The ABO blood group system revisited: a review and update

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The antigens of the ABO system were the first to be recognized as blood groups and actually the first human genetic markers known. Their presence and the realization of naturally occurring antibodies to those antigens lacking from the cells made sense of the erratic failure of blood transfusion hitherto and opened up the possibility of a safe treatment practice in life-threatening blood loss. Although initially apparently simple, the ABO system has come to grow in complexity over the years. The mass of knowledge relating to carbohydrate chemistry, enzymology, molecular genetics, and structural and evolutionary biology is now enormous thanks to more than a century of research using ABO as a principal model. This has provided us with data to form a solid platform of evidence-based transfusion and transplantation medicine used every day in laboratories and clinics around the globe. This review aims to summarize key findings and recent progress made toward further understanding of this surprisingly polymorphic system. ***Immunohematology* 2009;25:48–59.**

Key Words: ABO, blood group, antigen, allele, genotype

There have been many reviews of the ABO blood group system written throughout the years, covering different aspects of this fascinating topic. A limited selection of such reviews can be found in the reference list, particularly for readers who want to focus more on the discovery,[1] biochemistry,[2,3] enzyme structure,[4] and molecular genetics.[5,6] Our intention is not to reproduce them but to follow the guidelines of this new series of blood group systematic reviews in *Immunohematology* to provide a brief introduction to this amazingly complex blood group system.

History

The discovery of the ABO blood group system ranges from myth and folk legend all the way to the Nobel Prize. Karl Landsteiner, a Viennese pathologist, made the observation that when his serum and that of five colleagues were mixed individually with their saline-suspended RBCs, agglutination was observed with some mixtures but not with others. He reported this as a footnote to a paper published in 1900[7] followed by a more comprehensive paper in 1901.[8] Translations of both papers can be found in the review by Camp and Ellis.[1] In these early studies, he showed that two each of the six sera discriminated among three blood groups: A, B, and C (later renamed O from the German *ohne*, meaning *without*). Thus, Landsteiner demonstrated that a person's serum contained antibodies to the antigen(s) lacking from their RBCs. The fourth blood group, AB, was described a year later by Decastello and Sturli[9] in four individuals in a larger study of 34 healthy subjects and 121 patients.

Variation in A antigen expression was also recognized very early in the twentieth century (reviewed in Race and Sanger[10]), and the A blood group was divided into A_1 and A_2.[1,11] Other descriptions of weakened A antigen expression followed, and the A blood group was subdivided further based on characteristic reactivity with human polyclonal antisera, i.e., strength of reactivity and presence of mixed field agglutination; presence of anti-A_1; and whether A or H blood group substance was present in the saliva of secretor subjects (Table 1). Weak forms of B antigen were also found but are typically more difficult to define serologically into specific categories although some subgroups (e.g., B_{el}) are analogous to their A counterparts (A_{el}).

The frequency of the common ABO phenotypes (A_1, A_2, B, A_1B, A_2B, and O) varies greatly among different populations.[12,13] Populations with a high frequency of A phenotype are found mainly in Northern and Central Europe, and the

Table 1. Subgroups of A—agglutination reaction patterns adapted from textbooks

Subgroup of A	Reactions* with				Substances in saliva†	Anti-A1 in serum
	Anti-A	Anti-A,B	Anti-A1	Anti-H		
A_1	++++	++++	++++	0	A, H	No
A_2	++++	++++	0	++++	A, H	Sometimes
A_{int}	++++	++++	++(+)	+++	A, H	No
A_3	++(+) mf	++(+)mf	0	++++	A, H	Sometimes
A_x	0/+	++(+)	0	++++	H	Often
A_{end}	+	+	0	++++	H	Sometimes
A_m	0/+	0/+	0	++++	A, H	No
A_{finn}	+	+	0	++++	H	Yes
A_{bantu}	+(+)	+(+)	0	++++	H	Yes
A_{1ae}	0‡	0	+++**	++++	H	Yes***
A_y	0‡	0	0	++++	A, H	No
A_{el}	0‡	0	0	++++	H	Sometimes

*A negative reaction is denoted by 0. Positive reactions are denoted as from + (weak agglutination) to ++++ (maximal agglutination).
***Dolichos biflorus* only.
***Serum reactivity against both A_1 and A_2 RBC.
†Blood group ABO substances in saliva and other body fluids of secretors.
‡Despite lack of agglutination, anti-A can be adsorbed to and eluted from cells in this subgroup.
mf = mixed field agglutination.

A_2 phenotype reaches its peak among the Lapps in Northern Scandinavia but is very rare in Asia. The B phenotype is most frequent in Central Asia and almost absent in Amerindians. Blood group O is the most frequent phenotype in a global perspective, with Native American Indians being almost exclusively blood group O. Parts of Africa and Australia also show high frequencies of blood group O. The reason for the differences observed among populations is not fully understood although several theories have arisen. The concept of evolutionary selection based on pathogen-driven blood group changes will be discussed later (see section on pathogen interactions). The early importance of ABO diversity is supported by reports in which the group *O*-defining single base pair deletion at nucleotide position 261 (see section on molecular genetics) has been found in both Neandertal people[14] and ancient Egyptian mummies,[15] suggesting that selection pressure and survival of the fittest were indeed early features during our co-evolution with pathogens like malaria parasites and many others.

Nomenclature

The ABO system was the first to be discovered and has therefore been given the number 001 in the official International Society of Blood Transfusion (ISBT) terminology. Nomenclature for the ABO antigens is actually straightforward since the antigen status is determined by the presence or absence of specific carbohydrate molecules. This is particularly true for the A and B antigens but may be less clear for the two other antigens (A,B and A1) given official antigen status by the ISBT. The A,B antigen is thought to be an epitope not involving the A versus B–differentiating surfaces, but consists of a common recognition motif[16] found when either the A or B antigens are present. The debate surrounding the real identity of the A1 antigen is still ongoing,[17,18] but the A_1 phenotype reveals many differences compared with A_2, both quantitative and qualitative, so the question is quite complex (see section on biochemistry). Table 2 shows both the numerical and traditional nomenclature according to the ISBT Working Party on Terminology for Red Cell Surface Antigens.[19]

Table 2. ABO nomenclature

	System	ABO antigen notation			
ISBT number	001	ABO1 (001001)	ABO2 (001002)	ABO3 (001003)	ABO4 (001004)
ISBT name	ABO	A	B	A,B	A1

Blood group allele nomenclature including ABO is under consideration by a subcommittee of the aforementioned ISBT Working Party. In the absence of an officially agreed-on terminology, several different variants have evolved (see Table 7 in Chester and Olsson[6]). Typically, alleles are referred to by their serologic activity and a number. An unofficial but often used terminology is found at the Blood Group Antigen Gene Mutation Database, also known as the dbRBC Web site (http://www.ncbi.nlm.nih.gov/projects/gv/rbc[20]). In this review, alleles will be referred to in the traditional way but with dbRBC terminology given in brackets, e.g., A^1 [*A101*] and O^1 [*O01*].

It can be specifically noted that it is important for clarity and consistency to use subscripts, superscripts, and italics appropriately. For example, A1, A_1, and A^1 mean the antigen, the phenotype, and the allele, respectively.

Inheritance and Molecular Genetics

The A and B antigens are inherited as Mendelian characteristics in a codominant autosomal fashion. In 1908, Epstein and Ottenberg[21] were the first to suggest in a short case report that ABO blood groups might be inherited. This was later proved by von Dungern and Hirszfeld in 1910 (translated by Pohlmann[22]). In fact, ABO inheritance was one of the first genetic markers to be used in paternity testing and in forensic medicine.[23,24]

Unlike the majority of blood groups, the antigens of the six currently known carbohydrate systems are not coded by genes directly. Instead, these blood group genes encode glycosyltransferases that in turn synthesize the oligosaccharide epitopes (Fig. 1). Thus, the A and B antigens are made by A and B glycosyltransferase, respectively, encoded by the *ABO* gene carried on the long arm of chromosome 9 (9q34). As with many of the blood group genes, the position of the *ABO* locus was known for many years before the gene was cloned.[25] The genes encoding the A-synthesizing 3-α-*N*-acetylgalactosaminyltransferase and the B-synthesizing 3-α-*N*-galactosaminyltransferase were cloned by Yamamoto and colleagues in 1990[26,27] after purification and partial amino acid sequencing of A transferase from lung tissue.[28] Probing cDNA libraries obtained from human adenocarcinoma cell lines of different ABO types, the main alleles were defined.[26] They determined that the *B*-specific mRNA differed from the *A*-specific gene by only 7 of 1062 coding nucleotides, of which 4 result in amino acid differences in the enzyme product. The difference between the A^1 [*A101*] and O^1 [*O01*] genes was shown to be a single guanosine (G) deletion, which alters and severely truncates the open reading frame (ORF) as shown in Figure 2. The A_2 phenotype was shown to depend on a cytosine deletion in the 5′ end of the gene, resulting in elongation of the ORF.[29] The organization of the *ABO* locus[30,31] is shown in Figure 3. The gene consists

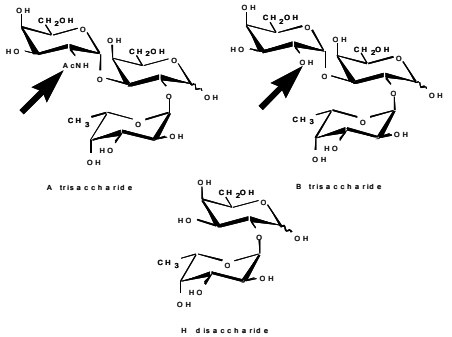

Fig. 1 Principal structure of the A, B, and H oligosaccharides. The difference between the A and B structures is highlighted by arrows.

of seven exons (plus an alternative exon 1a located upstream of exon 1[32]), with the majority of the catalytic domain of the enzyme encoded by exon 7.

Since the groundbreaking cloning paper, 215 *ABO* sequence entries have been submitted to the dbRBC Web site[20] (accessed on April 15, 2009) and curated by the dbRBC staff and experts, but new alleles are constantly being identified. Figure 4 shows a breakdown of the 181 alleles in the dbRBC categorized by their association with normal or altered phenotypes: these include 65 different *A* alleles, 47 *B* alleles, 58 *O* alleles, and 11 "*AB*" alleles. The remaining 34 sequences listed in the current dbRBC consist of 20 sequences encompassing the 5′ noncoding region including the repetitive and polymorphic CCAAT box binding factor/nuclear factor Y (CBF/NF-Y) motif and 14 intronic or overlap sequences. Of the *A* alleles, 6 and 11 encode normal A_1 or A_2 phenotypes on RBCs, respectively, whereas 48 describe mutated *A* alleles (A^{weak}) associated with different variants of weak A antigen expression (Table 1); the *B* alleles include 9 normal and 38 weak alleles; and 11 alleles

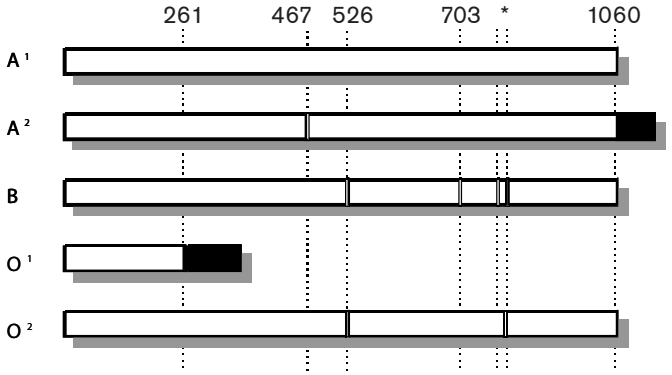

Fig. 2 Schematic representation of predicted open reading frames for some common *ABO* alleles. White rectangles represent translated A^1 [*A101*] consensus; black rectangles represent translated non-A^1 consensus (nonsense). Vertical bars indicate nucleotide (nt) positions (given above bars) of mutations leading to amino acid changes. * indicates *B* [*B01*] allele is mutated at nt 796 and 803; O^2 [*O03*] allele at nt 802.

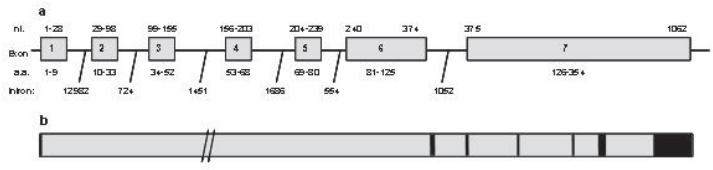

Fig. 3 Organization of the *ABO* gene. **A:** The seven exons and six introns are not drawn to scale. The numerals above boxes represent the first and last nucleotides (nt) of the coding region in each exon, and those below boxes show the corresponding amino acid (a.a.) numbers. The size of each intron is indicated with a thin oblique bar. **B:** The *ABO* gene drawn to scale (except intron 1); exons are black and introns gray.

have been described that encode glycosyltransferases capable of synthesizing easily detectable levels of both A and B antigens. This group includes alleles conveying the two unusual phenotypes cisAB and B(A). Fifty-eight alleles are predicted to give rise to proteins without enzymatic activity of which 45 contain 261delG, the mutation that alters the translational ORF and predicts a shortened protein product with no transferase activity. This large group of *O* alleles includes four principally important evolutionary lineages: O^1 [*O01*], O^{IV} [*O02*], *Otlse09* or $O1^{(467T;318T)}$ [*O09*], and O^{1bantu} [*O54*].[33,34] Numerous minor variants of these main alleles[35] as well as hybrid *O* alleles combining A^2 or *B* with different 261delG-containing *O* sequences[36] have also been found, most notably in individuals of African ancestry. Of the remaining 13 "nondeletional" alleles, 3 suffer similar fates to that caused by 261delG because they contain nonsense mutations resulting in altered ORFs caused by nucleotide insertions. Another 3 have nonsense mutations introducing immediate stop codons, thereby truncating the ORF at the same codon where they occur. Finally, 7 recorded nondeletional *O* alleles are crippled by at least one missense mutation each, giving rise to critical amino acid substitutions, the most famous example of which is 802G>A resulting in 268Arg. For a recent review about this latter group of interesting *O* alleles designated O^2 [*O03*], see Yazer and Olsson.[37] What is most intriguing about these nondeletional *O* alleles is that they are all attributable to mutations in *A* allele backbone sequences. This has two main practical consequences: first, virtually all *ABO* genotyping methods will signal the

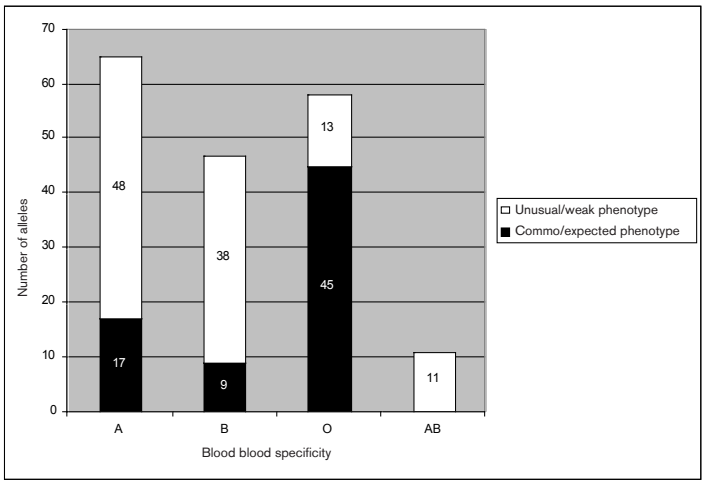

Fig. 4 Graphic representation showing the number of *ABO* alleles currently listed in the dbRBC database. The black bars represent alleles that encode a glycosyltransferase with normal activity; the white bars represent alleles encoding glycosyltransferases that have altered activity or specificity. The AB bar includes alleles encoding glycosyltransferases capable of synthesizing both A and B antigens, i.e., cisAB and B(A). The group O bar has been divided into the 45 O alleles that contain the mutation 261delG (black) and the 13 "nondeletional" alleles without it (white). The latter group is often associated with weak expression of A antigen.

presence of A^1 or A^2 alleles unless specifically designed to detect rare *O* alleles, which will result in the potentially catastrophic prediction of group A in a group O person. Second, the phenotype actually conveyed by inheritance of one of these so-called *O* alleles is not always O but often weak A or O-like but without anti-A present in plasma. The reason for this is currently unclear, but there has recently been a series of papers investigating the serologic pattern and mechanisms behind this interesting phenomenon.[38–42] There is currently no *O* allele described that is based on a *B* sequence, but we predict that when such an allele is found, it will show the opposite serology, i.e., either weak B expression or O-like without anti-B in plasma.

It is the molecular genetics that make this system so fascinating because mutations ranging from single nucleotide changes to more complex hybrid gene formations can alter the specificity of the enzyme, the efficacy of the enzyme, or both; changes that, at the phenotypic level, are manifested simply by altered antigen expression. Good examples are the various molecular bases of the A_x phenotype, which can either result from a single missense mutation in exon 7 of an A^1 [*A101*] allele or be based on different hybrids, for instance between the 5′ end of *B* and the 3′ end of *O* alleles.[43,44] The B_3 phenotype in the Taiwanese population has also been shown to result from different molecular backgrounds, one a missense mutation but more commonly, a principally interesting mutation that was the first one shown to affect splicing of *ABO* mRNA and cause exon skipping.[45] In addition to altered specificity or enzymatic efficacy, aberrant intracellular trafficking of ABO transferase may cause weak subgroup phenotypes.[46]

As alluded to previously, this added layer of complexity, in which mutations affect enzyme activity and not the antigen directly, provides difficulties in designing genotyping assays that will detect rare variants, which if misinterpreted can have serious consequences in ABO group determination.[47]

There are problems associated with all the more than 30 *ABO* genotype screening methods published or commercially available so far (reviewed in Olsson and Chester[48]). The vast majority is only designed to determine between three and six of the common alleles, although additional alleles can often surface if their polymorphisms interfere with the detection system. However, virtually all methods will fail to predict the phenotype of a sample in the presence of most A/B subgroup alleles, rare nondeletional null alleles, cis*AB* and *B*(*A*) alleles, or hybrid alleles, which can lead to serious consequences. Because this is particularly dangerous if blood or transplant recipients are typed (as suggested, e.g., by Procter et al.[49]) we recently developed and implemented an improved *ABO* genotype screening method that addresses these problems and can be used in a clinical laboratory setting.[47] In summary, the complex genetics of ABO is a major challenge for *ABO* genotyping efforts, not least because of the disturbing fact that a certain allele can lead to more than one phenotype and seemingly identical phenotypes can have more than one molecular genetic background.

The regulatory mechanism of the *ABO* gene has been investigated extensively. An enhancer element located approximately 4 kbp upstream of exon 1[50] was found to contain four 43-bp repeats in all alleles except A^1 [*A101*] and the infrequent O^2 [*O03*], which have only one copy.[51] This may play a role in expression.[52] The enhancer region contains a CBF/NF-Y binding site; mutations in this site decrease enhancer activity in a gastric cancer cell line,[50] and alterations in this region may even cause rare B subgroup phenotypes.[53] However, it was recently shown that A^1 [*A101*] or A^2 [*A201*] transcripts are virtually undetectable in peripheral blood whereas *B* and *O* (including O^2 [*O03*]) mRNA is readily found.[54,55] This appears to speak against a critical role for CBF repeats in erythroid *ABO* regulation, but more work is required. There is also an Sp1-binding site in the proximal promoter that may be important for erythroid *ABO* regulation.[56]

Although much is understood regarding the cause of weak A/B antigen expression on RBCs when it comes to inherited weak ABO subgroups,[5,6] less is known about altered ABO expression in hematologic disorders. Erythrocytes losing A, B, or H antigen have been noted in patients with hematologic malignancy, especially in the myeloid lineage.[57,58] Very recently it was suggested that methylation of the *ABO* proximal promoter is the reason for such leukemia-associated downregulation.[59] Reduced A and B antigen expression in bladder and oral carcinomas is partially attributable to loss of heterozygosity or hypermethylation.[60,61] Furthermore, urothelial tumor tissue contains decreased amounts of A/B antigens that correlated to decreased levels of ABO mRNA compared with cells from normal tissue.[62] Transient depression of A antigens has also been observed in some pregnant women,[44] but the reason for this is still unknown.

Biochemistry

The biochemistry of the A and B antigens was elucidated by the astonishingly early and brilliant work from the groups of Morgan and Watkins, and Kabat (reviewed by Watkins[2] and Kabat[63]). The A, B, and H determinants were hypothesized to reside on water-soluble glycoproteins able to inhibit agglutination of RBCs by antibodies or lectins. A precursor substance, H, was hypothesized as a building structure for A and B, and the terms *O*- (or *H*-) *substance* and *anti-H* were introduced in 1948.[64]

Owing to difficulties in obtaining sufficient quantities of blood group active material after extraction from RBCs, most of the early studies were performed on ovarian cyst or animal mucin, rich in secreted blood group substances. Before isolation and chemical identification of these substances, inhibition with simple sugars indicated *N*-acetyl-D-galactosamine (GalNAc), D-galactose (Gal), and L-fucose (Fuc) as the defining sugars in blood groups A, B, and O, respectively.[65,66]

Independent of the blood group of the individual investigated, a surprisingly similar composition of carbo-

hydrate residues (mainly Fuc, Gal, GalNAc and *N*-acetyl-D-glucosamine [GlcNAc]) was found. This was taken as a sign of large similar precursor molecules carrying small residues that differentiate blood groups.[67] The minimal determinant structures were subsequently shown to be trisaccharides as shown in Figure 1. The important concept of precursor-product relationship between H and A/B was formulated,[67,68] and the critical role of nucleotide-bound sugars as substrates in oligosaccharide synthesis was appreciated.[69] The biosynthetic pathway of the ABO blood group structures is as outlined in Figure 5, and the presence of A and B glycosyltransferases was first predicted[70] and then experimentally established.[71–73] Thus, the A and B glycosyltransferases use UDP-GalNAc and UDP-Gal, respectively, as substrates. Both require the H determinant as acceptor. The O protein is nonfunctional, leaving the H-defining terminal Fuc unaltered.

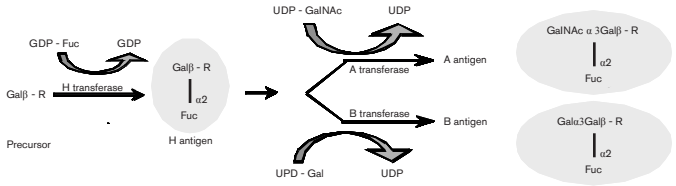

Fig. 5. Schematic depiction of the biosynthetic pathways for conversion of H determinants to A or B determinants. R represents the core structure. See text for enzyme abbreviations.

Although the composition of the A and B antigens is apparently straightforward, the biochemistry behind the shared A,B antigen recognized by many group O plasmas has only recently been elucidated experimentally. Bovin and his colleagues[16] synthesized a deacetylated A trisaccharide structure and bound it to the precursor in such a way as to expose what they hypothesized would be the common A,B epitope. They were able to demonstrate binding of both monoclonal and polyclonal anti-A,B to the synthetic structure.

Although it is well known that there are approximately five times fewer A antigens on A_2 than on A_1 RBCs, the underlying basis for the qualitative differences between them is less well defined. The A_2 transferase is 10 times less efficient than the A_1 transferase and has a different pH optimum and pI.[74,75] The A_2 enzyme is also less able to use chains other than type 1 or 2 carbohydrate precursors like the extended type 3 (repetitive A) and type 4 (globo-A) chains on RBC glycolipids.[17,76] More recently, Svensson et al.[18] have produced somewhat contradictory data indicating that although the A_2 transferase can readily use H type 3 chains to synthesize A antigen, the low levels of type 4 chains remain unconverted.

The ABH sugars are found on glycolipids (approximately 10%) and glycoproteins (approximately 90%) on the RBC as well as on many different tissues and cell types, including epithelial cells that line the lumen of the gastrointestinal, respiratory, and reproductive tracts as well as in salivary glands and skin. This wide distribution is a common feature for many of the carbohydrate blood groups, which has resulted in the term *histo-blood group* often being used to reflect this wide distribution. A and B antigen synthesis occurs during normal glycosylation of proteins and lipids in the Golgi compartment.[77] The precursor H substance is synthesized by one of two fucosyltransferases depending on the acceptor substrate used. The *FUT1* gene that encodes the 2-α-fucosyltransferase (α2FucT1) is responsible mainly for the synthesis of the H antigen on type 2 (and type 4) carbohydrate precursors found on RBCs.[78] The closely related *FUT2* gene encodes a very similar 2-α-fucosyltransferase (α2FucT2) that is expressed in epithelial cells and synthesizes H antigen mainly on type 1 and type 3 chains.[77] The major precursor types present in different tissues and secretions are shown in Table 3.

Table 3. Peripheral core structures and their principal tissue distribution (modified from Clausen and Hakomori[3])

Peripheral core type	Structure	Distribution
Type 1	Galβ1→3GlcNAcβ1→R	Endodermal, secretions, plasma
Type 2	Galβ1→4GlcNAcβ1→R	Ecto- and mesodermal (e.g., erythrocytes)
Type 3	Galβ1→3GalNAcα1→R	O-linked mucin-type, repetitive A
Type 4	Galβ1→3GalNAcβ1→R	Glycolipids in kidneys (and erythrocytes)

R = inner core structure or linkage.

In the Bombay phenotype (O_h), a silenced *FUT1* gene is present together with a silenced *FUT2* gene. Because H antigen is the precursor substrate for both A and B antigens, neither antigen can be synthesized without α2FucT activity, independent of the *ABO* genotype. The para-Bombay phenotype results from either (1) a silenced *FUT1* gene present together with an active *FUT2* gene, which permits the synthesis of H type 1 (and therefore A/B antigens) that may be adsorbed onto the RBC from the plasma; or (2) a mutated *FUT1* gene in which the encoded enzyme activity is greatly diminished, so that very low amounts of H antigen (and A/B antigen) are produced. It may be present with or without an active *FUT2* gene. In both cases, H antigen (and A/B antigen) is very weakly expressed and is often only detected by adsorption and elution tests with the appropriate blood group reagents. The H blood group system (ISBT 018) will be the subject of another review later in this *Immunohematology* series.

The A and B glycosyltransferases are type II membrane proteins located in the Golgi compartment,[79,80] although soluble forms are found in plasma and other body fluids. The enzyme consists of a short transmembrane domain, a stem region, and a catalytic domain that extends into the Golgi lumen (Fig. 6). The crystal structure elucidated by

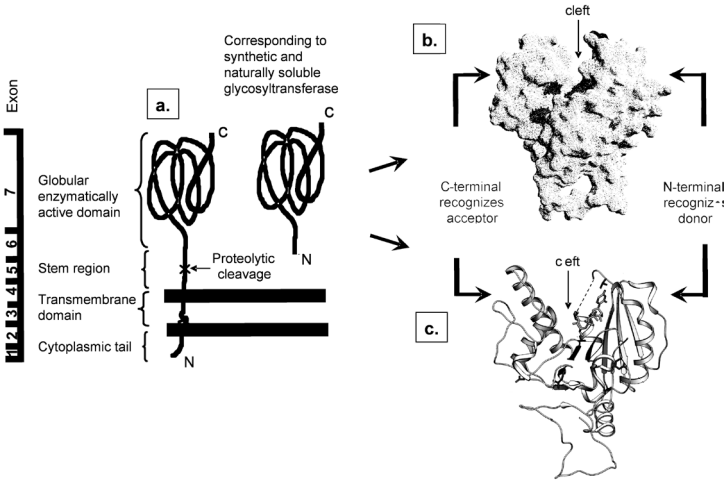

Fig. 6 A: The topology of the transferase is shown as a Golgi-localized, membrane-bound enzyme with a cytoplasmic *N*-terminus and catalytic *C*-terminal domain (modified from Paulson and Colley[79]). **B and C**: The three-dimensional surface model **(B)** was created with the Deep View Swiss Pdb Viewer version 3.7, and the three-dimensional ribbon structure **(C)** was generated using SETOR and SetoRibbon (unpublished). The vertical bar to the left illustrates the approximate exon usage for the different glycosyltransferase domains.

Patenaude and colleagues[81] showed that the catalytic site is divided into two domains; the *N*-terminal domain recognizes the nucleotide sugar donor substrate (UDP-Gal or UDP-GalNAc), whereas the acceptor substrate is held by the *C*-terminal domain. The DXD motif (DVD in these enzymes) that serves to capture Mn^{2+}, essential to the reaction because of its ability to bind the UDP part of the donor substrate, lies between the two domains. In addition, two so-called disordered loops have been identified by the crystal structural studies. One is located at the *C*-terminal of the enzyme. The other disordered loop lies close to the catalytic site of the enzyme. Its role is unclear[4]; however, mutations in this region have been shown to reduce enzyme activity and are often associated with weak subgroup phenotypes.[82]

Antibodies in the System

Anti-A and anti-B are naturally occurring antibodies that are produced by immunocompetent individuals from the age of approximately 6 months. The widely held dogma is that these antibodies are in fact mimicking antibodies produced against terminal carbohydrates on bacterial cell walls as a response to our normal intestinal microbial flora, and that these glycotopes share structural homology with A and B antigens.[83] In a modern follow-up to this original concept, three children with different congenital immune defects were studied after apparent ABO grouping discrepancies.[84] These patients (age range, 1.7 to 8 years) were limited to total parenteral nutrition and tube feeding. Their RBCs typed as group A, but each child lacked the expected anti-B by routine testing, although anti-B was weakly detectable in the serum of one of them after prolonged incubation at 4°C. The authors concluded that the absence of dietary exposure to bacteria prevented the production of anti-B despite normal immunoglobulin levels in the patients' sera. Recognition of self prevents an individual from making antibodies to antigens shared by the bacteria and may also partially explain disease susceptibility of one blood group over another (discussed briefly in the next section).

Anti-A and anti-B are predominantly IgM antibodies although IgG or IgA components are often found.[85,86] Class-switching to IgG does not occur unless there is a "hyperimmunizing" event such as an ABO-incompatible pregnancy or transfusion. Absence of the expected antibodies occurs rarely, although antibody titers vary considerably among individuals and have been shown to diminish with age. Patients who have immunoglobulin deficiencies will also lack anti-A or anti-B. Otherwise, most often the absence of an agglutinin in a healthy person should be taken as an indication that there may be a weak antigen or even (micro) chimerism present, perhaps detectable by adsorption and elution or by flow cytometry.

Individuals of the A_2 and A_2B phenotypes as well as those whose RBCs carry certain A subgroups (Table 1) can also produce anti-A1, generally reactive at room temperature and below. Although ABO antibodies of IgG type can cross the placenta, severe ABO-related hemolytic disease of the newborn is not common. The mechanisms behind this are discussed in more detail in the section on clinical consequences.

Pathogen Interactions

Bacteria, viruses, and parasites have been proposed as important driving forces for the geographic distribution of ABO blood group phenotypes because different pathogens demonstrate blood group–identical or –cross-reactive molecules on their surfaces. These are the probable targets for "blood group" antibodies, and their existence is the leading hypothesis as to why we make naturally occurring antibodies against the carbohydrate blood groups we lack. In addition, many pathogens show selective binding to blood group carbohydrate moieties via lectins (reviewed by Garratty[87]). More recent but perhaps anecdotal evidence for this theory was provided after severe hemolytic transfusion reactions in two group B patients receiving apheresis platelets from the same group A platelet donor.[88] The donor had donated regularly for a period of 20 years, during which time no adverse effects of transfusion had been observed, but had recently begun to supplement his diet with high-dose probiotics and his anti-B titer was shown to be greater than 8000. Inhibition studies performed with plasma from random group A donors and solubilized probiotic tablets demonstrated a reduction in titer, hinting at a role for ABO antibodies in neutralizing pathogens. This line of thinking is supported further by data from studies on viral glycosylation in host cells. HIV cultured in vitro with peripheral blood mononuclear cells (PBMC) from donors of different

ABO groups demonstrated specific neutralization with anti-A of those isolates grown in group A PBMCs but not those cultured with group B or group O cells.[89] Measles virus, when cocultured in a system expressing ABH glycosyltransferases (to mimic an in vivo environment), expressed A or B epitopes, or not, according to the enzymes expressed. Viral particles could then be neutralized by normal polyclonal anti-A or anti-B sera in the presence of complement if the corresponding glycans were present.[90] Conversely, the A and B (and H) antigens are also used as receptors for pathogen invasion by attachment to host cells as mentioned previously, so the equilibrium between invasion and evasion is a fine balance on both sides.

Furthermore, growing evidence for a leading role of *Plasmodium falciparum* as the major force shaping the human genome including the distribution of the blood groups has emerged.[91,92] The parasite-induced RBC surface protein pfEMP1 is known to bind to the A antigen trisaccharide,[93] and it has been shown that the severity, including mortality, of malarial disease is significantly lower in group O children than in other groups, mainly through the mechanism of reduced rosetting.[94,95] It is the focus on pediatric disease that is thought to make this pathogen particularly effective in exerting a selection pressure on our genome.

The general theme here is the concept of herd immunity, i.e., the fact that differences in the population will keep at least a fraction of all individuals protected from most if not all pathogens for humanity to survive. ABO differences as part of our innate immunity appear to be one of the better examples of this idea.[96]

Clinical Significance

Of all antibodies to RBC blood group antigens, anti-A and anti-B are arguably the most clinically important. On the other hand anti-A1 and anti-H are very seldom found to correlate with hemolytic adverse events but often show lower thermal optima. Before the discovery of ABO, transfusion between humans had been attempted with mixed success. In some cases, the patient survived and got better. In others the patient died rapidly, which we now realize was attributable to rapid intravascular hemolysis of ABO-incompatible RBCs (reviewed by Mollison et al.[97]). It has been reported that transfusion of as little as 30 mL of ABO-mismatched blood can result in a rapid fatal intravascular transfusion reaction (reviewed in Issitt and Anstee[98]). Together with transfusion-related acute lung injury (TRALI) and bacterial contamination of blood components, ABO incompatibility is still one of the main risks for major morbidity and mortality associated with transfusions, after erroneous transfusions.[99,100] The latest FDA report on transfusion-associated fatalities (found at http://www.fda.gov/cber/blood/fatal08.htm) shows that blood group incompatibility was judged as the cause of death in 37 percent of all cases during 2008, with TRALI at 35 percent. Among the hemolytic fatalities ABO was the cause in 59 percent. Surprisingly, however, many reports show that approximately 50 percent of patients who are inadvertently transfused with a major ABO-mismatched unit of blood tolerate the blood without any apparent signs of a transfusion reaction.[101] The mechanisms underlying the ability of some individuals to tolerate ABO-mismatched blood are not well understood but are very interesting as identification of resistance markers could be exploited in transplantation therapy. Despite our relative sophistication in providing appropriately ABO-matched blood for patients, there is recent clinical evidence that even the concept of ABO-compatible (as opposed to ABO-identical) products may not be as safe as it would appear. A study that followed the posttransfusion mortality among more than 86,000 patients receiving plasma showed that exposure to ABO-compatible but non-ABO-identical plasma was associated with an increased risk of death.[102] Whether this is associated with immune complexes between anti-A/B and soluble A/B antigen in AB plasma, for instance, remains to be studied. Furthermore, reports of the usage of platelet concentrates in cardiac surgery has also demonstrated less favorable outcomes in those patients receiving ABO-mismatched products.[103] The authors hypothesized that the formation of immune complexes may trigger cellular and inflammatory changes that adversely affect patient outcome.

Hemolytic disease of the fetus and newborn (HDFN) as a result of ABO incompatibility between mother and baby is a relatively common event in group O mothers carrying a group A or group B fetus. However, the disease is generally mild and rarely requires treatment, most often by phototherapy,[97] although inhibition of antibodies by administration of soluble A or B trisaccharides has been used successfully.[104] Mild HDFN is a consequence of the comparatively low levels of IgG ABO antibodies (which are often mainly IgG2 or IgG4) capable of crossing the placenta and also is related to the immaturity of the glycosylated structures on fetal RBCs. In addition, ABH antigens are present on many other cell types so the antibody concentration on RBCs is limited and the DAT often only weakly positive.

The clinical significance of anti-A and anti-B extends beyond transfusion medicine and is important in both solid organ and hematopoietic transplantation. Although ABO-mismatched hematopoietic stem cell transplantation is standard practice with a favorable outcome in most cases, transplantation of ABO-incompatible solid organs has been established only relatively recently with moderately successful outcome.[105] Children younger than 3 years of age have been shown to tolerate mismatched organs better, and West and colleagues have demonstrated good posttransplant survival in infants undergoing ABO-incompatible heart transplants,[106] showing that the relatively immature B-cell response in these patients can be exploited.[107] In a study of 46 patients undergoing ABO-mismatched renal transplants, Tobian et al.[108] demonstrated that therapeutic apheresis to reduce anti-A and anti-B titers to less than

16 together with standard immunosuppression protocols results in successful and stable engraftment. Progress continues in this field, driven by a shortage of appropriate organs for transplant. Yazer and Triulzi[109] conclude that immune hemolysis remains a major complication in ABO-mismatched transplantation of solid organs and to a lesser extent hematopoietic progenitor cells. However, passenger lymphocyte syndrome attributable to ABO or other blood group antibodies can now often be ameliorated by monoclonal antibody therapy, once clinicians realize the problem.

Summary and Future Perspectives

Despite the relative simplicity of the A and B antigens, perhaps especially considering the minor biochemical difference between them, the ABO blood group system remains one of the most interesting, both clinically and scientifically, dividing the world's population including patients and donors into four groups irrespective of origin or creed. Figure 7 summarizes the four principal levels at which the ABO system can be considered and shows how immunohematologists have been able to exploit the steadily increasing knowledge of this system by devising tests taking advantage of each of the different levels. It also shows some of the natural consequences generated and their relation to the microbes surrounding us.

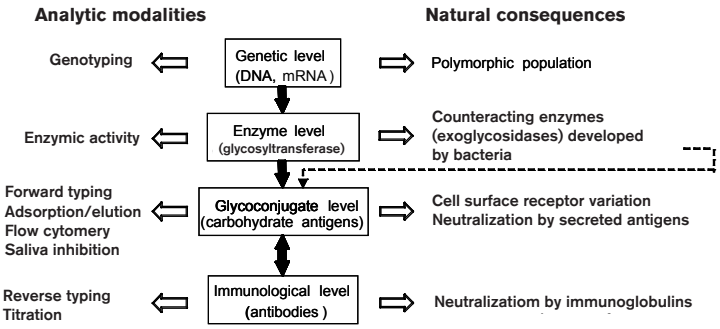

Fig. 7 Principles of the ABO system at four molecular levels and their modes of interaction are schematically represented by arrows in black. On the left side, the laboratory analytical modalities currently exploited at each level are listed; the right side shows some of the innate mechanisms used between host and pathogen.

A whole array of ABO-related research and other projects is currently in progress. Even if a surprising number of *ABO* alleles has been discovered already, no doubt there are more to come. ISBT is currently working to facilitate communication and reporting by introducing an official allele nomenclature. Although *ABO* genotyping cannot yet be used as a stand-alone analysis to support clinical decisions, improvement of the current status is highly desirable for its use as an independent addition to serology in the reference laboratory. Together with colleagues across Europe, we recently introduced the first microarray-based ABO typing system that takes the first steps toward this goal on a higher throughput system.[110] Efforts to renew methods for serologic typing are also ongoing. For instance, the young field of microfluidics holds promise for alternative blood grouping technology being more rapid, using smaller volumes of reactants, and being applicable to broader testing platforms.[111]

The temptation to create ABO-universal blood for transfusion purposes has kept scientists busy since the early 1980s when the first successful report of deliberate transfusion with modified RBCs across the ABO barrier was published.[112] Progress from those early days, using a poorly effective B-converting exoglycosidase from green coffee beans, has continued with the aims of eliminating ABO-associated hemolytic transfusion reactions and simplifying blood logistics and inventory management. Not only has the process taken important strides toward becoming a reality with both A- and B-degrading enzymes recombinantly available and necessary clinical trials in progress or being designed, but the project has also brought with it the scientifically exciting discovery of a large new class of bacterially derived exoglycosidase enzymes unknown hitherto and without clear homology or resemblance to any other group of molecules.[113,114] It is unknown at this point why so many different bacterial species have developed these blood group–converting enzymes. Speculation includes that it would simply be a means of digesting potential nutrient saccharides, but it also may be yet another way for microbes to make sure they are able to attach to the host cell surface, even if the histo-blood group of the current host happens not to fit the bacterial lectins initially. It is actually quite likely that yet other glycosidase specificities will be found in this extended arsenal of newly discovered enzymes. A recently exploited example is the Galα3Gal (also known as the straight B, fucose-less B, or Galili antigen) -degrading enzyme subfamily of galactosidases that can, for instance, be used to degrade xenograft antigenicity in pig tendons for use in knee surgery.[115]

Finally, it is easy to make the mistake of sitting back and looking at the ABO system as close to complete when it comes to knowledge and discovery. We predict that both the antigenic diversity and the way we look at antibody specificities in this and related systems will change dramatically during the next few years based on recent and future findings made possible by new technologies. ABO has for a long time served as a great model for genetics, enzymology, and biochemistry thanks to our deep understanding of the interindividual variation in this system, and there is nothing to suggest that it will stop now when high-throughput genetics and glycotope arrays are here to help us. On the contrary, it should be expected that there are still more surprises waiting around the corner.

Acknowledgments

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