ENSURING BLOOD COMPATIBILITY: UPDATE ON CANINE TYPING & CROSSMATCHING

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INTRODUCTION

Transfusion therapy has taken an increasingly important role in the life support of canine patients. Over the past decade the use of blood products in treating critically ill dogs has drastically increased. Furthermore, the need for blood typing and crossmatching of patients and donors, as well as testing of donors for transmittable diseases has now been recognized in order to assure safe and more efficacious transfusions in dogs. Different view points exist regarding the extent and methods used for compatibility testing and various techniques for laboratory or point-of-care use have been applied or are being developed. Recent advances in blood typing and compatibility testing in dogs are reviewed and practical recommendations are offered based on our experience at the University of Pennsylvania.

CANINE BLOOD TYPES

Blood types are genetic markers on erythrocyte surfaces that are antigenic and species specific. A set of blood types of two or more alleles makes up a blood group system. Based upon the presence of antibodies directed against red cell surface antigens, more than a dozen blood group systems have been described in dogs. For some blood groups certain glycoproteins have been proposed by immunoprecipitation methods and gel electrophoresis, but much more work is needed to better characterize them at the biochemical and molecular levels. Many systems are referred to as Dog Erythrocyte Antigens using the abbreviation DEA followed by a number. Red blood cells from a dog can either be positive or negative for any blood group system other than the DEA 1 system. For instance, for the DEA 3 system, a dog's red cells can be DEA 3 positive or DEA 3 negative. These blood types appear to be codominantly inherited. The DEA 1 system, however, has at least two subtypes: DEA 1.1 (also known as A1) and DEA 1.2 (A2). Thus, a dog's red cells can be DEA 1.1 positive or negative, and DEA 1.1 negative cells can be DEA 1.2 positive or negative. Recently, an A3 subtype has been described in Australia, but no reagents are available for comparative studies. Only very limited surveys on the frequency of these blood types have been reported (Table 1), which suggested possible geographic and breed-associated differences. Some of the blood types are seen rarely (DEA 3), while others occur commonly (DEA 4). In Japan an additional blood grouping system has been proposed, but its association to the DEA systems and its clinical importance have not been documented. Recently, we have identified an apparently new common red cell antigen that seems to be missing in some Dalmatians, hence named Dal antigen.

Table 1. Blood type frequencies in dogs (based upon limited surveys)

Blood types	% Positive	% Negative	Antigen size
DEA 1			
1.1 (A1)	33-45	55-67	50 & 200 kD
1.2 (A2)	7-20	35-60*	85 kD
DEA 3 (B)	5-10	90-95	34-71 kD
DEA 4 (C)	87-98	2-13	32-40 kD
DEA 5 (D)	12-22	78-88	NA
DEA 7 (Tr)	8-45	55-92	53, 58 & 63 kD

* DEA 1.1 *and* 1.2 negative dogs.
NA: not available

ALLOANTIBODIES

Strongly antigenic blood types are of great clinical importance, because they can elicit a potent alloantibody response. These alloantibodies may be of the IgG or IgM class and may be hemagglutinins or hemolysins. Clinically the most antigenic blood type in dogs appears to be DEA 1.1. Based upon experimental and clinical data, dogs can become sensitized after receiving a mismatched transfusion, i.e., a blood unit positive for one or more blood types not found on the recipient's red blood cells. Sensitizing dogs in experimental studies in the 1950's led to the documentation of some transfusion reactions caused by blood group incompatibilities and to the characterization of new blood types.

Transfusions of DEA 1.1 positive cells to a DEA 1.1 negative dog invariably elicit a strong alloantibody response. Following a first transfusion, anti-DEA 1.1 antibodies develop after >9 days and may cause a delayed transfusion reaction. However, a previously sensitized DEA 1.1 negative dog can develop an acute hemolytic reaction after transfusion of DEA 1.1 positive blood. Transfusion reactions may also occur after a sensitized dog by prior blood transfusion receives blood that is mismatched for any other red blood cell antigen than DEA 1.1, and may occur as early as 4 days after being sensitized. For instance, a previously sensitized DEA 4 negative dog experienced an acute hemolytic transfusion reaction while receiving DEA 4 positive blood. Furthermore, a previously transfused whippet developed an alloantibody against a common red cell antigen, resulting in a general incompatibility with any donor except a littermate. Finally, a Dalmatian sensitized to an antigen other then DEA 1.1,1.2 ,3, 4, 5 or 7 became incompatible to all potential donors except for a few other Dalmatians. However, despite the variety of blood types and the limited degree of compatibility testing in clinical practice, transfusion reactions are rarely reported. In surveys of blood product usage, transfusion reactions were observed in 2-13%, but none of them were definitely associated with blood group mismatches and hence could have been caused by other factors.

BLOOD TYPING PROCEDURES

Canine blood typing is generally based on serologic identification by agglutination reactions. Originally serum from sensitized dogs has been used for typing, but such polyvalent alloantibodies vary from batch to batch and are therefore not optimal. Recently monoclonal antibodies against DEA 1.1 have been developed at Kansas State University and at the University of Lyon. Because of the strong antigenicity of DEA 1.1, typing of donors for DEA 1.1 is strongly recommended. Whenever possible, the recipient should also be typed to allow the use of DEA 1.1 positive blood for DEA 1.1 positive recipients. A blood typing card has been available for a decade as a simple standardized in-practice kit (DMS Laboratories, 2 Darts Mill Road, Flemington, NJ 08822, 1-800-567-4DMS), in order to classify dogs as DEA 1.1 positive or negative. This assay requires a small amount of anticoagulated blood (0.1 ml) and is based on an agglutination reaction that occurs within 2 minutes when DEA 1.1 positive erythrocytes interact with a murine monoclonal DEA 1.1-alloantibody. Based upon limited data, it appears possible that this card may give a weak positive agglutination reaction with DEA 1.2 positive red cells. Typing for DEA 1.1 is also available through most commercial and veterinary school laboratories. These laboratories are using either the card method or reagents from Midwest Animal Blood Services, which requires the addition of a Coombs reagent. Furthermore, a unique gel column technology, widely used in human blood banking, has recently been found to be an excellent laboratory method based on a monoclonal alloantibody for DEA 1.1 typing, without cross-reaction with DEA 1.2 positive cells (DiaMed, Cressier, Switzerland).

Caution should be exercised whenever the patient's blood is autoagglutinating or has a very low hematocrit (<10%). It is recommended to check for autoagglutination of blood with buffer/saline on a slide or the card. Autoagglutinating blood may be first washed three times with saline to overcome the apparent autoagglutination. However, true (persistent) autoagglutination after three washes precludes typing, because it will always look like DEA 1.1 positive blood. In such circumstances, DEA 1.1 negative blood should be used, until the patient does not agglutinate anymore and can be retyped. DEA 1.1 positive blood from very anemic animals may not agglutinate when exposed to the DEA 1.1 or other reagents because of the prozone effect. In these cases some of the patient's plasma may be discarded before applying a drop of blood onto the card. Finally, recently transfused dogs may display a mixed field reaction, with only the transfused or recipient cells agglutinating.

Typing service and polyclonal antisera are available for DEA 1.1, 1.2, 3, 4, 5, and 7 (Midwest Animal Blood Services, Michigan). Use of these blood typing products, however, requires some expertise and experience. Some veterinarians recommend use exclusively of canine donors that are negative for all testable DEA except DEA 4 (up to 98% of dogs are DEA 4 positive) in order to prevent sensitization against these blood types. However, we do not support the routine typing for other blood types then DEA 1.1 for the following reasons:

1) This protocol unnecessarily eliminates many active and potential donors. Based upon published frequencies less than 1 in 10 dogs would be acceptable.
2) This extended blood typing protocol would be cost prohibitive because many dogs would need to be typed for every negative dog.
3) Generally, humans are only typed for the ABO and Rh blood group system, although >2 dozen other blood group systems are known.
4) Typing for more than DEA 1.1 does not eliminate the need for crossmatching following the first transfusion. Crossmatching may also identify incompatibilities against yet unknown types.
5) There are no supporting published clinical reports that transfusion reactions could be substantially reduced by extended blood typing.

BLOOD CROSSMATCHING TESTS

Whereas blood typing tests reveal the blood group antigens on the red blood cell surface, blood crossmatching tests assess the serologic compatibility or incompatibility between donor and recipient. Thus, the crossmatch tests check for the presence or absence of naturally occurring and induced alloantibodies in serum (or plasma); these antibodies may be hemolysins and/or hemagglutinins and can be directed against known blood groups or other red cell surface antigens. A standardized tube crossmatching procedure has been proposed and is used by many laboratories. Furthermore, we have evaluated the novel gel column technique and found it in preliminary studies to be a promising simple, sensitive and standardized method.

The major crossmatch tests for alloantibodies in the recipient's plasma against donor cells, whereas the minor crossmatch test looks for alloantibodies in the donor's plasma against the recipient's red blood cells. The presence of autoagglutination or severe hemolysis may preclude the crossmatch testing. A major crossmatch incompatibility is of greatest importance because it predicts that the transfused donor cells will be attacked by the patient's plasma, thereby causing a potentially life-threatening acute hemolytic transfusion reaction. As fatal reactions may occur with <1ml of incompatible blood, compatibility testing by administering a small amount of blood is not appropriate. A minor crossmatch incompatibility should not occur if donors have not been previously transfused and is of lesser concern because donor's plasma volume is small, particularly in packed red cell products, and will be markedly diluted in the patient.

The initial blood crossmatch between two dogs that have never before received a transfusion should be compatible, because dogs do not have naturally occurring alloantibodies. Therefore, one might omit a crossmatch before the first transfusion in clinical practice. Because the crossmatch does not determine the blood type of the patient and donor, a compatible crossmatch does not prevent sensitization of the patient against donor cells within 1 to 2 weeks. Therefore, previously transfused dogs should always be crossmatched, even when receiving blood from the same donor. The time span between the initial transfusion and incompatibility

reactions may be as short as 4 days and lasts for many years (i.e., years after the last transfusion alloantibodies may be present). Obviously, a blood donor should never have received a blood transfusion.

Simple blood typing and crossmatching procedures are now available to assure compatible transfusions. Only DEA 1.1 negative blood should be given to DEA 1.1 negative recipients, but DEA 1.1 positive dogs may receive DEA 1.1 negative or positive blood. Any previously transfused (>4 days ago) dog needs to be crossmatched before receiving additional blood even when getting it from the same donor.

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