In Vivo Cross-Match by Chromium-51 Urinary Excretion from Labeled Erythrocytes: A Case of Anti-Gerbich

Teruhito Mochizuki, W. Newlon Tauxe, and Glenn Ramsey

Department of Radiology, Division of Nuclear Medicine, Central Blood Bank, and Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

We studied a patient with an alloantibody to the high-frequency red blood cell (RBC) antigen Gerbich. A nationwide search for rare Gerbich-negative blood (<1:45,000 donors) located only seven units, and our supply was quickly exhausted. By using an in vivo cross-matching method, we demonstrated that this anti-Gerbich did not cause RBC destruction. Regular Gerbich-positive transfusions could then proceed without hemolysis. This cross-match test was based on the determination of the urinary excretion rates of injected radioactive chromium-labeled donor erythrocytes by which it was possible to determine compatibility only 24 hr after the test was begun. The procedure provides an easy and accurate means for in vivo cross-matching of conventionally incompatible donor blood.

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Based on conventional in vitro cross-matching techniques, blood banks are sometimes confronted with the problem of not being able to locate donor blood that is compatible for a particular recipient. In some of these red blood cell (RBC) antibody problems, patients who are transfused with incompatible blood may not necessarily react adversely (*1*). However, a reliable prediction of immune hemolysis cannot be made using currently available in vitro techniques.

Recently, we faced this problem with a patient who presented with anti-Gerbich (anti-Ge) antibody (*2–4*) and for whom no compatible blood was available in our blood bank. We speculated whether in vivo cross-matching would predict how the patient would respond to incompatible blood. We performed a previously described in vivo cross-matching technique by counting urinary excretion of radioactive chromium (^{51}Cr) from labeled erythrocytes (*5–7*) in an attempt to find compatible blood and to assess the hemolytic potential of this patient's rare and difficult antibody. The purpose of this paper is to present our results of anti-Ge in vivo cross-matching.

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For reprints contact: W. Newlon Tauxe, MD, Division of Nuclear Medicine, Department of Radiology, Presbyterian-University Hospital, DeSoto at O'Hara Streets, Pittsburgh, PA 15213.

MATERIALS AND METHODS

Case Report

The patient was a group A Rh+ 75-yr-old man undergoing chemotherapy for lymphoma. After initial blood transfusions, he developed antibody to the high-frequency RBC anti-Ge. It reacted in vitro to all tested Ge(+) RBCs at room temperature (2+), at 37°C in saline (1+), and with anti-Ge (1 to 2+). The patient's RBCs were Ge(−) and did not have the elliptocytosis seen in the Leach phenotype, a subset of Ge(−) subjects (*2*). In special testing kindly performed by the American Red Cross National Reference Laboratory, the anti-Ge was determined to be probably anti-Ge2, IgG1 and IgG2 in nature, and strongly reactive in the monocyte monolayer assay (MMA), an in vitro test result usually predictive of immune hemolysis (*1*).

Less than one person in 45,000 people has Ge(−) blood (*3*). Seven units of compatible Ge(−) RBCs were obtained through a nationwide search of the rare blood registries of the American Association of Blood Banks and American Red Cross. These seven units were transfused successfully over a 10-day period, but no additional Ge(−) blood was available. The in vivo cross-match was then performed using an aliquot of a random ABO-compatible Ge(+) unit of RBCs that was incompatible in vitro as above.

RBC Labeling Technique

Under sterile technique for reinjection, 50 μCi of ^{51}Cr was added to 10 ml of donor blood. This was swirled and allowed to stand with occasional mixing over the next 15 min. A 0.5-ml of ascorbic acid solution (50 mg) was added and the solution was swirled again. After 5 min, 20 ml of sterile saline were added, swirled, and centrifuged at 3,000 rpm at 4°C for 10 min. The supernatant was removed with a 3.5-in. 20-gauge sterile needle without stirring the red cells. The supernatant was discarded. Another 20 ml of sterile saline were added, swirled, and recentrifuged, as above. This washing procedure was repeated until the red cells had been washed three times. At the end of third wash, the volume was reconstituted to ~10 ml with cold saline to constitute the mother solution. One part would be reinjected into the patient; the other would be

made into a urine counting standard. Before injection, 5 ml were carefully and thoroughly mixed and drawn into a calibrated syringe for quantitative injection into the patient.

Preparation of Standard

Five milliliters of the mother solution were carefully transferred to a urine-collecting flask (2 liters), which was filled with tap water to the level of 1,800 ml. A small amount of hyamine for cell lysis and a droplet of octanoic acid (saponin) were added to quell foaming.

Urine Counting

Using a bottle similar to that used for preparing the standard, 24-hr specimens collected for six consecutive days were brought to 1,800 ml. If the specimens exceeded 1,800 ml, the sample was divided and each was brought to 1,800 ml. The standard, urine, and water background (BKG) were counted daily in a large well counter with the window of 320 keV ± 10% (288 to 352 keV). The excreted ^{51}Cr in the urine was calculated as:

$$\frac{(\text{urine counts} - \text{BKG}) \times 100}{(\text{standard counts} - \text{BKG})}.$$

The daily percentage of excretion was subtracted cumulatively from 100 and plotted on semi-logarithmic paper. A line was fit through the data points by the least-square's method and half-time and S_{y-x} were calculated.

RESULTS

The urine appearance data are summarized in Table 1 and the ^{51}Cr-RBC survival curve is plotted in Figure 1. The daily individual excretion in percent injected dose over the six days were 1.81, 1.18, 1.21, 1.36, 0.69, and 1.20. Therefore, the daily cumulative residual counts (expressed sequentially as percentages at time 0) were 98.19, 97.01, 95.08, 94.44, 93.75, and 92.55, respectively. As a result, the survival curve fit the following formula:

$$y(\%) = 99.59e^{-0.0123t},$$

where y = residual % and t = days. The half-life of the ^{51}Cr-RBC ($T_{50}{}^{51}Cr$) was 56 days [normal, 44 to 62 (*7*)]

The patient was then given 16 more units of incompatible (by conventional testing) RBCs over the next 3 wk, with no transfusion reactions or clinical evidence of hemolysis.

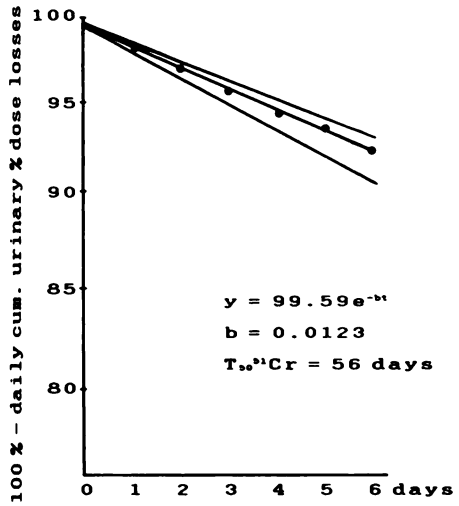

FIGURE 1
Semi-logarithmic plot of total-body retention of injected ^{51}Cr-labeled donor erythrocytes as measured by total urinary losses against time. The half-value of this line of 56 days is within normal limits (44–62 days), indicating no excessive red cell destruction. This curve is based on data presented in Table 1. The envelope of 95% confidence limits is indicated.

TABLE 1
Daily Urinary Excretion of ^{51}Cr-Labeled Erythrocytes

Day	Δ%*	Survival (Cumulative) (%)
0	0	100
1	1.81	98.19
2	1.18	97.01
3	1.21	95.80
4	1.36	94.44
5	0.69	93.75
6	1.20	92.55

* A Δ% shows a daily urinary excretion of ^{51}Cr-labeled erythrocytes.

DISCUSSION

In immunohematology, there are a number of RBC blood groups of very high frequency for which the transfusion significance of alloimmunization is uncertain (*8*). The corresponding alloantibodies in antigen-negative patients cause incompatibility with virtually all routine blood donors studied in vitro. Because of the rarity of these antibodies, usually only a few in each group have been studied for their clinical significance. Even then, conflicting data may exist within the same blood group. Wider use of more convenient in vivo cross-matching would be of great benefit in this situation.

Such is the case for Gerbich antibodies. We found only five previous studies in the literature of Ge(+) red cell transfusions in the presence of anti-Ge. Two patients' antibodies were clinically significant (*9,10*), but three others were not (*1,9*). Our case is unusual in that the antibody proved to be clinically insignificant despite a positive MMA.

We applied the urine-counting ^{51}Cr-RBC survival test instead of the blood-counting method (*11*). The urine-counting method has several advantages over the blood-counting method: it is accurate, does not need numerous venipunctures, and can differentiate gastrointestinal bleeding from hemolysis. The first day's urine count may have been slightly higher than the others because of a minute amount (<0.5%) of free ^{51}Cr present, even though we washed the donor cells. For an in vivo cross-

match, however, the first day's urine counting seemed to provide enough information to predict the compatibility of the blood. Although all urine samples were grouped into 24-hr collections in this case, if earlier results had been designed as in blood sampling method, it would have been possible to quantitate the first voided specimen separately to rule out major incompatibility and hemolysis.

A potential problem that may cause underestimation of hemolysis is sequestration of the tagged RBC, i.e., disappearance from the blood stream without hemolysis and subsequent ^{51}Cr appearance in the urine. However, in practice, this is unlikely to cause much of a problem, since this test was developed on the basis of differentiation of a series of hemolytic patients with sequestration, normal subjects, and patients with gastrointestinal bleeding.

The urine-counting method can also provide an easy way to monitor the ^{51}Cr-labeled transfused red cells by subtracting the expected counts of the previous cross-match.

We presented a rare case of anti-Ge in which in vivo cross-matching was successfully performed by counting ^{51}Cr from labeled erythrocytes excreted in the urine. This RBC survival test provided an easy and accurate means for in vivo cross-match of donor blood that was incompatible when tested in vitro.

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Editorial
In Praise of the Mighty Red Cell

In the course of examining the formulary of diagnostic radiopharmaceuticals, a few agents stand out for their demonstrated versatility and durability. The radiolabeled human erythrocyte is one such agent. In the 48 years that have elapsed since Nobel Laureate George de Hevesy introduced phosphorus-32- (^{32}P) labeled erythrocytes for the determination of blood volume in patients, investigation of the mechanistic aspects of labeling produced advances in methodology which led to thoughtful application of radiolabeled red cells to routine, as well as unique clinical problems. The fact that this level of interest continues today is demonstrated by two articles in this issue of the *Journal*: Mochizuki et al. describe a case in which the creative application of a diagnostic study with chromium-51-labeled red blood cells (RBCs) was possibly lifesaving for the patient by making available a source of blood for transfusion that would have otherwise been thought to be unusable in this patient, and the second describes the role of anion transport on technetium labeling.

The literature reveals a total of 13 radionuclides that have been used to label human erythrocytes: phosphorus-32, chromium-51, carbon-14, iron-55, iron-59, mercury-197, mercury-203, rubidium-81, carbon-11, indium-111, technetium-99m, gallium-67 and gallium-68. A small number of these have been used in humans for:

1. The measurement of RBC volume.
2. Measurement of RBC survival time.
3. Identification of sites of RBC destruction.
4. Blood pool imaging.
5. Selective spleen imaging with damaged RBCs.
6. In vivo cross-matching of donor blood.

It was the introduction of the chromium-51 technique by Sterling and Gray in 1950 that brought radiolabeled red cells into the clinical arena, paved the way for routine evaluation of red cell volume and

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For reprints contact: Ronald J. Callahan, PhD, Massachusetts General Hospital, 32 Fruit St., Boston, MA 02114.