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## CHAPTER 10

### BLOOD GROUPS: ABO AND Rh

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The success of human blood transfusions requires compatibility for the two major blood group antigen systems, namely ABO and Rh. The ABO system is defined by two red blood cell antigens, A and B, whose presence or absence is determined by three alleles (A, B, O) segregating at a single genetic locus. An unusual feature of this system is the presence of serum IgM antibodies in healthy adults to whichever antigen (A or B) is *absent* from that individual's cells. The presence or absence of Rh antigens on red blood cells is determined by two alleles at another locus, Rh. Rh INCOMPATIBILITY between mother and infant may result in ERYTHROBLASTOSIS FETALIS, which can be prevented by passive immunization of the mother with anti-Rh antibodies (RHOGAM).

Many lives are saved throughout the world each year through the use of blood transfusions, by preventing death from loss of blood due to trauma, and by allowing performance of surgical procedures which would otherwise be impossible. However, it was not until the early nineteenth century that routine blood transfusion between humans became possible, following the discovery that genetically determined differences exist between the blood of different individuals. These differences must be identified and compatibility ensured before transfusions can be safely carried out.

Many genetic systems controlling blood groups in humans are known, and they have been of considerable importance in our understanding of human genetics, as well as in both clinical and forensic medicine. We will discuss briefly the major features of the two most important of these systems, those controlling the **ABO blood groups** and the **Rh factor**.

### ABO BLOOD GROUPS

#### *A and B antigens*

The ABO blood groups are defined by the presence of two alternative antigens on red blood cells, determined by three alternative alleles at a single genetic locus. Two basic rules governing this system are as follows:

- 1) The blood "type" is defined by the presence of **two red blood cell antigens, "A" and "B."** RBCs of type A have the A antigen on their surface, those of type B have antigen B, type AB red cells bear *both* antigens, while type O cells bear *neither* antigen.
- 2) "Natural" antibodies called **isoagglutinins** exist in an individual's serum, directed against whichever of the A and B antigens is *not* present on that person's red cells (we will examine the source of these antibodies later).

The presence of ABO antigens and antibodies (*isoagglutinins*) in the four blood types is summarized below:

BLOOD TYPE	RBC ANTIGENS	SERUM ANTIBODIES	FREQUENCY
A	A	anti-B	40%
B	B	anti-A	10%
AB	A and B	none	5%
O	none	anti-A and anti-B	45%

The success of blood transfusions depends on ensuring the compatibility of the blood types between donor and recipient. If the *recipient* has antibodies to the infused red cells, these red cells will be rapidly destroyed, resulting in a potentially lethal **transfusion reaction**. Type A blood given to a type B recipient, for instance, can result in such a reaction, since the recipient's serum contains anti-A antibodies.

#### *Blood typing & crossmatching*

The presence of these antigens and antibodies can be readily detected by the **agglutination** reaction; mixing type A plasma (which contains anti-B antibodies) with type B red cells, for instance, results in agglutination of the red cells which can be easily observed. The blood type of any given individual can be determined in this manner, carrying out the agglutination reaction with a set of standard antibody-containing sera. Before a blood transfusion is given, in addition to choosing donor blood only of the same ABO type, direct **crossmatching** of donor and recipient is also generally carried out. This involves mixing donor RBCs with the recipient's serum to guarantee that the original typing of donor and recipient was correct, and to detect any possible unexpected agglutination reactions. (This is important because the complexity of the ABO system is considerably greater than we are discussing here, and many other relevant blood group systems exist as well.)

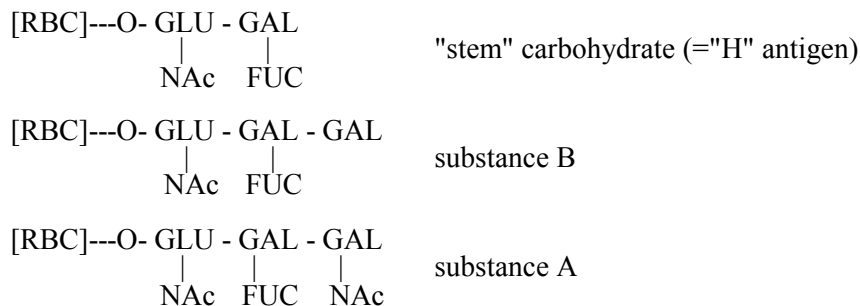
The primary cause of ABO mismatched transfusion reactions results from *destruction of donor red cells by the recipient's antibodies*; the reaction between donor antibodies and recipient cells is of less importance, since the small amount of antibody contained in the transfusion is generally diluted to harmless levels in the recipient. As a result, a type O individual (whose red cells bear neither antigen) is classically referred to as a **universal donor**, since his blood can fairly safely be given to a recipient with any ABO type; conversely, a person of blood type AB has been called a **universal recipient**, since his blood will contain no AB antibodies to damage any transfused red cells. Modern terminology more accurately refers to type O as a **universal red cell donor**, and to type AB as a **universal plasma donor**. In general, however, every effort is made to assure complete compatibility

between donor and recipient ABO blood groups (as well as the Rh type which we will discuss below) provided that an appropriate donor is available.

The safety of blood transfusions which are not perfectly matched can be enhanced by transfusing packed red blood cells only, without the antibody-containing plasma; type O red blood cells are safe when given to any recipient, and the potential damage done by the donor's anti-A and anti-B antibodies may be avoided in this manner. But two relevant points should be noted. First, transfusions of whole human blood are actually now fairly uncommon, and the *vast majority of transfusions involve the use of separated blood components* such as packed RBC's, plasma, platelets, leukocytes or purified plasma proteins. Second, perfect matching of donor and recipient for ABO and Rh, even for transfusion of packed RBCs, is still the norm, except in unusual or emergency situations.

*Structure of ABO antigens; "natural antibodies?"*

Why are there so-called "natural" antibodies to A and B blood group antigens? A description of the nature and distribution of these antigens will help answer this question. The blood group substances A and B represent two modified forms of a "stem" carbohydrate present on red blood cells and other tissues. Their structures are shown below (where GLU is glucosamine, GAL is galactose or galactosamine, FUC is fucose, and NAc represents an N-acetyl group):



*These same carbohydrates are also a common component of many foods we eat and many microorganisms in our intestinal tract.* The immune system is therefore constantly exposed to these antigens, and responds by making an effective humoral response. Since the immune system does not in general respond to antigens which are a normal part of "self" (see Chapter 18, TOLERANCE), a type B individual does not make antibodies to the B blood group substance, although the response to the type A antigens is robust. The net result is the production of antibodies, mostly of the **IgM** class, to whichever of these substances is *not* present on an individual's red blood cells. (It should be noted that such complex carbohydrates are a typical example of *thymus-independent antigens*, which generally elicit only IgM antibodies, discussed in Chapters 13 and 15.)

It is important to remember that the A and B blood group substances are present not only on red blood cells, but also in *virtually every other tissue*. They are therefore **important transplantation antigens** and must be taken into account together with HLA tissue-typing (see Chapter 11) when organ transplantation is performed. This is particularly important

given the fact that substantial levels of anti-A and anti-B antibodies (isoagglutinins) may be normally present, depending on the recipient's blood type.

### *Genetics of ABO*

The presence of A and B carbohydrates in our tissues is determined by three alleles at a single genetic locus. One allele encodes an enzyme which produces the A substance, another the B substance; and when both of these alleles are present in a heterozygote both carbohydrates are made. The third allele, O, behaves essentially as a "null" allele, producing neither A nor B substance. Thus, while the ABO system yields only *four blood types (phenotypes)*, there are six possible *genotypes*:

<u>Genotype</u>	<u>Blood Type (Phenotype)</u>
A/A	A
A/O	A
B/B	B
B/O	B
A/B	AB
O/O	O

Only a single genotype can produce the phenotype AB, namely the heterozygous state A/B. Likewise, type O individuals must be homozygous O/O. However, type A or type B individuals can be either homozygous or heterozygous, the O allele being effectively recessive since it does not contribute either of the two antigens.

The inheritance of the ABO blood groups follows simple Mendelian rules. For instance, a homozygous type A mother and a type AB father (below, left) can yield only two kinds of offspring, type A (genotype A/A) or type AB (genotype A/B). A heterozygous type A and a heterozygous type B, on the other hand (below, right), can yield four genotypes and four corresponding phenotypes.

A/A	X	A/B	A/O	X	B/O	<i>parents</i>
A/A		A/B	A/B	A/O	B/O	O/O
0.50		0.50	0.25	0.25	0.25	0.25
						<i>offspring frequency</i>

## **Rh BLOOD GROUPS**

### *Genetics*

While many blood group systems are known other than the ABO system, the Rh system is of special importance. This was originally defined by a rabbit antibody directed against the red blood cells of Rhesus monkeys, an antibody which turned out to be capable of distinguishing between the red blood cells of different human individuals. In simple terms, this system is defined by the presence or absence of a single red blood cell antigen, representing the two blood types Rh<sup>+</sup> and Rh<sup>-</sup>. These are determined by two alleles at a single locus, which segregate independently of the ABO blood group locus. Thus an Rh<sup>+</sup> individual may be homozygous (+/+) or heterozygous (+/-), while an Rh<sup>-</sup> individual must be homozygous (-/-).

The Rh<sup>-</sup> blood type is relatively uncommon, representing less than 15% of the population. Since Rh segregates independently of ABO, one can readily calculate the frequency of any given combination of ABO type and Rh type. If type A represents 40% of the population, and Rh<sup>-</sup> only 15%, then the frequency of type A, Rh<sup>-</sup> individuals is given by:

$$0.40 \times 0.15 = 0.06$$

or ~6% of the population.

So-called "natural" antibodies to Rh do not exist in humans, as they do for the AB antigens. However, Rh<sup>+</sup> cells infused into an Rh negative recipient can give rise to a strong antibody response, mainly of the IgG class, which can result in dangerous reactions to subsequent transfusions. Blood typing and cross-matching are therefore important to ensure compatibility for the Rh factor as well as ABO. However, unlike the A and B antigens, the *Rh antigens are present only on red blood cells*. Therefore, while they are important for blood transfusion, *they do not normally play a role in organ transplantation*, and Rh typing of organ donors and recipients therefore not a significant consideration.

#### *Rh-incompatibility; RhoGAM Therapy*

The Rh factor assumes a special importance in maternal-fetal interactions. A mother who is Rh<sup>-</sup> can bear an Rh<sup>+</sup> child if the father is Rh<sup>+</sup> (either homozygous or heterozygous). Since there are no natural anti-Rh antibodies, this generally poses no special risk for the first pregnancy. At the time of birth, however, tissue damage resulting from the separation of the placenta from the uterine wall can result in a significant amount of fetal blood entering the maternal circulation; which may stimulate a *strong IgG anti-Rh response* in the mother.

If the same mother then bears a *second* Rh<sup>+</sup> child, the existing anti-Rh antibodies can cross the placenta during the pregnancy and destroy fetal red blood cells. The ensuing damage to various organs results in the potentially dangerous condition **Erythroblastosis Fetalis** (also known as *Hemolytic Disease of the Newborn*, or **HDN**). This can be diagnosed prenatally by carrying out amniocentesis, and examining the amniotic fluid for the presence of free hemoglobin and its degradation products. Various approaches can be used during and after birth to rescue the infant, including **exchange transfusion**, complete replacement of the infant's blood to remove the anti-Rh antibodies and provide undamaged red blood cells.

However, the production of anti-Rh antibodies in an Rh<sup>-</sup> mother can often be prevented by administering anti-Rh immune globulin (*e.g.* **RhoGAM**) into the mother, typically at around 28 weeks of gestation and again within 72 hours of the birth of her Rh<sup>+</sup> baby. By mechanisms which are still not fully understood, these antibodies greatly reduce the likelihood of sensitization of the mother's immune system by the Rh<sup>+</sup> erythrocytes. If this procedure, developed in the 1960's, is successfully carried out *during each Rh<sup>+</sup> pregnancy*, anti-Rh antibodies are not produced by the mother, and subsequent pregnancies will not be at risk.

While Rh incompatibility is of considerable clinical significance, it should be noted that *not all untreated incompatible pregnancies result in disease*. Only a small fraction of

incompatible pregnancies actually result in the production of maternal anti-Rh antibodies, and in only a fraction of these cases is there significant damage to the newborn.

***What about ABO incompatibility?***

If maternal antibodies to red blood cells can damage the developing fetus, why is incompatibility for ABO blood groups not as dangerous as Rh-incompatibility, particularly since ABO isoagglutinins normally exist in mothers which could potentially damage the infant even during a first pregnancy? The answer lies in the isotype of antibody produced in the two cases. Anti-Rh-antibodies are mainly IgG (typical of anti-peptide responses) which is capable of crossing the placenta and entering the fetal circulation. The natural antibodies (isoagglutinins) to A and B blood group substances, however, are mostly of the IgM class (typical of anti-carbohydrate responses) and therefore do not cross the placenta.

IgG antibodies to the A and B blood group antigens may develop in some individuals, and the resulting ABO incompatibility actually accounts for about two thirds of all *discernable* cases of HDN; such cases, however, are generally very mild and require little or no treatment. Thus, while ABO incompatibility is actually much more common than Rh incompatibility, it is much less likely to cause significant disease. In fact, *it appears that the presence of ABO incompatibility between mother and fetus may confer some protection against the development of clinical Rh incompatibility.*

CHAPTER 10, STUDY QUESTIONS:

1. How many potential genetic crosses exist in which parents with *identical* ABO phenotypes can produce children who *differ* from one another in their phenotypes?
2. Is the Rh antigen a T-DEPENDENT or T-INDEPENDENT ANTIGEN? (See Chapter 15).
3. Maternal-fetal ABO incompatibility, which is much more common than Rh incompatibility, may *protect* against the development of anti-Rh antibodies in Rh-incompatible pregnancies. Suggest an explanation for this phenomenon.