Agglutination testing for human erythrocyte product in the rhesus macaque

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INTRODUCTION: There has been interest in using human blood products in nonhuman primate models of trauma to supplement human studies and to provide evidence to guide novel trauma resuscitation strategies. The compatibility of human RBCs has not been extensively studied in nonhuman primate species.

METHODS: Whole blood samples were collected from five healthy, nontransfused, not previously pregnant Chinese-bred rhesus macaques. The whole blood was centrifuged, and the plasma was decanted from each sample. Group O-negative human RBCs were mixed with the plasma from the rhesus macaque monkeys. Compatibility testing was performed by an immediate spin test and polyspecific and monospecific anti-human globulin (AHG) tests in glass tubes.

RESULTS: Immediate spin testing revealed three out of five plasma samples (60%) from rhesus macaques caused at least 1+ agglutination with the human RBCs. Polyspecific anti-human globulin (AHG) tests demonstrated that two of five plasma samples (40%) from rhesus macaques caused at least 1+ agglutination with the human RBC, while the monospecific AHG testing revealed that the incompatibility was caused by C3d, not IgG.

CONCLUSION: Human RBCs are not compatible with the plasma of some, but not all, Chinese-bred rhesus macaques.

The most common cause of preventable deaths after traumatic injury is severe bleeding.[1,2] There are an estimated 30,000 preventable deaths due to traumatic bleeding per year in the United States.[3] Traumatic injury is also the most common cause of death for people between 1 and 46 years of age, and trauma also accounts for the most years of life lost until the age of 75.[3] Improvement in trauma outcomes is therefore of great importance, and efforts need to focus on improving outcomes from traumatic hemorrhagic shock in particular.

Human trials examining the therapeutic modalities for traumatic hemorrhagic shock have provided evidence on how to improve outcomes.[4–6] Eliminating crystalloids early in the resuscitation and using plasma have been proven in large randomized controlled trials to improve outcomes.[4,6] Empiric ratios of RBCs to plasma and platelets in a 1:1 ratio also reduce death from bleeding in the first 24 hours after injury.[5] However, human trials are expensive and difficult to perform due to the heterogeneity in trauma populations and the many sources of potential confounders and effect modifiers. There has been recent interest in using nonhuman primate (NHP) models of trauma to supplement human studies to provide evidence to guide novel trauma

ABBREVIATIONS:: AHG = anti-human globulin; NHP = nonhuman primate; PS = polyspecific; RT = room temperature.

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resuscitation strategies.[7] One such approach is to use human blood products in NHP trauma models to allow for the direct examination of the efficacy of these products based on a variety of quality metrics. The use of NHP blood products in NHP models is also possible, but it would require developing methods of storing NHP RBCs, plasma, and platelets that have not been established. In addition, many of the current processing methods for blood products, such as pathogen reduction and different storage solutions, would need to be validated in NHPs before they could be examined for efficacy and safety in NHP models. Therefore, it is important to determine if human blood RBCs are compatible with NHP models, as this would allow for direct examination of efficacy and safety in traumatic bleeding models where there would be minimal heterogeneity, confounders, and effect modifiers compared to human studies.

A lyophilized human platelet product has been transfused to rhesus macaque monkeys. Thus, it might be possible to transfuse human RBCs to these monkeys.[8] However, an in vitro study reported that approximately one-third of this species of monkeys have antibodies against human RBCs,[9] while an earlier study demonstrated that almost all of the human RBCs that were transfused to the same type of monkey were cleared within 15 minutes of administration.[10] The goal of this study was to extend the previous experiments on the naturally occurring rhesus macaque anti-human RBC antibodies using routine pretransfusion testing methods.

MATERIALS AND METHODS

Blood samples

Whole blood samples were collected from five healthy, 15 to 20 years of age, nontransfused, not previously pregnant, Chinese-bred rhesus macaques, by the Institute for Human Virology at the University of Maryland School of Medicine. Samples were collected in ethylenediaminetetraacetic acid anticoagulant under an animal use protocol approved by the Institutional Animal Use and Care Committee at the University of Maryland School of Medicine, Baltimore, Maryland. One sample of group O-negative human whole blood was purchased from BioIVT. All of the monkey whole blood samples were maintained at refrigerator temperature (1–6 °C) until use, which was within 72 hours of collection. To separate the RBCs from the human and monkey whole blood samples, each sample was centrifuged (ST 16R centrifuge, Thermo Scientific) at 200× *g* for 10 minutes at room temperature. The plasma was decanted from the centrifuged sample for each rhesus macaque sample, while the separated human RBCs were suspended in 0.85% saline (Thermo Scientific) to make a 3% cell suspension.

Immediate spin test for compatibility between rhesus macaque plasma and human RBCs

Three drops (approximately 75 μL) of each rhesus macaque plasma sample was individually mixed with 50 μL of the 3% human RBCs in a glass tube. The plasma and RBC mixture was centrifuged at 200× *g* for 3 minutes at room temperature (RT) and graded macroscopically for agglutination using the standard scale ranging from 0 to 4+. One technologist performed all of the agglutination strength interpretations. Separately, human RBCs were mixed with 0.85% saline at the identical volumes listed above to serve as a negative agglutination control.

Polyspecific and monospecific anti-human globulin tests for compatibility between rhesus macaque plasma and human RBCs

Rhesus macaque plasma was mixed with human RBCs in glass tubes as described above for the immediate spin (IS) test and incubated at RT for 20 minutes. The plasma and RBC mixture was centrifuged at 200× *g* for 3 minutes at RT. Two washes were performed after centrifugation; 0.85% saline was added to each tube until approximately two-thirds full. The samples were again centrifuged at 200× *g* for 3 minutes at RT (ST 16 R centrifuge, Thermo Scientific), and the liquid component was decanted. Two drops of polyspecific (PS) anti-IgG/-C3d reagent (IMMUCOR) was then added to the washed RBCs. The mixture was centrifuged for a third time as described above and graded macroscopically for agglutination using the standard scale. A positive control, one drop (approximately 25 μL) of control cells (Checkcell IgG, IMMUCOR), was added to samples with no (0+) agglutination to exclude reagent neutralization. For all rhesus macaque plasma/human RBC combinations that demonstrated any degree of macroscopic positivity with the PS reagent, the tests were repeated using anti-IgG monoclonal reagent (IMMUCOR) and anti-C3d monoclonal reagent (IMMUCOR) as described above.

RESULTS

Immediate spin test for compatibility between rhesus macaque plasma and human RBCs

Three of five plasma samples (60%) from rhesus macaques caused at least 1+ agglutination with the human RBC sample. The negative controls demonstrated no agglutination (Table 1).

Polyspecific and monospecific anti-human globulin tests for compatibility between rhesus macaque plasma and human RBCs

Two of five plasma samples (40%) from rhesus macaques caused at least 1+ agglutination with the human RBC sample using the PS anti-human globulin (AHG) reagent (Table 1). The negative controls demonstrated no agglutination, and in all cases the check cells revealed that the AHG reagent had not been neutralized in the samples that did not demonstrate agglutination with the PS reagent.

The two rhesus macaque plasma/human RBC combinations that demonstrated agglutination with the PS AHG

TABLE 1. Summary of results by animal ID

	Immediate spin test					Indirect anti-globulin (polyspecific) test					Indirect anti-IgG monospecific test					Indirect anti-C3d monospecific test				
Animal ID	0	1+	2+	3+	4+	0	1+	2+	3+	4+	0	1+	2+	3+	4+	0	1+	2+	3+	4+
108Y	◆					◆					*	*	*	*	*	*	*	*	*	*
M083		◆				◆					*	*	*	*	*	*	*	*	*	*
M084		◆					◆				◆						◆			
DC9A	◆					◆					*	*	*	*	*	*	*	*	*	*
PP0638		◆					◆				◆						◆			

* Samples did not react to the indirect antiglobulin polyspecific test and were therefore not tested using the monospecific anti-IgG or anti-C3d test.

reagent were then tested using anti-IgG and anti-C3d monospecific AHG reagents. Both of these samples demonstrated 1+ agglutination with the anti-C3d AHG reagent only; no agglutination was observed when these samples were tested using the anti-IgG AHG reagent, and the check cells revealed that the AHG reagent had not been neutralized (Table 1).

DISCUSSION

These results indicate that some rhesus macaque monkeys have naturally occurring antibodies against human RBCs. These antibodies appear to be of the IgM isotype because agglutination was observed only in the IS phase of testing, and no reactivity was observed in the anti-IgG monospecific AHG test. Furthermore, detecting C3d on the human RBCs after incubation with rhesus macaque plasma also supports the notion that these naturally occurring antibodies are IgM in nature, as this isotype can directly fix complement. Some IgM antibodies are clinically significant in that they can lead to the premature destruction of the RBCs, and a previous in vivo study demonstrated that human RBCs are cleared within 15 minutes from the rhesus macaque's circulation,[10] thereby suggesting that these antibodies are indeed clinically significant. Unless in vivo studies can prove that human RBCs have normal survival in Chinese-bred rhesus macaques, or if the anti-human RBC antibodies can be removed from their circulation long enough to facilitate a clinical study of their efficacy in resuscitating these animals, human RBCs do not seem suitable for use in this species. However, another possibility would be to use the rhesus macaques that do not demonstrate agglutination in any of the phases of testing in trials of human RBCs, as their plasma would appear to be compatible with human RBCs. However, whether their plasma continues to demonstrate compatibility over time and after exposure to human RBCs remains to be determined. It would be interesting to further investigate these animals to determine why some produce anti-human RBC antibodies and why others do not.

This study has several limitations. A small number of rhesus macaque plasma samples were tested against the human RBCs; thus, the frequency of the naturally occurring anti-human RBC cannot be established. Agglutination testing has the advantages of being rapid and simple to perform, and the reagents are commercially available. The main limitation to this testing is the subjectivity of interpreting its output; for example, the plasma of monkey M083 produced 1+ agglutination in the IS test but was negative in the PS AHG test. While it is possible that the antibody that produced the 1+ agglutination in the IS test did not fix complement, it is also possible that the relatively weak agglutination in the IS test could have been interpreted as negative by a different operator.

In conclusion, human RBCs, when mixed with the plasma of some rhesus macaques, cause agglutination likely due to IgM naturally occurring anti-human antibodies. The clinical significance of this incompatibility requires further study, but based on previous literature it appears that it may not be appropriate to test human RBCs in monkeys of this species with these antibodies.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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