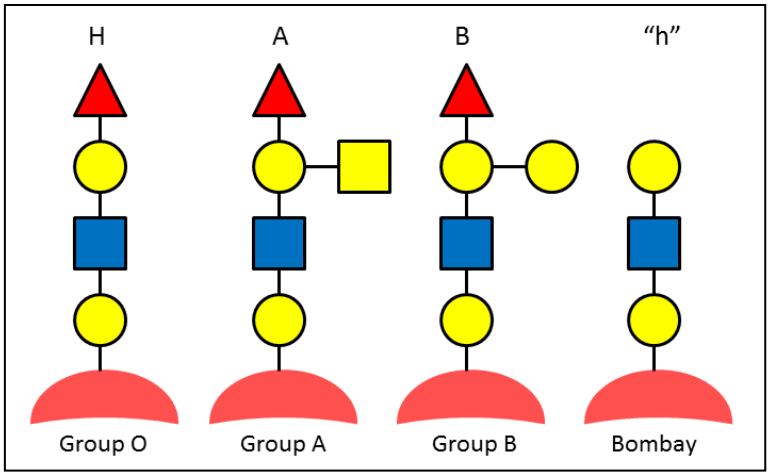
# IMMUNOHEMATOLOGY

**GENERAL PRINCIPLES.** Blood transfusion has been practical since the 1920s, when its relatively simple rules were worked out. It is possible because what you're really doing most of the time 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 soon 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, because 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 terminal 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 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 (red triangle) on the "core", and thus do not express even the H antigen, so there is no substrate for the A or B glycosyltransferases to modify. This is the Bombay phenotype ($O_h$,) and it is rare. All blood, *even type O*, 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.**

| | | | | USA Prevalence | |
|---|---|---|---|---|---|
| **Group** | **Cells** | **Plasma** | **Genotype** | **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, Ax, Bx, 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, O the most useful as blood donors, and group B people are cheerful, though irresponsible[1].

[1] **In Japan and some other parts of Asia, people interpret blood types (***ketsueki-gata)* **just as reliably as we do zodiac signs.** So Type *A* people are earnest, creative, and sensible, but fastidious and overearnest. Type *B* people are wild, active, and cheerful, but selfish and irresponsible. Type *AB* people are cool, controlled, and rational, but critical and indecisive. Type *O* people are agreeable, sociable, and optimistic, but vain and careless.

**Rh.** The second most important blood group system (there are over 600 identified blood group antigens!)[2] 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.

**When you donate blood.** 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 most blood banks the red cells are also typed for a select 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. Plasma and cells are then separated, and banked.

**When you get a transfusion.** Prior to transfusion, *recipients* are typed for ABO and Rh, and their plasma screened for expected and "unexpected" antibodies (using a panel of normal, phenotyped red cells). The computer can then tell which donor units are probably compatible with the recipient—this is called the "electronic crossmatch." They must be identical or compatible 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 **major crossmatch**, and if the answer is yes, and you give the blood anyway, in the worst-case scenario there will be generalized complement-mediated hemolysis, and free hemoglobin deposited in the kidneys which can lead to acute renal failure.

The crossmatch therefore is a lab test in which plasma from the prospective recipient is mixed with red cells from the prospective donor. [Visualize and you won't have to memorize. The big question is, will this recipient's plasma destroy the incoming red cells? Because if so, that could be catastrophic.] Donor red cells are first suspended in saline and a drop of the recipient's plasma added. The tube is then centrifuged gently and the supernatant checked for redness, which would indicate that hemolysis had occurred (the plasma contains complement, of course). The pellet is resuspended and examined for clumping of the red cells. If either test is positive that unit cannot be used.

> Usually this is enough testing for a typical, uncomplicated recipient. There are further tests that push the system to increase its sensitivity, which can be used for recipients whose electronic screen indicates they have made unexpected antibodies, perhaps due to previous transfusions.

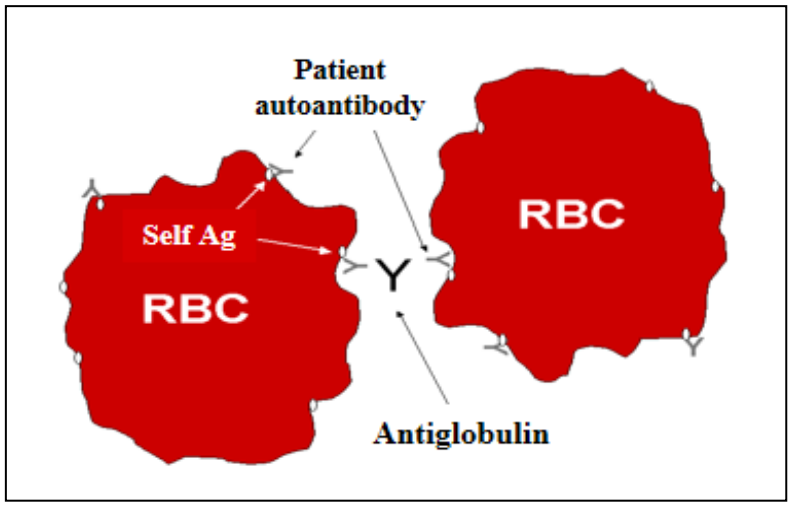
If there is no hemolysis or agglutination, the blood and the recipient are considered "compatible."

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

[2] "Blood group antigens" means red blood cell surface proteins or carbohydrates that are known to have allelic variants in the human population.

**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 (direct test) or in plasma (indirect).




Let's say we have a patient who, we've determined, has hemolytic anemia. Could it be due to an autoantibody she's making against her own red cells? We are sure the antibody wouldn't be enough (or of high enough avidity) to agglutinate her cells *in vivo*, because she'd be dead; but there could be enough to decrease RBC survival from 120 to 30 days, say, and that would make her anemic. So we take some of her RBC, wash them, and then add antibody against human IgG to them. If they had some human IgG sticking to their surface, this "antiglobulin" could cross-link it, and the cells would agglutinate. We've just described a *direct* antiglobulin test.

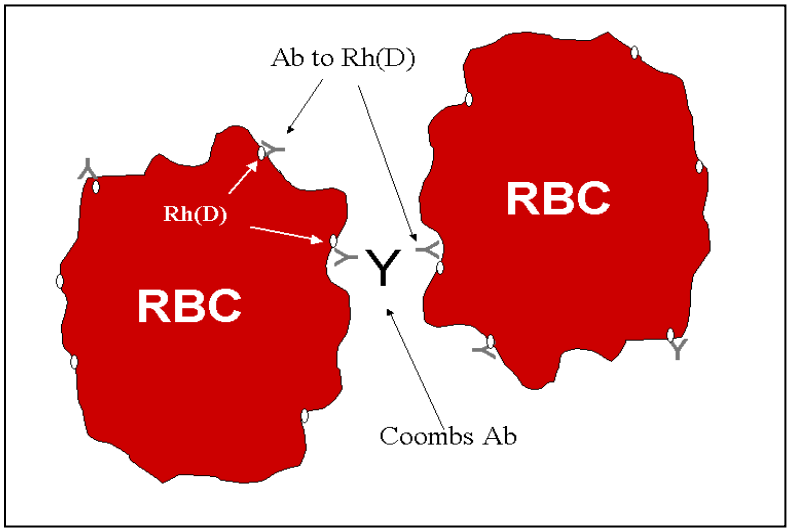
Back to the crossmatch described on the previous page: To really push for the detection of recipient antibody against potential donor RBC, we take the cells, add recipient plasma, and then wash off any unbound proteins. If there was antibody against the cells, it might bind but not agglutinate them. **But if now we added the antiglobulin,** it will cross-link the bound antibodies, and that would agglutinate the cells. This is an *indirect* antiglobulin test, and is done whenever there are questions about the major crossmatch. Even with adding this test the crossmatch can be completed in an hour.

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*.

The ►**indirect antiglobulin test (IAT)** 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. [Memory aid: There's always one more step in an indirect test.]

**HEMOLYTIC DISEASE OF THE NEWBORN (HDN).** This is also called erythroblastosis fetalis. It occurs in $Rh(D)^+$ babies of $Rh(D)^-$ mothers. In the last trimester, and especially at the time of delivery, some red cells from the fetus normally enter the mother's circulation. If she is $Rh(D)^-$ and the baby is $Rh(D)^+$, she may make anti-Rh(D). No problem for the baby, who soon isn't there anymore. But in a subsequent pregnancy with another $Rh(D)^+$ fetus, the mother's antibodies, made by B cell clones expanded in the first pregnancy and boosted by the second, 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 *RhoGAM*®. These antibodies combine with the fetal red cells, opsonizing them, and they are destroyed before they get a chance to immunize her. Note: she is not made 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, miscarriages, abortions, fetal manipulations, amniocenteses, etc.




Let's say we're visited by an $Rh(D)^-$ woman who's pregnant; she had two previous miscarriages. Did they immunize her against Rh(D)? We take some of her plasma and add it to ABO-compatible, $Rh(D)^+$ cells. We see nothing, but did it bind? So we now wash the cells and add antiglobulin; we see agglutination. She is, in fact, already immunized.

**ASK YOURSELF:** Was that a direct or indirect test?

> **ASK YOURSELF:** A newborn has a high bilirubin. He's $Rh(D)^+$ and mother is $Rh(D)^-$. Hemolytic disease of the newborn is possible, but there are other causes of hyperbilirubinemia. We take some of his red cells, wash then, and add antiglobulin. No agglutination is seen. How do you interpret these findings?

A later development in the prevention of HDN is the now universal 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 within 72 hours of 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?) Watch the movie. 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?

**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 refreshing frothy 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. Define heterophile antibody, and identify a common disease in which one type is increased enough to be useful diagnostically.

7. 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.

8. Explain the situation in which *ABO* hemolytic disease of the newborn can occur.