Toward universal donor blood: Enzymatic conversion of A and B to O type

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Peter Rahfeld and Stephen G. Withers

From the Department of Chemistry, University of British Columbia, Vancouver, British Columbia V6T 1Z1, the Michael Smith Laboratories, University of British Columbia, Vancouver, British Columbia V6T 1Z4, and the Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada

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Transfusion of blood, or more commonly red blood cells (RBCs), is integral to health care systems worldwide but requires careful matching of blood types to avoid serious adverse consequences. Of the four main blood types, A, B, AB, and O, only O can be given to any patient. 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 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, conversion of A, B, and AB RBCs to O-type RBCs should be achievable by removal of that sugar with an appropriate glycosidase. The first demonstration of a B-to-O conversion by Goldstein in 1982 required massive amounts of enzyme but enabled proof-of-principle transfusions without adverse effects in humans. New α-galactosidases and α-N-acetylgalactosaminidases were identified by screening bacterial libraries in 2007, allowing improved conversion of B and the first useful conversions of A-type RBCs, although under constrained conditions. In 2019, screening of a metagenomic library derived from the feces of an AB donor enabled discovery of a significantly more efficient two-enzyme system, involving a GalNAc deacetylase and a galactosaminidase, for A conversion. This promising system works well both in standard conditions and in whole blood. We discuss remaining challenges and opportunities for the use of such enzymes in blood conversion and organ transplantation.

Blood transfusion is an indispensable part of the health care system, saving many thousands of lives annually. To make this possible, ~85 million units of packed red blood cells (RBCs) are obtained annually around the world, according to the Red Cross, largely via donation. This requires a massive organization to secure supplies, and yet, according to the World Health Organization, demand for RBCs is expected to grow further (2). This growth is fueled in part by the demographically aging population and consequent rise in blood-intensive procedures, such as solid-organ transplants, hematopoietic stem-cell transplants, hemorrhage resuscitation, and aggressive chemotherapy against cancer. To counter this demand, significant improvements have been made in the collection and management of blood-component supply, including reassessment of policies concerning when blood is needed and in what quantities. This has helped to reduce the usage of blood products, somewhat stabilizing supply versus demand. However, problems remain, especially given the challenge of correctly matching blood types on all occasions.

The human blood group system is complex; 30 discrete blood groups are known, defined by 270 antigens plus 38 that have not been assigned to a particular group (3). These blood group antigens are based either on oligosaccharide epitopes (ABO, P, and Lewis antigens) or on specific amino acid sequences of proteins (Rh, Kell, and Duffy antigens). The majority of the antigens are integrated into the cell membrane, but some, like the Lewis system, are plasma antigens that are adsorbed onto the red cell surface (4). The A, B, and H (O-type) carbohydrate antigens of the ABO blood group system are the most important clinically (3, 5), with about 1 million antigens present on the surface of each RBC (6). Careful matching of the host and donor ABO blood types is essential to avoid transfusion incompatibility events, which are fatal in 10% of all cases (7). This applies to transfusion of whole blood, RBCs, or platelets as well as tissue or organ transplants, because ABO antigens are not only present on RBCs but also on most other tissues in the human body. The only exceptions are the so-called nonsecretors for whom a mutation in one of the fucosyl transferases leads to the absence of ABH antigens on secretory epithelial cells in the salivary glands, gastrointestinal tract, and respiratory cavities (8).

Under unhurried circumstances, transfusion is therefore performed with donor blood for which the antigens match those of the recipient as closely as possible. To confirm compatibility, cross-matching studies are performed in which small samples of donor and recipient blood are mixed and monitored for possible agglutination reactions that would indicate antigen/antibody interaction. In emergency situations “universal” O-type blood (preferably O negative) is employed because, as explained below, it is compatible with A, B, AB, and of course O-type blood. Minor incompatibilities due to other antigen
mismatches are not typically life-threatening. This review provides a brief background to blood antigens then describes attempts to convert A, B, and AB blood to O-type blood by enzymatic removal of their differentiating glycan antigens. Technologies developed must be compatible with current procedures for collection, storage, and handling of blood. Typically, after collection in sterile bags, blood is stored at room temperature for 24 h and then cooled to 4 °C and can be stored for up to 42 days. Most blood is separated into its plasma, red blood cell, and platelet components early in the process, and most commonly transfusion is performed with red blood cells rather than whole blood.

In this report, we review methods that have been employed to identify enzymes that work efficiently at the neutral pH conditions required by RBCs, culminating in the recent identification of a highly efficient enzyme pair from the human gut microbiome. For excellent reviews providing more detail on the earlier stages of this path of discovery, we refer the reader to the following publications: Olsson (9), Olsson and Clausen (10), and Garratty (11).

The ABO blood group system defined

The base structure of the ABO blood group system is the fucosyl galactose H-antigen of O-type blood (Fig. 1A), which is attached to glycoproteins and lipids on the surface of RBCs and other tissues. The A- and B-antigens differ from this H-antigen through the addition of an extra sugar moiety, this being α-GalNAc for A-type or α-galactose for B-type RBCs and both in the case of AB type (Fig. 1A). Individuals with A-type blood harbor anti-B antibodies, with the result that transfusion of an A individual with B-type RBCs would lead to agglutination of the RBCs and subsequent hemolysis. Likewise, B-type blood contains A antibodies; thus, the complementary transfusion may not be performed. People with O-type blood harbor both anti-A and anti-B antibodies; thus, they may receive only O-type blood.

O-type RBCs have a special status, because the H-antigens on their surface are not recognized by the anti-A or anti-B antibodies, and no specific H (O) antibody is formed, likely because A- and B-antigens are synthesized via the H-antigen, which is thus recognized as “self.” Consequently, O-type RBCs can be used as a universal donor for any other ABO blood type and thus are in great demand (Fig. 1B). As can be seen in Table 1, the distribution of blood types varies with race demographic. O type is the most common in all cases, followed by A and then B, with only small numbers of AB. The second most clinically important antigen is the Rh (D) factor, which is a transmembrane protein and renders the individual Rh-positive (Rh+) (3, 13, 14). The vast majority of people are Rh+, with only 18% of Caucasians being Rh-negative (Rh−) and other racial groups even less, as shown in Table 1 (15). Rh− patients may produce antibodies to the Rh protein, in which case they cannot receive blood from Rh-positive individuals. This is a particular concern during childbirth if the fetus is Rh-positive and the mother Rh-negative because, should fetal blood enter the maternal bloodstream, it would induce anti-Rh antibodies. Although these are not a problem at that time, this could be an issue during a subsequent pregnancy if the fetus is Rh+. In that case, maternal antibodies could cross the placenta and destroy fetal RBCs. Second pregnancies after an initial mismatch are thus especially closely watched by measurement of anti-Rhesus antibody levels. If problems are suspected, an injection of Rh immune globulin is provided at 28 weeks to suppress Rh antibody formation.

**Figure 1.** A, overview of basic A-, B-, and H-antigens on the surface of Type A, B, and O RBCs. B, representation of blood transfusion compatibility (black arrows, can be transfused; gray lines, no transfusion). O RBCs are the only ones lacking a recognized blood group antigen and can therefore be universally donated. Sugars are shown using the Consortium for Functional Glycomics notation (12).
Some 1–8% (O−) of the population in the United States are thus “true” universal donors, whereas 37–53% (O+) are universal donors within the (very large) Rh (D)-positive group. Consequently, O-type blood is always in high demand, especially in emergency situations where there is no time for blood group typing or just no possibility to perform it. Notably, in emergency settings in Canada at least, Rhesus-positive units are transfused to all recipients except women of childbearing age, irrespective of the Rhesus status of the recipient. Thus, although it is the largest of the blood groups within the worldwide population, there is a constant shortage of O-type blood.

### Subtypes of ABH antigens and their biosynthesis

The ABH antigens of the ABO blood group are composed of carbohydrate chains bound to either glycolipids (~10%) or glycoproteins (~90%). These antigens exist as a number of subtypes that differ in internal linkages within the linking oligosaccharide, as shown in Fig. 2. Interestingly, expression of these antigens on tissues can be organ-specific, with important implications for organ transplantation (16, 17).

These glycans are built up from two main groups of carbohydrates. Lactotriaosylceramide (GlcNAc-β-3-Gal-β-4-Glc-β-Cer) forms the core structure of the Type 1, 2, and 3 antigen linkage types (18–21). Transfer to this of galactose in a β-3 linkage or β-4 linkage followed by fucose in an α-2 linkage to the galactose yields the Type 1 and Type 2 H-antigens, respectively. From these, the A- and B-antigens are derived through the transfer of GalNAc or galactose by the A-transferase (GTA) or B-transferase (GTB), respectively (22). Further extension of the Type 2 A-antigen by transfer of galactose and fucose produces the Type 3 H-antigen, which is also called repetitive A, due to the repetition of the trisaccharide structure (21). Around 50% of the A Type 2 antigens synthesized on glycolipids are modified further to these Type 3 antigens (21).

The other core structure is that of globotriaosylceramide (Gal-α-1,4-Gal-β-1,4-Glc-β-Cer,Gb3 or Pk), from which the Type 4 antigen linkage is built (20). The initial addition of β-3-GalNAc yields the P-antigen; the further addition of galactose and fucose produces the Type 4 H-antigen, from which the Type 4 A-antigen is derived by transfer of GalNAc (23) (Fig. 2).

The 2-α-fucosyltransferases (FUT) play key roles in defining which tissues within the body present ABO blood group antigens. FUT1, which produces the Type 2 and Type 4 H-antigens, is the most important in terms of RBC-based antigen production (24, 25), whereas FUT2 is specifically required for formation of the Type 1 and Type 3 H-antigens. This latter enzyme is inactive in 20% of the Caucasian population, leading to the previously mentioned nonsecretor phenotype, in which most nonsecretors no longer present the ABH antigens (8). Loss of FUT1 and FUT2 leads to the rare Bombay phenotype, which is characterized by an absence of H-antigens on RBCs. Bombay patients can only receive autologous transfusions or blood from another Bombay blood group individual. FUT3 to FUT7 are responsible for the production of Lewis antigens by the addition of α-1,4- or α-1,3-fucose to GlcNAc/galactosamine residues of the appropriate precursor. The presence of the ABO blood antigens and their different linkage types will vary between different tissues in the body (26).

A blood types are further classified as A1 or A2 based on the density of the antigens on the RBC surface and specifically on the presence of the Type 3A repetitive structure on A2 RBCs. This difference is rooted in GTA enzymes that have higher activity in A1 RBCs than in A2. As a consequence, in the more prevalent A1 RBCs, more H-antigens are modified, and each of the Type 1, 2, 3, and 4 subtypes is formed. By contrast, in Type A2 RBCs, most of the H-antigens are unmodified, and only Type 2 antigens are formed at significant levels, with very little formation of Type 1 and 4 linkages and no formation of Type 3. Indeed, experimental distinction of A1 from A2 RBCs is based on antibodies to the Type 3 structure. Other A and B subgroups are known but are not as common as A2.

### Enzymatic blood type conversion, first generation

The finding that the only apparent difference between A, B, and O RBCs resides in the presence or absence of either GalNAc or Gal on the terminal H-antigen raised the possibility that A and B RBCs could be converted to O RBCs if that sugar could be selectively removed (Fig. 3). Such enzyme-converted O RBCs (ECO-RBCs) might then be usable as universal donor blood in place of normal O-type blood, although this procedure would clearly not affect the RBC Rh status. Accordingly, conversion of A+ and B+ RBCs would yield O+, whereas A− and B− RBCs would yield O−.

The first full reports demonstrating the feasibility of such enzymatic conversion were published by Goldstein and colleagues (27, 28) in the early 1980s, based on preliminary findings by Sharon and his group (29). Goldstein’s group focused upon the conversion of B RBCs rather than A, primarily because at that time, the only commercially available enzyme that might be applicable to such a task was the α-galactosidase from green coffee beans. The low pH optimum of this enzyme required that conversion be performed under suboptimal conditions for RBCs (pH 5.7) and using large amounts of enzyme. Nonetheless, full conversion to the H-antigen was confirmed, and RBC structure and viability were established. After demonstrating that converted B-erythrocytes from gibbons could be safely transfused back into the donor gibbons and that the transfused erythrocytes enjoyed normal circulation times, the team moved into a small human trial with three patients of blood type A, B, and O. RBCs from the B-type donor were converted, and then, after washing to remove enzyme, 5-ml samples of packed ECO-RBCs were injected into volunteers. Again,
the ECO-RBCs were shown to exhibit a normal half-life and to be well-tolerated. This same group followed up on these studies in the early 1990s, showing that, initially, a full unit (200 ml) of such ECO-RBCs and ultimately 2 or 3 units could be transfused into A- and O-type patients with no ill effects and normal RBC circulatory survival times. It was, however, noted that higher galactosidase levels (2 g per packed RBC bag (6 mg/ml for 80% hematocrit)) were needed for samples transfused into O than A volunteers to avoid a small increase in anti-B titer, although the reason why was not clear (30–32).

A larger, Phase 2 clinical trial carried out in 2000 by a second group came to similar conclusions (33). Using a recombinant form of the coffee bean enzyme to generate their ECO-RBCs, 21 patients were given ECO-RBCs, and no adverse events were recorded, although small increases in B-titer were again seen with some patients. Cross-matching studies, wherein ECO-RBCs were mixed with sera from group A and group O patients, resulted in some level of agglutination in 20% of serum samples from A patients and 40% from O, although the ECO-RBCs could not be agglutinated by murine anti-A and anti-B. While somewhat perplexing, this would appear not to be clinically significant, given the lack of adverse effects upon transfusion. The study concluded that, pending the development of suitable enzymes for conversion of A-type blood, enzymatic conversion could indeed be used to create a universal (group O) donor blood supply. However, it was evident that enzymes of improved pH optimum and activity were needed. Further research was done in an attempt to improve the efficiency of the enzymes. Here a key player was ZymeQuest (now Velico Medical), who developed a kinetically superior soy bean α-galactosidase (34). This enzyme was able to work at a higher pH of 5.8, and the amount used per packed RBC bag was reduced to 0.5 g (9). However, this was still not suitable for serious usage. Further, given the relatively small percentage of the North American and European population of B blood type, it was clear that...
such conversion approaches would not be viable until efficient A-cleaving enzymes were identified.

**Toward A-cleaving glycosidases and better B-antigen cleavers**

Success in the production of B ECO-RBCs inspired the search for enzymes that can convert the A-antigen to O. In considering other glycosidases that might be useful in blood type conversion, it is useful to review the broader classification of such enzymes. Glycoside hydrolases have been grouped into over 160 sequence-related families within the Carbohydrate-Active enZYmes (CAZy) database (35). Because sequence dictates structure, mechanism, and ultimately substrate specificity, this database, along with the sister CAZYPEDIA site has provided an extremely valuable organizing principle to glycoenzymology. The coffee bean \(\text{H}9251\)-galactosidase belongs to CAZy family GH27, a grouping of bacterial and eukaryotic \(\text{H}9251\)-galactosidases and \(\text{H}9251\)-N-acetylgalactosaminidases, including the human lysosomal enzymes, whose deficiency leads to the Fabry and Schindler lysosomal storage disorders. The low pH optimum of the coffee bean enzymes is perhaps thus unsurprising.

Another family of closely related \(\text{H}9251\)-N-acetylgalactosaminidases and \(\text{H}9251\)-galactosidases is that of GH36; enzymes from these two families have in common a catalytic domain with an \((\alpha/\beta)_8\) fold, along with an associated \(\beta\) sheet domain.

Early attempts to identify suitable \(\alpha\)-N-acetylgalactosaminidases focused on the bacterium *Clostridium perfringens*, which had earlier been identified as an “A-destroying” organism (36 – 39). The enzyme responsible for this activity was identified (and later shown to be a member of CAZy family GH36), but whereas it was able to convert the A-antigens to H-antigens on A\(_2\) RBC membrane fragments, it was not active on intact RBCs and therefore was not useful. Around the same time, the company ZymeQuest started to use a GH27 \(\alpha\)-N-acetylgalactosaminidase from chicken liver to convert the A-antigens (34). However, it was only able to fully convert A\(_2\) RBCs; conversion of A\(_1\) RBCs, with their much higher antigen content, was incomplete. Further, its optimal pH range was not really compatible with RBCs (3.8 – 5.7), meaning that up to 3 g of enzyme per packed RBC unit was needed (40). Other \(\alpha\)-N-acetylgalactosaminidases from different sources were found in the next few years (41 – 43), but unfortunately, none showed full conversion activity on A\(_1\) RBCs (41 – 43).

**Enzymatic blood type conversion, second generation**

A key publication describing the discovery of new enzymes for blood type conversion was that of Liu *et al.* in 2007 (44). TLC-based screening of a library of some 2500 fungal and bacterial lysates using fluorescently tagged tetrasaccharides that represent the A- and B-antigens identified new groups of \(\alpha\)-N-acetylgalactosaminidases and \(\alpha\)-galactosidases that function at pH 7. The \(\alpha\)-N-acetylgalactosaminidase activity was similar to that of the *Elizabethkingia meningosepticum* \(\alpha\)-N-acetylgalactosaminidase that had been identified by similar means previously (45). BLAST analysis then identified a family of enzymes that were subsequently assigned to GH109 and shown to hydrolyze their substrates with net retention of anomeric configuration. Members of this family use an NAD cofactor and cleave the glycosidic bond through an unusual mechanism involving transient redox-assisted elimination and addition steps, as had first been shown for members of GH4 (46). A three-dimensional structure of the enzyme confirmed the loca-
tion of the NAD cofactor near H3 of the substrate and the absence of any metal ion requirement. It also provided structural insight into the strict specificity of the enzyme for A-antigens and its broad specificity therein for all A subtypes. They also discovered a new family of inverting α-galactosidases that cleave the B-antigen efficiently at pH 7 and assigned these enzymes to GH110. A subfamily (GH110B) that also cleaves the linear B-antigen (missing the fucose) was reported in the following year (47), but at present, no three-dimensional structures are available to explain these findings.

The *E. meningosepticum* enzyme was indeed shown to cleave A-antigens from red blood cells, but only if buffers of low ionic strength were employed. This behavior was attributed to the need for the enzyme, which is predominantly cationic around the active site, to interact with the net negatively charged red blood cell surface (48). Under such conditions, they were able to use as little as 300 μg ml⁻¹ of enzyme to fully convert A red blood cells to O, as measured using standard typing agents. This corresponds to ~60 mg enzyme per unit of RBCs. Conversion of B-type RBCs by the α-galactosidases was considerably more efficient. Using the GH110A enzyme from *B. fragilis* in the same low-ionic strength buffer, a full unit of B-type RBCs could be converted using only 2 mg of enzyme. Indeed, by combining the two enzymes, they were also able to convert Type AB RBCs. The conditions employed were further studied by other groups, who showed that a standard low ionic strength glucose-containing buffer was equally good for use in conversion of A, B, and AB RBCs, but that the enzymes were not stable to storage at low ionic strength (49, 50). Considerably improved cleavage performance at higher ionic strengths could be attained by inclusion of molecular crowders such as dextrans in the reaction mixtures (51). These polymers effectively increase the enzyme concentration by decreasing the reaction volume available to them, thereby bringing them closer to the RBC surface. Given their long history as blood expanders that are used to maintain plasma volume in emergency situations, dextrans can probably be used safely in this manner.

Inspired by these studies, our laboratory explored the potential of a class of bacterial endo-β-gal that cleaves the entire A or B trisaccharide off red blood cells in the hope that a single enzyme could be used for conversion of both blood types. These GH98 enzymes, discovered by the Li group (52), had been shown to cleave the dominant β-1,4-linked Type 2 antigens effectively, but to have relatively low activities on the β-1,3-linkages of other subtypes. By use of iterative steps of directed evolution, guided by available crystal structures, we were able to increase the activity of a GH98 enzyme from *Streptococcus pneumoniae* some 170-fold in cleaving Type 1 linkages without significant loss of Type 2 cleavage activity (53). Crucial to our success was our development of an efficient coupled assay in which the methylumbelliferoyl glycoside of the Type A1 pentasaccharide, synthesized chemoenzymatically, was employed. Cleavage of the terminal α-GalNAC residue exposed the oligosaccharide to sequential degradation by the α-fucosidase, exo-β-gal, and β-hexosaminidase that were included in the assay mixture, resulting in release of the fluorescent methylumbelliferone. Overall, this provided an important demonstration of the potential for improving the activities of these enzymes (53, 54). However, the reality that this process would have to be repeated to create good enzymes for Type 3 and 4 linkages and the concern that the presence of a terminal GlcNAc residue could lead to RBC clearance led us to reconsider the wisdom of this specific approach. This was especially true in light of new opportunities presented by our development of high-throughput screens suitable for metagenomic analyses.

**Enzymatic blood type conversion, third generation**

The advent of functional metagenomic analysis has opened up new opportunities for discovery of novel enzymes because it allows the interrogation of the genomic repertoire of potentially all microorganisms within an environmental sample, not just those that can be cultured (55). By extracting all of the DNA from the sample and then expressing fragments thereof within a host organism such as *Escherichia coli*, a library is generated that can be assayed for the desired activity. Crucial to this approach, which can be performed with either small (3–10 kb) or large (30–40 kb) DNA inserts, is the availability of a suitable high-throughput screen.

So armed, we first considered what environments might harbor organisms that degrade A- and B-antigens. After rejecting ideas based on parasites, such as mosquitoes or leeches, that feed on human blood (because only primates have the ABO system), we settled on the human gut microbiome. The mucins that line the gut wall display many sugar structures on their termini, including the ABH antigens (56). Because gut bacteria are known to forage upon these mucin glycans, it seemed probable that some of the bacteria therein would produce glycosidases that could cleave the A- and B-antigens. Based on this and on a study showing a correlation of human microbiome content with blood type (57), we generated a large insert metagenomic library from a donor of AB blood type. Screening of these 20,000 clones identified several new GH109 enzymes as well as a GH36 α-N-acetylgalactosaminidase, although none of these exhibited properties superior to those of the *Em*GH109 found previously (44). However, one particularly active clone contained a pair of genes encoding two enzymes that work in concert to cleave the A-antigen. The first of these is a metallo-GalNac deacetylase (DeAc) that is highly specific for the A-antigen but works on all subtypes of A, generating a galactosamine (GalN) at the terminus in place of GalNAc. Crystallographic analysis of a product analogue complex revealed the metal-containing active site as well as a set of hydrogen-bonding interactions with the fucose moiety that explain the specificity for A-antigen (Fig. 4). Its C-terminal carbohydrate binding module binds tightly to LacNAc structures, consistent with its docking to the RBC cell wall, while acting on the A-antigen. The second enzyme is a GH36 α-galactosaminidase that efficiently cleaves the monosaccharide from the terminus, generating the H-antigen (58) (Figs. 3 and 4). This latter enzyme is much less specific, but because no other GalN is likely present on the cell surface, this is not likely to be a problem. Indeed, testing of this enzyme on O and B RBCs revealed no release of GalN. These two enzymes, working together, cleave the A-antigen very efficiently, requiring some 15–30-fold less enzyme than the previous best.
(EmGH109) and doing so in standard buffers without the need for molecular crowders, although their addition further enhances activity. Indeed, they work particularly well in whole blood, either at room temperature or at 4 °C, and can be removed from RBCs by simple washing during the centrifugation steps that are employed in normal RBC processing. As little as 1 mg per unit of RBCs may be needed for conversion. These properties should facilitate the use of these enzymes within the current structure for blood processing with minimal disruption. Interestingly, good evidence for the probable existence of such GalNAc deacetylases that work on A-type RBCs was already present in the medical and forensic science literature. Some sepsis patients had been observed to undergo a transient change in their blood type from A to B until the infection cleared up, when their A blood type returned. Kabat had in fact suggested that this process, known as the acquired B phenomenon (59), might be caused by a bacterial deacetylase generating a galactosamine moiety that was recognized as galactose by the polyclonal antibodies in use at that time, although no enzyme was purified. Similar phenomena of variable blood type had also been seen during attempts to type body parts recovered from the River Thames, again presumably due to river-borne bacteria (60).

**Current status and future prospects for enzymatically converted RBCs**

Despite the early success of converting B- to O-type RBCs and their subsequent transfusion into A- and O-type patients (33) without adverse effects, the technology has not yet moved into clinical practice. One hold-up has been the efficiency and efficacy of the A-cleaving enzymes available. This was in part alleviated by ZymeQuest’s development of the GH109 enzymes, although 15–60 mg of enzyme per unit of RBCs was still required. The other problem, noted earlier, is the low-level agglutination observed when ECO-RBCs are cross-matched with serum from Type A (in 20% of cases) or Type O (in 40% of cases) serum from other patients. The cause of this has not been determined, but it could arise from low levels of residual B-antigen caused by incomplete cleavage. Similar problems have also been seen after cleavage of the A-antigen (9), and, interestingly, agglutination was more frequent when using serum drawn from patients with previous infections (61). Again, the cause of this cross-matching agglutination, which does not seem to result in hemolysis, is not clear. Importantly, however, the presence of small amounts of residual A-antigen does not appear to be an issue for transfusion because it is known that some Type B individuals also express a low level of A-antigen.
due to their B-galactosyltransferase having a loose specificity. Blood from such individuals has been used for transplants to other B patients for years with no issue. As a consequence, blood-typing reagent manufacturers now adjust antibody concentrations to avoid detecting this phenomenon and unnecessarily rejecting blood (33). Importantly, the residual A-antigen site density of A-ECO-RBCs that were converted by GH109 enzymes is lower than that present on these B-type RBCs, and indeed the low levels of A-antigen on these B-type RBCs can be removed by treatment with GH109 enzymes (10). Consequently, it is likely that the ECO A-type RBCs are indeed safe for transfusion.

Another suggested explanation revolves around the way in which Type 3 chain A-antigens are cleaved by an exo-GalNAcase because only the terminal GalNAc would be cleaved, not the internal ones (Fig. 2). This generates an H-antigen capping the internal A-antigen. If the internal GalNAc residue is recognized by anti-A or other antibodies, this could result in the agglutination observed (11, 44, 61–63). A further possibility is that removal of the A- or B-antigens from the surface might lead to a rearrangement of other clustered antigens in the vicinity, exposing antigens that were previously hidden. Although there is no direct evidence for this, changes in glycan clustering around the ABH antigens upon their enzymatic removal was shown to differentially affect the recognition of cell surface sialic acids by cognate receptors, most likely through altered clustering (65). The only clinical trials reported on A-ECO-RBCs are Phase 1 trials conducted by ZymeQuest in 2005, wherein small volumes of A-ECO-RBCs were re-infused into the original donor with no ill effects (NCT00261274) (10). Clearly, further trials are needed.

As we move forward, a primary concern is clearly to understand the basis of these agglutinations during cross-matching and to evaluate whether these are of clinical significance. This most likely requires identification of the antibodies causing such agglutination and their specificity while also investigating the possible causes suggested above. Some clarity on these issues would greatly improve prospects of moving into further clinical trials, given the high activity of the newest enzymes and consequent low enzyme loadings needed. Prospects of removal of the Rh antigens are more limited, given that it is associated with a transmembrane protein. However, the much more invasive antigen-masking approach using cell surface polymers is one method that has been proposed (66).

Meanwhile, another possible application of these enzymes is in the area of organ transplantation. Of great importance among the antigens that need to be matched in identifying suitable organs for transplantation to an individual patient are the ABO antigens. Removing the A/B antigen enzymatically may seem impractical at first because the organ will regenerate the cleaved antigen. However, the greatest challenge with incompatible transplants is during the first few days after transplantation. If the immune response during this time can be reduced substantially, then success rates are expected to improve. Indeed, when wait times have become dangerously long, kidney transplantations across the ABO barrier are already performed at some centers. Typically, plasmapheresis is performed on the recipient to remove antibodies, along with immunosuppression and possibly splenectomy (67, 68). Removal of the A/B antigens from the donor organ prior to transplant would greatly reduce the need for immunosuppression and possibly plasmapheresis. Several papers relate the use of glycosidases to remove tissue surface antigens from whole organs during perfusion (1, 64, 67), but studies have not progressed beyond that point. The advent of improved enzymes and improved perfusion procedures give new life to this field.

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