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Serological and molecular analysis of ABO and Rh blood group chimeras

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ABSTRACT

The serological examination, blood transfusion strategies and the molecular analysis to blood group chimera were conducted to demonstrate existent of chimera in blood group. The blood grouping of ABO or/and RhD, newborn red blood cells separated by capillary centrifugation. Aabsorption tests and DTT treated agglutination erythrocyte tests were implemented in four patients. Further molecular biological research was conducted on one patient´s sample. The results showed that for patient 1: ABO blood group was AB/B chimera, Rh blood cells contained the RhCE chimera gene; Patient 2: Rh blood cells contained the RhD chimera gene; Patient 3: ABO blood group was AB/B chimera, Rh blood cells contained the RhD chimera gene; Patient 4: ABO blood group was O/B chimera, Rh blood cells contained the RhCE chimera gene. The study suggests that the individuals categorized as chimeras are likely to be more common than existing literature reports. According to the serological tests, in the absence of a history of recent blood transfusion or disease to cause reduced antigen, the phenomena of hybrid aggregation of the ABO and Rh blood system were the main feature. In terms of transfusion strategy, the selection of ABO and Rh blood groups should be depended on the group of cells with more antigens.

Keywords: blood group chimera, blood transfusion, ABO, Rh

INTRODUCTION

The *chimera* is genetically defined as "a single organism composed of cells with distinct genotypes" – which means an individual derived from two or three zygotes in very rare cases, where the organism possesses blood cells of different blood types. In genetics, chimerism refers to individuals exhibiting two or more different sets of genes. Similarly, in immunology, it means the existence of different cell lines from two or more chromosomes, residing in one individual. These cell lines are able to tolerate each other, which means immunotolerance, do not stimulate rejection, and result in what is known as a *chimeric state*. According to different purposes and meanings, chimeras can be classified as homologous chimera (also called "mosaic chimera"), heterogenous chimera (also called "camilla chimera"); congenital or acquired chimerism; blood group chimera or organ chimera.

The present studies of chimerism mainly focus on bone marrow hematopoietic diseases, stem cell transplantation (especially in bone marrow transplantation in patients with leukemia), and the diagnosis of prenatal genetic diseases. However there still remain few reports on chimera in blood transfusion in China. The four cases of blood group chimerism recently discovered in our laboratory were reported in this study.

MATERIALS AND METHODS

Patients

Four patients were enrolled in our study. Patient 1 was 53 years old, female, with meningioma, hav–

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ing a history of pregnancy and no history of blood transfusion; Patient 2 was 81 years old, female, with ovarian and uterine leiomyoma, having a history of pregnancy but no history of blood transfusion; Patient 3 was 73 years old, male, with prostatic hyperplasia but no blood transfusion history; Patient 4 was 27 years old, female, with pregnancy edema, having no history of blood transfusion but pregnancy history was unknown. All samples were sent for the identification of ABO blood type using 2 mL of EDTA anticoagulant.

Reagents and instruments

Anti-A(batch No. 102141), Anti-B(batch: 204141) were supplied by Immucor Co. Ltd. U.S.A. Anti-stereotype cells (batch: 20185309) were supplied by Shanghai Blood Biological Medicine Co. Ltd. IgM anti-D (batch: V187175) was provided by Baso Company of China. Anti-A1(batch: 8000215024) and anti-H (lot: 8000230758) were provided by Sanquin Company of the Netherlands. The direct anti-typing card (batch number: 50560.78.04) and ABD re-examination card (lot number: 50063.83.01) were provided by Bio-Rad Company of America. The Rh blood type card (batch number: 201802008) was provided by Jiangsu Libo Pharmaceutical Biotechnology Co. Ltd. 0.01mol/L DTT and human ABO antibody were made by our laboratory; Other instruments included centrifuge KA–2200 (Kubauta, Japan), capillary centrifuge Kubota 3220 (Kubauta, Japan); incubator, centrifuge (Bio-Rad, USA); fluorescent PCR instrument (ABI, USA)

Identification of ABO, RhD blood groups

Test tube method and blood group re-examination cards were used for identification of ABO, RhD blood groups. Monoclonal anti-A, anti-B and anti-D (Immucor Inc, USA) were used for forward serotyping and A, B, O cells (Shanghai Blood Biological Medicine Co.Ltd, China) were used for reverse serotyping. Anti-A1 and anti-H (Sanquin, Switterland) were used for serial forward ABO typing. The direct comb test, forward ABO and RhD tests were depended on micro column test (Bio-Rad, USA).

Rh typing

Rh typing cards (Jiangsu Libo Pharmaceutical Biotechnology Co. Ltd) were used for Rh blood group typing.

Absorption test (separation of two groups of red blood cells)

The test of red blood cells from patient 1 and patient 3 was conducted by human anti-A reagent, patients 2 by IgM anti-D reagent, patient 4 by anti-B reagent. Briefly, non-agglutinating erythrocytes after IgM type anti-A, anti-B or anti-D absorption were performed ABO and Rh typing. DTT treatment was carried out on the agglutinating erythrocytes to re-suspend the IgM Abs reaction before ABO and Rh typing.

Capillary centrifugation

The red blood cells near the white membrane were washed with normal saline three times, before being transferred to capillary pipet. Then the red hematocrit blood cells were collected, mixed well, and centrifuged in the capillary tube. The ABO and Rh blood groups were identified by using red blood cells taken from 5 mm of proximal end of the capillary tube. The blood cells pooled at the end of the pipet were older or transfused blood cells, while the buffy coat at the other end represented younger blood cells or reticulocyte.

Confirmation of ABO and Rh blood group chimeras

The DNA was extracted from blood sample of patient 1 and samples of R1R2, R1R1, R2R2, AA, AO, BO, AB, BB which were confirmed by blood group serology and molecular biology. The standard DNA specimens of AA, AO, BB, BO, AB of ABO allele and R1R2. R1R1, R2R2 of RH allele were regarded as related standard in sanger sequence and pedigree analysis. The DNA of all samples had a consistent concentration of 70 ng/μL. Homozygous AA, BB and AB have a melting curve at a specific temperature, which relates the chimera specimen to its allele status. For example, the signal ratio of A´s primer is 200 for AA, 100 for AB and 5 for BB. If the chimera ratio is 150, we can conclude that the chimera allele is likely to be over 50% (for allele A). For further analysis we used an ABO and Rh genotyping kit (using a cyber green I melting curve) with an internal and positive control (Jiangsu ZhongJi Wantai Biopharmaceutical Co. Ltd, China).

RESULTS

The results of ABO and Rh blood groups

The AB0 and Rh blood groups are shown in ***Table 1*** and ***Table 2***.

The results of capillary centrifugation

The serological typing of ABO and RhD were same between younger and older erythrocyte for each chimera through capillary pipet centrifugation (show in ***Table 3*** and ***Table 4***).

Table 1 **The results of ABO blood group in patient 1, 2, 3 and 4**

Patients	anti-A	anti-B	anti-A1	anti-H	A cells	B cells	O cells	auto-anti
1	4+mf	4+	4+mf	1+	0	0	0	0
2	0	4+	/	1+	4+	0	0	0
3	4+mf	4+	4+mf	1+	0	0	0	0
4	0	4+mf	/	2+	4+	0	0	0

Table 2 **The results of Rh blood group in patient 1, 2, 3 and 4**

Patients	anti-C	anti-c	anti-D	anti-E	anti-e
1	4+	4+dcp	4+	4+dcp	4+
2	4+	4+dcp	4+dcp	4+dcp	4+
3	4+	4+	4+mf	0	4+
4	4+	4+dcp	4+	4+dcp	4+

dcp: double cell population.

The results of absorption test

The absorption test results are shown in ***Table 5***. Patients 1 and 3 were absorbed with anti-A; patient 2 with anti-D; and patient 4 with anti-B. ABO and Rh blood typing was carried out on non-agglutinated erythrocytes after absorption. At the same time, ABO

Table 3 **Results of ABO blood group card after capillary centrifugation in patients 1, 2, 3 and 4**

Patients	Capillary centrifugation	anti-A	anti-B
1	before	4+dcp	4+
	after	4+dcp	4+
2	before	0	4+
	after	0	4+
3	before	4+dcp	4+
	after	4+dcp	4+
4	before	0	4+dcp
	after	0	4+dcp

dcp: double cell population.

Table 4 **Results of Rh typing card after capillary centrifugation**

Patients	Capillary centrifugation	anti-C	anti-c	anti-D	anti-E	anti-e
1	before	4+	4+dcp	4+	4+dcp	4+
	after	4+	4+dcp	4+	4+dcp	4+
2	before	4+	4+dcp	4+dcp	4+dcp	4+
	after	4+	4+dcp	4+dcp	4+dcp	4+
3	before	4+	4+	4+dcp	0	4+
	after	4+	4+	4+dcp	0	4+
4	before	4+	4+dcp	4+	4+dcp	4+
	after	4+	4+dcp	4+	4+dcp	4+

dcp: double cell population.

and Rh blood groups were carried out on agglutination erythrocytes which were treated with DTT. The results showed that patient 1 was a chimera of BCCDee/ABCcDEe blood groups; patient 2 was a chimera of BCCDee /BCcDEe blood groups; patient 3 was a chimera of BCCDee/ABCcDee blood groups, and patient 4 was a chimera of BCCDeeO/CcDEe blood groups (***Table 5***).

Confirmation of ABO blood group chimeras

We used specific primers of gene A to conduct quantitative amplification (use the same concentration of DNA) towards the standard AA type, AB type, OO type and ABB type(patient 1). The results showed that the fluorescence value was AA > AB > ABB > OO(***Fig. 1***). We used specific primers of gene B to conduct quantitative amplification (as above) towards the standard BB type, AB type, OO type and ABB type(***Fig. 2***). The results showed that the fluoresc

Table 5 **ABO and Rh blood groups of the agglutination and non-agglutination erythrocytes after absorption in patients 1, 2, 3 and 4**

Patients	Absorption reagent	Red blood cells	anti-A	anti-B	anti-C	anti-c	anti-D	anti-E	anti-e	Conclusion
1	anti-A	non-agglutination	0	4+	4+	0	4+	0	4+	B/CCDee
		agglutination	4+	4+	4+	4+	4+	4+	4+	AB/CcDEe
2	anti-D	non-agglutination	0	4+	4+	0	0	0	4+	B/CCdee
		agglutination	0	4+	4+	4+	4+	4+	4+	B/CcDEe
3	anti-A	non-agglutination	0	4+	4+	4+	0	0	4+	B/Ccdee
		agglutination	4+	4+	4+	4+	4+	0	4+	AB/CcDee
4	anti-B	non-agglutination	0	0	4+	4+	4+	4+	4+	O/CcDEe
		agglutination	0	4+	4+	0	4+	0	4+	B/CCDee

Confirmation of Rh blood group chimeras

cDNA from Patient 1, R1R1,R2R2 and R2R2 were amplified using specific primers of C, c, E, e (***Fig. 3A~D***). The results showed that there was chimerism in the RhCE gene. The results showed that the gene

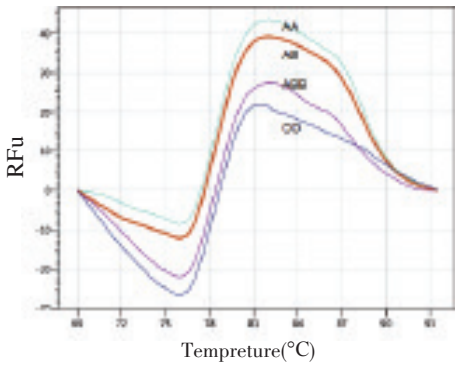

Fig. 1 Amplification curves for A gene specific primers

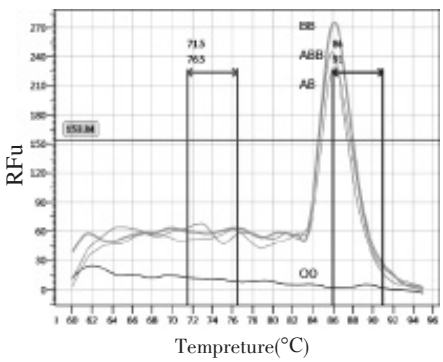

Fig. 2 Amplification curves for B gene specific primers

expression of patient 1 was approximately the same as that of control samples as shown in ***Fig. 3E***.

DISCUSSION

The phenomenon of blood group chimerism was first discovered in 1952, but until now, only 70 cases of congenital chimera have been confirmed in the world[1–3]. Most of them were found in the process of blood type detection and HLA typing in an extremely large number of specimens. At the same time, due to the influence of the examiners´ experience, require–ments and limitations in testing techniques, some chimerism phenomena may have been missed or not adequately investigated [4–5]. Therefore the frequency of chimeric blood groups in the population may be underestimated compared with current reports. This report investigates four blood group chimeras found in our department. Patients 1, 2, and 4 had blood group chimerism of both ABO and Rh systems, pa–tient 2 was found with Rh blood group chimerism.

The phenomenon of blood group chimerism mainly refers to the existence of two cell lines in one patient´s body at the same time. This often cre–

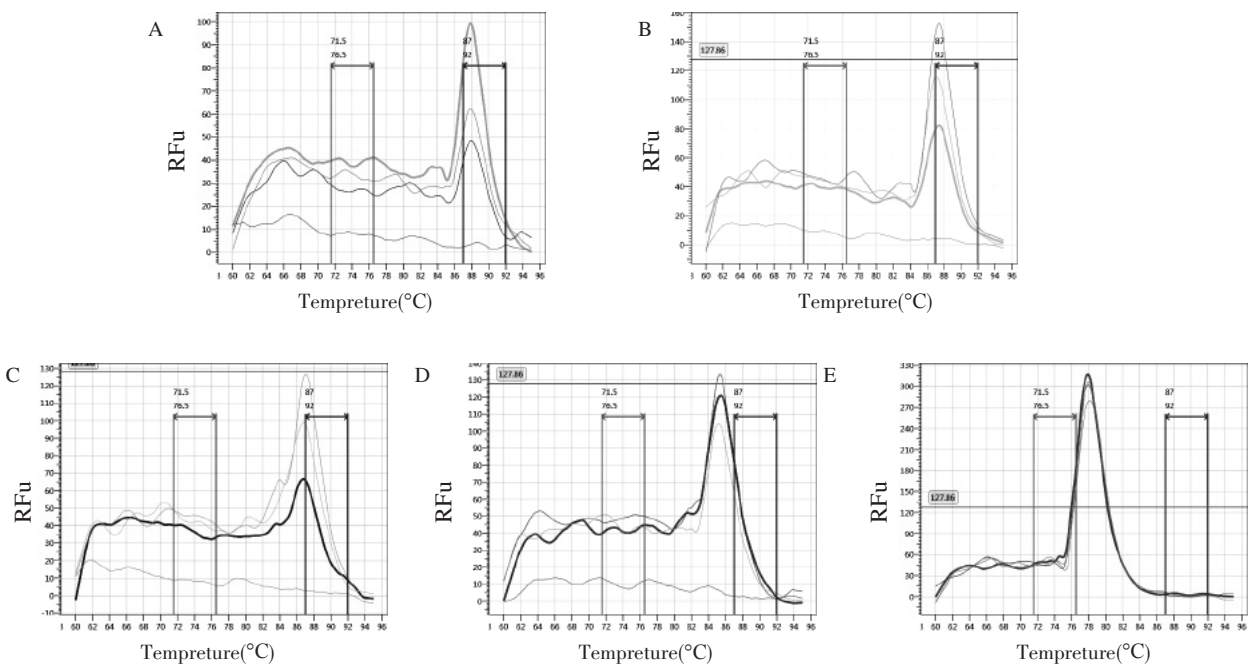

Fig. 3 **cDNA from Patient 1, R1R1,R2R2 and R2R2 were amplified using specific primers of C, c, E, e.**

A :Amplification curves for C gene specific primers; B:Amplification curves for c specific primers; C:Amplification curves for E specific primers; D:Amplification curves for e specific primer; E:DNA expression of R1R1, R1R2 and R2R2 DNA (Control).

ates a mixed field of vision in the process of ABO blood group identification. However the use of capillary centrifugation can effectively distinguish the patient´s own cells from the input cells. This is currently widely used in the identification of blood typing of red blood cells and in the investigation of

adverse reactions in clinical blood transfusions. The underlying principle is to separate the erythrocytes by different specific gravity of newborn cells and old red blood cells. In this report all cases of chimerism were confirmed using the above technique. In the pre-examination survey, it was confirmed that none of the four patients had a history of recent blood transfusion. Patient 1 had meningioma, patient 2 had gynecologic tumor, patient 3 had prostatic hyperplasia, and patient 4 was pregnant with edema. However, none of these diseases leads to the weakening of A or B antigen, nor do they lead to the weakening of RhD antigen. Therefore, after the blood type serological tests on-clinic, the four patients were suspected to have chimeric blood group. A chimeric blood group refers to the presence of two cell lines in one patient. We selectively used anti-A, anti-B and anti-D for absorption tests, and then detected the ABO and Rh blood groups of agglutinated and non-agglutinated red blood cells, respectively. The results were as follows: patient 1 was the chimera of B CCDee/AB CcDEe blood groups, patient 2 was the chimera of B CCDee/B CcDEe blood groups, patient 3 was the chimera of B CCDee/AB CcDee blood groups, and patient 4 was the chimera of B CCDee/O CcDEe blood groups.

In the same PCR reaction system, if the concentration of DNA is the same, the allele of type A is 100% while the genotype is AA; the allele of type A is 50% while the genotype is AB; and the allele of type A is 0% while the genotype is BB. If the genotype was ABB, the A allele was 33%, while the B allele was 0%, 50%, 100% and 67% in genotypes of AA, AB, BB and ABB respectively. Using this principle, we were able to amplify the A and B genes using specific primers, and compared the dissolution curve´s fluorescence values for specific AA, AB, OO types and suspected chimeras in the same PCR reaction system to confirm whether the patients were positive for ABO blood group chimera. In the same way, we amplified the C, c, E, e, by specific primers and compared the dissolution curve´s fluorescence values among R1R2, R1R1, R1R1, R2R2 and suspected Rh blood group chimera patients, and evaluated whether there was Rh blood group chimerism. In this study we also made further molecular identification for patient 1. After the amplification of allele A, the solubility curve result was AA > AB > patient 1 > OO, indicating that the allele A percent was lower than 50. After the amplification of specific primers RhC, Rhc, RhE, Rhe, the number of alleles of RhC, Rhe was higher than 50%, while the number of alleles of Rhc RhE was lower than 50%. All results showed that chimerism existed in both ABO and Rh blood groups. The present study demonstrated that the usage of PCR to identify chimeras in everal blood groups was easy and convenient [6–8].

For blood type chimera detection, our experience was as follows: ① Capillary centrifugation was used to determine whether the patient had a recent history of blood transfusion. If the patient´s blood type (before and after centrifugation) was consistent with mixed visual field agglutination, it is highly suspicious to exist blood group chimerism. ② After absorbing erythrocytes from patients with human anti-A, anti-B and monoclonal IgM anti-D, two groups of erythrocytes with different antigens were separated. From this, the ABO and Rh blood groups of the agglutinated and non-agglutinated red blood cells could be identified simultaneously. The ABO and Rh blood group genes are located on two different chromosomes, and the existence of two chromosomal mutations at the same time is extremely rare. In the four cases with blood group chimeras found in our laboratory, there were 3 cases with ABO chimera and Rh blood group chimera [9]. However, this needs to be further studied in subsequent experiments to confirm the ABO chimera subtype. The reports also suggest that in addition to ABO and Rh blood groups, chimerism may also exist in other blood group systems such as Kidd, MNS and Duffy [10]. In the strategy of blood transfusion, ABO blood type and Rh blood type should be transfused according to the group with more blood type antigens. In the four cases of this report, we suggest that patient 1 should be transfused with AB, RhD positive blood; patient 2 with AB, RhD positive blood; patient 3 with AB, RhD positive blood; and patient 4 with B, RhD positive blood.

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