26 CHAPTER 26 Red Cell Antigens and Type II Hypersensitivity

26.1 KEY POINTS

- Type II hypersensitivity, also called cytotoxic hypersensitivity, occurs when an immune response destroys normal cells.
- The destruction of transfused red blood cells when administered to a mismatched recipient is an example of type II hypersensitivity. The disease is a result of the lysis of the transfused red cells by antibodies and complement.
- Mothers may become sensitized by their fetus during pregnancy and so make antibodies against fetal red cells. These antibodies, if ingested in colostrum, may cause destruction of a newborn animal's red cells. This is called hemolytic disease of the newborn.
- Some drugs may bind to blood cells and make them targets of a type II hypersensitivity reaction.

Red cells, like nucleated cells, have cell surface molecules that can act as antigens. However, unlike the major histocompatibility complex (MHC) molecules, red cell surface antigens are not involved in antigen processing, although they do influence graft rejection. (Allografts between blood group–incompatible animals are rapidly rejected.) Most red cell–surface antigens are either glycoproteins or glycolipids and are integral components of the cell membrane that serve key cellular functions. For example, the ABO antigens in humans are anion and glucose transporter proteins, whereas the antigens of the M and C systems of sheep red cells are associated with the membrane potassium pump and amino acid transport, respectively.

If blood is transfused from one animal to another, genetically different individual, the red cell antigens will stimulate an antibody response in the recipient. These antibodies will cause the rapid elimination of the transfused red cells as a result of intravascular hemolysis by complement, and extravascular destruction through opsonization and removal by the mononuclear phagocyte system. Cell destruction by antibodies in this way is classified as a type II hypersensitivity reaction.

26.2 BLOOD GROUPS

The antigens expressed on the surface of red blood cells are called blood group antigens or erythrocyte antigens (EAs). There are many different blood group antigens, and they vary in their antigenicity, some being more potent and therefore of greater importance than others. The expression of blood group antigens is controlled by genes and inherited in conventional fashion. Thus for each blood group system there exists a variable number of alleles. (If blood group alleles are invariably inherited together in groups of two or more, they are called phenogroups.) The alleles, in turn, control a variable number of EAs. The complexity of erythrocyte blood group systems varies greatly. They range from simple systems like the L system of cattle, which consists of two alleles controlling a single antigen, to the highly complex B system of cattle. The B system contains several hundred alleles or phenogroups that, together with the other cattle blood groups, may yield millions of unique blood group combinations. Although most blood group antigens are integral cell membrane components, some are soluble molecules found free in serum, saliva, and other body fluids and passively adsorbed onto red cell surfaces. Examples of such soluble antigens include the J antigens of cattle, the R antigens of sheep, the A antigens of pigs, and the dog erythrocyte antigen (DEA) 7 antigens of dogs.

Animals may make antibodies against foreign blood group antigens, even though they may never have been exposed to foreign red cells. For example, J-negative cattle have anti-J antibodies in their serum, and A-negative pigs have anti-A antibodies. These “natural” antibodies (or isoantibodies) are derived not from previous contact with foreign red cells but from exposure to cross-reacting epitopes that commonly occur in nature (see Chapter 7, Figure 7-8). Thus many blood group antigens are also common structural components of plants, bacteria, protozoa, and helminths. The presence of these natural antibodies is not, however, a uniform phenomenon, and not all blood group antigens are accompanied by the production of natural antibodies to their alternative alleles.

26.3 BLOOD TRANSFUSION AND INCOMPATIBLE TRANSFUSIONS

Blood is easily transfused from one animal to another. If the donor red cells are identical to those of the recipient, no immune response results. If, however, the recipient possesses preexisting antibodies to donor red cell antigens, they will be attacked immediately. These preexisting antibodies are usually of the immunoglobulin M (IgM) class. When these antibodies bind red cell antigens, they may cause agglutination or hemolysis, or stimulate opsonization and phagocytosis of the transfused cells. In the absence of preexisting antibodies, foreign red cells will stimulate an immune response in the recipient. The transfused cells then circulate until antibodies are produced and immune elimination occurs. A second transfusion with identical foreign cells results in their immediate destruction.

The rapid destruction of large numbers of foreign red cells can lead to serious illness. The severity of transfusion reactions varies from a mild febrile response to death and depends on the amount of incompatible blood transfused. Early recognition of a problem may avert the most severe consequences. The most severe reactions occur when large amounts of incompatible blood are transfused to a sensitized recipient. This results in complement activation and hemolysis of the transfused cells. Large amounts of free hemoglobin escape, resulting in hemoglobinemia and hemoglobinuria. Large numbers of lysed red cells may trigger blood clotting and disseminated intravascular coagulation. Complement activation also results in anaphylatoxin production, mast cell degranulation, and the release of vasoactive molecules and cytokines. These molecules provoke circulatory shock with hypotension, bradycardia, and apnea. The animal may show sympathetic responses such as sweating, salivation, lacrimation, diarrhea, and vomiting. This may be followed by a second stage in which the animal is hypertensive, with cardiac arrhythmia as well as increased heart and respiratory rates.

If a reaction is suspected, the transfusion must be stopped immediately. It is important to maintain urine flow with fluids and a diuretic because accumulation of hemoglobin in the kidney may cause renal tubular destruction. Recovery follows elimination of the foreign red cells.

Transfusion reactions can be prevented by prior testing of the recipient for antibodies against the donor's red cells. The test is called cross-matching. Blood from the donor is centrifuged and the plasma discarded. The red cells are then resuspended in saline and recentrifuged. This washing procedure is repeated (usually three times), and eventually a 2% to 4% suspension of red cells in saline is made. These donor red cells are mixed with recipient serum and then incubated at 37° C for 15 to 30 minutes. If the red cells are lysed or agglutinated by the recipient's serum, then no transfusion should be attempted with those cells. It is occasionally found that the donor's serum may react with the recipient's red cells. This is not of major clinical significance because transfused donor antibodies are rapidly diluted within the recipient. Nevertheless, blood giving such a reaction is best avoided.

26.4 HEMOLYTIC DISEASE OF THE NEWBORN

Female animals may become sensitized to foreign red cells not only by incompatible blood transfusions given for clinical purposes but also by leakage of fetal red cells into their bloodstream through the placenta during

pregnancy. In sensitized females, these anti–red cell antibodies may then be concentrated in their colostrum. When a newborn animal suckles, these colostral antibodies are absorbed through the intestinal wall and so reach its circulation. These antibodies, directed against the blood group antigens of the newborn, cause rapid destruction of their red cells. The resulting disease is called hemolytic disease of the newborn (HDN) or neonatal isoerythrolysis.

Four conditions must be met for HDN to occur. The young animal must inherit a red cell antigen from its sire that is not present in its mother. The mother must be sensitized to this red cell antigen. The mother's response to this antigen must be boosted repeatedly by transplacental hemorrhage or repeated pregnancies. Finally, a newborn animal must ingest colostrum containing high-titered antibodies to its red cells.

26.5 BLOOD GROUPS, BLOOD TRANSFUSION, AND HEMOLYTIC DISEASE IN DOMESTIC ANIMALS

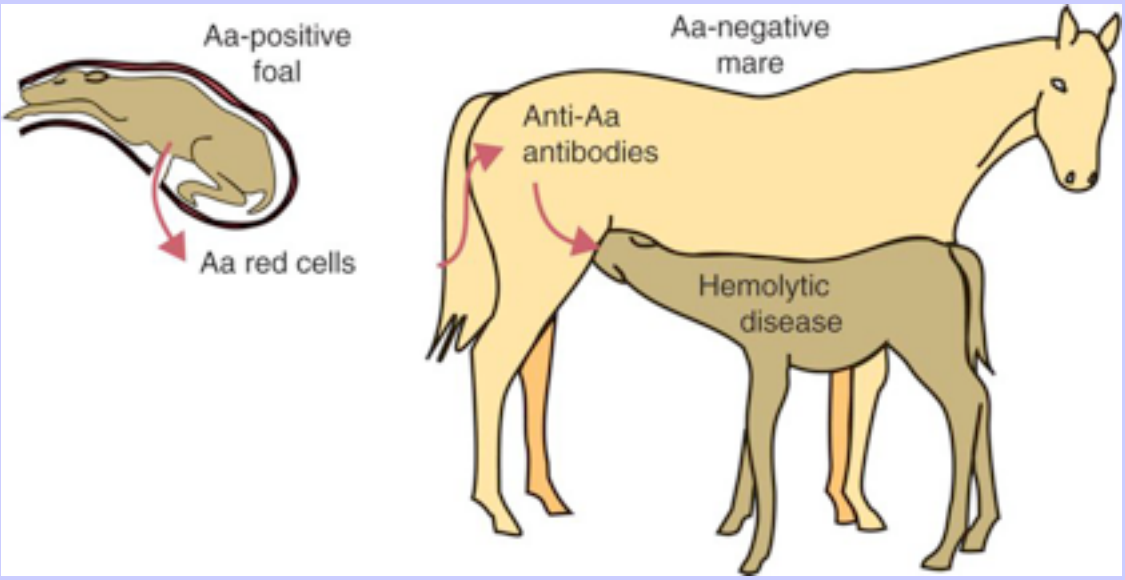
All mammals possess red cell antigens that can interfere with blood transfusions and on occasion cause hemolytic disease in newborn animals (Table 26-1). Although historically they were named alphabetically in order of their discovery, there is a growing tendency to add the prefix EA (erythrocyte antigen) to reduce confusion with MHC antigens.

26.5.1 Horses

Horses possess seven internationally recognized blood group systems (EAA, EAC, EAD, EAK, EAP, EAQ, and EAU). Some, such as EAC, EAK, and EAU, are simple, one-factor, two-allele, two-phenotype systems. On the other hand, the EAD system is very complex, with at least 25 alleles identified to date. Their major significance lies in the fact that HDN in foals is relatively common (Figure 26-1). In mules, in which the antigenic differences between dam and sire are great, about 8% to 10% of foals may be affected. In thoroughbreds and standardbreds, the prevalence is considerably less, ranging from 0.05% to 2% of foals. This is in spite of the fact that in up to 14% of pregnancies the mare and the stallion have incompatible red cells.

HDN may occur in foals from mares that have been sensitized by previous blood transfusions or by admin

FIGURE 26-1 The pathogenesis of hemolytic disease of the newborn in foals. In the first stage, fetal lymphocytes leak into the mother's circulation and sensitize her. In the second stage, these antibodies are concentrated in colostrum and are then ingested by the suckling foal. These ingested antibodies enter the foal's circulation and cause red cell destruction.



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istration of vaccines containing equine tissues. Most commonly, however, mares are sensitized by exposure to fetal red cells through repeated pregnancies. The mechanism of this sensitization is unclear, but fetal red cells are assumed to gain access to the maternal circulation as a result of transplacental hemorrhage. Mares have been shown to respond to fetal red cells as early as day 56 after conception. The greatest leakage probably occurs during the last month of pregnancy and during foaling as a result of the breakdown of placental blood vessels.

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Table 26-1 Domestic Animal Blood Groups

Species	Blood Group Systems	Serology
Horse*	A, C, D, K, P, Q, U	Agglutination
		Hemolytic
Bovine	A, B, C, F, J,† L, M, R,† S, Z, T′	Hemolytic
Sheep	A, B, C, D, M, R†	Hemolytic
		Agglutination (D only)
Pig*	A,† B, C, D, E, F, G, H, I, J, K, L, M, N, O, P	Agglutination
		Hemolytic
		Antiglobulin
Dog	DEA 1.1, 1.2, 3, 4, 5, 6, 7,† 8	Agglutination
		Hemolytic
		Antiglobulin
Cat	AB	Agglutination
		Hemolytic

* In these species there is growing acceptance of the convention to denote red cell antigens with the prefix "EA" (erythrocyte antigen).

† Soluble blood group substances.

Maternal sensitization is usually minimal following a first pregnancy. However, if repeated pregnancies result in exposure to the same red cell antigens, then the maternal response will be boosted. Hemolytic disease is therefore usually only a problem in mares that have had several foals. The most severe form of the disease results from the production of antibodies directed against the Aa antigen of the EAA system. Anti-Qa (EAQ system) produces a less severe disease of slower onset. All in all, 90% of clinical cases are attributable to anti-Aa and anti-Qa, although other minor antigens, such as Pa, Ab, Qc, Ua, Dc, and Db, have also been implicated. Mares that lack Aa and Qa are therefore most likely to produce affected foals. Pregnant mares may also produce antibodies to Ca (EAC system), but these are rarely associated with clinical disease. Indeed preexisting antibodies to Ca may reduce sensitization by Aa. The presence of this anti-Ca in a mare may cause the rapid elimination of any foal red cells that enter its bloodstream and so prevent further sensitization. A red cell antigen that is found in donkeys and mules but not horses causes hemolytic disease in mules. Thus horse mares can readily make antibodies to this donkey antigen.

Antibodies produced by mares do not cross the placenta but reach the foal through the colostrum. Affected foals are therefore born healthy but sicken several hours after suckling. The severity of the disease is determined by the amount of antibody absorbed and by the sensitizing antigen. The earliest signs are weakness and depression. The mucous membranes of affected foals may be pale and may eventually show a distinct jaundice. Some foals sicken by 6 to 8 hours and die from shock so rapidly that they do not have time to develop jaundice. More commonly the disease presents as lethargy and weakness between 12 and 48 hours of age, although it may be delayed for as long as 5 days. Icterus of the mucous membranes and sclera is consistent in foals that survive for

at least 48 hours. Hemoglobinuria, although uncommon, is pathognomonic in a newborn foal. As a result of anoxia, some foals in the terminal stages of the disease may convulse or become comatose.

Hemolytic disease is readily diagnosed by clinical signs alone. Hematological examination is of little diagnostic use but may be of assistance in indicating appropriate treatment. Definitive diagnosis requires that immunoglobulin be demonstrated on the surface of the red cells of the foal. In the case of anti-Aa or anti-Qa, addition of a source of complement (fresh normal rabbit serum) causes rapid hemolysis. If hemolytic disease is anticipated, the serum of a pregnant mare can be tested for antibodies by an indirect antiglobulin test. Using red cells from horses with a major sensitizing blood group, it is possible to show that the antibody titer increases significantly in the month before parturition, when sensitization is occurring.

A test that may be useful for detecting the presence of antierythrocyte antibodies in colostrum is the jaundiced foal agglutination test. This involves making serial dilutions of colostrum in saline. A drop of anticoagulated foal blood is added to each tube and the tubes are centrifuged so that the red cells form pellets at the bottom. In the presence of antibodies, the cells clump tightly and the pellets remain intact when the tubes are emptied. Unagglutinated red cells, in contrast, flow down the side of the tube. Concentrated colostrum is viscous and tends to induce rouleaux formation that mimics agglutination. However, if the mare's blood is used as a negative control, this can be accounted for. The foal's blood should also be diluted in saline to ensure that the foal has not already absorbed antibodies and that false-positive results are not obtained.

Mildly affected foals (with a packed cell volume [PCV] of 15% to 25% and a red cell count greater than 4×10^6) will continue to nurse. Those with a PCV of less than 10% will stop nursing and become recumbent. Marked icterus is suggestive of HDN in foals, but mild icterus may be seen in septicemia despite the fact that septic foals are not anemic.

The prognosis of uncomplicated hemolytic disease is good provided the condition is diagnosed sufficiently early and the appropriate treatment instituted rapidly. Management of HDN includes prevention of further antibody absorption, adequate nutrition, oxygen therapy, fluid and electrolyte therapy, and maintenance of the acid-base balance. Warmth, adequate hydration, and antimicrobial therapy are also critically important. In acute cases, blood transfusion is necessary. A red cell count less than 3×10^6/mL or a PCV less than 15% warrants a blood transfusion. Transfused equine red cells have a half-life of only 2 to 4 days, so that transfusion is only a temporary life-saving measure. Compatible blood may be difficult to find because of the high prevalence of Aa and Qa in the normal equine population. A donor should not only be Aa or Qa negative but should also lack antibodies to these antigens. Exchange transfusion, though efficient, requires a donor capable of providing at least 5 L of blood as well as a double intravenous catheter and an anesthetized foal. A much simpler technique that avoids many difficulties is transfusion of washed cells from the mare. About 3 to 4 L of blood is collected in sodium citrate and centrifuged, after which the plasma is discarded. The red cells are washed once in saline and transfused slowly into the foal. The blood is usually given in divided doses about 6 hours apart. Milder cases of hemolytic disease may require only careful nursing.

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If hemolytic disease is anticipated as a result of either a rising antibody titer or the previous birth of a hemolytic foal, stripping off the mare's colostrum and giving the foal colostrum from another mare may prevent its occurrence. The foal should not be allowed to suckle its mare for 24 to 36 hours. Once suckling is permitted, the foal should only be allowed to take small quantities at first and should be observed carefully for adverse side effects.

Neonatal thrombocytopenia has been recorded in the foal. Immunoglobulins can be identified on the foal's platelets, and antibodies to these platelets can be found in the mare's serum.

26.5.1.1 Serological Testing

Horse blood groups may be identified by agglutination, hemolytic, and antiglobulin tests. Each blood group system has a preferred test system. The complement used in the hemolytic test comes from rabbits, but it must be absorbed before use to remove any antihorse antibodies.

26.5.2 Cattle

Eleven blood group systems—A, B, C, F, J, L, M, S, T′, Z, and R′—have been identified in cattle. Two of these (B and J) are of the greatest importance. The B blood group system is one of the most complex systems known, since it is estimated to contain more than 60 different alleles. These alleles are not inherited independently but in combinations called phenogroups. Because of the complexity of the B system, it is practically impossible to obtain absolutely identical blood from any two unrelated cattle. Indeed, it has been suggested that the complexity of the B system is such that there exist sufficient different antigenic combinations to provide a unique identifying character for each bovine in the world. Naturally, such a system provides an ideal method for the accurate identification of individual animals, and many breed societies use blood grouping to check the identity of registered animals. The C system is also complex, with 10 alleles combining to form about 90 phenogroups.

The J antigen is a lipid found free in body fluids and passively adsorbed onto red cells. It is absent from the red cells of newborn calves but is acquired within the first 6 months of life. J-positive cattle are of two types. Some possess J antigen in high concentration, and this may be detected both on their red cells and in serum. Other animals may have low levels of J antigen in serum, and it is only with great difficulty detected on red cells. (It is probable that a secretor gene controls the expression of J in cattle.) J-negative cattle, lacking the J antigen completely, may possess natural anti-J antibodies, although the level of these antibodies shows a marked seasonal variation, being highest in the summer and fall. Because of the presence of these antibodies, transfusion of J-positive red cells into J-negative recipients may result in a transfusion reaction even in the absence of known previous sensitization.

HDN in calves is rare but has resulted from vaccination against anaplasmosis or babesiosis. Some of these vaccines contain red cells from infected calves. In the case of *Anaplasma* vaccines, for example, the blood from a large number of infected donors is pooled, freeze-dried, and mixed with adjuvant before being administered to cattle. The vaccine against babesiosis consists of fresh, infected calf blood. Both vaccines cause infection and, consequently, the development of immunity in the recipient animals. They may also stimulate the production of antibodies against blood group antigens of the A and F systems. Cows sensitized by these vaccines and then mated with bulls carrying the same blood groups can transmit colostral antibodies to their calves, which may then develop hemolytic disease.

The clinical signs of HDN in calves are related to the amount of colostrum ingested. Calves are usually healthy at birth but begin to show symptoms from 12 hours to 5 days later. In acute cases, death may occur within 24 hours after suckling, with the animals developing respiratory distress and hemoglobinuria. On necropsy these calves have severe pulmonary edema, splenomegaly, and dark kidneys. Less severely affected animals develop anemia and jaundice and may die during the first week of life. The red cells of affected calves have antibodies on their surface (detected by an antiglobulin test) and may sometimes be lysed by the addition of complement in the form of fresh normal rabbit serum. Death is due to disseminated intravascular coagulation as a result of activation of the clotting system by red cell ghosts.

26.5.2.1 Serological Testing

Bovine blood groups are detected by hemolytic tests. Washed red cells are incubated in specific antisera, and rabbit serum is used as a source of complement.

26.5.3 Sheep

The blood groups of sheep resemble those of cattle. Six blood group systems (A, B, C, D, M, and R) are

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FIGURE 26-2 The regulation of expression of R blood group antigens in sheep. The I gene controls expression of the R system.

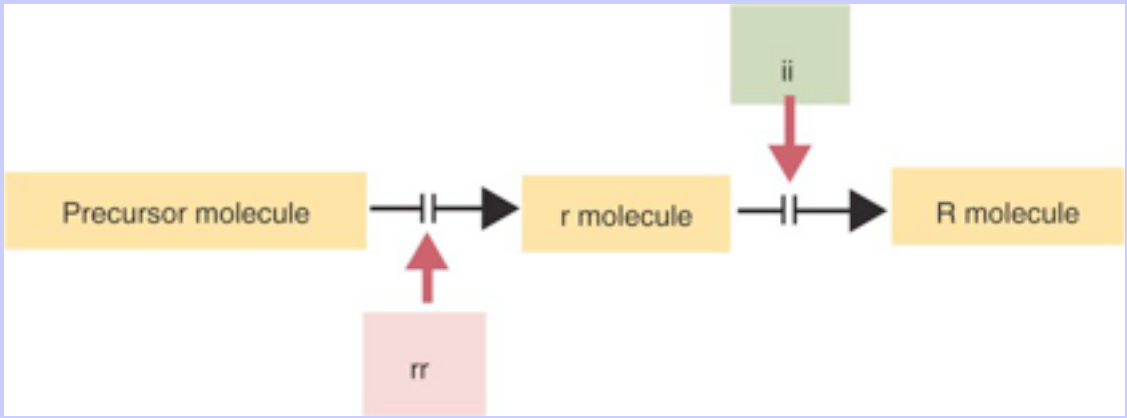
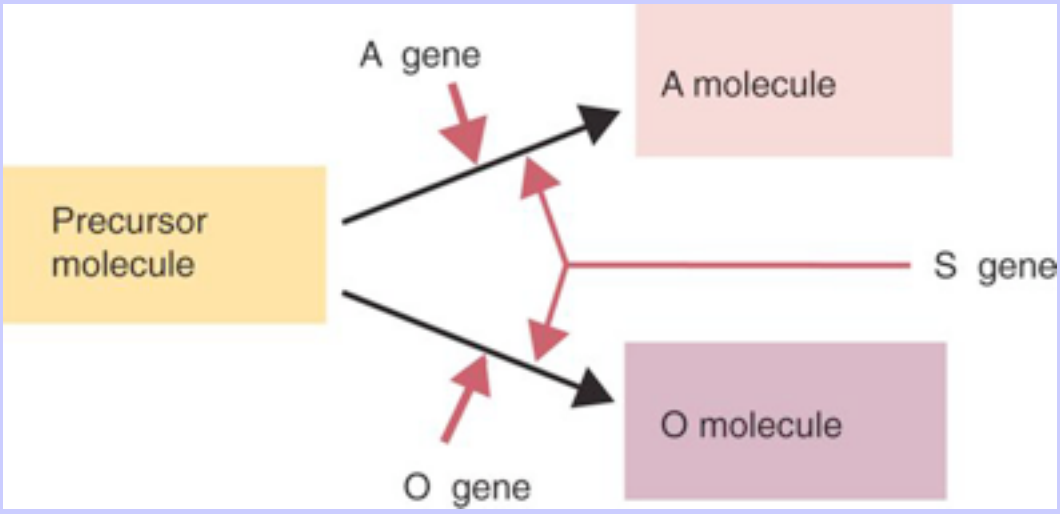


FIGURE 26-3 The production of A or O blood group substances by a pig requires the presence of the S gene. Pigs that lack this gene (ss animals) produce neither of these blood group substances.



currently recognized. The ovine equivalent of bovine B is also termed B and, like the bovine system, is relatively complex, containing at least 52 different alleles. Sheep also possess an ovine equivalent of the bovine J system, called the R system. Two soluble antigens are found in this system, R and O, coded for by alleles R and r. The production of R and O substances is controlled by a gene called I and its recessive allele i. If a sheep is homozygous for i, it expresses neither R nor O antigens. This interaction between the I/i genes and the R-O system is called an epistatic effect (Figure 26-2). R and O antigens are soluble antigens found in the serum of II or Ii sheep and are passively adsorbed onto red cells. Natural anti-R antibodies may be found in R-negative sheep. Sheep also fall into two groups according to whether their red cells have high or low potassium levels. This is regulated by the M blood group system. The Mb antigen acts as an inhibitor of potassium transport.

26.5.3.1

Box 26-1 The Inheritance of the A Blood Group System in Pigs

In pigs, the expression of the A blood group is regulated by two gene loci. One locus, the A locus, contains two alleles A and O, of which A is dominant. The other, the S locus, also contains two alleles, S and its recessive allele s. The S locus controls the expression of the A system so that A or O blood groups can only be expressed if the animal carries at least one S gene. Possible genotypes are therefore AA, AO, and OO as well as SS, Ss, and ss.

These may be combined thus:

- Animals that are AASS, AASs, AOSS, or AOSs will have A red cells.
- Animals that are OOSS or OOSs will have O red cells.
- Animals that are AAss, Aoss, or Ooss will express neither A nor O and so will have “null” red cells.

26.5.3.2

Serological Testing

Sheep blood groups are detected by hemolytic tests. The only exception to this rule is the D system, which is detected by agglutination.

26.5.4

Pigs

Sixteen pig blood group systems have been identified (EAA-EAP). Of these, the most important is the EAA system. The EAA system controls the expression of two antigens, A and O. Their expression is regulated by a gene called S (secretor) with two alleles S and s. In the homozygous recessive state (ss) this gene can prevent the production of the A and O substances (Figure 26-3). As a result, the amount of these antigens bound to red cells in these animals is reduced to an undetectable level (Box 26-1). A and O, like J in cattle and R and O in sheep, are not true red cell antigens but soluble molecules found in serum and passively adsorbed onto red cells after birth. Natural anti-A antibodies may occur in A-negative pigs, and transfusion of A-positive blood into such an animal may cause transient collapse and hemoglobinuria.

HDN in piglets formerly occurred as a result of the use of hog cholera vaccine containing pig blood. This vaccine consisted of pooled blood from viremic pigs inactivated with the dye crystal violet. Sensitization of sows by this vaccine led to the occasional occurrence of hemolytic disease of their offspring. There appeared to be a breed predisposition to this disease, which was most commonly seen in the offspring of Essex and Wessex sows. Affected piglets did not necessarily show clinical disease, although their red cells were sensitized by antibody. Other piglets showed rapidly progressive weakness and pallor of mucous membranes preceding death, and those

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animals that survived longest showed hemoglobinuria and jaundice. The severity of the reaction did not appear to be directly related to the anti–red cell antibody titer in the piglet serum. Since the withdrawal of all live hog cholera virus vaccines, the problems associated with their use have disappeared.

True HDN has also been recorded in the pig. The antibodies responsible are usually directed against antigens of the very complex EAE system. In addition to the development of hemolytic anemia in newborn piglets, the presence of antibodies to platelet antigens may cause a thrombocytopenia. This is seen clinically as a bleeding problem on tail docking and a tendency to bruise easily (neonatal purpura). On blood smears, the platelets may be clumped, and antiglobulin testing of them will yield a positive result. Deprivation of colostrum in an attempt to prevent piglets from absorbing anti–red cell antibodies may result in the newborn animals being highly susceptible to infection.

26.5.4.1 Serological Testing

Pig blood groups are detected by agglutination, hemolytic, and antiglobulin tests.

26.5.5 Dogs

In dogs, eight red cell antigens are internationally recognized (DEA 1.1, 1.2, 3, 4, 5, 6, 7, and 8), but five others have been described. (An older nomenclature called them by the traditional alphabetic system, A, Tr, B, C, D, F, J, K, L, M, and N.) The majority of these appear to be inherited as simple Mendelian dominants. Only the DEA 1 antigens are sufficiently antigenic to be of clinical significance. These include the alleles 1.1, 1.2, and 1.3. About 60% of dogs express a DEA 1 antigen. There are no naturally occurring antibodies to DEA 1.1 and 1.2. Antibodies to DEA 7 may occur in 20% to 50% of DEA 7–negative dogs. Antibodies to DEA 1.3, 3, and 5 are found in about 10% of negative dogs, but these are usually of low titer and not of clinical significance. Therefore it is recommended that canine blood donors be negative for DEA 1.1, 1.2, 3, 5, and 7. More than 98% of the canine population is DEA 4 positive. A universal donor would be an animal negative for all the DEA groups except DEA 4. Unless the blood type of the recipient is known, only universal donor blood should be used and a cross-match performed on all recipients, even if universal blood is used. In practice, the most important canine blood type is DEA 1.1. About 33% to 45% of the dog population are DEA 1.1 positive and in general can be considered to be universal recipients. Dogs that are DEA 1.1 negative can also be considered to be universal donors. DEA 1.1–positive blood should never be transfused into a DEA 1.1–negative dog. If so, the recipient will become sensitized to DEA 1.1 blood and high-titered antibodies produced. Subsequent transfusion of positive blood into such an animal could lead to a severe reaction. Similarly, if a negative bitch is sensitized by incompatible transfusions and mated to a positive dog, hemolytic disease may occur in her puppies. Natural HDN in dogs is extremely rare. It occurs when a DEA 1.1–negative breeding bitch is transfused with DEA 1.1–positive blood and subsequently bred to a DEA 1.1–positive male. The puppies develop a hemolytic anemia after 3 to 10 days.

The DEA 7 system (Tr system) is a soluble antigen system antigenically related to the human A, cattle J, sheep R, and pig A systems. Two antigens belong to the system, Tr and O. An epistatic secretor gene controls their expression. Anti–DEA 7 occurs naturally in some DEA 7–negative dogs.

A blood group antigen called Dal has been identified on the basis of antibodies produced in Dalmatians following blood transfusion. Presumably some Dalmatians lack this antigen, which is present in other dog breeds.

26.5.5.1 Serological Testing

Agglutination at 4° C and hemolytic and antiglobulin tests have all been used for the detection of canine blood groups. The source of complement can be either fresh dog or rabbit serum.

26.5.6 Cats

In cats, only one major blood group system, the AB system, has been reported. The AB antigens are glycolipids. Cats may be A, B, or AB. A is completely dominant over B. About 75% to 95% of cats are A positive, about 5% to 25% are B positive, and less than 1% are AB. However, this distribution differs among countries and among different purebred cat breeds. Thus in the United States more than 99% of domestic short-hair and long-hair cats are type A, whereas in the British short-hair breed only about 40% are type A. Severe transfusion reactions have been described in group B cats that received very small quantities of group A blood since 95% of B cats possess IgM anti-A. (Interestingly, only about 35% of A cats possess anti-B, and it is of the IgG and IgM classes and of much lower titer.) If completely matched blood is transfused into cats, its half-life is about 4 to 5 weeks. If, however, group B blood is transfused into cats of blood group A, its half-life is only a few days. If group A blood is transfused into a cat of blood group B, its half-life is just over 1 hour. It is this very rapid destruction that results in severe clinical reactions. Thus a group B cat given as little as 1 mL of group A blood will go into shock, with hypotension, apnea, and atrioventricular block, within a few minutes. Cross-matching is therefore essential in this species.

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Occasionally, hemolytic transfusion reactions occur between AB blood group–matched cats. These appear to be due to natural antibodies against a blood group antigen called Mik. Its mode of inheritance is undefined.

HDN has been recorded in Persian and related (Himalayan) breeds but is very rare. It occurs in kittens from queens of blood group B bred to sires of blood group A. The queens subsequently develop high-titered anti-A antibodies. Although healthy at birth, these kittens develop severe anemia as a result of intravascular hemolysis. Affected kittens show depression and possibly hemoglobinuria. Necropsy may reveal splenomegaly and jaundice. Antibodies to the sire's and the kitten's red cells are detectable in the queen's serum.

26.5.6.1 Serological Testing

Agglutination and hemolytic tests are used for feline blood typing.

26.5.7 Humans

In humans, HDN is due almost entirely to immunization of the mother against the antigens of the Rhesus (Rh) system (now classified as CD240). The condition is, or should be, of historical interest only because a very simple but effective technique is available for its prevention. This depends on preventing an Rh-negative mother from reacting to the Rh-positive fetal red cells that escape from the placenta into her circulation at birth. Strong human anti-Rh globulin is obtained from male volunteers and given to mothers at risk soon after birth. It acts by specifically inhibiting the B cell response to that antigen (see Chapter 17). Routine use of this material therefore prevents maternal sensitization, antibody production, and hemolytic disease. The use of a similar system in the domestic mammals is unnecessary because deprivation of colostrum is sufficient to prevent the disease.

26.5.8 Chickens

Chickens have at least 12 different blood group systems with multiple alleles. The red cell B system is also the major histocompatibility system in the chicken. A hemolytic disease may be artificially produced in chicken embryos by vaccinating the hen with cock red cells.

26.6 PARENTAGE TESTING

Under some circumstances it is necessary to confirm the parentage of an animal. One way of doing this is by examining the blood group antigens of an animal and its alleged parents (Table 26-2). The method is based on the principle that since blood group antigens are inherited, they must be present on the red cells of one or both parents. If a blood group antigen is present in a tested animal but absent from both its putative parents, then parentage must be reassigned. Similarly, if one parent is homozygous for a specific blood group antigen, this antigen must appear in the offspring. However, it must be recognized that blood typing procedures can only exclude, never prove, parentage.

26.7 HEMOPHAGOCYTIC SYNDROME

Hemophagocytic syndrome is a benign proliferative disorder of activated macrophages associated with multiple cytopenias in the blood. These cytopenias result from hemophagocytosis and probably reflect excessive phagocytic activity by macrophages. The syndrome has been described in humans, dogs, and cats. In humans it may be either inherited or acquired. In dogs, the syndrome has been reported as secondary to infectious, neoplastic, or immune-mediated diseases. Diagnostic criteria include the presence of pancytopenia or bicytopenia and the presence of greater than 2% hemophagocytic macrophages in a bone marrow aspirate. Most of these dogs have an underlying disease condition. Thus about a third of canine cases are associated with immune-mediated diseases such as lupus or immune-mediated thrombocytopenia. These animals are commonly anemic, neutropenic, and thrombocytopenic and it may be argued that autoantibodies opsonized the blood cells leading to their phagocytosis. Other affected dogs suffer from infectious diseases such as pyometra, pleuritis, ehrlichiosis, blastomycosis, or lyme disease. In some cases affected dogs recover once their underlying infection is treated. The disease is also associated with some neoplastic diseases such as malignant lymphoma or myelodysplastic syndrome.

Table 26-2 The Use of Blood Groups to Assign Paternity

	Blood Group				
	1.1	1.2	6	7	8
Sire 1 ?	+	+	–	+	–
Sire 2 ?	+	+	–	–	+
Dam	–	–	+	+	–
Puppy 1	+	+	–	–	–
2	+	+	–	+	–
3	–	–	–	+	+ *
4	–	–	+	+	–

Courtesy Dr. D Colling.

* This puppy possesses DEA 8, which could not have come from sire 1 or its dam. Sire 1 could not have sired this litter.

Cases of canine hemophagocytic syndrome also occur in the absence of any obvious associated disease. Affected dogs are anemic, neutropenic, thrombocytopenic, febrile, anorexic, and lethargic. In humans this syndrome may result from an NK cell deficiency or as a result of excessive macrophage activation resulting from oversecretion of Th1 cytokines.

26.8 TYPE II HYPERSENSITIVITY REACTIONS TO DRUGS

Red cells may be destroyed in drug hypersensitivities by three mechanisms. First, the drug and antibody may combine directly and activate complement, and red cells will be destroyed in a bystander effect as activated complement components bind to nearby cells.

Second, some drugs may bind firmly to cells, especially those in the blood. For example, penicillin, quinine, L-dopa, aminosalicylic acid, and phenacetin may adsorb onto the surface of red cells. Since these cells are then modified, they may be recognized as foreign and eliminated by an immune response, resulting in hemolytic anemia. Penicillin-induced hemolytic anemia is not uncommon in horses. These conditions can be suspected based on recent treatment with penicillin and improvement when its use is discontinued. It may also be possible to detect antibodies against penicillin or penicillin-coated red cells in these animals. Sulfonamides, phenylbutazone, aminopyrine, phenothiazine, and possibly chloramphenicol may cause agranulocytosis by binding to granulocytes, and phenylbutazone, quinine, chloramphenicol, and sulfonamides may provoke thrombocytopenia. If the cells from animals experiencing these reactions are examined using a direct antiglobulin test, antibody may be demonstrated on their surface. If these antibodies are eluted, they can be directed not against the blood cells but against the offending drug.

Third, drugs such as the cephalosporins may modify red cell membranes in such a way that the cells passively adsorb antibodies and then are removed by phagocytic cells.

26.9 TYPE II HYPERSENSITIVITY IN INFECTIOUS DISEASES

Just as drugs can be adsorbed onto red cells and render them immunologically foreign, so also can bacterial antigens such as the lipopolysaccharides, viruses such as equine infectious anemia virus and Aleutian disease virus, rickettsia such as *Anaplasma,* and protozoa such as the trypanosomes and *Babesia*. These altered red cells are regarded as foreign and are either lysed by antibody and complement or phagocytosed by mononuclear phagocytes. Clinically severe anemia is, therefore, characteristic of all these infections.

26.10 SOURCES OF ADDITIONAL INFORMATION

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