

*Letter to the Editor***Comment on "An enzymatic pathway in the human gut microbiome that converts A to universal O type blood"^[1]**

Dear Editor,

The ABO blood group plays an important role in genetic research, clinical blood transfusion, and transplantation immunity. The transfusion of blood, or more commonly red blood cells (RBCs) requires careful matching of blood types to avoid serious adverse consequences. A, B, and O blood types share their antigen carbohydrate chain structure but with a different ending in the glycosyl of the carbohydrate chain. Specifically, A type blood contains N-acetylgalactosamine (GalNAc); B type blood contains galactose (Gal); AB type blood contains both N-acetylgalactosamine and galactose; O type blood contains neither of these terminal glycosyls. This universal donor O type blood is crucial for emergency situations where time or resources for typing are limited, so it is often in short supply. A and B type blood differ from the O type in the presence of an additional sugar antigen (GalNAc and Gal, respectively) on the core H antigen found on O type RBCs. Thus, the conversion of A, B, and AB type RBCs to O type RBCs should be achievable by the removal of the sugar antigens with an appropriate glycosidase.

Historically, Goldstein first discovered a way to convert B antigen into O antigen, using an enzyme from green coffee beans called α -galactosidase, which has a low optimal pH 5.7, but requires a large number of enzymes^[2]. Because of the relatively small percentage of the North American and European population of B type blood, the search for enzymes that can convert A antigen to O became necessary. The crucial discovery of new enzymes for blood type conversion was made in 2007^[3]. New α -galactosidases and α -N-acetylgalactosaminidases were identified by screening bacterial libraries, and they were found able to fully convert A type RBCs to O type RBCs at pH

7.0. This became the first useful conversion of A type RBCs, however, subsequent studies were not able to improve the efficiency of enzyme conversion until recently. This improvement was made possible by functional metagenomic analysis, resulting in the discovery of new enzymes^[4].

In 2019, Rahfeld *et al.* used enzymes from the human gut microbiome to convert A antigen to H antigen of O type blood^[1]. A metagenomic library derived from the feces of an AB donor was screened and a significantly efficient two-enzyme system was found for A conversion. Two enzymes were identified from the obligate anaerobe *Flavonifractor plautii* that work in concert to efficiently convert A antigen to H antigen of O type blood. These two enzymes were referred to as FpGalNAc deacetylase (FpGalNAcDeAc) and FpGalactosaminidase (FpGalNase). Compared with previous studies, FpGalNAcDeAc and FpGalNase had obvious advantages. Only a small amount of the enzyme was required to completely remove the A antigen from red blood cells, with a conversion rate of 30 times higher than that of any other enzymes previously found. Importantly, the research showed that 26 different A⁺ RBC donors were successfully converted with no detectable residual A antigen, and these enzymes worked equally as well in blood as in a buffer system. The researchers believed that more work is needed to definitively clear all traces of A antigen that might be recognized by human polyclonal anti-A antibody. However, these enzymes had high activity and specificity in both buffer solutions and whole blood, which makes them cost-effective in existing automated routines for blood collection, processing, and storage. They also found that the enzymes used in the conversion could be removed by simple centrifugation washing procedures during normal

RBC processing. This looks likely to be a major breakthrough in terms of improving the efficiency of enzyme conversion, and this study was the first glimmer of light for the artificial method for universal blood.

With the deepening of research, researchers found that agglutination reaction in the process of cross-matching was an urgent problem to be solved. Low level agglutination was observed when enzyme-converted type O RBCs were cross-matched with serum from type A or O. The cause of this phenomenon has not been determined, but it may arise from low level of residual A antigen caused by incomplete cleavage^[5]. Similar problems have also been found after cleavage of the B-antigen. This is likely to require the identification of the antibody and its specificity that caused this agglutination reaction. There are currently 43 different blood group systems containing 345 red cell antigens identified by ISBT, however, most of these antigens are not glycoproteins. The research by Rahfeld *et al.* has only been applicable to the modification of glycosylated proteins. Many problems are needed to be further studied and solved, including methods for measuring the dynamic equilibrium of enzyme reaction during blood type conversion and the likely impact of residual enzymes in the human body. Clarifying these issues will greatly improve the prospects for further clinical trials which may be applied in the field of platelet glycoprotein conversion.

For patients with blood diseases, especially those with leukemia, aplastic anemia, idiopathic thrombocytopenic purpura, and myelodysplastic syndromes, repeated blood transfusions are required. Repeated platelet transfusion is often prone to produce platelet antibodies and cause platelet transfusion refractoriness (PTR)^[6-7]. The main reason for this is anti-donor classic human leukocyte antigen class I (HLA-I) allogeneic immunity. According to Rahfeld's research, it may be possible to screen HLA-I degrading enzymes in human gut microbiota, and use them to produce the mutation-free HLA-I deficient universal platelets. These techniques require further study, but may represent a significant breakthrough

and an example of the extraordinary success of humankind in exploring the mysteries of blood.

Yours Sincerely,

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