Comparison of tube and gel red blood cell agglutination techniques in detecting chimeras after major ABO-mismatched allogeneic hematopoietic stem cell transplantation

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We compared the ability of tube and gel red blood cell (RBC) agglutination techniques to follow erythroid engraftment in a patient who received a major ABO-mismatched peripheral blood stem cell transplant and bone marrow transplant. Tube and gel RBC agglutination techniques were used to detect mixed-field reactivity in cell mixtures containing A/O and c+/c– RBCs and the ability of these two technologies to detect RBC chimeras were compared. We detected c+ RBCs in c+/c– RBC populations microscopically at 1% by the tube RBC agglutination technique, but not until 10% by the gel technique. Group A RBCs in A/O RBC populations were detected at 10% by both techniques. In the patient studied, group A RBCs and c+ RBCs were detected on Days 20 and 14, respectively, with the tube RBC agglutination technique, but neither marker was detected until Day 26 with the gel technique. Tube and gel RBC agglutination techniques comparably identified ABO mixed fields. Although the tube RBC agglutination technique showed greater sensitivity than the gel technique in detecting the c antigen, the gel technique was easier to use and allowed more reliable interpretation of mixed fields by the technologist. ***Immunohematology* 1998;14:63–67.**

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Patients with hematologic malignancies who undergo ablative therapy involving total-body irradiation and cytotoxic drugs are often rescued from such treatment with a transplantation of allogeneic stem cells from bone marrow. Collecting the donor's bone marrow, however, is a painful procedure usually requiring hospitalization and general anesthesia.[1] This process now can be avoided because of the efficacy of a synthetic preparation of the growth factor, granulocyte colony–stimulating factor (G-CSF), in increasing the number of stem cells in the peripheral circulation.[2] Peripheral blood stem cells (PBSCs) are an easily accessible alternative to bone marrow as a source of stem cells for transplantation in treating hematologic malignancies. Recent studies indicate that by optimizing stem cell and lymphocyte doses, an allogeneic PBSC transplantation performed in place of an allogeneic bone marrow transplant (BMT) results in faster engraftment, fewer posttransplant red blood cell (RBC) and platelet transfusions, and no greater incidence of acute or chronic graft-versus-host disease (GVHD).[3] Thus, transplant outcome can be improved.

Regardless of matched donor-recipient pair ABO types, PBSCs from HLA-identical sibling donors have been used to treat patients with severe hematologic malignancies including chronic myelogenous leukemia (CML), acute lymphoblastic leukemia, and multiple myeloma. In fact, 15 percent to 20 percent of allogeneic BMT cases involve ABO-incompatible transplants,[4] and studies indicate that ABO incompatibility does not affect the incidence of graft rejection,[4] engraftment,[5] GVHD,[6] or patient survival in BMT.[7]

This study compared the ability of tube and gel RBC agglutination techniques to follow erythroid engraftment. We compared their efficacy in detecting antigen markers in prepared mixtures of RBC populations and in mixtures resulting from in vivo engraftment of erythroid cells. A 37-year-old male patient with CML received major ABO-mismatched PBSCs as well as bone marrow from an HLA-identical sibling. Because the donor and the recipient were mismatched in both the ABO and c phenotypes, it was possible to analyze the sensitivity of the

traditional tube RBC agglutination technique and the gel RBC agglutination technique in detecting mixed chimerism as RBC engraftment occurred in the weeks following transplantation.

Case Report

Serologic and historical data were collected on a 37-year-old male patient with CML who was treated by hematopoietic stem cell transplantation. The patient, group O, c–, received major ABO-mismatched PBSC and bone marrow transplants from a 58-year-old, HLA-matched group A, c+ sister.

The PBSC donor was given six doses of G-CSF on each of the 6 days before the transplant to increase the stem cells (identified by a CD34+ marker) in her peripheral circulation from normal levels of less than 2×10^6 CD34+ cells per liter to 16×10^6 CD34+ cells per liter. The PBSC collection was performed by apheresis on both the day before transplant (Day –1) and the day of the transplant (Day 0). The collected products were pooled on Day 0.

To isolate CD34+ stem cells from the pooled product, biotinylated anti-CD34 was used. The treated product was then run through a column containing avidin (CellPro, Inc., Bothell, WA), from which the CD34+ rich product was removed and collected in a bag. The PBSC product then underwent a negative selection procedure using biotinylated anti-CD2 and a second avidin column (CellPro, Inc.). Because T-lymphocytes are implicated in acute GVHD, the goal of the negative selection was to reduce their number to levels below 1×10^5 CD3+ cells per kilogram of the patient's weight. After processing, the PBSC product contained 1.63×10^6 CD34+ cells per kilogram of the patient's weight and 0.26×10^5 CD3+ cells per kilogram of the patient's weight.

Because the minimum transplant dose established by the transplant protocol was 3×10^6 CD34+ cells per kilogram of the patient's weight, additional donor stem cells were collected by bone marrow aspiration on Day 1. The marrow was processed only to select for CD34+ cells, as described for the PBSC product (CellPro, Inc.). After processing, the bone marrow product contained 1.06×10^6 CD34+ cells per kilogram of the patient's weight and 0.25×10^6 CD3+ cells per kilogram of the patient's weight.

The patient was given a preparative regimen of 1,360 rads of TBI given in eight doses on pretransplant Day –7 through Day –4 and 60 mg/kg doses of cyclophosphamide on pretransplant Day –3 and Day –2. He was then transfused with the PBSCs on Day 0 and additional marrow on Day 1, for a total of 2.69×10^6 CD34+ cells and 0.5×10^5 CD3+ cells.

Materials and Methods

To establish the baseline sensitivities of the tube and gel RBC agglutination techniques in detecting mixed chimeras, an initial analysis was performed with RBC mixtures prepared in the laboratory. Six chimeric samples were created by mixing the appropriate volumes of group A, c+ RBCs with group O, c– RBCs, such that the concentration of the group A, c+ population sequentially increased from 1 percent to 50 percent. Cell mixtures of 1, 10, 20, 30, 40, and 50 percent were prepared. Each mixture was then typed simultaneously by both the tube and gel RBC agglutination techniques (Micro Typing Systems, Inc., Pompano Beach, FL).[8]

Samples of the PBSC transplant recipient's RBCs and plasma were collected in EDTA tubes and tested on Days 8, 14, 20, 26, 34, and 42. To detect mixed chimeras, each sample was typed for ABO and c by both the gel and the traditional tube RBC agglutination techniques using mouse monoclonal antisera. Standard methods were followed to perform isohemagglutinin titrations and direct antiglobulin tests (DATs) on samples collected from the patient.[8] When the DAT was positive, antibody specificity was determined by performing a rapid acid elution from intact RBCs according to the manufacturer's directions (Elu-Kit II, Gamma Biologicals, Inc., Houston, TX). All agglutination reactions were graded as indicated in Table 1.[8] All testing was performed by one person.

Table 1. Grading guide for agglutination reactions

Grade	Tube Technique	GEL Technique
4+	Solid agglutinate + clear background	Agglutination band at top of column
3+	Several large agglutinates + clear background	Agglutination restricted to upper half of column
2+	Dispersed agglutinates + clear background	Agglutination dispersed throughout column
1+	Dispersed agglutinates + cloudy background	Agglutination restricted to lower half of column
W+	Microscopic agglutination only	Not applicable
0	No agglutination	No agglutination
mf = mixed field	Microscopic evaluation of two distinct RBC populations	Macroscopic assessment of band at top of column + RBC pellet at bottom

Results

Analysis of in vitro mixed chimeras

The tube RBC agglutination technique was more sensitive than the gel technique in detecting the c marker when a mouse monoclonal antibody was used. In prepared group A, c+ and group O, c– RBC mixed popula-

tions, the tube technique detected cells expressing the c antigen at a concentration of 1 percent; the graded strength of the agglutination increased with increasing concentrations of cells expressing c (Table 2). The gel RBC agglutination technique, however, first detected cells expressing the c marker at a concentration of 10 percent; the agglutination of the c+ RBCs was graded as a 4+. The 4+ agglutination remained constant as concentration of RBCs expressing c increased. Only subtle differences were apparent in the thickness of the agglutination band at the top of the gel column (Table 2).

The tube and gel RBC agglutination techniques were equally sensitive for group A when using a mouse monoclonal antibody. In prepared group A, c+ and group O, c– RBC mixed populations, the A blood group was detected at a 10 percent concentration by both the tube and the gel RBC agglutination techniques (Table 2).

Table 2. Agglutination grade of mixed-chimeric red blood cells (RBCs) prepared in the laboratory

RBC composition	Tube RBC agglutination technique		Gel RBC agglutination technique	
A,c+/O,c–	anti-A	anti-c	anti-A	anti-c
50%	4+^{mf*}	4+^{mf}	4+^{mf} heavy	4+^{mf} heavy
40%	4+^{mf}	4+^{mf}	4+^{mf} heavy	4+^{mf} heavy
30%	4+^{mf}	3+^{mf}	4+ ^{mf} moderate	4+^{mf} moderate
20%	4+^{mf}	3+^{mf}	4+^{mf} moderate	4+^{mf} moderate
10%	1+^{mf}	1+^{mf}	4+^{mf} light	4+^{mf} light
1%	0	w+^{mf}	0	0

*Mixed field

Mixed chimeras were more reliably detected by the gel than by the tube RBC agglutination technique. The tube technique required a specific awareness of the posssibility of a mixed field for the technologist to accurately detect it, whereas the gel technique displayed an indisputable mixed field when it was present, whether the technologist was alerted to its potential presence or not. For example, in a prepared mixture of 10 percent group A, c+ RBCs with group O, c– RBCs, the gel technique responded with its strongest grade (4+), while the tube technique revealed a weaker grade reaction (1+) (Table 2).

Analysis of the patient's mixed populations

Both assays were able to detect mixed populations containing group A, c+ RBCs posttransplantation. The detection of a mixed field using mouse monoclonal anti-A test sera occurred on Day 20 with the tube RBC agglutination technique but not until Day 26 with the gel RBC agglutination technique (Table 3). The detection of a mixed field using mouse monoclonal anti-c test sera occurred on Day 14 with the tube RBC agglutination technique but not until Day 26 with the gel technique (Table 3). It should be noted, however, that macroscopic detection of mixed fields by the tube technique did not appear for either marker until Day 26 (Table 3).

Recipient isohemagglutinin levels were measured to confirm donor stem cell engraftment. As expected, before PBSC transplantation, the patient's serum (genetically type O) contained both anti-A and anti-B. Anti-A titers steadily decreased from a level of 64 pretransplantation to 1 by Day 42 posttransplantation. Anti-B titers decreased at a more gradual rate, from a level of 64 pretransplantation to 32 by Day 42 posttransplantation (Table 3).

These results show that RBC engraftment began on Day 14. Myeloid cell engraftment also occurred by Day 14, but evidence of platelet engraftment was not present until Day 26.

As a consequence of the ABO-mismatched transplant engraftment, the DAT first tested positive with polyspecific anti-IgG,C3d on Day 11 and remained positive through Day 42, which was the last day tested in this study (Table 3). When the patient's chimeric (A/O) RBCs were eluted, anti-A was detected on Day 11. The strength of anti-A that was eluted increased in successive weeks from a grade of w+ on Day 14 to a grade of 2+ on Day 42 (Table 3).

Discussion

The gel RBC agglutination technique is currently approved by the Food and Drug Administration for reverse ABO typing, DATs, and indirect antiglobulin tests, and it is being reviewed by the FDA for forward ABO typing. Although it has failed to detect several clinically insignificant antibodies, the gel RBC agglutination technique has increased sensitivity for some antibodies that may be missed by traditional tube technology.[9,10] It is as good or better than the tube RBC agglutination technique for the above tests because of its simplicity, reliability, and practicality.[11]

We found that both the tube and the gel RBC agglutination techniques can be used to assess mixed populations of RBCs. Using monoclonal anti-c, the tube RBC agglutination technique made earlier detection of mixed fields possible in both prepared and clinical samples, but this was contingent on *microscopic* evaluation. However, microscopic findings are often subject to interpretation by the technologist. An analysis of *macroscopic* tube RBC agglutination readings revealed c antigen mixed-field detection at the same time posttransplantation as the gel RBC agglutination technique.

Table 3. Transplant recipient blood counts and results of serolgic analysis

Day	Blood Counts					Tube Agglutination				Gel Agglutination				Titers		AHG† Assays	
	Hgb g/dL	Hct %	Plts 10^9/L	WBCs 10^9/L	ANC* 10^9/L	Anti-A	A_1 red cells	B red cells	Anti-c	Anti-A	A_1 red cells	B red cells	Anti-c	Anti-A‡	Anti-B§	DAT	Eluate
–7	15	43	597	23.4	22.77									64	64	NT‖	NT
0	10	29	243	0.38	0.314												
2	10	29	69	0.08	0.044									32	32	0	NT
8	6.8	20	33	0.02	0.000	0	4+	4+	0	0	4+	4+	0	8	32	0	NT
11	6.9	20	28	0.03	0.014											AHGvw+	anti-A vw+¶
14	8.4	25	26	1.10	0.854	0	2+	4+	vw+^{mf}	0	3+	4+	0	2	16	AHGw+	anti-A W+**
20	9.1	26	23	3.35	2.982	w+^{mf}	1+	4+	w+^{mf}	0	0	4+	0	2	16	AHGw+	anti-A 2+
26	8.8	24	73	1.17	0.538	3+^{mf}	1+	4+	1+^{mf}	4+^{mf}	0	4+	4+^{mf}	2	32	AHG2+	anti-A 1+
34	9.9	27	87	1.18	0.843	3+^{mf}	1+	4+	2+^{mf}	4+^{mf}	0	4+	4+^{mf}	1	32	AHGw+	anti-A 2+
42	8.5	24	112	2.27	1.185	4+^{mf}	1+	3+	3+^{mf}	4+^{mf}	0	4+	4+^{mf}	1	32	AHG1+	anti-A 2+

* Absolute neutrophil count
† Antihuman globulin
‡ Normal = 8 to 2,048
§ Normal = 8 to 256
‖ Not tested
¶ Very weak
** Weak

The two techniques were comparable when using ABO antigens to detect engraftment. As with the c antigen agglutination reactions, microscopic evaluation of ABO types by the tube RBC agglutination technique detected erythroid engraftment earlier than did the gel RBC agglutination technique. Yet a comparison of macroscopic data alone for both techniques revealed detection of ABO mixed chimeras at identical times posttransplantation. Thus, the two techniques demonstrated comparable sensitivity in detecting ABO-mismatched mixed chimeras.

It is interesting to note that the patient's B isohemagglutinin titer decreased slightly despite the transplantation of stem cells from the A donor (Table 3). This decrease can be attributed to the conditioning regimen as well as to the lag time before recipient engraftment of donor lymphocytes and the subsequent production of anti-B.

In conclusion, if it is critical to detect the earliest production of donor RBCs in a particular patient, the tube RBC agglutination technique may prove the best choice. After a major ABO-mismatched allogeneic transplant, however, we found gel RBC agglutination easier to use and less susceptible to interpretation discrepancies by the technologist in the detection of mixed fields.

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