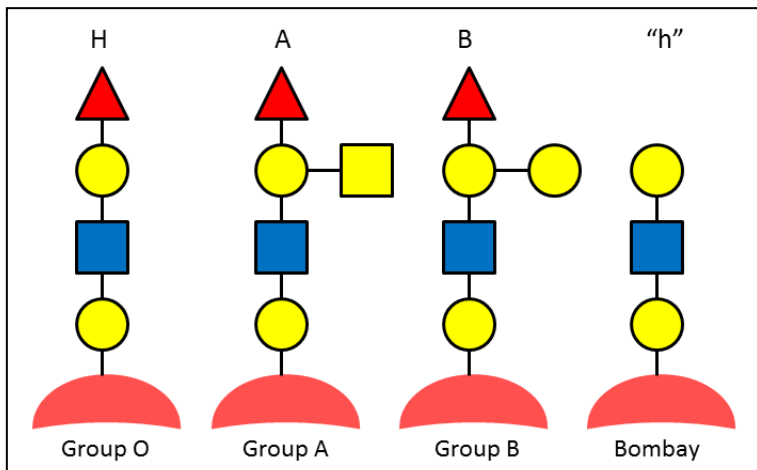


IMMUNOHEMATOLOGY

GENERAL PRINCIPLES. Blood transfusion has been practical since the 1920s, when its relatively simple rules were worked out. It is possible because most of the time what you really are doing is transplanting red blood cells. Red cells do not carry MHC antigens in humans, and the antigens they do carry are much less polymorphic in the population (that is, many fewer alleles). The white cells that come along as passengers are promptly recognized and destroyed. When people repeatedly need platelets, which *do* bear Class I HLA, they may develop an “alloimmunization” problem, in which case HLA typing as well becomes necessary. This isn’t trivial; HLA typing is expensive, and good matches are much harder to find.

The ability to transfuse red cells from one person to another depends on avoiding immune responses. When problems do arise, they are often immunological.

ABO (ABH) BLOOD GROUP ANTIGENS AND SUBSTANCES. Blood group **antigens** are glycolipids found on the surface of **all** body cells, including of course red cells. The lipid backbone spans the plasma membrane, and the sugars confer the antigenic specificity, A, B or O. Blood group **substances** are glycoproteins with the same sugars, found in the body fluids of people who have the *Secretor (Se)* phenotype. About 80% of people are secretors; their blood type can be determined from sweat stains, cigarette butts, etc. There are no particular advantages to being a secretor. People who are O are somewhat protected from pancreatic cancer and much less likely to develop venous thromboembolic disorders.



The boxes are standard symbols for different sugars—you can look them up in a book if you want to. A set of glycosyl transferases assemble the basic “core” sugar chain which almost everybody has—it is called the “H” antigen. Then a final glycosyl transferase, of which there are three alleles, can act. The O allele is an “amorph;” it does not code for a working transferase, and so group O people have only the basic core, the H antigen. People who are group A

have a glycosyl transferase allele which puts an additional sugar on the core chain, and people who are B have a different allelic form of this enzyme which adds a different sugar. Group AB individuals have both the A and B antigens on their red cells, because they got both the A and B transferases from their parents.

There are some people who lack the transferase gene that puts the final sugar on the “core,” and thus do not express even the H antigen. This is the Bombay phenotype ($O_{h,}$) and it is very rare. *Even type O blood* is foreign to such people.

ASK YOURSELF: What blood type would a Bombay individual appear to be, upon routine typing? If a Bombay person needed a blood transfusion, what kind of blood would you give her?

As they are very simple carbohydrate structures, it is not surprising that ABO antigens are found in many places in nature. This leads to an interesting and important observation:

You will inevitably have come into contact with these carbohydrates in the environment during your infancy; those which are not the same as your own are, of course, foreign to you, and you will become immunized to them. Thus a person who is group A will make antibody to B, but not to A; a person who is O (meaning she has neither A nor B) will be expected to have antibody to both A and B; and so on. These antibodies are sometimes called “naturally-occurring,” although like all antibodies we believe that their production is in response to antigenic stimulation. They are called **ISOHEMAGGLUTININS**. Incidentally, measuring their titer can be of use in the diagnosis of B cell immunodeficiency, since they should begin to appear in blood between 3 and 6 months of age, as antigen exposure occurs. **Isohemagglutinins are of the IgM class.**

ASK YOURSELF: A child with a history of repeated infections is found to have neither anti-A nor anti-B in her blood. Her T cells, including Tfh, are normal. Does this mean she has B cell immunodeficiency?

ABO (ABH) GROUPS.

Group	Cells	Plasma	Genotype	USA Frequency	
				White	Black
A	A	anti-B	AA or AO	42%	27%
B	B	anti-A	BB or BO	9	21
AB	AB	none	AB	3	4
O	O	anti-A and B	OO	46	48

Notes:

1. Some relatively isolated groups have very different distributions; e.g. certain Peruvian Indians are 100% group A, others 100% O, group O is rare in China, and group B is very common in Vietnam. This is interesting for anthropologists, who use these data to estimate relatedness of groups and times of migrations. It is important if your donor and recipient populations are different; it can be a problem for travelers.
2. There are variant A and B types (A₂, A₃, A_x, B_x, etc.) in which the A or B antigen is expressed rather weakly; such people may be typed incorrectly or with difficulty in the blood bank. Suppression of ABO antigens can be seen in some diseases such as leukemia. In addition, titers of isohemagglutinins can be low in the elderly and in hypogammaglobulinemia. Any of these conditions can lead to an “ABO discrepancy” (lack of correlation between ABO phenotype as determined by cell and serum typing) which must be resolved. DNA typing is possible.
3. AB is the rarest blood group, but group B people are better looking.

Rh. The second most important blood group system (there are over 600 identified blood group antigens!) and the only other one we will concern ourselves with here, is Rh, so called because it was discovered by workers studying rhesus monkeys. Rh antigens are on *proteins* coded for at two loci; one is for the alleles d/D, and the other for c/C and e/E. By far the most important allele here is **D**, so that if you say you are “Rh positive” you really mean “Rh(D) positive.” D is dominant over d (the “d” allele, another amorph, is heavily mutated and does not make a protein) so people who are DD or Dd type as Rh⁺. Ninety-two percent of U.S. blacks are Rh⁺; 85% of whites. Rh(D)⁻ is rare in sub-Saharan Africa. There are no “naturally occurring” isohemagglutinins for Rh; it’s a protein, and not ubiquitous in nature, so you don’t make antibody to it unless you’re Rh(D)⁻ and become immunized with Rh(D)⁺ red cells.

TYPING AND CROSS-MATCHING.

In the blood bank, the first process is “Type and Screen.” All donor units of blood are typed for ABO and Rh, and tested for syphilis, hepatitis B and C, HIV, and West Nile Virus antibody. In many blood banks the red cells are also typed for quite a long list of “minor” blood group antigens as well. Reverse typing is also performed, making sure that the isohemagglutinins in the plasma are appropriate for the determined red cell type, and screening for a list of other RBC antibodies. Units are then banked.

Prior to transfusion, the recipient is typed for ABO and Rh, and his plasma screened for expected and “unexpected” antibodies. The computer can then tell which donor units are compatible with the recipient. They must ordinarily be identical at ABO and Rh. Now you must ask: are there nevertheless antibodies in *this* recipient’s plasma which can react with antigens on *this* donor’s red blood cells? This question is called the **crossmatch**, and if the answer is yes, and you give the blood anyway, there will be shortened red cell survival and the recipient may be harmed, occasionally even fatally.

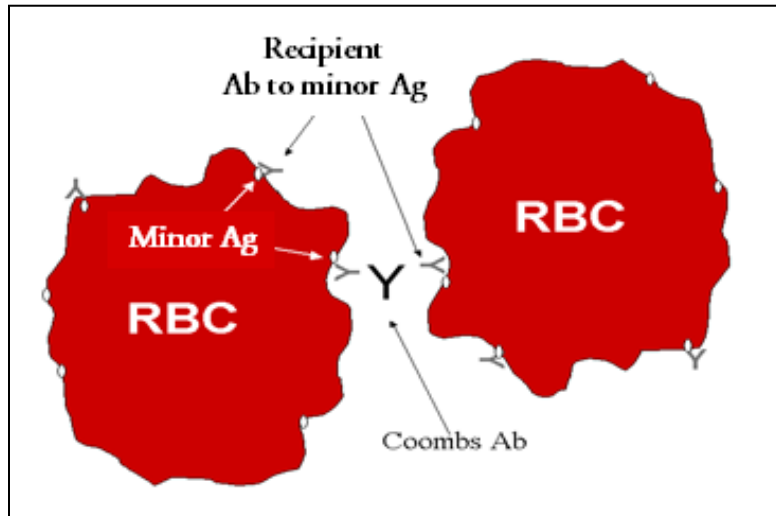
The crossmatch therefore is a lab test in which plasma from the prospective recipient is mixed with red cells from the donor. [Visualize and you won’t have to memorize.] The cells are first suspended in saline and a drop of the recipient’s plasma added. If there is agglutination, it means there are lots of high-avidity antibodies in the serum (probably IgM). These are most dangerous because of their potent complement-activating ability. If there is no agglutination, there may still be antibody, not enough to agglutinate but enough to opsonize the red cells and cause their destruction in the patient. So you try to enhance chances of agglutinating the cells, by doing the test in a low ionic strength diluent. Finally, to maximize the detection of clinically significant amounts of IgG antibody, you do an indirect antiglobulin (Coombs) test (below). If there is no agglutination, the blood and the recipient are considered “compatible.”

A “compatible” crossmatch does not guarantee normal survival of transfused cells; does not prevent immunization of the recipient to other blood groups; may not detect all unexpected antibodies in the recipient; will not prevent delayed hemolysis from a secondary antibody response in the recipient; will not detect all ABO or Rh typing errors. And a compatible crossmatch is useless if the blood is given to the wrong patient!

Group O red blood cells can be given to recipients of almost any phenotype (universal donor). Type AB people can be universal recipients.

ASK YOURSELF: Although Group O is the universal donor, this applies to red cells; whole blood should not be given. Can you think why?

ANTIGLOBULIN (COOMBS) TEST. This is a test which uses antibody-against-human-Ig to detect human Ig on the surface of red blood cells, or in plasma.



Imagine that a potential recipient has antibodies in her plasma, say against a minor antigen, which can react with the donor's red blood cells, but that the antigenic determinants are scarce, or maybe not easily accessible. The patient's antibody might not cross-link the cells, so you wouldn't know it was there, but it could lead to dangerous cell lysis if transfused. By adding antiglobulin serum, the bound human antibody can be revealed. In the blood bank, Polyspecific Antiglobulin Serum (Coombs serum) consists of

animal anti-human IgG plus anti-complement C3, since if one is present the other will probably be, too. For cross-matching, the indirect antiglobulin test is used.

The **indirect antiglobulin test** asks, is there unexpected antibody to red cell antigens in the plasma of this potential recipient? You take red cells, add the plasma, rinse the cells (we assume they haven't agglutinated,) and then add antiglobulin. If the cells now agglutinate, there must have been antibody to them in the plasma, because antiglobulin alone won't react with red cells.

The **direct antiglobulin test (DAT)** asks, is there antibody *already* on these cells I am interested in? You rinse off the cells and add antiglobulin to find out. The direct test detects cells that were coated with antibody *in vivo*. HDN, below, offers an example of its use.

[Memory aid: There's one more step in an indirect test.]

HEMOLYTIC DISEASE OF THE NEWBORN (HDN). This is also called erythroblastosis fetalis. It occurs in Rh(D)⁺ children of Rh(D)⁻ mothers. In the last trimester, and especially at the time of delivery, some red cells from the baby enter the mother's circulation. If she is Rh(D)⁻ and the baby is Rh(D)⁺, she will usually make anti-Rh(D). No problem for the baby, who isn't there anymore. But in a subsequent pregnancy with another Rh(D)⁺ fetus, the mother's antibodies, formed after the first pregnancy, can cross the placenta and destroy the fetus' red blood cells. In addition, each subsequent pregnancy with an Rh(D)⁺ fetus boosts her response. The fetus will be born jaundiced. This can be dangerous: high levels of bilirubin (a breakdown product of hemoglobin) can cross the blood-brain barrier and damage the basal ganglia, resulting in cerebral palsy or, if there is very severe damage, fetal death.

The disease is preventable if, at the time that the mother delivers her first Rh(D)⁺ baby, she is given IgG antibody to Rh(D) (Rh-immune globulin), the most familiar brand being Ortho's *RhoGAM*[®]. These antibodies combine with the fetal red cells, opsonizing them, and they are destroyed by phagocytes before they get a chance to immunize her. Note: she is not tolerant—just not immunized. She must receive Rh immune globulin *each time* there is a chance of being immunized by Rh(D)⁺ cells: this includes all subsequent normal deliveries, abortions, fetal manipulations, amniocenteses, etc.

A later development in the prevention of HDN is the practice of giving a shot of RhoGAM to Rh negative women at 28 weeks gestation, to prevent immunization by small transplacental bleeds during the third trimester. The Rh-immune globulin would also be given at or shortly after delivery, if the child turns out to be Rh(D)⁺.

ASK YOURSELF: All normal people except Group AB have isohemagglutinins (antibodies to A or B antigens), so a Group A mother has anti-B. If the father is BB, the fetus will be AB or B; why does her anti-B not destroy the fetus's red cells? Think this through before reading on.

This question is so important that we'd better reiterate the science now. Normally, isohemagglutinins are IgM, probably because the ubiquitous antigens that stimulate them are T-independent (carbohydrates are, remember?). So they don't cross the placenta. Anti-Rh antibodies are IgG (being anti-proteins), and do cross the placenta.

Note: Occasional people **do** make IgG isohemagglutinins. This is especially true of Group O people. So A or B fetuses of these women are at some risk of *ABO* hemolytic disease. There is no "RhoGAM" for this—are you clear on why?

ASK YOURSELF: There is a tendency, especially in isolated breeding populations, for blood group O to increase with time (although in groups that are pure "A," like some Native Peruvians, this doesn't happen). Can you think of a possible reason?

ASK YOURSELF: Can you think of a couple of situations in which it would be useful to do a direct antiglobulin test? an indirect antiglobulin test?

HETEROPHILE ANTIBODIES. These are antibodies to one antigen which bind, fortuitously, to another; a fancy name for cross-reactive antibodies. The best example is an antibody that appears in the serum of a patient with infectious mononucleosis; it is really in response to a viral antigen, but it happens to react also with sheep red blood cells, giving us a quick and cheap presumptive test (the Monospot) for mono. Another example is an antibody that people with syphilis make; there is a similar phosphodiester group in the bacterium *Treponema pallidum* and the phospholipids that can be extracted from beef heart (cardiolipin), so a simple test can be performed that doesn't require *Treponema*.

ASK YOURSELF: If you get this one right, buy yourself a beer or non-alcoholic beverage of your preference: A man is group A, his wife group O. They have a baby who turns out to be AB. The baby looks so much like him that he accuses her of non-maternity! But she really *is* the mother, and he really *is* the father. How could this be?

When you give blood
you give another birthday.
Another day at the beach,
Another night under the stars,
Another talk with a friend.
Another date, another dance,
Another laugh,
Another hug,
Another chance.

From the Federation of Bombay Blood Banks.

Learning Objectives for Immunohematology

1. For persons of the A, B, AB and O blood groups, give the following data: most and least common groups; red cell antigens; specificities of the ABO antibodies in their plasma; safe donors to that type; safe recipients of blood from that type; possible genotypes.
2. Name the antibody class of most ABO isohemagglutinins.
3. Explain the ABO antigen situation in a person of Bombay blood type, and the consequences of a transfusion of non-Bombay blood into such a patient.
4. Define the crossmatch, and explain why it is important. Explain how red cells are destroyed following a mismatched transfusion, and why this may be devastating to the recipient.
5. Compare and contrast the techniques of the direct and indirect antiglobulin tests and the questions they are designed to answer.
6. Identify two situations in which the direct antiglobulin test would be of value in diagnosis.
7. Identify two situations in which the indirect antiglobulin test would be of value in diagnosis.
8. Define heterophile antibody, and identify a common disease in which one type is increased enough to be useful diagnostically.
9. In Hemolytic Disease of the Newborn, explain:
 - a. The consequences of severe hemolysis in the newborn.
 - b. The way in which the mother becomes sensitized.
 - c. The class of antibody to Rh(D) the mother makes.
 - d. The consequences of sensitization to subsequent fetuses.
 - e. The role of Rh-immune globulin.
10. Explain the situation in which ABO hemolytic disease of the newborn can occur.