# CASE 44
# HEMOLYTIC DISEASE OF THE NEWBORN

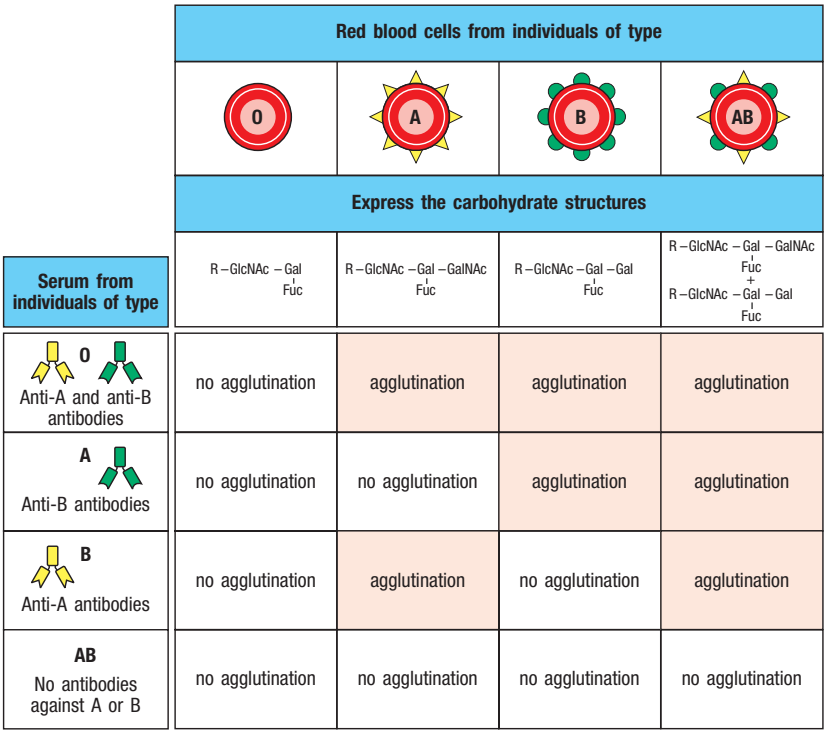
The adaptive immune response distinguishes genetic differences between individuals of the same species.

Adaptive immune responses evolved to protect vertebrate species against the world of microorganisms. However, anything discerned as 'nonself' may become a target of such responses, which can include molecular differences between individuals within a species. An antigenic determinant present in some members of a species but not in others is said to show polymorphic variation, and antibodies directed against such determinants are called alloantibodies. Perhaps the best-known alloantibodies are those that are used to determine our blood groups. Individuals who have red cells of type A have alloantibodies that react with the red blood cells of individuals who are type B and vice versa. Individuals who have red blood cells of type AB have no alloantibodies, and those with type O red blood cells have antibodies against both A and B red blood cells (**Fig. 44.1**). These alloantibodies arise because the capsules of Gram-negative bacteria, which inhabit our gut, bear antigens that stimulate antibodies that cross-react with the carbohydrate antigens of the ABO blood groups.

Alloantibodies induced by a fetus in the pregnant mother frequently cause serious problems. Alloimmunization most often results from Rhesus (Rh) incompatibility between mother and fetus. Approximately 15% of women are Rh-negative; that is, their red blood cells do not bear the Rh antigen. A woman who is Rh-negative has an 85% chance of marrying an Rh-positive man, and their chances of having an Rh-positive baby are very high. Not infrequently, during delivery of the newborn infant, some blood escapes from the fetal circulation into the maternal circulation, and as a result of this, the mother develops alloantibodies against the Rh antigen. During a subsequent pregnancy with an Rh-positive fetus, the maternal IgG alloantibodies cross the placenta and cause destruction of the fetal red cells, causing anemia. As we shall see, the consequences of this can be very serious and result in fetal death or severe damage to the newborn infant.

TOPICS BEARING ON THIS CASE:

- Antibody suppression of B-cell activation
- ABO blood groups
- Alloantibodies
- Rhesus blood group
- Coombs test
- Hemagglutination assays



| Serum from individuals of type | Red blood cells from individuals of type: O | A | B | AB |
|---|---|---|---|---|
| | Express the carbohydrate structures: R–GlcNAc–Gal (Fuc) | R–GlcNAc–Gal–GalNAc (Fuc) | R–GlcNAc–Gal–Gal (Fuc) | R–GlcNAc–Gal–GalNAc (Fuc) + R–GlcNAc–Gal (Fuc) |
| O — Anti-A and anti-B antibodies | no agglutination | agglutination | agglutination | agglutination |
| A — Anti-B antibodies | no agglutination | no agglutination | agglutination | agglutination |
| B — Anti-A antibodies | no agglutination | agglutination | no agglutination | agglutination |
| AB — No antibodies against A or B | no agglutination | no agglutination | no agglutination | no agglutination |

**Fig. 44.1 Hemagglutination is used to type blood and match compatible donors and recipients for blood transfusion.** Common gut bacteria bear antigens that are similar or identical to blood group antigens, and these stimulate the formation of antibodies against these antigens in individuals who do not bear the corresponding antigen on their own red blood cells (left column); thus, type O individuals, who lack A and B antigens, have both anti-A and anti-B antibodies, whereas type AB individuals have neither. The pattern of agglutination of the red blood cells of a transfusion donor or recipient with anti-A and anti-B antibodies reveals the individual's ABO blood group. Before transfusion, the serum of the recipient is also tested for antibodies that agglutinate the red blood cells of the donor, and vice versa, a procedure called a cross-match, which may detect potentially harmful antibodies against other blood groups that are not part of the ABO system.

The Rh antigenic determinants are spaced very far apart on the red cell surface. As a consequence, IgG antibodies against the Rh antigen do not fix complement and therefore do not hemolyze red blood cells *in vitro*. For reasons that are less well understood, IgG antibodies against the Rh antigen do not agglutinate Rh-positive red blood cells. Because of this it was very difficult to detect Rh antibodies until Robin Coombs at the University of Cambridge devised a solution to the problem by developing antibodies against human immunoglobulin. He showed that Rh-positive red blood cells coated with IgG anti-Rh antibodies could be taken from a fetus and agglutinated by antibodies against IgG. Furthermore, he showed that when the serum of an alloimmunized woman was incubated with Rh-positive red blood cells, these red blood cells could then be agglutinated by antibody against IgG (Fig. 44.2). The former is called the direct Coombs test and the latter the indirect Coombs test. This application of immunology to a vexing clinical problem led ultimately to treatment and prevention of the problem.

## The case of Cynthia Waymarsh: a fetus in immunological distress.

**Mrs Waymarsh was 31 years old when she became pregnant for the third time. She was known to have blood group A, Rh-negative red cells. Her husband was also type A but Rh-positive. Their first-born child, a male, was healthy. During her second pregnancy Mrs Waymarsh was noted to have an indirect Coombs titer at a 1:16 dilution of her serum. The fetus was followed closely, and the delivery of a healthy baby girl was induced at 36 weeks of gestation.**

**Five years later, Mrs Waymarsh became pregnant again. At 14 weeks of gestation her indirect Coombs titer was 1:8, and at 18 weeks it was 1:16. Amniotic fluid was obtained at 22, 24, 27, and 29 weeks of gestation and was found to have increasing**

*30.5 weeks: hematocrit up to 16.3%. Further transfusion requested.*

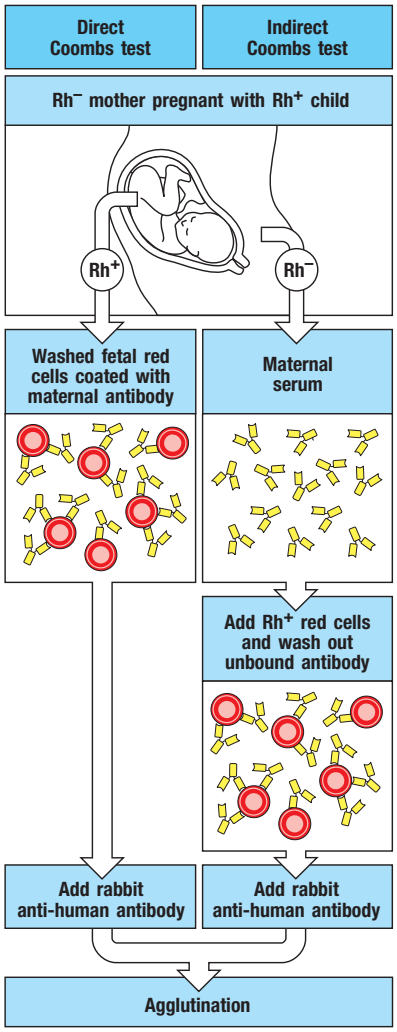



**Fig. 44.2 The Coombs direct and indirect anti-globulin tests for antibody to red blood cell antigens.** An Rh-negative ($Rh^-$) mother of an Rh-positive ($Rh^+$) fetus can become immunized to fetal red blood cells that enter the maternal circulation at the time of delivery. In a subsequent pregnancy with an $Rh^+$ fetus, IgG anti-Rh antibodies can cross the placenta and damage the fetal red blood cells. In contrast to anti-Rh antibodies, anti-ABO antibodies are of the IgM isotype; they cannot cross the placenta, and so do not cause harm. Anti-Rh antibodies do not agglutinate red blood cells, but their presence can be shown by washing the fetal red blood cells and then adding antibody against human immunoglobulin, which agglutinates the antibody-coated cells. The washing removes unrelated immunoglobulins that would otherwise react with the anti-human immunoglobulin antibody. Anti-Rh antibodies can be detected in the mother's serum in an indirect Coombs test; the serum is incubated with $Rh^+$ red blood cells, and once the antibody has bound, the red cells are treated as in the direct Coombs test.

**amounts of bilirubin (a pigment derived from the breakdown of heme, indicating that the fetus's red blood cells were being hemolyzed). At 29 weeks of gestation a blood sample was obtained from the umbilical vein and found to have a hematocrit of 6.2% (normal 45%). (The hematocrit is the proportion of blood that is composed of red cells, and because the volume of white cells is comparatively negligible, this is simply ascertained by centrifuging whole unclotted blood in a tube.) On finding that the fetus was profoundly anemic, 85 ml of type O, Rh-negative packed red blood cells were transfused into the umbilical vein. At 30.5 weeks of gestation another sample of blood from the umbilical vein was obtained; the hematocrit was 16.3%. The fetus was transfused with 75 ml of type O, Rh-negative packed red blood cells.**

**The fetus was examined at weekly intervals for the appearance of hydrops (see below), and none was observed. At 33.5 weeks of gestation, the hematocrit of a blood sample from the umbilical vein was 21%, so 80 ml of type O, Rh-negative packed red blood cells were again transfused into the umbilical vein. At 34.5 weeks of gestation it was determined that the fetus was sufficiently mature to sustain extrauterine life without difficulty; labor was induced and a normal female infant was born. The hematocrit in the umbilical vein blood was 29%. The baby did well and no further therapeutic measures were undertaken.**

## Hemolytic disease of the newborn.

*33.5 weeks: hematocrit up to 21%. Further transfusion requested.*

Although hemolytic disease of the newborn is most commonly the result of alloimmunization with Rh antigen, other red blood cell alloantigens, such as Lewis, Kell, Duffy, Kidd, and Lutheran, may cause alloimmunization. In each case, the maternal IgG antibodies cross the placenta in increasing amounts during the second trimester of pregnancy and hemolyze the fetal red blood cells. The resulting anemia may become so severe that, if untreated, the fetus goes into heart failure and develops massive edema; this is called *hydrops fetalis* and results in fetal death. The risk of fetal development of hydrops rises from 10% when the indirect Coombs titer of the mother is 1:16 to 75% when the maternal titer is 1:128. If the anemia is not so severe as to cause hydrops, the affected infant at birth is still massively hemolyzing red blood cells. The newborn must dispose of the heme breakdown pigments rapidly, because an excessive accumulation of bilirubin results in the deposition of this pigment in the brain and severe neurological impairments. In response to the profound anemia, the number of red blood cell precursors (erythroblasts) in the spleen, liver, and bone marrow expands rapidly; for this reason, hemolytic disease of the newborn has also been called erythroblastosis fetalis.

As we have seen in this case, the extent of hemolysis can be determined easily by obtaining amniotic fluid, into which the fetus begins to urinate by 20 weeks of

gestation. The quantity of bilirubin excreted into the amniotic fluid correlates with the amount of hemolysis in the fetus. Second, the fetus can be followed by ultrasonography for the development of hydrops. Third, the degree of anemia can be ascertained directly, but with some difficulty and risks, by obtaining a sample of blood from the umbilical vein.

It has become possible in the past few decades to eliminate hemolytic disease of the newborn to a very great extent. All Rh-negative women are given 300 μg of purified polyclonal IgG antibody derived from alloimmunized donors specific for the Rh antigen (RhIG) at 28 weeks of gestation, then again at 34 weeks and within 48 hours after delivery to prevent sensitization. The amount of RhIG in one vial (300 μg) is sufficient to neutralize 30 ml of fetal blood in the maternal circulation. This procedure fails to prevent alloimmunization in only 0.1% of Rh-negative women. It must be presumed in such failures that the fetus bled more than 30 ml of blood into its mother. Removal of the Rh antigen from the maternal circulation has been presumed to be the mechanism of action of RhIG. However, the limited efficacy of monoclonal antibodies generated against the Rh antigen in preventing alloimmunization suggests that RhIG has important effects in addition to Rh antigen clearance. The hypothesis that RhIG antibodies suppress the priming of maternal B cells, an early stage in B-cell activation, and prevent maternal anti-Rh antibody production is the focus of current investigation.

## Questions.

**1** *It was stated that the Rh antigens are so sparsely scattered on the red cell surface that IgG molecules bound to the Rh antigens are too far apart to fix C1q. Therefore, complement-mediated hemolysis cannot be invoked to explain hemolytic disease of the newborn. By what mechanism are the red cells destroyed?*

**2** *When an Rh-negative woman is ABO-compatible with her husband, as Mr and Mrs Waymarsh are, the risk of Rh alloimmunization is 16%. When they are ABO incompatible the risk falls to 7%. How do you explain this difference?*

**3** *Why were Rh-negative red blood cells used for the intrauterine transfusion?*

**4** *Do you have concerns about administering RhIG to women at 28 weeks of gestation?*

**5** *The serum of an Rh-negative woman who is pregnant gives a negative indirect Coombs test but her serum agglutinates Rh-positive cells suspended in saline. What is your interpretation of this phenomenon and what do you do about it?*