Anti-M causing delayed hemolytic transfusion reaction

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A 52-year-old gravida 1, para 1 woman with M– red cells experienced a delayed hemolytic transfusion reaction and exhibited an anti-M antibody following the infusion of four units of M+ red cells. Measurements of erythrocyte survival using ^{51}Cr-labeled donor M+ and M– red cells and in vitro studies of monocyte-macrophage phagocytosis of sensitized reagent red cells implicate anti-M in the pathogenesis of hemolysis. **TRANSFUSION** 1983;23: 322–324.

SEVERAL REPORTS describe anti-M causing a delayed hemolytic transfusion reaction (DHTR).[1-5] Most, however, lack data correlating anti-M with the immunologic destruction of transfused red cells (RBC) because clear signs of DHTR were not described[1,3,4] or because other antibodies, also capable of producing hemolysis, were present in addition to anti-M.[1-3] A woman who developed anti-M while experiencing a DHTR provided a unique opportunity for investigation. The studies performed correlate survival of ^{51}Cr-labeled M+ and M– donor RBC in this patient with in vitro studies of monocyte-macrophage phagocytosis of RBC sensitized with her anti-M.

Case Report

A 52-year-old gravida 1, para 1 Caucasian woman had been transfused with eight units of blood for gastrointestinal hemorrhage 10 years ago. For treatment of a new episode of gastrointestinal hemorrhage, she was given five units of packed RBC that quickly raised her hematocrit from 11 to 27 percent. Twelve days later, without signs of rebleeding, her hematocrit was 17 percent, and her spleen, not felt at the time of admission, was palpable 8 cm below the left costal margin. Other laboratory data included reticulocytes 16.7 percent, total bilirubin 1.8 mg per dl, direct reacting bilirubin 0.4 mg per dl, lactic dehydrogenase 539 IU per l, haptoglobin 15 mg per dl, and urine sediment negative for hemosiderin.

A suspected DHTR was investigated using standard serologic techniques[6] and commercially available reagents. A direct antiglobulin test (DAT) was negative. An indirect antiglobulin test (IAT), previously negative, became 3+ positive. The patient's serum contained an anti-M reactive at room temperature and at 37°C, with a titer of 8 in an IAT using anti-IgG. Treating the patient's serum with 0.01 M dithiothreitol (DDT) failed to reduce anti-M reactivity in an IAT performed at 37°C. Anti-M activity at room temperature, however, was reduced by DTT, indicating the antibody possessed properties of IgG and IgM. Other antibodies detected included an anti-K with an IAT titer of 4 and a weakly reactive antibody of undetermined specificity that had a high titer but low avidity (HTLA). We observed no other unexpected antibodies in the patient's serum.

After the DHTR, the patient's RBC were phenotyped as group A, R$_1$r and negative for M, Le^a, S, K, and Jk^b. No mixed-field reactions were noted, indicating the absence of any of the transfused RBC. Of the five units of RBC transfused, four were M+; three were Jk^b+; two were S+; two were N+; one was Le^a+; and one was K+. Furthermore, two of the transfused units were homozygous for the M antigen; two were heterozygous for the M and N antigens; and one was homozygous for the N antigen. The latter unit was also K+.

No more RBC transfusions were administered. The patient was given 2 grams of iron dextran intravenously. Five weeks later, when her hematocrit measured 42 percent and her spleen was no longer palpable, special studies were initiated.

Methods and Materials

^{51}Chromium Survival Studies

Two studies of erythrocyte survival were performed 13 days apart using 5-ml aliquots of donor RBC each labeled with 20 μCi ^{51}Cr.[7,8] Both units of donor RBC were collected in citrate-phosphate-dextrose-adenine (CPDA-1) and were 4 to 5 days old when the survival studies were conducted. In each study, blood samples were collected from the opposite arm at 10 and 60 min and 3, 6, and 24 hours following the injection of washed radiolabeled RBC. Radioactivity in RBC collected at 10 min was designated as 100 percent for calculating RBC survival at other time intervals. In the first study, donor cells were M+, N–, Le^a–, K–, S+, and Jk^b+. In the second study, donor cells were M–, N+, Le^a–, K–, S+, and Jk^b+. The patient's serum reacted with the donor cells in the first study but not with the donor cells used in the second study. While measuring RBC survival, the patient's serum exhibited an anti-M IAT titer of 2 and an anti-K IAT titer of 4.

Monocyte-Macrophage Assays

Techniques previously described were used to investigate monocyte-macrophage interaction with RBC.[9-11] Mononuclear cell layers, isolated from heparinized samples of normal (allogeneic) and the patient's (autologous) blood, were incubated with selected reagent RBC sensitized with anti-M from the patient's serum. These reagent RBC, however, were not obtained from donors whose erythrocytes were used in the survival studies. The mononuclear cell layers were then stained with Wright-Giemsa and examined microscopically. Interaction of RBC with monocyte-macro-

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Submitted for publication July 20, 1982; revision received September 11, 1982, and accepted September 13, 1982.

0041-1132/83/0700/0322 $00.95

phages was expressed two ways. The number of RBC attached to or phagocytized by 100 monocyte-macrophages represents the total association index (ARBC). The number of RBC phagocytized per 100 monocyte-macrophages represents the phagocytic index (PRBC). Testing 41 normal monocyte-macrophage monolayers against normal unsensitized allogeneic RBC gave the following normal values ± 2 standard deviations (2 SD): ARBC 6.7 ± 10.2 and PRBC 0.2 ± 1.0.[10] Values for ARBC greater than 17 and values for PRBC greater than 2 are considered significantly elevated when compared to normal.

Results

Figure 1 shows that the M+N− erythrocytes (M+ cells) exhibited a rapid disappearance from the circulation with only 17.2 percent of radioactivity remaining after 1 hour and 1.4 percent remaining after 24 hours. The M−N+ erythrocytes (M− cells) exhibited a slower disappearance from the circulation with 95 percent remaining after 1 hour and 81 percent remaining after 24 hours.

Table 1 illustrates the results obtained when reagent RBC were sensitized with the patient's serum and then interacted with either allogeneic or autologous monocyte-macrophage monolayers. The first allogeneic monolayer failed to demonstrate either significant ARBC or PRBC interactions with sensitized M+N− erythrocytes. Two other unrelated allogeneic monolayers (#2 and #3) demonstrated significant PRBC, but only #2 demonstrated significant ARBC using homozygous M reagent RBC. One of the monolayers (#3) demonstrated specificity by showing significant PRBC using sensitized M+N− and M+N+ erythrocytes but not with M−N+ erythrocytes. Using sensitized M+N+ reagent RBC this last allogeneic monocyte-macrophage monolayer showed significant ARBC, but results were insignificant using M+N− reagent RBC. Studies using autologous monocyte-macrophages, however, showed both ARBC and PRBC to be clearly elevated with either sensitized M+N− or M+N+ erythrocytes but not with M−N+ erythrocytes. The weakly positive antiglobulin test showed the latter RBC were weakly sensitized by the HTLA antibody present in the patient's serum. Studies using unsensitized RBC as controls uniformly fell within normal limits and all PRBC values were zero. Reagent RBC were K− in all monocyte-macrophage studies.

Discussion

Anti-M has been implicated in the pathogenesis of DHTR, but most published reports have not demonstrated conclusively that anti-M caused RBC destruction.[1-4] Initial studies performed on our patient, following RBC transfusions for the second episode of gastrointestinal hemorrhage, meet criteria for a DHTR as defined by Pineda et al.,[12] except the rapid destruction of sensitized RBC resulted in a negative DAT. The appearance of anti-M in her serum suggested this antibody was responsible for the loss of donor RBC. The presence of anti-K in her serum may have contributed to destruction of one unit of transfused RBC, but this antibody could not be responsible for the loss of all donor cells.

Data in Figure 1 demonstrate rapid destruction of

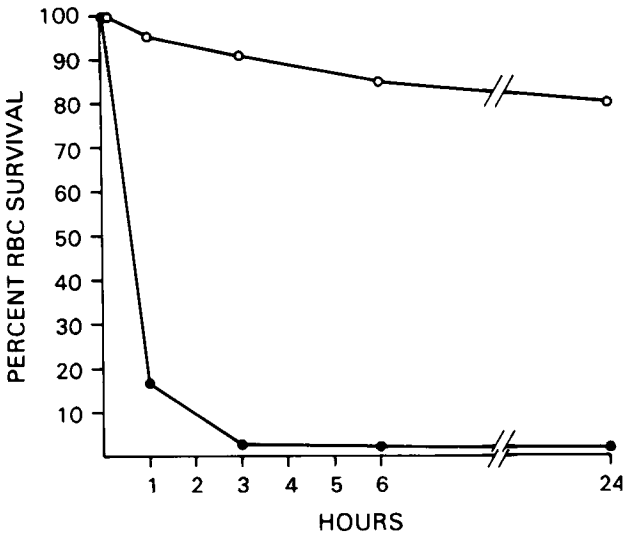

FIG. 1. Survival of ^{51}Cr-labeled M+ and M− donor red cells. ●——● M+ cells; O——O, M− cells.

M+ donor cells transfused into the patient. The M− donor RBC, however, exhibit a much slower rate of disappearance from the circulation when injected into this same patient. Indeed, the 1-hour survival of M− cells (95%) falls within the published normal range.[5,7,8,13] The unexplained reduction in the 24-hour survival (81%) of M− donor RBC is probably insignificant; this slight decrease does not indicate a high degree of RBC destruction compared to that caused by anti-M. All measurements of RBC survival occurred while the patient had an anti-M titer of only 2. Results of these measurements support the view that anti-M played an

Table 1. *Interaction of monocyte-macrophages with red cells sensitized with anti-M*

Monocyte-macrophage sources	RBC Phenotypes	Monocyte-macrophage Interaction		
		AGT*	ARBC†	PRBC‡
Allogeneic 1	M+N−	2½+	10	1
Allogeneic 2	M+N−	2½+	18	3
Allogeneic 3	M+N−	3+	13	8
	M+N+	2½+	22	2
	M−N+	½+	5	0
Autologous	M+N−	3+	31	4
	M+N+	2½+	40	3
	M−N+	½+	4	0

* Antiglobulin test using a standard technique employing anti-IgG.[6]

† Total Association Index, i.e., the average number of attached or phagocytized RBC per 100 monocyte-macrophages. Normal mean ± 2 SD = 6.7 ± 10.2.

‡ Phagocytic Index, i.e., the average number of phagocytized RBC per 100 monocyte-macrophages. Normal mean ± 2 SD = 0.2 ± 1.0.

important role in the pathogenesis of RBC destruction and the DHTR. Mollison[5] has shown greatly decreased RBC survival due to anti-M antibodies that were nonreactive in an IAT but did react at 30°C by agglutination. Although HTLA antibodies were present, they have little if any clinical significance.[13] We doubt that this antibody contributed to our patient's DHTR.

Studies with monocyte-macrophage assays suggest this technique may be useful in predicting the clinical significance of alloantibodies.[9,10] The results of in vitro studies with monocyte-macrophage assays reported here are supported by the donor RBC survival. The patient's (autologous) monocyte-macrophages exhibited significant elevations of ARBC and PRBC when incubated with M+ reagent RBC sensitized by the anti-M in the patient's serum. Results with normal, allogeneic monocyte-macrophages were not as definite, although they too suggest that anti-M in the patient's serum sensitized M+ erythrocytes with resultant phagocytosis by mononuclear cells. These monocyte-macrophage assays showed that M+ red cells, sensitized with the patient's serum, were more reactive with the patient's own mononuclear cells than with some normal, allogeneic mononuclear cells. These preliminary data provide additional evidence that in vitro studies of mononuclear phagocytosis of sensitized RBC may prove useful in the diagnosis and evaluation of antibody-mediated hemolytic disease or in the selection of compatible units of RBC for transfusion.[9,10] These observations suggest that mononuclear cells from a patient are more sensitive to immunologically induced phagocytosis than mononuclear cells from normal allogeneic sources. This was observed previously in three patients with DAT-negative acquired hemolytic disease.[10]

Phagocytosis of antibody-coated RBC is the usual mechanism of immune-mediated extravascular hemolytic anemia. Fudenberg and Allen[14] described three patients with hemolytic transfusion reactions in whom splenic sequestration of incompatible donor RBC indicated an extravascular site of erythrocyte destruction. The sudden, transient splenic enlargement observed in our patient suggests RBC destruction occurred in splenic macrophages. This correlates with the results of monocyte-macrophage assays performed with the patient's own mononuclear cells (Table 1).

Anti-M occurs in about 2 percent of patients reported to have DHTR.[15] Why rare examples of anti-M cause hemolytic reactions while others do not is unknown. We should not assume that RBC destruction will or will not occur necessarily in the presence of anti-M. The use of in vitro assays may be predictive of antibody significance in vivo; however, further evaluation of these assay systems is required.

Acknowledgments

The authors are indebted to Vicky Smith who assisted in the selection of the donor RBC used in the measurement of erythrocyte survival and to Brenda Berry who typed the manuscript.

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