Tailor-made red cells: the long road towards blood safety

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Attempts to create modified red cells that could be used for clinical or diagnostic purposes date back to the 1980s.[1] These attempts initially involved enzymatic modification of red cell membrane antigens, with the primary goal of converting A/B into O red cells by the use of glycosidase enzymes. Group B red cells enzymatically converted to O cells were amenable to larger-scale production (i.e., one or more units of blood) and were tested in phase I and II clinical trials.[2] However, despite successful proof-of-principle trials and advances in enzymology, wider clinical usage of these cells has been hindered by feasibility issues and also by incompletely understood residual cross-match agglutination.[3]

Recent advances in molecular genetics opened another avenue of research, aiming to create tailor-made red cells by gene editing. In this issue of *Haematologica*, Funato *et al*.[4] publish their results on the use of gene editing for the purpose of producing red cells devoid of selected blood group antigens. These "designer" red cells can then be used as part of the panel for identification of allo-antibodies against high frequency antigens and, with additional genome editing, those against low-frequency antigens. Following on their team's work on establishment of erythroid cell lines,[5] Funato *et al*. started with peripheral blood CD34-positive cells immortalized with tetracycline-inducible human papilloma virus V16 E6/E7 genes. The resulting cell line, peripheral blood stem cell-derived erythroid progenitor-4 (PBDEP-4), differentiates to late (orthochromatic) erythroblasts in an erythropoietin-containing medium after withdrawal of doxycycline. Although they rarely expel the nucleus to become reticulocytes, these erythroblasts express the blood group antigens of the original donor and can be readily identified by flow cytometry or, being hemoglobinized, visually by tube agglutination.

The next step was to edit out the genes encoding antigens of major blood groups from the PBDEP-4 cell line. Selection of genes for editing was governed by the frequency of allo-antibodies in the Japanese population. The authors used a CRISPR/Cas9 system in a painstaking, one-by-one editing process, starting with *RHD/RHCE* genes and continuing with *ACKR1* (corresponding blood group: Fy), *SLC14A1* (Jk), *GYPA/GYPB* (MN/Ss), Jr, and *A4GALT* (P1PK) genes. Overall, they were able to delete 11 antigens without evident adverse effects on cell line proliferation and differentiation. The final product were late erythroblasts strongly expressing the Diego Di^b (DI2) antigen (PBDEP-Di^b) and lacking the blood group antigens present on the PBDEP-4 cells (RhD, C, e, Fy^a, Jk^a, Jk^b, M, N, s, Jr^a, and P1).

Because of the limited proliferation potential of human erythroid progenitor cells, Funato *et al*.,[4] as well as Hawksworth *et al*.,[6] used immortalized erythroid cell lines, which have the unlimited potential to differentiate into late erythroblasts and reticulocytes and are amenable to gene editing. Other researchers used induced pluripotent stem cells (iPSC) and CRISPR/Cas9 gene editing: An *et al*.[7] reprogrammed mononuclear cells from donors with rare *RH* phenotypes into iPSC; alternatively, they used the CRISPR/Cas9 system to generate a Rh null cell line into which they then inserted cDNA of conventional or variant *RHD* alleles to create red cells with rare phenotypes such as D--, Go^{a+}, or DAK^+. Petazzi *et al*. also used iPSC to convert A2/O group cells into O group cells; editing of the *ABO* gene by the CRISPR/Cas9 method caused a frameshift mutation, abrogating the expression of A-transferase and resulting in a group O phenotype.[8]

Clearly, the primary interest in red cells engineered to lack their naturally occurring antigens was driven by attempts to improve blood safety, by preventing transfusions of wrong ABO blood. Transfusion of ABO-incompatible red cells, a potentially fatal event, still happens due to errors at blood collection and administration points,[9] despite heightened awareness and improvements in patient and blood unit identification. Additionally, large-scale manufacturing of universal donor (O) red cells is desirable because it could reduce the demand for donor O red cells and help the management of O red cell stocks, especially at times of increased demand (natural catastrophes, accidents and, sadly, wars). With regard to non-ABO blood groups, conversion of erythrocytes to their "null" counterparts[10] can help the selection of the right blood for allo-immunized patients with frequent transfusion requirements, such as patients with sickle cell disease or

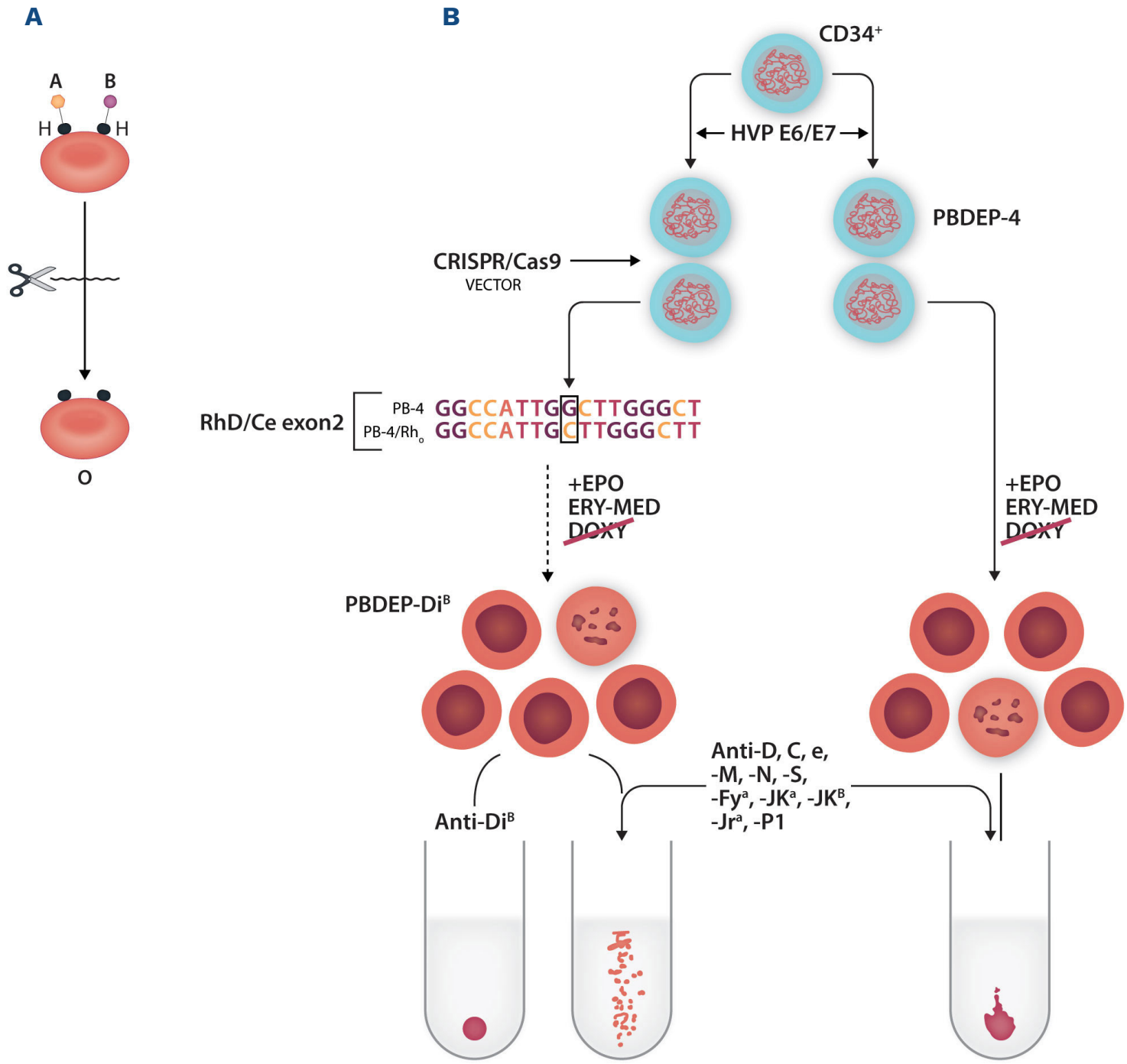

Figure 1. Methods to produce tailor-made red cells. (A) Enzymatic cleavage of sugar moieties defining blood groups A and B. (B) CD34+ cells are immortalized into the PBDEP-4 line. These cells are differentiated into late erythroblasts or subjected to gene editing to remove selected blood group antigens (dotted line: subsequent editing steps after *RhD/Ce* gene editing). Edited cells (PBDEP-Di^{b}) are devoid of antigens of the RH, FY, JK, MNS, JR and P1PK groups. CRISPR/Cas9: clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9; PBDEP-4: peripheral blood stem cell-derived erythroid progenitor-4; EPO: erythropoietin; ERY-MED: medium supporting erythroid differentiation; DOXY: doxycycline.

β-thalassemia, as well as transfusion-dependent patients with acquired anemias (e.g., myelodysplastic syndromes).

Having reagent red cells with rare phenotypes is of paramount importance for identification of rare red cell antibodies. These cells are currently sourced from rare donors. Generating such cells from immortalized cell lines or from reprogrammed somatic cells, in conjunction with the possibilities offered by gene "knock-in" and "knock-out", would curtail the need to use a finite source such as donors. Moreover, by targeted deletion of blood group genes, scientists can learn about the function of proteins encoded by these genes, and of the physical properties of the red cells generated in this manner. In this sense, it is fascinating that the cell line generated by Funato *et al.*, PBDEP-Di^{b}, even though lacking 11 red cell antigens, had normal erythroid differentiation (apart from enucleation) and cell morphology, and a minimal cell death rate. Most significantly, PBDEP-Di^{b} cells were suitable for their intended use, i.e., antibody identification, at least by the tube technique. *Quod erat demonstrandum*.

There are still, however, questions to be answered, mainly concerning the production and distribution costs, application of the technique to gel column automated systems, and optimal storage conditions. Notwithstanding these hurdles, tailor-made reagent red cells may be on laboratory benches before too long.

Disclosures

No conflicts of interest to disclose.

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