A confusion in antibody identification: anti-D production after anti-hr^B

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It is well known that certain combinations of alloantibodies are frequently found together. Patients with sickle cell disease (SCD) are mostly of African ancestry, and they may make anti-hr^B. A transfusion of hr^B– blood is often achieved by using e– (R_2R_2) RBCs; it is generally believed that hr^B– patients readily make anti-E or a "broad-spectrum" anti-Rh34 (-Hr^B). We describe two multiply transfused D+ patients with SCD and a history of anti-hr^B who subsequently produced anti-D. This raises the question whether anti-hr^B together with anti-D is a more common antibody combination than anti-hr^B with anti-E or anti-Rh34. ***Immunohematology* 2007;23:158–60.**

Key Words: alloantibody, blood group incompatibility, crossmatch, Rh blood group system

It is well known by immunohematologists that certain combinations of alloantibodies are frequently found together, for example, anti-C with anti-e; anti-E with anti-c; and anti-Le^a with anti-Le^b. Patients of African ancestry with sickle cell disease (SCD) often require chronic transfusion therapy, and they may have variant antigens that make them prone to produce unusual antibody combinations. For example, patients with variant *RHCE* genes may make alloanti-e-like antibodies, including anti-hr^B. After anti-hr^B has been identified, transfusion of hr^B– blood is often achieved by using e– (R_2R_2) RBC components because haplotypes that lack e are hr^B–, and such blood is more readily available than e+, hr^B– blood.[1] However, it is generally believed that these patients readily make anti-E with or without a "broad-spectrum" anti-Rh34 (-Hr^B).[2,3] If such patients receive frequent transfusions, additional antibodies are likely to be formed, making antibody identification and finding compatible blood complicated. We describe here two multiply transfused D+ patients with SCD and a history of anti-hr^B (plus other alloantibodies) who subsequently made anti-D. As certain D variant haplotypes are often associated with hr^B– haplotypes,[4,5] this raises the question as to whether anti-hr^B together with anti-D, in D+ patients, is a more common combination than anti-hr^B with anti-E or anti-Rh34.

Case Reports

Patient 1

An African American woman with clinical sepsis and a history of SCD β-thalassemia was admitted for the surgical removal or drainage of a left subphrenic abscess. Although her baseline Hct level was normally 30% to 36%, her admission Hct was 18.9%. Before admission, she was known to be D+ and to have anti-hr^B, -E, -M, and a warm autoantibody. A sample was submitted to the American Red Cross Northern Ohio Region Reference Laboratory. It was determined that the patient's serum contained the previously identified anti-hr^B and anti-E; however, the previously detected anti-M and warm autoantibody were no longer detectable. Attempts were made to obtain compatible blood, including the procurement of D–– units. Because of the urgency to proceed with surgery and the unavailability of hr^B– units, least incompatible blood was selected to support this patient in the perioperative period. She received two units of crossmatch compatible and four units of crossmatch incompatible blood on the day after her admission. During the following 12 days, her Hct stabilized at 30% to 35%. Surgery was delayed because the patient was clinically stable on broad-spectrum antibiotic therapy. On the 13th day after transfusion, her Hct precipitously fell to 20%. During the next 5 days, despite transfusion of six least incompatible units, including one that was E–, hr^B–, her Hct further declined to 13%. Her reticulocyte count diminished to essentially zero, and a bone marrow aspiration performed at this time demonstrated pure RBC aplasia. When D+, hr^B–, and D–– RBC components were subsequently obtained, they were strongly incompatible with the patient's posttransfusion serum, thereby ruling out the possibility of anti-E and anti-Rh34 as the sole additional specificities. Repeat testing of her posttransfusion serum revealed 4+ reactivity with the antibody screening RBCs. The

patient's RBCs were positive in the DAT with anti-IgG. A sample was investigated for the possible development of an additional antibody to a high-prevalence antigen. In a selected RBC panel, Rh_{null}; D–, hr^B–; and some D+, hr^B– RBCs that lacked the other relevant antigens were compatible. Because DIIIa RBCs are frequently hr^B– (and VS+),[1] patients who make anti-hr^B are likely to have the DIIIa phenotype and be at risk of making anti-D. Thus, we considered the possibility that the patient had made anti-D. The compatible D+, hr^B– RBCs were either D–, or were determined by DAK typing (they were DAK+)[6] and by DNA (prepared from WBCs) analysis using PCR-RFLP as described[6,7] to have the partial D phenotype, DIIIa. Anti-D made by a DIIIa person does not react with DIIIa RBCs and explains the compatibility of some D+, hr^B– RBCs. Transfusion was withheld from the patient for 1 week, during which time her Hct remained less than 15%. An episode of chest pain prompted the transfusion of a D–, E–, hr^B– compatible RBC component slowly in two aliquots. After this transfusion her Hct stabilized at 20%. During the next 3 weeks her reticulocyte count increased to 10%, and she achieved a Hct of 30% with no further transfusion therapy.

Patient 2

An African American man with SCD was admitted to the hospital with low hemoglobin and hematocrit levels and a painful sickle cell crisis. His serum was known to contain anti-hr^B, -C, -E, and -K. He had received transfusions on multiple occasions before his serum strongly agglutinated some RBCs lacking these antigens. Attempts to locate compatible blood were unsuccessful. When tested with a panel of hr^B– RBC samples that lacked C, E, and K, his serum did not agglutinate eight samples (one of these samples was E+) and did agglutinate four samples (two of which were E+). One of the eight nonreactive samples was D–. It was shown that the compatible D+ samples and the patient's RBCs were DIIIa. With the experience that had been gained from Patient 1, anti-D was quickly identified in this patient's serum.

Discussion

Given the reputed immunogenicity of D, it surprised us that these patients produced anti-D after anti-hr^B and other alloantibodies. One explanation for this unexpected result is that the patients with a partial D on their RBCs produce an anti-D to a part, but not all, of D. In both cases presented here, many transfusions of D+, hr^B– RBCs (including R_2R_2) were tolerated before anti-D was produced. The partial D phenotype, DIIIa, which may be present in approximately 4 percent of Americans with African ancestry,[6] is not readily identified because DIIIa RBCs are strongly agglutinated by reagent and single-clone monoclonal anti-D. The presence of anti-D may not be readily apparent when a panel of D+, hr^B– RBCs is tested because some, but not all, may be DIIIa. DNA analysis has shown that altered *RHCE* genes are often in *cis* with genes encoding partial D phenotypes.[4,5] Thus, patients whose RBCs possess a partial D phenotype with a partial e phenotype can type positive for these antigens and have an alloantibody apparently of the same specificity, that is D+ with alloanti-D, and e+ with alloanti-e. This can dramatically complicate antibody identification, as well as our ability to provide compatible blood components to such patients. It is important to remember that providing compatible blood components for patients with anti-e-like antibodies can be confounded by the presence of anti-D, and that a panel of RBCs lacking a high-prevalence e-variant antigen (such as hr^B) is likely to include some RBCs with a partial D phenotype. Thus, the pattern of reactivity will not be that expected for anti-D. In these patients, reactivity with R_2R_2 RBCs may be attributable to anti-D and not anti-E or anti-Rh34. We have since tested other patients whose serum first contained anti-e-like antibodies and then anti-D, which shows that in certain patients this phenomenon is not especially rare. When testing serum from patients of African ancestry, think anti-D!

Providing blood for transfusion to patients with complex serologic problems has always been challenging; however, providing blood for the patients described in this paper is particularly difficult. They require unique antigen combinations that are found only in donors of African descent. A nationwide search in the United States for blood for Patient 2 failed to identify any compatible donors. Hemoglobin substitutes might be useful in these cases, but they are rarely available for compassionate use, and none is currently approved by the U.S. Food and Drug Administration. Because the only reagent RBCs that were serologically compatible with the patient had been received on a RBC exchange from South Africa, the blood center in Durban, South Africa, was contacted. They generously provided two units of blood from one of the compatible donors; however, there is an ongoing concern that such blood should not be used because the blood center in South Africa had not performed nucleic acid testing for viral markers.

The practice of prophylactically matching patients with SCD for Rh and K is widespread; however, such matching would not prevent this infrequent type of alloimmunization. Patients, as described in these case studies, who have partial Rh antigens will likely type D+, even though they are at risk of being sensitized to anti-D. Because the Rh phenotype that is compatible with these patients can only be found in donors of African ancestry, an ongoing effort to recruit, type, and retain these minority donors must become a priority in the nation's blood centers.

Acknowledgments

We dedicate this paper to Ragnhild Øyen (deceased January 2007), whose insightful analysis of the data and antibody identification skills were instrumental in solving these cases. We are grateful to Robert Ratner for preparation of the manuscript. We also thank Elizabeth Smart of the South African National Blood Service, East Coast Region, for providing two units of compatible blood for Patient 2.

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