBCs from normal ABO phenotypes (i.e., groups O, A, B, and AB). The individual's RBCs appeared to lack all of the ABO blood group antigens plus an additional antigen that was previously unknown.

In 1952, a paper about the "new blood group character related to the ABO blood group" was published (2). This new blood group character is the H antigen and it is the building block for the antigens of the ABO blood group.

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. 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.

Nomenclature

- Number of H antigens: 1
- ISBT symbol: H
- ISBT number: 018
- Gene symbol: FUT1
- Gene name: Fucosyltransferase 1

Basic biochemistry

The biosynthesis of the H antigen and the A and B antigens involves a series of enzymes (glycosyltransferases) that transfer monosaccharides. The resulting antigens are oligosaccharide chains, which are attached to lipids and proteins that are anchored in the RBC membrane.

The H antigen is produced by a specific fucosyltransferase. Depending upon a person's ABO blood type, the H antigen is converted into either the A antigen, B antigen, or both. If a person has blood group O, the H antigen remains unmodified. Therefore, the H antigen is present in the highest amounts in blood type O and in the least amounts in blood type AB.

Two regions of the genome encode two enzymes with very similar substrate specificities—the H locus (FUT1) and the Se locus (FUT2).

The H locus contains the FUT1 gene, which is expressed in RBCs. At least one functioning copy of FUT1 needs to be present (H/H or H/h) for the H antigen to be produced on RBCs. If both copies of FUT1 are inactive (h/h), the Bombay phenotype results.

The Se locus contains the FUT2 gene, which is expressed in secretory glands. Individuals who are "secretors" (Se/Se or Se/se) contain at least one copy of a functioning enzyme. They produce a soluble form of H antigen that is found in saliva and other bodily fluids. "Non-secretors" (se/se) do not produce soluble H antigen. The enzyme encoded by FUT2 is also involved in the synthesis of antigens of the Lewis blood group.

Common H phenotypes

The two common H phenotypes are "secretor" and "non-secretor".

Secretor (common)

- H antigen is expressed on RBCs.
- H antigen is expressed in saliva.
- No anti-H is produced.
- Genotype: H/H or H/h; Se/Se or Se/se

Non secretor (common)

- H antigen is present on RBCs.
- H antigen is absent from saliva.
- No anti-H is produced.
- Genotype: H/H or H/h; se/se

Uncommon H Phenotypes

The Bombay phenotype and para-Bombay phenotype are relatively rare. In India, where H deficiency was first discovered, the frequency of both phenotypes combined is 1 in 10,000 (1). H deficiency is slightly more common in Taiwan, affecting 1 of 8,000 people (1). A relatively large number of H-deficient individuals were found on

Reunion Island, which is a small French Island 800 km east of Madagascar in the Indian Ocean (3). Both the classical Bombay phenotype and a new variant type of partial H deficiency was seen in the islanders (4). In Europe, 1 per million people are H deficient (1).

Bombay phenotype

- H antigen is not expressed on RBCs.
- H antigen is not found in saliva.
- Serum contains anti-H.
- Genotype: h/h se/se

Para-Bombay phenotype

- H antigen is weakly expressed on RBCs.
- H antigen may be present or absent in saliva.
- Serum contains anti-H.
- Genotype: (H), Se/Se or Se/se or se/se

Expression of the H antigen

The H antigen shares the same broad tissue distribution as the A and B antigens. Likewise, in individuals who are "secretors", a soluble form of the H antigen is found in saliva and all fluids except cerebrospinal fluid.

Function of the H antigen

The function of the H antigen, apart from being an intermediate substrate in the synthesis of ABO blood group antigens, is not known although it may be involved in cell adhesion (5). People who lack the H antigen do not suffer any deleterious effects, and being H-deficient is only an issue if they were to need a blood transfusion because they would require H-deficient blood.

Clinical significance of H antibodies

Transfusion reactions

If patients with anti-H in their circulation receive transfusions of blood that contains the H antigen (e.g., blood group O), they are at risk of suffering an acute hemolytic transfusion reaction.

Hemolytic disease of the newborn

In theory, the maternal production of anti-H during pregnancy could cause hemolytic disease in a fetus who did not inherit the mother's Bombay phenotype. In practice, cases of HDN caused in this way have not been described, possibly because of the rarity of the Bombay phenotype.

Molecular information

The H blood group locus (containing FUT1) and the secretor locus (containing FUT2) are located on chromosome 19 at q.13.3. FUT1 and FUT2 are tightly linked, being only 35 kb apart. Because they are highly homologous, they are likely to have been the result of a gene duplication of a common gene ancestor.

The H locus contains four exons that span more than 8 kb of genomic DNA. Both the Bombay and para-Bombay phenotypes are the result of point mutations in the FUT1 gene (6, 7).

The classical Bombay phenotype is caused by a Tyr316Ter mutation in the coding region of FUT1 (1, 8). The mutation introduces a stop codon, resulting in a truncated enzyme that lacks 50 amino acids at the C-terminal

end, rendering the enzyme inactive. In Caucasians, the Bombay phenotype may be caused by a number of mutations (9, 10). Likewise, a number of mutations have been reported to underlie the para-Bombay phenotype (11).

References

1. Reid ME and Lomas-Francis C. The Blood Group Antigen Facts Book. Second ed. 2004, New York: Elsevier Academic Press.
2. Bhende YM, Deshpande CK, Bhatia HM, Sanger R, Race RR, Morgan WT, Watkins WM. A "new" blood group character related to the ABO system. Lancet. 1952;1:903–4. PubMed PMID: 14918471.
3. Gerard G, Vitrac D, Le Pendu J., Muller A, Oriol R. H-deficient blood groups (Bombay) of Reunion Island. Am J Hum Genet. 1982;34:937–47. PubMed PMID: 7180848.
4. Fernandez-Mateos P, Cailleau A, Henry S, Costache M, Elmgren A, Svensson L, Larson G, Samuelsson BE, Oriol R, Mollicone R. Point mutations and deletion responsible for the Bombay H null and the Reunion H weak blood groups. Vox Sang. 1998;75:37–46. PubMed PMID: 9745152.
5. Zhu K, Amin MA, Kim MJ, Katschke K. J., Jr, Park CC, Koch AE. A novel function for a glucose analog of blood group H antigen as a mediator of leukocyte-endothelial adhesion via intracellular adhesion molecule 1. J Biol Chem. 2003;278:21869–77. PubMed PMID: 12672794.
6. Kelly RJ, Ernst LK, Larsen RD, Bryant JG, Robinson JS, Lowe JB. Molecular basis for H blood group deficiency in Bombay (Oh) and para-Bombay individuals. Proc Natl Acad Sci U S A. 1994;91:5843–7. PubMed PMID: 7912436.
7. Kaneko M, Nishihara S, Shinya N, Kudo T, Iwasaki H, Seno T, Okubo Y, Narimatsu H. Wide variety of point mutations in the H gene of Bombay and para-Bombay individuals that inactivate H enzyme. Blood. 1997;90:839–49. PubMed PMID: 9226185.
8. Koda Y, Soejima M, Johnson PH, Smart E, Kimura H. Missense mutation of FUT1 and deletion of FUT2 are responsible for Indian Bombay phenotype of ABO blood group system. Biochem Biophys Res Commun. 1997;238:21–5. PubMed PMID: 9299444.
9. Wagner FF, Flegel WA. Polymorphism of the h allele and the population frequency of sporadic nonfunctional alleles. Transfusion. 1997;37:284–90. PubMed PMID: 9122901.
10. Wagner T, Vadon M, Staudacher E, Schmarda A, Gassner C, Helmberg W, Lanzer G, Flegel WA, Wagner FF. A new h allele detected in Europe has a missense mutationin alpha(1,2)-fucosyltransferase motif II. Transfusion. 2001;41:31–8. PubMed PMID: 11161242.
11. Yu LC, Yang YH, Broadberry RE, Chen YH, Lin M. Heterogeneity of the human H blood group alpha(1,2)fucosyltransferase gene among para-Bombay individuals. Vox Sang. 1997;72:36–40. PubMed PMID: 9031499.

7. The Rh blood group

The Rh blood group is one of the most complex blood groups known in humans. From its discovery 60 years ago where it was named (in error) after the Rhesus monkey, 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).

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.

The significance of the Rh blood group is related to the fact that the Rh antigens are highly immunogenic. In the case of the D antigen, individuals who do not produce the D antigen will produce anti-D if they encounter the D antigen on transfused RBCs (causing a hemolytic transfusion reaction, HTR) or on fetal RBCs (causing HDN). For this reason, the Rh status is routinely determined in blood donors, transfusion recipients, and in mothers-to-be.

Despite the importance of the Rh antigens in blood transfusion and HDN, we can only speculate about the physiological function of the proteins, which may involve transporting ammonium across the RBC membrane and maintaining the integrity of the RBC membrane.

At a glance

Antigens of the Rh blood group.

Number of antigens	**49: D, C, E, c, and e are among the most significant**
Antigen specificity	**Protein** The sequence of amino acids determines the specificity of most of the Rh antigens.
Antigen-carrying molecules	**Proteins with unknown function** 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 RhD protein encodes the D antigen.
Molecular basis	**Two genes, RHD and RHCE, encode the Rh antigens.** The Rh genes are 97% identical, and they are located next to each other on chromosome 1. The D/d polymorphism most commonly arises from a deletion of the entire RHD gene. The C/c polymorphism arises from four SNPs that cause four amino acid changes, one of which (S103P) determines the C or c antigen specificity. The E/e polymorphism arises from a single SNP (676G→C) that causes a single amino acid change (A226P).
Frequency of Rh antigens	**D**: 85% Caucasians, 92% Blacks, 99% Asians **C**: 68% Caucasians, 27% Blacks, 93% Asians **E**: 29% Caucasians, 22% Blacks, 39% Asians **c**: 80% Caucasians, 96% Blacks, 47% Asians **e**: 98% Caucasians, 98% Blacks, 96% Asians (1)
Frequency of Rh phenotypes	**Rh haplotype DCe**: most common in Caucasians (42%), Native Americans (44%), and Asians (70%) **Rh haplotype Dce**: most common in Blacks (44%) **Rh D-negative phenotype**: most common in Caucasians (15%), less common in Blacks (8%), and rare in Asians (1%) (1)

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 (1).
Hemolytic disease of the newborn	**Yes—the most common cause of HDN**. The D antigen accounts for 50% of maternal alloimmunization (2). Anti-D and anti-c can cause severe disease. Anti-C, anti-E, and anti-e can cause mild to moderate disease.

Background information

History

In 1939, a mother who had just given birth to a still-born child needed a blood transfusion. The ABO blood group system had been discovered almost 40 years previously, and the importance of giving an ABO-compatible blood transfusion was well established. However, although the mother was transfused with ABO compatible blood from her husband, she still experienced an adverse reaction to the transfusion. Her serum was found to contain antibodies that agglutinated her husband's RBCs, even though they were ABO compatible. The death of the mother's fetus and her adverse reaction to a blood transfusion from her husband was related. During the pregnancy, the mother had been exposed to an antigen on the fetal RBCs that was of paternal origin. Her immune system attacked this antigen, and the destruction of the fetal RBCs resulted in fetal death. The mother re-encountered the same paternal antigen when she received a blood transfusion from her husband. This time her immune system attacked the transfused RBCs, causing a hemolytic transfusion reaction. The antibodies responsible led to the discovery of the Rh blood group.

It was wrongly thought that the agglutinating antibodies produced in the mother's serum in response to her husbands RBCs were the same specificity as antibodies produced in various animals' serum in response to RBCs from the Rhesus monkey. In error, the paternal antigen was named the Rhesus factor. By the time it was discovered that the mother's antibodies were produced against a different antigen, the rhesus blood group terminology was being widely used. Therefore, instead of changing the name, it was abbreviated to the Rh blood group.

Remarkably, only 20 years after the discovery of Rh incompatability in pregnancy, effective treatment became available. Today, the Rh status of mothers-to-be is checked during pregnancy to identify those at risk of HDN. In addition, all blood transfusions are matched for the Rh status.

Nomenclature

- Number of Rh antigens: 49
- ISBT symbol: Rh
- ISBT number: 004
- Gene symbols: RHD and RHCE
- Gene names: Rhesus blood group, D antigen; and, Rhesus blood group, CcEe antigens

Basic biochemistry

Common Rh phenotypes

The most common Rh haplotype in Caucasians, Asians, and Native Americans is DCe. In Blacks, the Dce haplotype is slightly more common (1).

In Caucasians, the Rh D-negative phenotype results from a deletion of the RHD gene. About 15% of Caucasians are Rh D-negative.

In Africans, there are three molecular backgrounds that give rise to the Rh D-phenotype which is found in 8% of the population. One is the RHD gene deletion that is common in Caucasians. The other two mechanisms are inheriting a RHD pseudogene (contains a duplication of nucleotides that introduces a premature stop codon) or inheriting a RHD hybrid gene (contains nucleotide sequences from the RHCE gene, produces no D antigen and abnormal C antigen) (3)

Uncommon Rh phenotypes

The D antigen contains over 30 epitopes. Variations of the D phenotype arise when these epitopes are only weakly expressed ("weak D phenotype") or when some are missing ("partial D phenotype").

Weak D: all D antigen epitopes are present but are underexpressed

"Weak D" is a Rh phenotype found in less than 1% of Caucasians and is only slightly more common in African Americans (2). It is typically caused by a single amino acid switch in the transmembrane region of the RhD protein. This disrupts how the RhD protein is inserted into the RBC membrane, reducing the level of expression of RhD. In most cases, adequate levels of D antigen are present and because there has been no change in D epitopes, the formation of anti-D is prevented. Therefore, individuals with the weak D phenotype can receive Rh D-positive blood.

Partial D: some D antigen epitopes are missing

In contrast, people who have been identified as having the "partial D" phenotype should not receive Rh D-positive blood but in practice, people with partial D are difficult to identify. This phenotype is usually caused by the creation of a hybrid RhD and RhCE protein. The hybrid protein is similar enough to RhD to be correctly inserted in the RBC membrane, but it lacks several epitopes found on the complete RhD protein. If a person with the partial D phenotype encounters the complete D antigen on transfused RBCs, they may form anti-D and suffer from a transfusion reaction.

Expression of Rh antigens

The Rh antigens are expressed as part of a protein complex in the RBC membrane. This complex is only expressed in cells of the erythroid line, and therefore Rh antigens are only expressed in RBCs. The composition of the complex is unknown, but it is thought to be a tetramer, consisting of two molecules of Rh-associated glycoprotein (RhAG) and two molecules of Rh proteins. The Rh proteins may be RhD (carrying the D antigen) or RhCE (carrying the C or c antigen and the E or e antigen). It is unknown whether both RhCE and RhD can be in a single complex, but in D-negative individuals the complex would only contain RhCE.

RhAG must be present to direct the Rh antigens to the RBC membrane. If it is missing, none of the Rh antigens are expressed. RHAG is related to the Rh proteins, sharing about 35% of their primary sequence and is the same type of transmembrane protein. However, it is not polymorphic and does not carry Rh antigens itself (3).

Function of Rh proteins

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.

Individuals with the rare Rh_{null} phenotype caused by the deletion of RHAG have RBCs that do not express any of the Rh antigens because they cannot be targeted to the RBC membrane. The absence of the Rh complex alters the RBC shape, increases its osmotic fragility, and shortens its lifespan, resulting in a hemolytic anemia that is usually mild in nature. These patients are at risk of adverse transfusion reactions because they may produce antibodies against several of the Rh antigens.

Rh antigens may also be involved in the transport of ammonium across the RBC membrane. Interestingly, the first member of a family of water channels (aquaporins) and the first member of a family of urea transporters were both found in blood group proteins (the Colton blood group and Kidd blood group, respectively).

Clinical significance of Rh antibodies

The Rh antigens are highly immunogenic, and most of the Rh antibodies should be considered as potential causes of hemolytic transfusion reactions and HDN.

Whereas most blood types are determined by red cell antigens that differ by one or two amino acids, the Rh blood group contains the D antigen which differs from the C/c and E/e antigens by 35 amino acids. This large difference in amino acids is the reason why the Rh antigens are potent at stimulating an immune response (4).

The majority of antibodies formed against the Rh antigens are of the IgG type. They are capable of causing significant HTR and HDN. Rh antibodies rarely, if ever, bind complement, and therefore RBC destruction is mediated almost exclusively via macrophages in the spleen (extravascular hemolysis).

There are a few examples of Rh alloantibodies that are naturally occurring and are of the IgM type, but they are in the minority.

Transfusion reactions

Anti-D, anti-C, anti-E, and anti-e have all been involved in hemolytic transfusion reactions, particularly delayed reactions (5).

Routine blood typing for Rh D status in both blood donors and transfusion recipients has reduced the incidence of transfusion reactions caused by anti-D. But sensitization to other Rh antigens can be a problem in transfusion medicine, particularly in patients with sickle cell anemia (SCA). SCA is more common in Blacks, and the treatment of SCA involves blood transfusions. Blacks are also more likely to express variants of the Rh e antigen, and therefore produce anti-e, along with other Rh alloantibodies, which increases the difficulty in finding Rh-compatible blood donors.

Hemolytic disease of the newborn

Anti-D causes the most severe form of HDN and it used to be a major cause of fetal death. Since the introduction of anti-D immunoglobulin along with careful monitoring of at-risk pregnancies, the prevalence of HDN because of Rh D incompatibility has decreased dramatically. However, all cases cannot be prevented, and RhD alloimmunization remains a major cause of disease (6).

Other Rh alloantibodies that are capable of causing severe HDN include anti-c (7, 8), which clinically is the most important Rh antigen after the D antigen.

Moderate disease can be caused by anti-C^W (9) and anti-C^X (10). Rh alloantibodies that are typically associated with mild HDN include anti-C (relatively common) (11), anti-E (12), and anti-e (13).

Molecular information

Gene

The Rh locus is located on the short arm of chromosome 1 (on 1p36-p34). It contains the RHD and RHCE genes, which lie in tandem. The RHD and RHCE genes are structural homologs and result from a duplication of a common gene ancestor.

RHD and RHCE each contain 10 exons and span a ~75-kb DNA sequence. The RHD gene is flanked by two 9-kb, highly homologous sequences called "Rhesus boxes" (14, 15). It is thought that unequal homologous recombination confined to the Rhesus boxes is a common cause of the deletion of the RHD gene, which is found in up to 40% of the population.

View the sequences of RHD and RHCE alleles at the
dbRBC Sequence Alignment Viewer

Protein

The RHD and RHCE genes each encode a transmembrane protein over 400 residues in length that traverses the RBC membrane 12 times. The RhD protein only differs from the common form of the RhCE protein by about 35 amino acids.

The RhD protein bears the D antigen which has over 30 epitopes. The RhCE protein carries the epitope for the C or c antigen on the second extracellular loop, and the epitope for the E or e antigen on the fourth extracellular loop. A number of nucleotide substitutions in the RHCE gene in turn cause a number of amino acid changes in the RhCE protein, but two polymorphisms are thought to be key in producing the polymorphic antigens on this protein, i.e., the S103P polymorphism (produces the C or c antigen, respectively), and the P226A polymorphism (produces the E or e antigen, respectively).

References

1. Reid ME and Lomas-Francis C. The Blood Group Antigen Facts Book. Second ed. 2004, New York: Elsevier Academic Press.
2. Avent ND, Reid ME. The Rh blood group system: a review. Blood. 2000;95:375–87. PubMed PMID: 10627438.
3. Daniels G. The molecular genetics of blood group polymorphism. Transpl Immunol. 2005;14(3-4):143–153. PubMed PMID: 15982556.
4. Westhoff CM. The Rh blood group system in review: a new face for the next decade. Transfusion. 2004;44:1663–73. PubMed PMID: 15504174.
    5. Daniels GL. Human Blood Groups. 2nd ed. 2002: Blackwell Science.
6. Urbaniak SJ, Greiss MA. RhD haemolytic disease of the fetus and the newborn. Blood Rev. 2000;14:44–61. PubMed PMID: 10805260.
7. Hackney DN, Knudtson EJ, Rossi KQ, Krugh D, O'Shaughnessy RW. Management of pregnancies complicated by anti-c isoimmunization. Obstet Gynecol. 2004;103:24–30. PubMed PMID: 14704240.
8. Appelman Z, Lurie S, Juster A, Borenstein R. Severe hemolytic disease of the newborn due to anti-c. Int J Gynaecol Obstet. 1990;33:73–5. PubMed PMID: 1974537.

9.   Bowman JM, Pollock J. Maternal CW alloimmunization. Vox Sang. 1993;64:226–30. PubMed PMID: 8517051.

10. Finney RD, Blue AM, Willoughby ML. Haemolytic disease of the newborn caused by the rare Rhesus antibody anti-CX. Vox Sang. 1973;25:39–42. PubMed PMID: 4198957.

11. Bowman JM, Pollock JM, Manning FA, Harman CR. Severe anti-C hemolytic disease of the newborn. Am J Obstet Gynecol. 1992;166:1239–43. PubMed PMID: 1566777.

12. Joy SD, Rossi KQ, Krugh D, O'Shaughnessy RW. Management of pregnancies complicated by anti-E alloimmunization. Obstet Gynecol. 2005;105:24–8. PubMed PMID: 15625137.

13. Chapman J, Waters AH. Haemolytic disease of the newborn due to Rhesus anti-e antibody. Vox Sang. 1981;41:45–7. PubMed PMID: 6798758.

14. Wagner F F, Flegel W A. RHD gene deletion occurred in the Rhesus box. Blood. 2000;95:3662–8. PubMed PMID: 10845894.

15. Wagner F F, Moulds J M, Flegel W A. Genetic mechanisms of Rhesus box variation. Blood. 2005;45(3):338–44. PubMed PMID: 15752150.

8. The Kell blood group

The Kell blood group system is complex and contains many antigens that are highly immunogenic. These antigens are the third most potent, after those of the ABO and Rh blood groups, at triggering an immune reaction.

Antibodies that target Kell antigens can cause transfusion reactions and hemolytic disease of the newborn (HDN). In the case of HDN, ABO and Rh incompatibility are more common causes. However, disease caused by maternal anti-ABO tends to be mild, and disease caused by maternal anti-Rh can largely be prevented. The infrequent cases of HDN caused by Kell immunization tend to result in severe fetal anemia because maternal anti-Kell target fetal red blood cell (RBC) precursors, suppressing the fetal production of RBCs.

At a glance

Antigens of the Kell blood group.

Number of antigens	**25** The K antigen is one of the most clinically significant Kell antigens.
Antigen specificity	**Protein** Amino acid sequence determines the specificity of Kell antigens
Antigen-carrying molecules	**Glycoprotein with enzymatic function** The Kell glycoprotein is a transmembrane, single-pass protein that carries the Kell antigens. It is an endothelin-3-converting enzyme; it cleaves "big" endothelin-3 to produce an active form that is a potent vasoconstrictor (1).
Molecular basis	**The KEL gene encodes the Kell antigens.** KEL is highly polymorphic. It has two major codominant alleles, k and K, which result from a SNP (698C→T), and the corresponding k and K antigens differ by a single amino acid change (T193M).
Frequency of Kell antigens	**~100%:** k, Kp^b, Ku, Js^b, K11, K12, K13, K14, K18, K19, Km, K22, K26, K27 **K antigen: 2%** in Blacks, **9%** in Caucasians, up to **25%** in Arabs **~2%:** Kp^a, U1^a **~0.01%:** Js^a (0.01% in Caucasians, 20% in Blacks), Kp^c, K23 Others: K17 (~0.3%), K24 (rare), VLAN (rare), K16 (unknown) (2)
Frequency of Kell phenotypes	**K-k+** in 91% Caucasians and 98% Blacks **K+k-** in 0.2% Caucasians and is rare in Blacks **K+k+** in 8.8% Caucasians and 2% Blacks **Kp (a-b+)** in 97.7% Caucasians and 100% Blacks **Js (a-b+)** in 100% Caucasians and 80% Blacks (2)

Antibodies produced against Kell antigens.

Antibody type	**IgG** IgM is uncommon
Antibody reactivity	**Does not bind complement** If hemolysis does occur, it is extravascular in nature.
Transfusion reaction	**Can cause a severe hemolytic transfusion reaction** Anti-K and anti-Ku are capable of causing a severe reaction. A milder reaction is caused by anti-k, anti-Kp^a, anti-Kp^b, anti-Js^a, and anti-Js^b.
Hemolytic disease of the newborn	**Can cause severe fetal anemia** Kell isoimmunization is the third most common cause of HDN after Rh and ABO. Anti-Kell causes severe fetal anemia by suppressing fetal RBC synthesis (3, 4).

Background information

History

The Kell blood group system was discovered in 1946. It was named for Mrs. Kelleher, a patient in whom anti-Kell antibodies had resulted in hemolytic disease of her newborn child (the child's RBCs expressed K antigen which were bound by anti-K in the mother's serum). Since this time, a total of 25 Kell antigens have been discovered and they are expressed in different frequencies in different populations. But the original K antigen remains of prime importance in transfusion medicine and HDN.

Nomenclature

- Number of Kell antigens: 25
- ISBT symbol: KEL
- ISBT number: 006
- Gene symbol: KEL
- Gene name: Kell blood group

Basic biochemistry

Common Kell phenotypes

The Kell blood group system is complex. The Kell locus is highly polymorphic and gives rise to many Kell antigens. There are, however, two major codominant allelic genes that produce two important antigens: K and k (previously known as Kell and Cellano, respectively), which differ by a single amino acid. The k antigen is more common than the K antigen in most populations, the K-k+ phenotype is found in 98% of Blacks and 91% of Caucasians (2).

Uncommon Kell phenotypes

Null phenotype

The Kell system has a rare null phenotype, K_O, in which RBCs lack all Kell antigens. Individuals with this phenotype are healthy but produce anti-Ku when they encounter RBCs that do express Kell antigens. Anti-Ku is capable of causing a mild to severe transfusion reaction with at least one fatal case being reported (5). Therefore, if K_O individuals ever require a blood transfusion, they should only be transfused with K_O blood products.

McLeod syndrome

In the RBC membrane, the Kell glycoprotein is covalently linked to the XK protein, a multipass membrane protein thought to have a role in transport. In the absence of XK, a condition called McLeod syndrome, Kell antigens are only weakly expressed and the RBCs are abnormal with spiky projections (acanthocytosis). Systemic findings include muscular dystrophy, cardiomyopathy, psychiatric disturbances, and neurological defects, such as loss of reflexes and movement disorders (1).

Expression of Kell antigens

Kell antigens were once thought to be restricted to blood cells of erythroid origin (i.e., RBCs and their precursors), but they have recently been found to be expressed in myeloid tissues (6, 7).

The Kell antigen is also expressed in a small amount on a number of organs including lymphoid organs, muscle (both cardiac and skeletal), and the nervous system (2).

Functions of the Kell glycoprotein

The Kell glycoprotein is an endothelin-3-converting enzyme. By cleaving an inactive precursor (big endothelin-3), it creates active endothelin-3, which is a potent constrictor of blood vessels.

Clinical significance of Kell antibodies

The K antigen is the most immunogenic antigen after the antigens of the ABO and Rh blood group systems.

Transfusion reactions

Anti-Kell antibodies are usually of the antibody class IgG (IgM is far less common). The antibodies that have been implicated in causing transfusion reactions, which can occasionally be severe in nature include, anti-K, anti-k, anti-Kp[a], and anti-Js[b] (2). The production of anti-Ku in patients with K[o] has resulted in a fatal hemolytic transfusion reaction (5).

Hemolytic disease of the newborn

Anti-Kell is an important cause of HDN. It tends to occur in mothers who have had several blood transfusions in the past, but it may also occur in mothers who have been sensitized to the Kell antigen during previous pregnancies.

In contrast to Rh and ABO sensitization, HDN attributable to Kell sensitization is caused by anti-K suppressing the fetal production of RBCs. Unlike Rh and ABO, Kell antigens are expressed on the surface of RBC precursors, and anti-K promotes the immune destruction of K+ erythroid early progenitor cells by macrophages in the fetal liver (rather than only mature fetal RBCs). Because the RBC precursors do not contain hemoglobin, less bilirubin is released during the hemolysis, and jaundice in the newborn period is less common. However, the underlying anemia may be severe (8).

Various case studies have reported the following antibodies causing HDN: anti-K (7, 9-12), anti-k (13), anti-Kp[a] (14), anti-Kp[b] (15), anti-Js[a] (16, 17), anti-Js[b] (16, 18), and anti-U1[a] (19).

Molecular information

Gene

The KEL gene is found on chromosome 7, at 7q33, and contains 19 exons that span more than 21 kbp of genomic DNA. The KEL gene is highly polymorphic, with different alleles at this locus encoding the 25 antigens that define the Kell blood group.

View the sequences of KEL alleles at the
dbRBC Sequence Alignment Viewer

The K/k blood group polymorphism represents a point mutation resulting in an amino acid switch from threonine 193 (in the k antigen) to methionine 193 (in the K antigen) in the Kell glycoprotein. The K antigen is more potent at triggering an immune reaction than the k antigen. Its higher level of antigenicity may be because, unlike other Kell antigens, it is *not* glycosylated at residue 191 (20).

Other common Kell blood group polymorphisms include Kp[b]/Kp[a] which arises from a 961C→T SNP causing the R281W amino acid change, and Js[b]/Js[a] which arises from a 1910T→C SNP causing the L597P amino acid change (21).

Protein

The Kell protein is a polypeptide chain of 732 amino acids in length that becomes glycosylated at five different sites. It makes a single pass through the RBC membrane.

The Kell protein is anchored to the surface of the RBC by being linked to an integral RBC membrane protein, XK, by a single disulfide bond. XK is a transmembrane protein that traverses the RBC membrane 10 times. If XK is absent, the multisystemic syndrome, McLeod's syndrome, results.

The Kell protein has both sequence and structural homology to a large family of zinc-dependant endopeptidases (enzymes that cleave proteins within the peptide chain, not near the N or C terminus). The Kell protein and other proteins in this family contain a pentameric sequence which is essential for zinc binding and catalytic activity.

References

1. Mohandas N, Narla A. Blood group antigens in health and disease. Curr Opin Hematol. 2005;12:135–40. PubMed PMID: 15725904.
2. Reid ME and Lomas-Francis C. The Blood Group Antigen Facts Book. Second ed. 2004, New York: Elsevier Academic Press.
3. Vaughan JI, Warwick R, Letsky E, Nicolini U, Rodeck CH, Fisk NM. Erythropoietic suppression in fetal anemia because of Kell alloimmunization. Am J Obstet Gynecol. 1994;171:247–52. PubMed PMID: 8030708.
4. Vaughan JI, Manning M, Warwick RM, Letsky EA, Murray NA, Roberts IA. Inhibition of erythroid progenitor cells by anti-Kell antibodies in fetal alloimmune anemia. N Engl J Med. 1998;338:798–803. PubMed PMID: 9504940.
5. Lin M, Wang CL, Chen FS, Ho LH. Fatal hemolytic transfusion reaction due to anti-Ku in a Knull patient. Immunohematol. 2003;19:19–21. PubMed PMID: 15373542.
6. Wagner T, Berer A, Lanzer G, Geissler K. Kell is not restricted to the erythropoietic lineage but is also expressed on myeloid progenitor cells. Br J Haematol. 2000;110:409–11. PubMed PMID: 10971399.
7. Wagner T, Resch B, Reiterer F, Gassner C, Lanzer G. Pancytopenia due to suppressed hematopoiesis in a case of fatal hemolytic disease of the newborn associated with anti-K supported by molecular K1 typing. J Pediatr Hematol Oncol. 2004;26:13–5. PubMed PMID: 14707704.
8. Daniels G, Hadley A, Green CA. Causes of fetal anemia in hemolytic disease due to anti-K. Transfusion. 2003;43:115–6. PubMed PMID: 12519439.
9. de Jonge N, Martens JE, Milani AL, Krijnen JL, van Krimpen C, Ponjee GA. Haemolytic disease of the newborn due to anti-K antibodies. Eur J Obstet Gynecol Reprod Biol. 1996;67:69–72. PubMed PMID: 8789754.
10. Fernandez-Jimenez MC, Jimenez-Marco MT, Hernandez D, Gonzalez A, Omenaca F, de la Camara C. Treatment with plasmapheresis and intravenous immunoglobulin in pregnancies complicated with anti-PP1Pk or anti-K immunization: a report of two patients. Vox Sang. 2001;80:117–20. PubMed PMID: 11348543.
11. Collinet P, Subtil D, Puech F, Vaast P. Successful treatment of extremely severe fetal anemia due to Kell alloimmunization. Obstet Gynecol. 2002;100:1102–5. PubMed PMID: 12423822.
12. Ahaded A, Brossard Y, Debbia M, Lambin P. Quantitative determination of anti-K (KEL1) IgG and IgG subclasses in the serum of severely alloimmunized pregnant women by ELISA. Transfusion. 2000;40:1239–45. PubMed PMID: 11061862.
13. Bowman JM, Harman FA, Manning CR, Pollock JM. Erythroblastosis fetalis produced by anti-k. Vox Sang. 1989;56:187–9. PubMed PMID: 2728396.
14. Costamagna L, Barbarini M, Viarengo GL, Pagani A, Isernia D, Salvaneschi L. A case of hemolytic disease of the newborn due to anti-Kp[a]. Immunohematol. 1997;13:61–2. PubMed PMID: 15387785.

15. Dacus JV, Spinnato JA. Severe erythroblastosis fetalis secondary to anti-Kp^b sensitization. Am J Obstet Gynecol. 1984;150:888–9. PubMed PMID: 6507516.
16. Gordon MC, Kennedy MS, O'Shaughnessy RW, Waheed A. Severe hemolytic disease of the newborn due to anti-Js(b). Vox Sang. 1995;69:140–1. PubMed PMID: 8585197.
17. Levene C, Rudolphson Y, Shechter Y. A second case of hemolytic disease of the newborn due to anti-Js^a. Transfusion. 1980;20:714–5. PubMed PMID: 7192023.
18. Stanworth S, Fleetwood P, de Silva M. Severe haemolytic disease of the newborn due to anti-Js(b). Vox Sang. 2001;81:134–5. PubMed PMID: 11555475.
19. Sakuma K, Suzuki H, Ohto H, Tsuneyama H, Uchikawa M. First case of hemolytic disease of the newborn due to anti-Ula antibodies. Vox Sang. 1994;66:293–4. PubMed PMID: 8079454.
20. Daniels G. The molecular genetics of blood group polymorphism. Transpl Immunol. 2005;14(3-4):143–153. PubMed PMID: 15982556.
21. Lee S, Russo D, Redman C. Functional and structural aspects of the Kell blood group system. Transfus Med Rev. 2000;14(2):93–103. PubMed PMID: 10782495.

9. The Duffy blood group

The Duffy glycoprotein is a receptor for chemicals that are secreted by blood cells during inflammation. It also happens to be a receptor for *Plasmodium vivax*, a parasite that invades red blood cells (RBCs) and causes malaria. RBCs that lack the Duffy antigens are relatively resistant to invasion by *P. vivax*. This has influenced the variation in Duffy blood types seen in populations where malaria is common.

Antibodies formed against the Duffy antigens are a cause of both transfusion reactions and hemolytic disease of the newborn.

At a glance

Antigens of the Duffy blood group.

Number of antigens	**6: Fy^a, Fy^b, Fy3, Fy4, Fy5, Fy6**
Antigen specificity	**Protein** Amino acid sequence determines the specificity of Duffy antigens.
Antigen-carrying molecules	**Glycoprotein that is a red cell receptor** The Duffy glycoprotein is a receptor that binds cytokines released during inflammation. It also binds the malaria parasite *Plasmodium vivax*, and RBCs that lack the Duffy Fy^a and Fy^b antigens are resistant to invasion. Structurally, the Duffy protein is similar to the family of G-protein coupled receptors, having 7 transmembrane domains.
Molecular basis	**The FY gene encodes the Duffy antigens.** FY has two major codominant alleles, FYA and FYB, which result from a SNP (125G→A), and the corresponding Fy^a and Fy^b antigens differ by a single amino acid (G42D). Individuals who are homozygous for a -33T→C SNP in the erythroid promoter region of the FYB allele have the phenotype Fy(a-b-) and do not express Duffy antigens on their RBCs.
Frequency of Duffy antigens	**Fy^a:** 66% Caucasians, 10% Blacks, 99% Asians **Fy^b:** 83% Caucasians, 23% Blacks, 18.5% Asians **Fy3:** 100% Caucasians, 32% Blacks, 99.9% Asians (1).
Frequency of Duffy phenotypes	**The Duffy null phenotype, Fy(a-b-), is very rare in Caucasians but is found in 68% of Blacks** (1). Fy(a+b+): 49% Caucasians, 1% Blacks, 9% Chinese Fy(a-b+): 34% Caucasians, 22% Blacks, <1% Chinese Fy(a+b-): 17% Caucasians, 9% Blacks, 91% Chinese

Antibodies produced against Duffy antigens.

Antibody type	**IgG** Mainly IgG, IgM is rare
Antibody reactivity	**Does not bind complement**
Transfusion reaction	**Typically a moderate, delayed transfusion reaction** Anti-Fy^a and anti-Fy^b can cause transfusion reactions that range from mild to severe in nature and may occur immediately after the transfusion (rarely) or more commonly, after a delay. Anti-Fy3 is a cause of mild to moderate, delayed transfusion reactions.
Hemolytic disease of the newborn	**Typically mild disease** Anti-Fy^a causes mild HDN (rarely, severe HDN can occur). Anti-Fy^b and anti-Fy3 are uncommon causes of mild HDN.

Background information

History

The Duffy blood group was discovered in 1950. It was named for a patient with hemophilia who had received multiple blood transfusions and was the first known producer of anti-Fy^a. A year later, anti-Fy^b was discovered in a woman who had had several children. The remaining Duffy antigens (FY3, FY4, FY5, and FY6) were discovered 20 years later, but from these, only FY3 appears to be clinically significant.

The frequency of the Duffy phenotypes varies in different populations. The Duffy null phenotype, Fy(a-b-), is rare among Caucasian and Asian populations, whereas it is the most common phenotype in Blacks, occurring in over two-thirds of the Black population. The racial variation in the distribution of Duffy antigens is a result of a positive selection pressure—the absence of Duffy antigens on RBCs makes the RBCs more resistant to invasion by a malarial parasite.

Worldwide, of the four *Plasmodium* species that routinely cause malaria in humans, *P. falciparum* is responsible for the majority of fatal cases (2). But in Asia and the Americas, *P. vivax* is a more common cause of malaria. To cause disease, *P. vivax* must first enter the human RBC, which it does by binding to the N-terminal extracellular domain of the Duffy glycoprotein through the cysteine-rich region of the Duffy binding protein (DBP) (3). Individuals with the Duffy null phenotype do not express the Duffy protein on their RBCs and therefore are immune to *P. vivax* infection.

Interestingly, the Fy(a-b-) phenotype is most common in areas where there is little *P. vivax* malaria (4). In areas of West Africa, there is a high frequency of the Fy (a-b-) phenotype and a low incidence of *P. vivax* malaria. This may be because the pre-existence of a high frequency of the Fy(a-b-) phenotype prevented *P. vivax* malaria from becoming endemic in West Africa (4, 5).

Nomenclature

- Number of Duffy antigens: 6
- ISBT symbol: FY
- ISBT number: 008
- Gene symbol: FY
- Gene name: Duffy blood group

Basic biochemistry

The Duffy glycoprotein is encoded by the FY gene, of which there are two main alleles, FYA and FYB. They are codominant, meaning that is the FYA is inherited from one parent and the FYB allele if inherited from the other, both gene products, Duffy Fy^a and Fy^b antigens, will be expressed on the RBCs.

Phenotypes

There are four main Duffy phenotypes:

- Fy(a+b-)
- Fy(a+b+)
- Fy(a-b+)
- Fy(a-b-)

The Fy^a and Fy^b antigens are found relatively frequently in Caucasians (Fy^a 66% and Fy^b 83%) and Asians (Fy^a 99% and Fy^b 18.5%) but are far less common in Blacks (Fy^a 10% and Fy^b 23%). In fact, the Fy(a- b-) phenotype is present in two-thirds of African-American Blacks but is very rare in Caucasians (1).

An important minor Duffy phenotype is the Fy^x [Fy(b+^{x})]. The FYX allele encodes the Fy^b antigen, but it is only weakly expressed because a reduced amount of Duffy protein, and it is not always detected by anti-Fy^b.

Expression of Duffy antigens

Duffy antigens are expressed on many different types of cells. Even Fy(a-b-) individuals who do not produce Duffy antigens on their RBCs do express Duffy antigens elsewhere, including endothelial cells that line blood vessels, epithelial cells of kidney collecting ducts, lung alveoli, and Purkinje cells of the cerebellum. Duffy antigens are also expressed in the thyroid gland, the colon, and the spleen.

Function of Duffy glycoprotein

The Duffy glycoprotein is also called the Duffy-Antigen Chemokine Receptor (DARC). As a chemokine receptor, it binds to the chemicals that are secreted by cells during inflammation and recruits other blood cells to the area of damage. These chemokines include C-X-R (acute inflammation chemokine) and C-C (chronic inflammation chemokine), IL-8 (interleukin 8), and RANTES (regulated on activation, normal T-expressed and secreted) (6).

Animal studies suggest that the function of Duffy as a chemokine receptor is not physiologically important because mice that lacked the mouse homolog of the Duffy gene (Dfy) were not more susceptible to infection than mice that expressed Dfy (7). Indeed, individuals with the null Duffy phenotype appear to have normal RBCs and a normal immune system.

Clinical significance of Duffy antibodies

Transfusion reactions

Antibodies against the Duffy antigens Fy^a (8), Fy^b (9, 10), Fy3 (1), and Fy5 (11,12) have all been implicated as the cause of a transfusion reaction. Anti-Fy^a is more commonly found in patients who are of African descent (in whom the Duffy null phenotype is more common) and have sickle cell anemia (and therefore may require multiple blood transfusions).

Hemolytic disease of the newborn

Maternal-fetal incompatibilities within the Duffy blood group system is an uncommon cause of HDN. The disease tends to be mild in nature. The Duffy antigens known to have caused maternal immunization and subsequent hemolytic disease are Fy^a (13-16), Fy^b (17), and Fy3 (1).

Molecular information

Gene

The Duffy locus, FY, is located on chromosome 1 at position q22-q23. It consists of two exons that span over 1,500 bp of genomic DNA. The two main alleles, FYA and FYB, differ by a single nucleotide at position 125 (G and A, respectively) and they likewise encode Fy^a and Fy^b antigens that differ by a single amino acid at residue 42 (glycine and aspartic acid, respectively).

View the sequences of FY alleles at the
dbRBC Sequence Alignment Viewer

There are two genetic backgrounds that give rise to the Duffy negative phenotype Fy(a-b-) (18). Most commonly, a mutation in the promoter region of the FYB allele abolishes the expression of the Duffy glycoprotein in RBCs, but the protein is still produced in other types of cells. This erythroid-specific mutation is found in African Americans (70%) and West Africans (approaching 100%) (19). Perhaps because the Duffy antigens are expressed in other tissues, these patients do not generally make anti-Fyb or anti-Fy3 (20).

Less commonly, the Fy(a-b-) phenotype is a result of point mutation that introduces a premature stop codon into the coding sequence. It is unlikely that the truncated Duffy protein is transported to the cell surface, and it is likely that the Duffy protein would be absent from all tissues in individuals who carry this type of mutation. There may be strong anti-Fy3 in these patients (20).

The molecular basis of the F^x [Fy(b+^{x})] phenotype is a mutation in the coding sequence 265C→T (Arg897Cys), which always occurs with another mutation, 298G→A (Ala100Thr) (18).

Protein

The Duffy glycoprotein is a transmembrane protein that spans the RBC membrane seven times and has an extracellular N-terminal domain and a cytoplasmic C-terminal domain. It shares structural similarity with G-protein coupled receptors but so far, it has not been shown to be a member of this family.

The binding site for chemokines, the binding site for *P. vivax*, and the major antigenic domains are all located in overlapping regions in the extracellular N-terminal domain.

References

1. Reid ME and Lomas-Francis C. The Blood Group Antigen Facts Book. Second ed. 2004, New York: Elsevier Academic Press.
2. Rayner J. Getting down to malarial nuts and bolts: the interaction between Plasmodium vivax merozoites and their host erythrocytes. Mol Microbiol. 2005;55:1297–9. PubMed PMID: 15720540.
3. VanBuskirk KM, Sevova E, Adams JH. Conserved residues in the Plasmodium vivax Duffy-binding protein ligand domain are critical for erythrocyte receptor recognition. Proc Natl Acad Sci U S A. 2004;101:15754–9. PubMed PMID: 15498870.
4. Livingstone FB. The Duffy blood groups, vivax malaria, and malaria selection in human populations: a review. Hum Biol. 1984;56:413–25. PubMed PMID: 6386656.
5. Carter R. Speculations on the origins of Plasmodium vivax malaria. Trends Parasitol. 2003;19:214–9. PubMed PMID: 12763427.
6. Mohandas N, Narla A. Blood group antigens in health and disease. Curr Opin Hematol. 2005;12:135–40. PubMed PMID: 15725904.
7. Luo H, Chaudhuri A, Zbrzezna V, He Y, Pogo AO. Deletion of the murine Duffy gene (Dfy) reveals that the Duffy receptor is functionally redundant. Mol Cell Biol. 2000;20:3097–101. PubMed PMID: 10757794.
8. Le Pennec PY, Rouger P, Klein MT, Robert N, Salmon C. Study of anti-Fya in five black Fy(a-b-) patients. Vox Sang. 1987;52:246–9. PubMed PMID: 3604183.
9. Talano J A, Hillery C A, Gottschall J L.et al. Delayed hemolytic transfusion reaction/hyperhemolysis syndrome in children with sickle cell disease. Pediatrics. 2003;111(6Pt1):e661–5. PubMed PMID: 12777582.
10. Kim HH, Park TS, Oh SH, Chang CL, Lee EY, Son HC. Delayed hemolytic transfusion reaction due to anti-Fyb caused by a primary immune response: a case study and a review of the literature. Immunohematol. 2004;20:184–6. PubMed PMID: 15373650.

11. Chan-Shu SA. The second example of anti-Duffy5. Transfusion. 1980;20:358–60. PubMed PMID: 7385332.

12. Bowen DT, Devenish A, Dalton J, Hewitt PE. Delayed haemolytic transfusion reaction due to simultaneous appearance of anti-Fya and Anti-Fy5. Vox Sang. 1988;55:35–6. PubMed PMID: 3420847.

13. Goodrick MJ, Hadley AG, Poole G. Haemolytic disease of the fetus and newborn due to anti-Fy(a) and the potential clinical value of Duffy genotyping in pregnancies at risk. Transfus Med. 1997;7:301–4. PubMed PMID: 9510929.

14. Babinszki A, Berkowitz RL. Haemolytic disease of the newborn caused by anti-c, anti-E and anti-Fya antibodies: report of five cases. Prenat Diagn. 1999;19:533–6. PubMed PMID: 10416968.

15. Weinstein L, Taylor ES. Hemolytic disease of the neonate secondary to anti-Fya. Am J Obstet Gynecol. 1975;121:643–5. PubMed PMID: 1115167.

16. Geifman-Holtzman O, Wojtowycz M, Kosmas E, Artal R. Female alloimmunization with antibodies known to cause hemolytic disease. Obstet Gynecol. 1997;89:272–5. PubMed PMID: 9015034.

17. 17.Vescio, L.A., et al., Hemolytic disease of the newborn caused by anti-Fyb. Transfusion, 1987. 27(4): p. 366.

18. Pogo AO, Chaudhuri A. The Duffy protein: a malarial and chemokine receptor. Semin Hematol. 2000;37:122–9. PubMed PMID: 10791881.

19. Daniels G. The molecular genetics of blood group polymorphism. Transpl Immunol. 2005;14(3-4):143–153. PubMed PMID: 15982556.

20. Rios M, Chaudhuri A, Mallinson G, Sausais L, Gomensoro-Garcia AE, Hannon J, Rosenberger S, Poole J, Burgess G, Pogo O, Reid M. New genotypes in Fy(a-b-) individuals: nonsense mutations (Trp to stop) in the coding sequence of either FY A or FY B. Br J Haematol. 2000;108:448–54. PubMed PMID: 10691880.

10. The Kidd blood group

The Kidd (JK) glycoprotein is the red blood cell (RBC) urea transporter. Situated in the membrane it rapidly transports urea into and out of RBCs, maintaining the osmotic stability and shape of the RBC in the process. The Kidd glycoprotein is also expressed in the kidney, where it enables the kidney to build up a high concentration of urea which is needed for the kidney to produce concentrated urine.

People who do not produce the Kidd glycoprotein tend not to be able to maximally concentrate urine, but despite this, they are healthy and their RBCs have a normal shape and lifespan.

Antibodies that target Kidd antigens are a significant cause of delayed hemolytic transfusion reactions. Anti-Kidd antibodies are also a cause of hemolytic disease of the newborn (HDN), the severity of the disease varies but tends to be mild in nature.

At a glance

Antigens of the Kidd blood group.

Number of antigens	**3: Jk1 (Jk^a), Jk2 (Jk^b) and Jk3**
Antigen specificity	**Protein** Amino acid sequence determines the specificity of Kidd antigens
Antigen-carrying molecules	**Glycoprotein that transports urea** The Kidd protein is a transmembrane, multi-pass protein that transports urea across the RBC membrane.
Molecular basis	**The SLC14A1 gene encodes the Kidd glycoprotein.** Located on chromosome 18 (18q11-q12), contains 11 exons that span more than 30 kbp of DNA. The SLC14A1 gene has two major codominant alleles, Jk^a and Jk^b, which result from a SNP (838G→A), and the corresponding Jk^a and Jk^b antigens differ by a single amino acid (D280N).
Frequency of Kidd antigens	**Jk^a**: 77% Caucasians, 92% Blacks, and 73% Asians **Jk^b**: 74% Caucasians, 49% Blacks, and 76% Asians **Jk3**: 100% in most populations, >99% in Polynesians (1)
Frequency of Kidd phenotypes	**Jk(a+b+): 50% Caucasians**, 41% Blacks, **49% Asians** **Jk(a+b-):** 26% Caucasians, **51% Blacks**, 23% Asians **Jk(a-b+):** 23% Caucasians, 8% Blacks, 27% Asians **JK(a-b-):** Rare in most populations, found in **0.9% Polynesians** (1)

Antibodies produced against Kidd antigens.

Antibody type	**IgG and IgM** IgG is more common
Antibody reactivity	**Capable of hemolysis** Can bind complement
Transfusion reaction	**Yes—common cause of delayed hemolytic transfusion reactions.** Anti-Jk^a and anti-Jk^b are dangerous antibodies because they can be difficult to detect in routine blood cross-matches. They are a common cause of delayed hemolytic transfusion reactions. Anti-Jk3 is rare and can cause immediate and delayed hemolytic transfusion reactions.
Hemolytic disease of the newborn	**Yes—typically mild disease.** Anti-Jk^a has been implicated in at least one severe case of HDN, but most cases of HDN caused by the anti-Kidd antibodies are mild in nature.

Background information

History

In 1951, a patient called Mrs. Kidd was found to have produced antibodies targeted against a then unknown red cell antigen during her pregnancy. The marker was present on the RBCs of her fetus, and the maternal antibodies targeted against it caused fatal hemolytic disease in her newborn child.

The protein was given the name Jk^a and was the first antigen to be discovered in the Kidd blood group system. Since this time, two other antigens, Jk^b and Jk3, have been found.

In 1959, the first example of the null phenotype, i.e., Jk(a-b-), was found in a woman who had become jaundiced after a blood transfusion. Her serum was found to contain an antibody that recognized both Jk^a and Jk^b. This antibody was subsequently named anti-Jk3.

Nomenclature

- Number of Kidd antigens: 3
- ISBT symbol: JK
- ISBT number: 009
- Gene symbol: SLC14A1
- Gene name: Solute carrier family 14, member 1

Basic biochemistry

Phenotypes

There are three common Kidd phenotypes: JK(a+b-), JK(a-b+), and JK(a+b+).

The Jk-null phenotype, JK(a-b-), is rare in most populations. Individuals with this blood type are often detected after they have been immunized to Kidd antigens during a previous blood transfusion or pregnancy. After immunization, JK(a-b-) individuals form anti-Jk3, which can cause HDN in subsequent pregnancies and hemolyse donor blood that contains Jk^a and/or Jk^b antigens during a subsequent blood transfusion.

Expression of Kidd antigens

The expression of the Kidd antigens is limited to RBCs and the kidney (in the vasa recta).

Function of Kidd protein

The Kidd protein is a major urea transporter in RBCs. It rapidly transports urea into and out of RBCs and in the process helps to maintain osmotic stability. The urea transport across Kidd null RBC membranes is ~1000 times slower than across normal RBC membranes (2, 3).

The transport of urea by the Kidd glycoprotein in the kidney enables the kidney medulla to maintain a high concentration of urea, which in turn enables the kidney to produce concentrated urine.

However, the absence of the Kidd glycoprotein is not associated with disease. The RBCs in Kidd null individuals have a normal shape and lifespan (3). Individuals with the Jk(a-b-) phenotype are unable to maximally concentrate urine, but it does not cause any other health problems (4).

Clinical significance of Kidd antibodies

The Kidd antibodies are often difficult to detect, making them hazardous in transfusion medicine, where they are suspected to be a common cause of delayed hemolytic transfusion reactions (DHTRs) (5).

Transfusion reactions

Anti-Jk^a can cause severe and fatal hemolytic transfusion reactions (6) but is more commonly associated with less severe DHTRs. It has been estimated that over one-third of DHTRs are caused by anti-Jk^a (7, 8). Case studies have also pointed to anti-Jk^b as being responsible for severe DHTR (9, 10). Anti-Jk3 has also been responsible for causing severe hemolytic transfusion reactions, both immediate and delayed (5).

Hemolytic disease of the newborn

During pregnancy, fetal Kidd antigens are capable of causing alloimmunization of the mother (11). But in contrast to the hemolytic activity of Kidd antibodies in incompatible blood transfusions, anti-Jk^a and anti-Jk^b are only rarely responsible for severe HDN (12). Likewise, anti-Jk3 is a rare cause of HDN, but the first documented case in Mrs. Kidd's newborn was fatal.

Molecular information

Gene

The SLC14A1 gene (Solute carrier family 14, member 1) is a member of the urea-transporter gene family and is located on chromosome 18 (18q12-q21). The gene is organized in 11 exons distributed across than 30kb of DNA. The first three exons and part of the fourth are not translated; exons 4-11 encode the mature Kidd protein.

The Jk^a and Jk^b antigens are the products of two alleles that are inherited in a co-dominant fashion. The Jk^a/Jk^b polymorphism results from a 838G→A transition, resulting in an D280N substitution (13). Based on this, several investigators have suggested different methods for JK genotyping (13–15).

The Jk(a-b-) phenotype is generally inherited as a recessive trait—a number of different mutations have been found to be responsible 16). In the Polynesian population where the null phenotype is less rare, a splice site mutation causes loss of exon 6 from mRNA transcripts and it is unlikely that the truncated Kidd protein produced is transported to the RBC membrane (17). A similar situation holds true in the Finnish population in which another genetic explanation causes the same phenotype (17, 18).

View the sequences of Kidd alleles at the
dbRBC Sequence Alignment Viewer

Protein

The Kidd protein urea transporter is an integral protein of the RBC membrane. It is a transmembrane protein containing 389 amino acid residues. The protein is predicted to span the membrane 10 times with both the N terminus and C terminus being intracellular. This membrane topology is shared by the anion exchanger that bears the Diego blood group antigens.

The Kidd protein consists of two hydrophobic domains that each span the membrane five times, and they are linked by a large glycosylated extracellular loop. The Asn211 on this third loop carries 1% of ABO antigens found on the RBC. The Jk^a/Jk^b polymorphism is found on the neighboring fourth extracellular loop (19).

References

1. Reid ME and Lomas-Francis C. The Blood Group Antigen Facts Book. Second ed. 2004, New York: Elsevier Academic Press.

2. Frohlich O, Macey R I, Edwards-Moulds J J.et al. Urea transport deficiency in Jk(a-b-) erythrocytes. Am J Physiol. 1991;260(4 Pt 1):C778–83. PubMed PMID: 1902060.

3. Mohandas J, Narla A. Blood group antigens in health and disease. Curr Opin Hematol. 2005;12:135–40. PubMed PMID: 15725904.

4. Sands J M, Gargus J J, Frohlich O, Gunn R B, Kokko J P. Urinary concentrating ability in patients with Jk(a-b-) blood type who lack carrier-mediated urea transport. J Am Soc Nephrol. 1992;2:1689–96. PubMed PMID: 1498276.

5. Daniels, G., Human Blood Groups. 2nd ed. 2002: Blackwell Scientific, Oxford, UK.

6. Daniels, G., Human Blood Groups. 1st ed. 1995: Blackwell Scientific, Oxford, UK.

7. Pineda A A, Taswell H F, Brzica S M. Transfusion reaction. An immunologic hazard of blood transfusion. Transfusion. 1978;18:1–7. PubMed PMID: 415392.

8. Ness P M, Shirey R S, Thoman S K, Buck S A. The differentiation of delayed serologic and delayed hemolytic transfusion reactions: incidence, long-term serologic findings, and clinical significance. Transfusion. 1990;30:688–93. PubMed PMID: 2219254.

9. Morgan P, Wheeler C B, Bossom E L. Delayed transfusion reaction attributed to anti-Jkb. Transfusion. 1967;7:307–8. PubMed PMID: 6031075.

10. Holland P V, Wallerstein R O. Delayed hemolytic transfusion reaction with acute renal failure. JAMA. 1968;204:1007–8. PubMed PMID: 5694777.

11. Geifman-Holtzman O, Wojtowycz M, Kosmas E, Artal R. Female alloimmunization with antibodies known to cause hemolytic disease. Obstet Gynecol. 1997;89:272–5. PubMed PMID: 9015034.

12. Matson G A, Swanson J, Tobin J D. Severe hemolytic disease of the newborn caused by anti-Jka. Vox Sang. 1959;4:144–7. PubMed PMID: 13669426.

13. Olives B, Merriman M, Bailly P, Barnett A, Todd J, Cartron J P, Merriman T. The molecular basis of the Kidd blood group polymorphism and its lack of association with type 1 diabetes susceptibility. Hum Mol Genet. 1997;Jul;6(7):1017–20. PubMed PMID: 9215669.

14. Irshaid N M, Thuresson B, Olsson M L.Genomic typing of the Kidd blood group locus by a single-tube allele-specific primer PCR technique. Br J Haematol. 1998 ;Sep;102(4):1010–4. PubMed PMID: 9734652.

15. Araujo F, Pereira C, Monteiro F, Henriques I, Meireles E, Lacerda P, Aleixo A, Rodrigues M J, Cunha-Ribeiro R M, Araujo F. Blood group antigen profile predicted by molecular biology-use of real-time polymerase chain reaction to genotype important KEL, JK,RHD, and RHCE alleles. Immunohematol. 2002;18(3):59–64. PubMed PMID: 15373552.

16. Irshaid N M, Eicher N I, Hustinx H, Poole J, Olsson M L. Novel alleles at the JK blood group locus explain the absence of the erythrocyte urea transporter in European families. Br J Haematol. 2002;116:445–53. PubMed PMID: 11841450.

17. Irshaid N M, Henry S M, Olsson M L. Genomic characterization of the kidd blood group gene:different molecular basis of the Jk(a-b-) phenotype in Polynesians and Finns. Transfusion. 2000;Jan;40(1):69–74. PubMed PMID: 10644814.

18. Sidoux-Walter F, Lucien N, Nissinen R, Sistonen P, Henry S, Moulds J, Cartron J P, Bailly P. Molecular heterogeneity of the Jk(null) phenotype: expression analysis of the Jk(S291P) mutation found in Finns. Blood. 2000;Aug 15;96(4):1566–73. PubMed PMID: 10942407.

19. Lucien N, Sidoux-Walter F, Roudier N, Ripoche P, Huet M, Trinh-Trang-Tan M M, Cartron J P, Bailly P. Antigenic and functional properties of the human red blood cell urea transporter hUT-B1. J Biol Chem. 2002;277:34101–8. PubMed PMID: 12093813.

11. The Diego blood group

The antigens of the Diego blood group are carried on an important protein, called the band 3 protein, which lies in the red blood cell (RBC) membrane. This protein is a chloride/bicarbonate exchanger involved in carbon dioxide transport from tissues to lungs. It also is found in the kidney, where it is involved in acid secretion.

Many mutations in the gene that encodes the Diego antigens, SLC4A1, are known. These mutations can result in RBCs with an abnormal membrane (hereditary ovalocytosis and spherocytosis) and kidneys that are defective in secreting acid (renal tubule acidosis). Other SLC4A1 mutations that do not give rise to disease may result in new blood group antigens that belong to the Diego blood group system.

At a glance

Antigens of the Diego blood group.

Number of antigens	21: Di^a, Di^b, and Wr^a are among the most significant
Antigen specificity	**Protein** Amino acid sequence determines the specificity of Diego antigens
Antigen-carrying molecules	**Glycoprotein that transports anions** The Diego protein is a transmembrane, multi-pass protein that is integral to the RBC membrane. It is an anion antiporter that exchanges Cl^- and $HCO3^-$ across the RBC membrane.
Molecular basis	**The SLC4A1 gene encodes the Diego antigens.** Located on chromosome 17 (17q21-22), the SLC4A1 gene contains 20 exons that span more than 18 kbp of DNA. The alleles Di^b and Di^a result from a SNP (2561C→T), and the corresponding Di^b and Di^a antigens differ by a single amino acid (P854L).
Frequency of Diego antigens	**Di^a is found mainly in populations of Mongolian descent.** It is found in 36% of South American Indians, 12% of Japanese, and 12% of Chinese, whereas it is rare in Caucasians and Blacks (0.01%). **Di^b is found universally in most populations** (1).
Frequency of Diego phenotypes	**Di(a-b+) is found in >99.9% of Caucasians and Blacks and >90% of Asians.** Di(a+b+) found in <0.1% of Caucasians and Blacks and in 10% Asians. Di(a+b-) found in <0.01% Caucasians, Blacks, and Asians. Di(a-b-) found in 1 case only (1).

Antibodies produced against Diego antigens.

Antibody type	**IgG or IgM** Anti-Di^a and anti-Di^b is IgG; anti-Wr^a is IgG or IgM (1).
Transfusion reaction	**Yes** Anti-Di^a and anti-Di^b are capable of causing a moderate to severe delayed transfusion reaction. Anti-Wr^a can cause an immediate hemolytic transfusion reaction (1).
Hemolytic disease of the newborn	**Yes** Anti-Di^a and anti-Wr^a can cause severe disease. Anti-Di^b tends to cause mild hemolytic disease (1).

Background information

History

The Diego blood group was discovered in 1955 and was named for the first patient to produce an antibody against the new blood system's antigens. The patient, Mrs. Diego, had given birth to a child affected by HDN.

Her serum was found to contain an antibody (now called anti-Di^a) which, during her pregnancy, had crossed the placenta to attack the RBCs of her fetus (which expressed the Di^a antigen).

In 1967, a second Diego antigen, Di^b, was discovered. It wasn't until 1995 that other Diego antigens began to be discovered.

At present, 21 Diego antigens are known, but it is the presence or absence of Di^a and Di^b that is of importance in determining a person's Diego blood type.

Nomenclature

- Number of Diego antigens: 21 (2)
- ISBT symbol: DI
- ISBT number: 010
- Gene symbol: SCLA1
- Gene name: Solute Carrier family 4, Anion exchanger, member 1

Note: The alternate gene symbol is AE1, which stands for Anion Exchanger 1. The alternate gene name is erythrocyte membrane protein band 3.

Basic biochemistry

Common phenotypes

The most common Diego phenotype is Di(a-b+), which is found in over 99.9% Caucasians and Blacks, and over 90% of Asians. The Di(a+b+) is found in 10% of Asians. Whereas the Di^a antigen is universally expressed in most populations, the prevalence of the Di^a antigen differs among races, making the Diego blood group of great interest to anthropologists (3).

In the USA, the Di^a antigen has not been found in Caucasian or Black blood donors (4). The Di^a antigen is more commonly found in Oriental people of Mongolian descent, being more common in the Japanese (12%) and the Chinese (5%). In South American Indians, up to 54% of the population carries the Di^a antigen (1).

Interestingly, the Di^a antigen is less rare in the Polish population (0.47%) (5) compared to most Caucasian populations (0.01%). This may reflect the gene admixture that resulted from the invasion of Poland by Tatars (Mongolian heritage) many centuries ago (6).

Expression of Diego antigens

The expression of Diego antigens is limited to RBCs and the kidney (in the distal tubule and the collecting tubule).

Function of Diego protein

Anion exchange across the RBC membrane

The SLCA41 protein is an anti-porter that plays an essential role in enabling the RBC to transport the waste product CO_2 to the lungs, where it can be removed from the body.

The SLCA41 protein exchanges one Cl$^-$ for one HCO3$^-$. The direction of the exchange depends on the concentration of the ions on either side of the RBC membrane. When levels of waste CO_2 are high, CO_2 diffuses across the RBC membrane and is converted into HCO3$^-$ which is transported out of the RBC in exchange for Cl$^-$. If anion exchange did not occur, HCO3$^-$ would accumulate inside the RBC and reach toxic levels, altering

the intracellular pH. In the lungs, the lower level of CO_2 encourages the direction of the exchange to reverse. Once inside the RBC, the $HCO3^-$ yields CO_2 which diffuses out of the RBC and is exhaled from the body.

Integral protein of the RBC membrane

The SLCA1 protein is an integral part of the RBC membrane. It helps anchor the membrane to the underlying spectrin skeleton. It helps the RBC to be stable and flexible, and maintain its biconcave shape.

Mutations of SLC4A1 can cause abnormally shaped RBCs that may be spherical (spherocytes, seen in hereditary spherocytosis), oval shaped (ovalocytes, seen in Southeast Asian ovalocytosis), or elliptical (elliptocytes). Because these RBCs are more fragile, they are prematurely removed from the circulation (hemolytic anemia).

Anion exchange across in the kidney tubule

SCLA1 is expressed in the kidney, where it also mediates the exchange of anions. Mutations that disrupt its function can cause a renal tubular acidosis in which the kidney fails to adequately excrete acid anions, allowing them to accumulate.

Clinical significance of Diego antibodies

Transfusion reactions

Anti-Di[a] and anti-Di[b] are more commonly associated with HDN than transfusion reactions. However, these antibodies are capable of causing immediate (9) and delayed hemolytic transfusion reactions (2, 8).

Hemolytic disease of the newborn

HDN caused by Diego antibodies are more common in South East Asia and South America.

Anti-Di[a] is capable of causing moderate to severe HDN, and cases have been reported in Japan (9), China (10, 11), and Poland (5).

Anti-Di[b] typically causes mild HDN. Cases have been reported in Japan (12), China (13), Poland (6), and in a mother of South American descent (14).

Molecular information

Gene

The SLC4A1 gene, also known as the AE1 gene, is a member of the anion exchanger (AE) gene family. SLC4A1 is located on chromosome 17q21-q22 and consists of 20 exons that are distributed over almost 18 kbp of genomic DNA.

The Di[a] and Di[b] antigens are produced as a result of a single nucleotide polymorphism (SNP) of the SLC4A1 gene. The result is at amino acid position 854; the common (wild-type) Di[b] antigen has a proline residue, and the Di[a] antigen has a leucine residue.

View the sequences of SLC4A1 alleles at the
dbRBC Sequence Alignment Viewer

Protein

The band 3 protein encoded by SLC4A1 is an important integral protein of the RBC membrane. It is 911 amino acids in length, and it loops across the RBC membrane 12 times.

The N terminal domain of the protein lies in the cytoplasm of the RBC, where it interacts with hemoglobin (influencing the exchange of anions) and also interacts with metabolic enzymes (influencing the metabolism of glucose inside the RBC).

Its C-terminal domain spans across the membrane of the RBC and mediates the exchange of chloride and bicarbonate anions across the membrane.

References

1. Reid ME and Lomas-Francis C. The Blood Group Antigen Facts Book. Second ed. 2004, New York: Elsevier Academic Press..
2. Daniels G L, Fletcher A.et al. Blood group terminology 2004: from the International Society of Blood Transfusion committee on terminology for red cell surface antigens. Vox Sang. 2004;87(4):304–316. PubMed PMID: 15585029.
3. Daniels G. Human Blood Groups, Second ed. 2002, Blackwell Science.
4. Komatsu F, Hasegawa K, Yanagisawa Y, Kawabata T, Kaneko Y, Watanabe S, Miyagi S, Sakuma M, Kagawa Y, Kajiwara M. Prevalence of diego blood group Dia antigen in Mongolians: comparison with that in Japanese. Transfus Apheresis Sci. 2004;30:119–24. PubMed PMID: 15062749.
5. Kusnierz-Alejska G, Bochenek S. Haemolytic disease of the newborn due to anti-Dia and incidence of the Dia antigen in Poland. Vox Sang. 1992;62:124–6. PubMed PMID: 1519367.
6. Lenkiewicz B, Zupanska B. The first example of anti-Diego(b) found in a Polish woman with the Di(a+b-) phenotype and haemolytic disease of the newborn not requiring treatment. Transfus Med. 2003;13:161–3. PubMed PMID: 12791084.
7. Hinckley M E, Huestis D W. Case report. An immediate hemolytic transfusion reaction apparently caused by anti-Dia. Rev Fr Transfus Immunohematol. 1979;22:581–5. PubMed PMID: 121166.
8. Yamane K, Yagihashi A, Sasaki M, Kuwashima K, Morio A, Watanabe N. A delayed hemolytic transfusion reaction (DHTR) with multiple alloantibodies (Anti-E, Jka, Dia, Fyb, and S) induced by E-antigen-negative, crossmatch-compatible blood. Immunopharmacol Immunotoxicol. 1998;20:531–9. PubMed PMID: 9805232.
9. Alves de Lima L M, Berthier M E, Sad W E, DiNapoli J, Johnson C L, Marsh W L. Characterization of an anti-Dia antibody causing hemolytic disease in a newborn infant. Transfusion. 1982;22:246–7. PubMed PMID: 7090038.
10. Ting J Y, Ma E S, Wong K Y. A case of severe haemolytic disease of the newborn due to anti-Di(a) antibody. Hong Kong Med J. 2004;10:347–9. PubMed PMID: 15479965.
11. Yung C H, Lin J S, Hu H Y, Lyou J Y, Chen Y R, Chen C R, Hao T C, Peng C S, Tzeng C H. [Hemolytic disease of the newborn caused by maternal anti-Di(a): a case report in Taiwan]. Zhonghua Min Guo Wei Sheng Wu Ji Mian Yi Xue Za Zhi. 1995;28:146–50. PubMed PMID: 9774993.
12. Uchikawa M, Shibata Y, Tohyama H, Mori H, Aisaka K, Nakagawa M. A case of hemolytic disease of the newborn due to anti-Dib antibodies. Vox Sang. 1982;42:91–2. PubMed PMID: 7199783.
13. Chen C C, Broadberry R E, Chang F C, Ding F, Lin M. Hemolytic disease of the newborn caused by maternal anti-Dib: a case report in Taiwan. Zhonghua Yi Xue Za Zhi (Taipei). 1993;52:262–4. PubMed PMID: 8258120.
14. Donato E, Guinot M, Vilar C, Garcia R, Canigral G. rHuEPO in the management of pregnancy complicated by anti-Dib. Transfusion. 2003;43:681–2. PubMed PMID: 12702197.

12. The MNS blood group

The antigens of the MNS blood group are carried on sugar-bearing proteins called glycophorins. These lie in the red blood cell (RBC) membrane. One end of a glycophorin is attached to the underlying cell, and the other end bears the sugars and determines a person's MNS blood type.

At a glance

Antigens of the MNS blood group.

Number of antigens	42: including M, N, S, and s
Antigen specificity	**Protein** Amino acid sequence determines the specificity of MNS antigens
Antigen-carrying molecules	**Glycophorins** Glycophorins are transmembrane, single-pass glycoproteins that contain carbohydrate, mostly in the form of sialic acid. Glycophorins A and B carry the MNS antigens, and they may also serve as receptors for cytokines and pathogens, including the malaria parasite, *Plasmodium falciparum*.
Molecular basis	**Two genes encode the MNS antigens, GYPA and GYPB.** Both genes are located on chromosome 4 (4q28.2-q13.1). A third gene, GYPE, may be involved in the creation of variant MNS antigens. GYPA has two codominant alleles, M and N, which result from three SNPs (59C→T, 71G→A, 72G→T), and the corresponding M and N antigens differ by two amino acids (S1L, G5E). The codominant alleles of GYPB, C and c, result from one SNP (143C→T), and the corresponding S and s antigens differ by a single amino acid (T29M).
Frequency of MNS antigens (%)	**M**: 78% Caucasians, 74% Blacks **N**: 72% Caucasians, 75% Blacks **S**: 55% Caucasians, 31% Blacks **s**: 89% Caucasians, 93% Blacks (1)
Frequency of MNS phenotypes (%)	**M+N+S-s+:** 22% Caucasians, **33% Blacks** **M+N+S+s+: 24% Caucasians,** 13% Blacks **M-N+S-s+:** 15% Caucasians, 19% Blacks **M+N-S+s+:** 14% Caucasians, 7% Blacks **M+N-S-s+:** 8% Caucasians, 16% Blacks **M-N+S+s+:** 6% Caucasians, 5% Blacks **M+N-S+s:** 6% Caucasians, 2% Blacks Less common phenotypes are M+N+S+s- (4% Caucasians, 2% Blacks) and M-N+S+s- (1% Caucasians, 2% Blacks). The phenotypes M+N-S-s-, M+N+S-s-, and M-N+S-s- are rare in Caucasians but are found in ~0.5% of Blacks (1).

Antibodies produced against MNS antigens.

Antibody type	**IgG and IgM** The Ig class depends upon which antigen is targeted.
Transfusion reaction	**Uncommon but potentially severe** Anti-S and anti-s are among the MNS antibodies implicated in causing transfusion reactions.
Hemolytic disease of the newborn	**Uncommon but potentially severe** Anti-S is more common that anti-s, but both are capable of causing severe-to-fatal HDN (2).

Background information

History

After the discovery of the first blood group, ABO, in 1900, Landsteiner and his colleagues continued to experiment with blood to identify other blood groups.

MNS was the second blood group, discovered in 1927, after immunizing rabbits with human RBCs. The M and N antigens were identified first, but it was another 20 years before the S and s antigens were named. Now, more than 40 antigens are known in this blood group, but the M, N, S, and s antigens remain the most common.

Nomenclature

- Number of MNS antigens: 43 (3)
- ISBT symbol: MNS
- ISBT number: 02
- Gene symbols: GYPA and GYPB
- Gene names: Glycoprotein A and Glycoprotein B

Note: A third locus, GYPE, lies adjacent to GYPB and is thought to be involved in the gene arrangements of the CYPB locus that result in the production of variant MNS alleles.

Basic biochemistry

Common phenotypes

In Caucasians, the most common phenotypes are M+N+S+s+ (24%), M+N+S-s+ (22%), and M-N+S-s+ (15%). The latter two phenotypes are common in Blacks also, occurring at a frequency of 33% and 19%, respectively (1).

Uncommon MNS phenotypes

Many of the uncommon MNS antigens result from mutations within the GYPA and GYPB genes. For example, the Mt^a antigen is produced by a single nucleotide polymorphism (SNP) in GYPA that results in a change of amino acid from threonine to isoleucine at position 58 in the GYPA protein. Likewise, the Vr antigen arises from a SNP that causes a Ser47Tyr change (4).

Other MNS antigens are created by swapping of DNA between the GYPA and GYPB genes, which lie close together on chromosome 4. The resulting hybrid glycoproteins bear new MNS antigens, e.g. the Stones antigen (St^a), Dantu antigen, Henshaw antigen (He), Mg, and the Miltenberger antigen (Mi^a).

The rare blood type En(a-) is characterized by RBC membranes that lack glycophorin A as a result of several different mutations. A deletion of the GYPB gene occurs in individuals with the rare blood type S-s-U- (also known as U-). A deletion of both GYPA and GYPB results in the MkMk phenotype. Such individuals lack expression of both glycophorin A and B on their RBCs.

Expression of MNS antigens

The MNS antigens are found mainly on RBCs. There are about 1 million copies of glycophorin A per RBC and 0.2 million copies of glycophorin B.

The MNS antigens are also expressed in the kidney (on the renal endothelium) and epithelium.

Function of the molecules that carry the MNS antigens

Glycophorins A and B may serve as receptors for cytokines, bacteria, and viruses, but the lack of the glycophorins does not result in disease, indicating that their function is not physiologically significant, at least in modern times.

Scientists are interested in these glycophorins because they bear the MNS antigens and because they may act as a receptor for *Plasmodium falciparum*. This is a parasite that causes malaria in humans. Individuals who have rare blood types in which either the glycophorin A or B is absent, e.g., phenotypes En(a-) and S-s-U-, have RBCs that are resistant to invasion by *Plasmodium*.

Clinical significance of MNS antibodies

Transfusion reactions

Anti-M and anti-N are not considered to be a cause of transfusion reactions, although rare cases of delayed transfusion reactions have occurred as a result of anti-M (5). Anti-M is fairly common and is thought to mostly be naturally occurring because it is frequently found in children who have never received a blood transfusion.

Mild to moderate transfusion reactions can be caused by the presence of anti-S and anti-s in the patient's serum (6, 7).

Severe transfusion reactions have been attributed to anti-U, anti-Vw, anti-Mur, and anti-En[a] (1, 8, 9).

Hemolytic disease of the newborn

Of the MNS antibodies, anti-S is more common than anti-s, and both are capable of causing severe hemolysis.

Less common causes of HDN include anti-M, anti-N, anti-U, anti-Mi[a], anti-Mt[a], and anti-En[a] (1, 10-15). Other MNS antibodies implicated in HDN are anti-Vw, anti-Mur, anti-Hut, anti-Hil, anti-Mv, anti-Far, anti-s[D], anti-Or, and anti-MUT. In addition, other antibodies to low-incidence MNS antigens should be considered as potentially harmful (1, 16-20).

Molecular information

Two genes encode the glycophorins that carry the antigens of the MNS blood group: GYPA and GYPB. Both are on the long arm of chromosome 4 in the region 4q28.2-q13.1. They are tightly linked, and recombination occurs between them.

A third gene, GYPE, is located next to GYPB and may play a role in the gene arrangements that result in new variant alleles.

GYPA and GYPB are similar, sharing up to 97% sequence homology. In fact, the 5'-GYPA-GYPB-GYPE-3' gene cluster is thought to have originated from a single ancestral gene that underwent two duplications.

View the alleles of GYPA, GYPB, and GYPE loci at the
dbRBC Sequence Alignment Viewer

The GYPA locus

The GYPA gene consists of 7 exons that span more than 60 kbp. It has two allelic forms called MNS1 and MNS2, which produce the M antigen and N antigen, respectively. The alleles are identical, except for two amino acid

substitutions. The MNS1 allele encodes serine at residue 1 and glycine at residue 5. The MNS2 allele encodes leucine at residue 1 and glutamate at residue 5.

The GYPB locus

The GYPB gene consists of five exons that span more than 58 kbp. It has two allelic forms called MNS3 and MNS4, which produce the S antigen and the s antigen, respectively. The alleles differ in one amino acid. The MNS3 allele encodes a methionine at residue 29, whereas the MNS4 allele encodes a threonine at this position.

Protein

Glycophorins A and B are single-pass, transmembrane proteins. Glycophorin A contains abundant sialic acid, which contributes to the negative surface charge of the RBC membrane. It has three main domains: an extracellular domain (70 amino acids), the membrane spanning domain (22 amino acids), and an intracellular domain (39 amino acids). The M and N phenotypes differ from each other by one amino acid at positions 1 and 5 (as described above) in the extracellular N-terminal domain.

Glycophorin B is structurally similar to glycophorin A, also consisting of three domains but with a shorter intracellular domain of six amino acids. The S and s phenotypes differ from each other by one amino acid at position 29 (as described above) (21).

References

1.   Reid ME and Lomas-Francis C. The Blood Group Antigen Facts Book. Second ed. 2004, New York: Elsevier Academic Press.
2.   Daniels G. Human Blood Groups, Second ed. 2002, Blackwell Science.
3.   Daniels G L, Fletcher A.et al. Blood group terminology 2004: from the International Society of Blood Transfusion committee on terminology for red cell surface antigens. Vox Sang. 2004;87(4):304–316. PubMed PMID: 15585029.
4.   Storry J R, Coghlan G, Poole J, Figueroa D, Reid M E. The MNS blood group antigens, Vr (MNS12) and Mt(a) (MNS14), each arise from an amino acid substitution on glycophorin A. Vox Sang. 2000;78:52–6. PubMed PMID: 10729812.
5.   Sancho J M, Pujol M, Fernandez F, Soler M, Manzano P, Feliu E. Delayed haemolytic transfusion reaction due to anti-M antibody. Br J Haematol. 1998;103:268–9. PubMed PMID: 9792320.
6.   Brandes W W, Cahan A, Jack J A. Incompatible transfusion caused by anti-S. J Am Med Assoc. 1954;156:836–7. PubMed PMID: 13201415.
7.   Guastafierro S, Sessa F, Cuomo C, Tirelli A. Delayed hemolytic transfusion reaction due to anti-S antibody in patient with anti-Jk(a) autoantibody and multiple alloantibodies. Ann Hematol. 2004;83:307–8. PubMed PMID: 15064858.
8.   Rothman I K, Alter H J, Strewler G J. Delayed overt hemolytic transfusion reaction due to anti-U antibody. Transfusion. 1976;16:357–60. PubMed PMID: 951732.
9.   Molthan L. Intravascular hemolytic transfusion reaction due to anti-Vw+Mia with fatal outcome. Vox Sang. 1981;40:105–8. PubMed PMID: 6165142.
10. Duguid J K, Bromilow I M, Entwistle G D, Wilkinson R. Haemolytic disease of the newborn due to anti-M. Vox Sang. 1995;68:195–6. PubMed PMID: 7625079.
11. Telischi M, Behzad O, Issitt P D, Pavone B G. Hemolytic disease of the newborn due to anti-N. Vox Sang. 1976;31:109–16. PubMed PMID: 986104.
12. Dopp S L, Isham B E. Anti-U and hemolytic disease of the newborn. Transfusion. 1983;23:273–4. PubMed PMID: 6679391.

13. Novaretti M C, Jens E, Pagliarini T, Bonif cio Sle O, Dorlhiac-Llacer P E, Chamone Dd Dde A. Hemolytic disease of the newborn due to anti-U. Rev Hosp Clin Fac Med Sao Paulo. 2003;58:320–3. PubMed PMID: 14762491.
14. Broadberry R E, Lin M. The incidence and significance of anti-"Mia" in Taiwan. Transfusion. 1994;34:349–52. PubMed PMID: 8178333.
15. Field T E, Wilson T E, Dawes B J, Giles C M. Haemolytic disease of the newborn due to anti-Mt a. Vox Sang. 1972;22:432–7. PubMed PMID: 5032416.
16. Taylor A. M., Knighton G J. A case of severe hemolytic disease of the newborn due to anti-Verweyst (Vw). Transfusion. 1982;22:165–6. PubMed PMID: 7071922.
17. Wu K H, Chang J G, Lin M, Shih M C, Lin H C, Lee C C, Peng C T, Tsai C H. Hydrops foetalis caused by anti-Mur in first pregnancy--a case report. Transfus Med. 2002;12:325–7. PubMed PMID: 12383339.
18. Wu K H, Chu S L, Chang J G, Shih M C, Peng C T. Haemolytic disease of the newborn due to maternal irregular antibodies in the Chinese population in Taiwan. Transfus Med. 2003;13:311–4. PubMed PMID: 14617342.
19. van den Bos A G, Steiner K. Haemolytic disease of the newborn caused by anti-MUT (MNS 35). Vox Sang. 2004;87:208–9. PubMed PMID: 15569075.
20. Reid M E, Sausais L, Oyen R, Storry J R, Shukla H, Hsu T, Lim S M. First example of hemolytic disease of the newborn caused by anti-Or and confirmation of the molecular basis of Or. Vox Sang. 2000;79:180–2. PubMed PMID: 11111238.
21. Chasis J A, Mohandas N. Red blood cell glycophorins. Blood. 1992;80:1869–79. PubMed PMID: 1391951.