Preparation of A_2 reverse grouping cells from A_2B red blood cells by alpha-galactosidase

Hong-Wei Gao[1], Da-Zhou Wu[2], Su-Bo Li[1], Man-Ni Wang[2], Ying-Li Wang[1], Guo-Qiang Bao[1], Shou-Ping Ji[1], Ying-Xia Tan[1], Hua Xu[2], Feng Gong[1]

[1]Department of Blood Biochemistry and Molecular Biology, Beijing Institute of Transfusion Medicine, Beijing; [2]Shaanxi Blood Center, Xi'an, People's Republic of China

Introduction

Correct typing of donors' and recipients' blood group is of paramount importance in transfusion services. There are two distinct parts in ABO typing, forward and reverse typing, both of which are routinely carried out[1]. Reverse typing is obligatory, because it can help to reveal mistyping, weak A subgroups with anti-A1 and unexpected IgM antibodies. Any discrepancy between the results of the tests with serum or plasma and red cells should be investigated. Reverse grouping cells, including A, B, O and A_2 type red blood cells (RBC), are important to resolve ABO discrepancies. Anti-A and/or anti-B antibodies can be easily detected by red blood cells with A and/or B blood group antigens. The presence of an anti-A1 should be confirmed by testing serum against A_1, A_2, and O red cells. This method necessitates A_2 reverse grouping cells. Very few people in China have the A_2 blood group. In the Xi'an area (north-western China) only about 0.0006% of the population have A_2 RBC, while about 0.003% of the population have the A_2B group are. The ratio of the prevalence of A_2B:A_2 is 5 (unpublished data). In the Shanghai area (eastern China), this same ratio is about 2.5. Thus, there are more people with A_2B group RBC than there are with A_2 RBC[2,3]. As it is known that α-galactosidase, a kind of exo-glycosidase, can remove the reducing end α-galactose residues of group B antigen by hydrolysis and based on the characteristics of the group B epitopes and α-galactosidase hydrolysis reaction, α-galactosidase has been used in B to O RBC conversion study since the 1980s[4]. Our group has been researching in this area for more than 10 years[5,6] and we recently obtained a novel α-galactosidase from *B. fragilis* (which belong to CAZy GH110) with highly specific activity, greatly restricted substrate specificity and a neutral pH optimum[7,8]. Our research showed that this novel α-galactosidase can convert B RBC to O RBC with high substrate specificity and at low cost[7,8]. We, therefore, started to study the A_2B to A_2 conversion using α-galactosidase in order to broaden the source of A_2 RBC.

Materials and methods

Enzymatic treatment of A_2B red blood cells with *B. fragilis* α-galactosidase

Fresh human whole blood (blood group A_2B) was obtained from Shaanxi Province Blood Centre (Xi'an, China), and the buffy coat was removed. Enzymatic conversion was performed in 1 mL reaction mixtures containing 200 mmol/L glycine and 3 mmol/L NaCl, at pH 6.8 (conversion buffer), with 30% packed RBC as described by Liu *et al.*[9,10]. Briefly, RBC were prewashed 1:1 and 1:4 (v/v) in conversion buffer before addition of α-galactosidase (0.005 mg/mL packed RBC) and incubation for 60 min with gentle mixing at 26 °C, followed by four repeat washing cycles with 1:4 (v/v) phosphate-buffered saline (PBS) by centrifugation at 2,000 rpm for 5 min. The washed, enzyme-converted RBC were stored in monoammonium phosphate nutrient solution at 4 °C.

Flow cytometry

Flow cytometry analysis of native and enzyme-converted RBC was performed using an FACScan flow cytometer (Cytomics FC 500 Beckman Coulter, Brea, United States of America) with anti-B monoclonal antibodies (Changchun Brother Biotech Co., Ltd., Changchun, China) and fluorescein isothiocyanate-conjugated affinity purified goat anti-mouse IgM (Jackson ImmunoResearch Laboratories, Inc., West Grove, United States of America). Briefly, 10 µL cells were fixed overnight at room temperature under gentle agitation by the addition of 100 µL of 2% paraformaldehyde (w/v, Sigma-Aldrich, St. Louis, United States of America) in PBS to prevent agglutination of antigen-positive cells. Next, 2 µL packed RBC were prewashed with 500 µL PBS twice and resuspended in 50 µL PBS. Then 50 µL of undiluted primary antibody was added and incubated for 60 minutes in the dark at 25 °C. After two washes and resuspension in 100 µL PBS, 2 µL of undiluted secondary antibody was added and incubated for 60 minutes in the dark at 25 °C. The cells were analysed after another two washes (as above) and resuspension in 500 µL PBS.

Blood Transfus 2013; 11: 305-7 DOI 10.2450/2012.0020-12

Blood group typing

The B status was detected by classical serological techniques and FACS analysis. Agglutination reactions were carried out with three commercial anti-B monoclonal reagents (from Changchun Brother Biotech Co., Ltd., Changchun, China, Shanghai Hemo-pharmaceutical & Biological Co., Ltd. Shanghai, China and Beijing Kinghawk Pharmaceutical Co., Ltd, Beijing, China), and anti-B sera from ten group A donors. A_2 RBC were confirmed by commercial anti-A1 monoclonal blend reagents (Shanghai Hemo-pharmaceutical & Biological Co., Ltd.) and anti-A1 sera from two donors who had been previously identified. The contrast control of A_2 RBC (native A_2 RBCs) was obtained from Medson Technology Co., Ltd., Atlanta, United States of America. The results were confirmed by direct observation under the microscope after centrifugation immediately and after 30 minutes at 4 °C.

Results

The enzyme-treated RBC did not agglutinate with the three kinds of commercial anti-B monoclonal antibodies or with the anti-B serum from ten group A donors in multiple observations of visual microscope fields in different conditions. All the results were rechecked using anti-B antibody: native A_2B showed a higher positive rate, 99.3%, but native A_2 and enzymatically converted A_2 RBC gave low positive rates of 0.3% and 0.7%, respectively (Figure 1). Moreover the enzymatically produced A_2 RBC, just like the A_2 control RBC, did not agglutinate with anti-A1 monoclonal antibodies or anti-A1 serum (Table I). These results indicate that we successfully obtained A_2 RBC from A_2B RBC through treatment with α-galactosidase. These enzymatically produced RBC could be used to prepare A_2 type panel cells.

Discussion

This study confirmed that the method of preparing A_2 reverse grouping cells from group A_2B RBC by α-galactosidase was feasible and some distinguishing features of converted A_2 could be detected. We had previously established the method of B antigen epitope cleaning by α-galactosidase and demonstrated that B epitopes could be removed completely[7,8]. In addition, we have confirmed that other antigenic characteristics of converted cells were not changed by the enzymatic hydrolysis[11]. In this study, we treated A_2B cells with α-galactosidase in accordance with our previous enzymatic hydrolysis technology, and detected B and A epitopes with anti-B/A/A1 monoclonal antibodies and FACS. In order to ascertain that A1 epitopes did not exist, human anti-A1 sera was used for further confirmation. Our results showed that A_2B RBC can be converted to A_2 cells successfully and that the converted A_2 RBC can have A_2 antigen traits.

As α-galactosidase and α-acetyl-galactosaminidase from bacteria with high activity were cloned and expressed, research was focused on their roles in increasing the number of red cell samples suitable for antibody investigations[12]. Here we describe a new method for preparing A_2 cells for reverse grouping, which can broaden the availability of such cells. As the treatment process is not complicated and the cost is low, the method could readily become widespread.

Besides α-galactosidase, there is also α-aectyl-galactosaminidase, a kind of exo-glycosidases, which

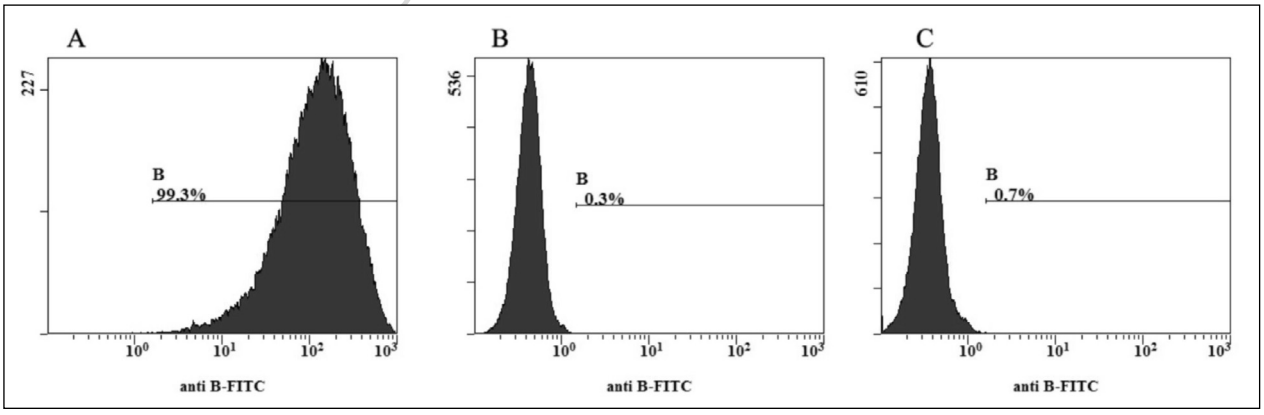

Figure 1 - FACS analysis of A_2 RBC converted from A_2B cells. The FACS histograms show the B antigen site density as measured by anti-B fluorescein isothiocyanate-labelled monoclonal antibody on A_2B (A), native (B) and enzymatically converted A_2 RBC (C). The x-axis represents the fluorescence intensity on a logarithmic scale, whereas the y-axis shows the number of RBC evaluated. The antibody diluting concentration was 1:128. The native A_2B showed a higher positive rate, 99.3%, but native A_2 and enzymatically converted A_2 RBC gave low positive rates of 0.3% and 0.7%, respectively.

Table I - Comparison of the antigenic properties of A_2B, A_2 and converted A_2 RBC.

	Typing results*				
	Monoclonal antibody			Human serum	
RBC	Anti-A	Anti-B	Anti-A1	Anti-A1	Anti-B
A_1	4+	0	4+	2+~3+	0
A_2B	4+	4+	0	0	2+~3+^{s}
A_2 control	4+	0	0	0	0
Converted A_2 from A_2B	4+	0	0	0	0

Legend *Typing with three kinds of licensed ABO typing reagents and methods as indicated. The agglutination score ranges from 0 to 4+.
s: strongly

can remove A epitopes[13]: we believe this strategy could be useful for preparing special B or O reverse ABO typing panel cells.

Acknowledgements

The Authors would like to thank the National Science Foundation of China for its financial support (Grant No. 30801063).

Keywords: red blood cells, reverse typing, α-galactosidase, enzymatic conversion.

The Authors declare no conflicts of interest.

References

1) Klein HG, Spahn DR, Carson JL. Red blood cell transfusion in clinical practice. Lancet 2007; **370**: 415-26.
2) Dashkova NG, Ragimov AA, Asoskova TK. Significance of the isoantigen A2 and immune anti-A1 antibodies in transfusiology. Anesteziol Reanimatol 2009; **6**: 62-5.
3) Xiang D, Liu X, Guo ZH, et al. The study of ABO subgroup in Shanghai. Chin J Blood Transfus 2006; **19**: 25-6.
4) Goldstein J, Siviglia G, Hurst R, et al. Group B erythrocytes enzymatically converted to group O survive normally in A, B, and O individuals. Science 1982; **215**: 168-70.
5) Zhang YP, Yang J, Gao X, et al. B→O blood conversion. Chinese Science Bulletin 2003; **48**: 269-73.
6) Zhang YP, Gong F, Bao GQ, et al. B to O erythrocyte conversion by the recombinant α-galactosidase. Chin Med J 2007; **120**: 1145-50.
7) Gao HW, Li SB, Bao GQ, et al. [A reconstructed *B. Fragilis*-derived recombinant α-galactosidase developed for human blood type B→O conversion.] Zhongguo Shi Yan Xue Ye Xue Za Zhi 2011; **19**: 503-7.
8) Gao HW, Li SB, Bao GQ, et al. Properties of a novel α-galactosidase from B. Fragilis and its potential for human blood-type B to O conversion. Scientia Sinica Vitae 2011; **41**: 1030-6.
9) Liu QP, Sulzenbacher G, Yuan H, et al. Bacterial glycosidases for the production of universal red blood cells. Nat Biotechnol 2007; **25**: 454-64.
10) Liu QP, Yuan H, Bennett EP, et al. Identification of a GH110 subfamily of alpha 1,3-galactosidases: novel enzymes for removal of the alpha 3Gal xenotransplantation antigen. J Biol Chem 2008; **283**: 8545-54.
11) Xu H, Yu CY, Zhang YP. Human RBCs blood group conversion from AB to O by the combination of α-galactosidase and α-N-acetylgalactosaminidase. Chin J Blood Transfusion 2008; **21**: 917-20.
12) Daniels G, Withers SG. Towards universal red blood cells. Nat Biotechnol 2007; **25**: 427-8.
13) Yu CY, Xu H, Wang LS, et al. Human RBCs blood group conversion from A to O using a novel α-N-acetylgalactosaminidase of high specific activity. Chinese Science Bulletin 2008; **53**: 2008-16.

Arrived: 8 February 2012 - Revision accepted: 30 April 2012
Correspondence: Feng Gong
Department of Blood Biochemistry and Molecular Biology
Beijing Institute of Transfusion Medicine
No. 27 Taiping Road
Beijing 100850, P.R. China
e-mail: gongfeng@nic.bmi.ac.cn