Assessing the clinical significance of anti-Cr^a and anti-M in a chronically transfused sickle cell patient

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Abstract: An alloantibody to a high-incidence antigen, associated with multiple other alloantibodies, made it impossible to supply antigen-negative red blood cells (RBCs) for a chronically transfused sickle cell anemia patient. Anti-Cr^a, -E, -K, -S, -Fy^a, -Fy^b, as well as anti-M reactive at 37°C and in the antiglobulin phase of testing, were identified in the patient's serum. An extensive search of rare donor files at the American Red Cross and at the American Association of Blood Banks (AABB) failed to identify Cr(a−), M−, E−, K−, S−, Fy(a−b−) donors. Various studies were performed to predict the clinical significance of the anti-Cr^a and anti-M. Results of ^{51}chromium survival studies showed 91.8 percent survival at 10 minutes and 87.2 percent survival at 60 minutes with Cr(a+), M−, E−, K−, S−, Fy(a−b−) red cells, suggesting that immediate destruction of transfused Cr(a+) red cells would be unlikely. However, further analysis revealed diminished long-term survival of the donor's red cells with only 60.1 percent recovery at six days (T 1/2 = 12 days) and 10.8 percent at 14 days (T 1/2 = 4.5 days). A monocyte-monolayer assay (MMA) indicated that both the anti-Cr^a (5.9%) and anti-M (18%) would probably be clinically significant (normal value 0–3%). Mass screening continues at several blood centers for Cr(a−), M−, E−, K−, S−, Fy(a−b−) donors. However, if no suitable donors are found, the results of the ^{51}chromium survival studies and the MMA support the decision to transfuse this patient with Cr(a+), M−, Fy(a−b−), S−, K−, E− red cells, if necessary.

We report a patient with sickle cell anemia whose serum contained anti-M reactive at 37°C and in the indirect antiglobulin test (IAT), and IAT-reactive anti-Cr^a, -E, -K, -S, -Fy^a, -Fy^b, and -Lu^a. The probability of finding donors negative for all of these factors was estimated as 1 in 40,000 ABO compatible black donors. All Cr(a−), E−, K−, S−, Fy(a−b−) donor units available through the Red Cross Rare Donor Registry, the AABB Rare Donor File, and the World Health Organization (WHO) were M+. Since the patient's medical condition necessitated transfusion, we considered the advisability of transfusing Cr(a+), M−, E−, K−, S−, Fy(a−b−), or Cr(a−), M+, E−, K−, S−, Fy(a−b−) units. Anti-Cr^a is considered to be a clinically significant alloantibody. The Cr(a−) phenotype is found almost exclusively in blacks, with the incidence of Cr(a−) individuals estimated at 1 in 5,000 black donors.[1] Although anti-M often is not clinically significant, it should be considered of hemolytic potential if the antibody is reactive at 37°C or in the IAT.[2] Various studies were performed to predict the clinical significance of the anti-Cr^a and -M in this patient.

Case History

A blood sample from a 37-year-old black male with sickle cell anemia was referred to a reference laboratory for investigation of multiple antibodies. The patient had received two units of blood 11 months earlier. The patient's RBCs were phenotyped as C+c+D+E−e+, M−N+, S−s+, K−, Fy(a−b−), Jk(a+b+), Le(a−b+) and the patient's serum contained anti-M, -S, and -Fy^a. Since the anti-M was not demonstrable in saline at 37°C or by the IAT, transfusion of M− red cells was not recommended. The patient was transfused with three units of S−, Fy(a−), crossmatch-compatible RBCs. Two weeks later, the hematocrit was 20 percent, and anti-M, -E, -K, -S, -Fy^a, -Fy^b, and a weak unidentified antibody were detected. The anti-M was now reactive 4+ at room temperature (RT), 37°C, and by the IAT using a prewarmed saline technique. The patient's clinical status necessitated transfusion of four units of M−, E−, K−, S−, Fy(a−b−), least incompatible RBCs. The hematocrit rose initially to 24 percent following transfusion, but

dropped to 18 percent within the next two weeks. The weak unidentified antibody was identified at Red Cross national headquarters reference laboratory as anti-Cr^a; anti-Lu^a and an autoanti-I were also detected. The patient's direct antiglobulin test (DAT) was negative and his cells were typed as Cr(a−) with two examples of anti-Cr^a. No compatible blood was available for transfusion. When a sample was submitted one month post-transfusion, microscopic antiglobulin reactivity was still evident with M−, E−, K−, S−, Fy(a−b−) reagent RBCs.

Following a monocyte monolayer assay (MMA) suggesting that both anti-Cr^a and -M would be clinically significant, the patient was treated with corticosteroids for two months. ^{51}Cr-survival studies were done near the end of the treatment period.

Materials and Methods

Serologic studies

Standard serologic methods[3] were used for all testing. Antibody detection and identification were performed with commercial reagent RBCs in LISS and Polybrene media. Ficin capillary techniques were used at Red Cross national headquarters to detect the anti-Cr^a. Red cell typings were performed using commercial blood grouping reagents according to manufacturer's directions.

Blood samples drawn at 60 minutes, 24 hours, 6 days, and 14 days post-injection of ^{51}cr-labeled RBCs were tested against the Cr(a+), M−, E−, K−, S−, Fy(a−b−) donor cells in LISS-IAT. A DAT was performed on each sample using polyspecific antihuman globulin, and an eluate (acid elution) was prepared when indicated.

Survival studies

Red cell survival studies were performed using a 5-mL aliquot of Cr(a+), M−, E−, K−, S−, Fy(a−b−) donor RBCs labeled with 100 μCi ^{51}chromium. The donor cells were phenotypically matched except for the Cr^a antigen. Blood samples were collected at 3 minutes, 10 minutes, 60 minutes, 24 hours, 48 hours, 6 days, and 14 days following injection of radiolabeled RBCs. Radioactivity in the red cell sample collected at three minutes was designated as 100 percent for calculating the percent of RBC survival.

Monocyte monolayer assay (MMA)

A MMA[4] was performed to assess the clinical significance of anti-M and -Cr^a. Normal donor mononuclear cell layers were incubated with Cr(a+), M− and Cr(a−), M+ reagent RBCs sensitized with the patient's antibodies. Monocyte-RBC interaction was reported as percent of reactive monocytes. Testing was done in duplicate with and without the addition of fresh complement and also on serum treated with dithiothreitol (DTT) to remove IgM activity. The total reactivity was measured as % adherence + phagocytosis/200 monocytes.

Results

The results of the monocyte monolayer assay are shown in Table 1. The study was performed on a one-month posttransfusion sample; anti-Cr^a was microscopically demonstrable at that time. Those results suggested that both the anti-M (18%) and anti-Cr^a (5.9%) would be clinically significant and that reactivity was enhanced in the presence of complement.

Table 1.
MMA results with Cr(a+), M+ or M−, E−, K−, S−, Fy(a−b−) cells

RBC phenotype	*Coated test RBCs* 37°C	anti-IgG	*Reactive* monocytes* %
Cr(a+), M− Complement present	0	micro	5.9
Cr(a+), M+ Complement present	+	++	18.0
Cr(a+), M− No complement present	0	micro	0.7
Cr(a+), M+ No complement present	+	++	5.9
Cr(a+), M+ Serum DTT-treated	0	+(weak)	11.0

* Normal levels are 0–3%.

Table 2 gives the results of the ^{51}chromium RBC survival study using Cr(a+), M− RBCs. Serologic studies were performed on a sample drawn four months post-transfusion. Anti-Cr^a was not demonstrable at that time. Red cell survival of 91.8 percent at 10 minutes and 87.2 percent at 60 minutes suggested that immediate destruction of transfused Cr(a+) RBCs would be unlikely. However, long-term survival of the donor's cells was diminished with only 60.1 percent recovery at six days (T 1/2 = 12 days) and 10.8 percent at 14 days (T 1/2 = 4.5 days).

Table 2.
Survival of ^{51}chromium-labeled Cr(a+), M−, E−, K−, S−, Fy(a−b−) donor cells

Time	Red cell recovery*%	^{51}Chromium plasma activity†%	T 1/2‡ (days)
10 min	91.8	0.48	
60 min	87.2	0.21	
24 hr	79.9		
48 hr	74.7		
6 days	60.1		12
14 days	10.8		4.5

*Acceptable = > 70% recovery at 60 minutes.
†Acceptable = < 5% ^{51}chromium plasma activity at 10 and 60 minutes.
‡Normal = 25–35 days.

Results of serologic testing following the ^{51}chromium survival study are given in Table 3. No serum reactivity was observed until 14 days later when microscopic reactivity was noted in the IAT and DAT. An acid eluate prepared from the 14-day sample was microscopically positive with both Cr(a+), M− and Cr(a−), M+ RBCs.

Table 3.
Serum testing following the ^{51}chromium survival study

Time	IAT	DAT
3 min	0	0
60 min	0	0
24 hr	0	0
48 hr	0	0
6 days	0	0
14 days	micro*	micro†

*Titer 1:4 with Cr(a+), M−, E−, K−, S−, Fy(a−b−) donor cells.
†Acid eluate reactive with CR(a+), M− and Cr(a−), M+ RBCs.

Discussion

The patient's combination of anti-Cr^a and other multiple alloantibodies, including a possibly clinically significant anti-M, made it impossible to provide antigen-negative RBCs for transfusion. In addition, the patient's diagnosis of sickle cell anemia further complicated the evaluation of his response to transfusion therapy.

Reports of ^{51}chromium-labeled red cell survival studies using Cr(a+) red cells in individuals with anti-Cr^a have shown varying results. Daniels[5] reported significant destruction of Cr(a+) RBCs at 15 minutes, and McSwain and Robins[6] reported only 57.7 percent of the test dose surviving at 4 hours and 36.2 percent at 21 hours. However, Smith et al.[7] found minimal immediate red cell destruction with 94 percent recovery at one hour and 82 percent at 17 hours, but long-term survival was decreased, with only 34 percent of the labeled RBCs remaining after 21 days. Ross and McCall[8] found similar results with 89 percent recovery at one hour, 82.6 percent at 24 hours, and 73 percent at 96 hours.

In 1980, the International Committee for Standardization in Haematology[9] recommended that a one-hour red cell survival > 70 percent with < five percent ^{51}chromium label in the plasma in both the 10- and 60-minute samples indicated that, in an emergency situation, a unit volume of those cells could be transfused with little likelihood of immediate red cell destruction. Silvergleid et al.[10] reported the use of the one-hour in vivo survival of ^{51}chromium-labeled RBCs to select units for transfusion in 38 patients for whom crossmatch-compatible blood was unavailable. His studies concluded that a one-hour survival of > 85 percent with a test dose of antigen-positive RBCs should not be associated with a hemolytic transfusion reaction. Davey et al.[11] suggested that 70 percent survival at 24 hours should be the standard for acceptable red cell survival. Thus, our patient's results of 87.2 percent survival at 60 minutes and 79.9 percent at 24 hours, and plasma values of 0.48 percent at 10 minutes and 0.21 percent at 60 minutes suggest that immediate red cell destruction would be unlikely in this case.

The initial slower rate of red cell removal was followed by accelerated red cell destruction during the first and second weeks, resulting in the survival curve exhibiting two slopes. The long-term survival of the donor red cells was decreased, with 60.1 percent and 10.8 percent of the cells recovered after 6 and 14 days, respectively. The T 1/2 of 12 days (normal 25–35 days), when calculated at 6 days, had decreased to 4.5 days during the second week of the study. The hematocrit decreased concomitantly from 33 percent to 27 percent. A microscopically positive IAT and DAT were detected when the 14-day sample was tested. Testing of the eluate was limited due to the amount of specimen, but no specificity for Cr^a was apparent, as both Cr(a−) and Cr(a+) cells were reactive.

One explanation for the development of a positive IAT and DAT could be an anamnestic response of the anti-Cr^a due to the test dose of Cr(a+) RBCs. However, our patient had experienced a similar decrease in hematocrit following transfusion with Cr(a+), M−, E−, K−, S−, Fy(a−b−) RBCs two months earlier with no increase in antibody reactivity on subsequent samples,

and the DAT remained negative. This was similar to our experience with other sickle cell patients transfused with phenotypically matched RBCs, who experienced apparent delayed hemolytic transfusion reactions several days posttransfusion. In these cases, no additional alloantibodies could be demonstrated even after exhaustive testing.

Another explanation may be the possibility of autoantibody formation in these patients. Petz et al.[12] have demonstrated the presence of IgG on the red cells of 62 percent of patients with sickle cell disease using the complement-fixing antibody consumption test. The authors described a patient with a life- threatening hemolytic transfusion reaction without demonstrable alloantibody whose red cells eluted an apparent autoantibody with undetermined specificity. They suggested that transfusion may precipitate the hemolysis by stimulating further production of autoantibody. Our patient's favorable response to corticosteroid treatment may support this theory of autoantibody formation.

Alloantibodies are not generally considered to be clinically significant unless they are active at 37°C or in the IAT. Anti-M was not demonstrable in saline at 37°C or in the IAT on initial investigation; thus, transfusion of M− red cells was not recommended. Subsequent transfusions two weeks later elicited an anamnestic response and anti-M was then strongly reactive in all phases of testing, including 37°C and in the IAT. Alperin et al.[13] correlated ^{51}chromium red cell survival studies with in vitro monocyte-macrophage phagocytosis, implicating anti-M in delayed hemolytic transfusion reactions.

The MMA has been used as an alternative method to ^{51}cr- survival studies for predicting the clinical significance of alloantibodies.[14] Our results indicated that Cr(a−), M+ red cells sensitized with the patient's serum were more reactive (18%) with the monocytes than the Cr(a+), M− red cells (5.9%). As the normal range for this test is 0–3 percent, both the anti-Cr^a and the anti-M appeared to be clinically significant. However, the Cr(a−), M+ cells, sensitized with the patient's serum, were more reactive with the monocytes. We considered this further support for the use of Cr(+), M− antigen-negative RBCs for transfusion of this patient because there were no Cr(a−), M− antigen-negative units of blood available.

We are currently working with many blood centers to screen for Cr(a−), M−, E−, K−, S−, Fy(a−b−) blood for this patient in the event that his condition necessitates transfusion in the future.

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