Review: ABO blood group system—ABH oligosaccharide antigens, anti-A and anti-B, A and B glycosyltransferases, and *ABO* genes

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Introduction

The ABO system is one of the most important blood group systems in transfusion medicine. The ABO system consists of A antigens, B antigens, and antibodies against these antigens. Landsteiner discovered the ABO system in 1900. As opposed to many other blood group systems such as the Rh system, in this system the presence of "naturally occurring" antibodies against A and B antigens in individuals who do not express those antigens (Landsteiner's Law) causes an adverse and occasionally fatal outcome at the first mismatched transfusion. The concept that "only matched donor blood that would not result in RBC agglutination could be transfused" set the path for safe blood transfusion.

The ABO system is of interest in a variety of scientific fields (Table 1). In addition to the four major groups (A, B, AB, and O), we now know that additional subgroups exist that exhibit different patterns and degrees of agglutination. The A and B antigens were initially identified on RBCs, and later they were found on the surfaces of other types of cells as well as in secretions. Therefore, the ABO system is occasionally called the histo-blood group system, rather than the blood group system. Because these antigens exist in cells other than RBCs, ABO matching is important not only in blood transfusion but also in cell, tissue, and organ transplantation. Forensic science utilizes the ABO blood groups for suspect exclusion in the analysis of crime scene evidence, such as blood, saliva, seminal fluid, and even hair.

The A and B antigens are carbohydrate antigens, and not protein antigens, the primary gene products.

Table 1. A wide variety of ABO studies

- ***ABO* Genes**
  - Polymorphic: three major (*A, B,* and *O*) and dozens of subgroup (A^2, A^x, B^3, etc.) alleles
  - Different allele frequencies among different races
    - ⇒ **genetics, population study, anthropology**
- **A and B Antigens**
  - Oligosaccharide antigens
    - ⇒ **carbohydrate chemistry, glycobiology**
  - RBC, various tissues and cell types, and secretion (saliva, seminal fluid, etc.)
    - ⇒ **blood transfusion, cell/tissue/organ transplantation, forensic sciences**
  - Human, chimp (A, O), gorilla (B), baboon (A, B, AB), pig (A, O), etc.
    - ⇒ **systematics**
  - Expression changes during development, cell differentiation, and carcinogenesis
    - ⇒ **molecular biology, developmental and cellular biology, cancer biology**
- **A and B Glycosyltransferases**
  - Different donor nucleotide-sugar substrate specificity
    - ⇒ **enzymology, structural biology**
  - Presence of transferases with similar specificity (α1,3GalT, Forssman synthase, iGb3 synthase)
    - ⇒ **evolution**
- **Anti-A and Anti-B Antibodies**
  - "Naturally occurring" antibodies
    - ⇒ **immunology**

The A and B antigens are synthesized by a series of enzymatic reactions catalyzed by enzymes called glycosyltransferases. The final step of their biosynthesis is catalyzed by A and B transferases encoded by the functional *A* and *B* alleles at the *ABO* genetic locus, respectively. The allele frequencies vary among different races, which furnishes interesting questions in population studies, anthropology, and

human genetics. A and B antigens are not restricted to humans. The identical or similar antigens have been identified in other species. Furthermore, glycosyltransferases other than A and B transferases exist that exhibit similar specificity in reactions. Therefore, ABO is also of evolutionary and enzymological interest. A/B antigen expression exhibits dynamic changes during development and pathogenesis. Loss of A/B antigen expression in cancer, such as prostate cancer, has been documented. Therefore, the expression of *ABO* genes is an interesting subject in cancer biology in addition to molecular, cellular, and developmental biology. Safer blood transfusion conceived by Landsteiner and improved upon by many others, primarily immunohematologists, has become a routine medical practice. Since the cloning of the *ABO* gene in 1990,[1] progress has been made in the structural and functional analyses of the *ABO* genes and A and B transferases at the molecular level. I hope that the readers of *Immunohematology* find this review article interesting and useful for a better understanding of the scientific basis of the ABO system, oligosaccharide ABH antigens, A and B transferases, and *ABO* genes, and for applying this information to clinical applications.

ABO blood grouping is crucial for safe blood transfusion.

The discovery of the ABO blood group system was made when Landsteiner separated the cellular components from the liquid components of blood and observed the agglutination of RBCs in certain combinations upon mixing (Fig. 1). Based on the agglutination patterns, he categorized the subjects into three groups. In the next year Decastello and Sturli discovered the fourth group, and these four groups became the ABO blood groups. In order to explain the agglutination patterns, Landsteiner postulated that there were two antigens (A and B) and two antibodies against those antigens (anti-A and anti-B). He assumed the presence of the antibodies in the sera of individuals who did not express those antigens, which was later named Landsteiner's Law. His understanding was an important step toward the safe practice of blood transfusion, where transfusion should be performed between individuals whose blood components would not agglutinate upon mixing. It was reasonable to assume that the hemagglutination due to mismatch would also occur inside the body if it occurs in the test tube. Therefore, ABO typing before any transfusion was logical. To crossmatch also was wise because unknown antigens or antibodies could be present. Because the readers of *Immunohematology* are familiar with safe practice of blood transfusion and the techniques used for ABO typing, they are not described in detail here. Please refer to several excellent books on transfusion medicine for information.[2–4] It is important to note that subgroups have been identified, based on the different degrees and patterns of agglutination, using reference RBCs and antibodies. Those subgroups include A_2, A_3, A_x, A_{el}, B_3, B_x, and B_{el}. The natural antibodies seem to occur due to constant or occasional immunologic stimulation by substances, such as food, pollen, and bacteria, that are ubiquitous in nature.

A and B antigens are not protein antigens but oligosaccharide antigens.

Because the antibodies against A and B antigens were available from human sera and later from monoclonal origins, those reagents were used for immunohistochemistry, in addition to hemagglutination for blood typing. Some plant lectins were found to have an affinity with A and B antigens and were used for immunostaining purposes. It soon became clear that the expression of A and B antigens was not limited to RBCs. Those antigens were also found on the surfaces of several different types of human cells and demonstrated in secretions by an inhibition test. The antigens reactive to those antibodies and lectins were also found to be present in other species of organisms, including bacteria and plants, although the chemical nature of those antigens remains to be characterized.

In the 1930s, Landsteiner and colleagues suggested that soluble substances in secretions that inhibit hemagglutination were some type of new carbohydrate-amino acid complex. Later, in the 1950s, a group led by Watkins and Morgan and another group led by Kabat played a major role in the final determination of the chemical nature of ABH antigenicity (H antigens were found to be abundant in individuals with group O type blood group). They initially showed that simple sugars inhibited lectin-mediated agglutination of RBCs and prevented the specific precipitation of soluble blood group substances. This suggested a link between certain sugars and blood group specificity; *N*-acetyl-D-galactosamine, D-galactose, and L-fucose for A, B, and H specificity, respectively. Watkins and Morgan later showed that glycosidase preparations from bacteria and mollusks abolished the antigenicity, confirming the

Blood grouping based on RBC agglutination (Landsteiner, 1900)

RBC Serum	Dr.St.Dr.	Plee.Dr.	Sturl. Dr.	Erdh. Mr.	Zar. Mr.	Land.
Dr. St.	-	+	+	+	+	-
Dr. Plee.	-	-	+	+	-	-
Dr. Sturl.	-	+	-	-	+	-
Dr. Erdh.	-	+	-	-	+	-
Mr. Zar.	-	-	+	+	-	-
Mr. Land.	-	+	+	+	+	-

(+ agglutination - no agglutination)

A/B antigens on RBC and anti-A/B antibodies in serum

Blood group	*Antigen*	*Antibody*
A	A	anti-B
B	B	anti-A
AB	A + B	-
O	-	anti-A + anti-B

Inherited characteristics (Bernstein, 1924)

One gene locus-three (A, B, and O) alleles model

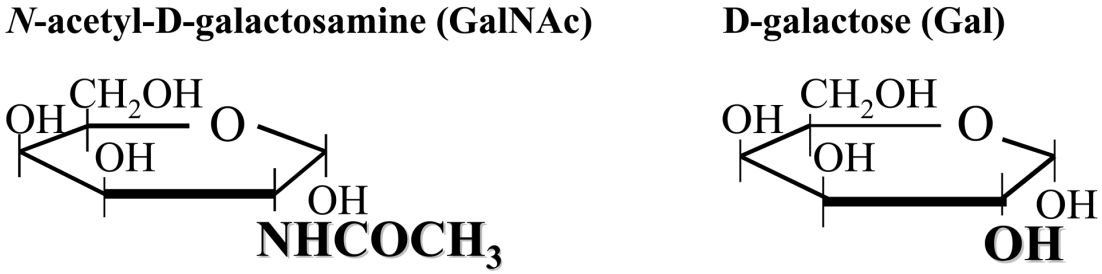
Phenotype	A	B	AB	O
Genotype	AA, AO	BB, BO	AB	OO

A and B antigens (Watkins, Morgan, Kabat, 1920-1960)

A antigen: GalNAc α1-3 (Fuc α1-2) Gal-

B antigen: Gal α1-3 (Fuc α1-2) Gal-

Immunodominant sugars



A and B genes & A and B glycosyltransferases

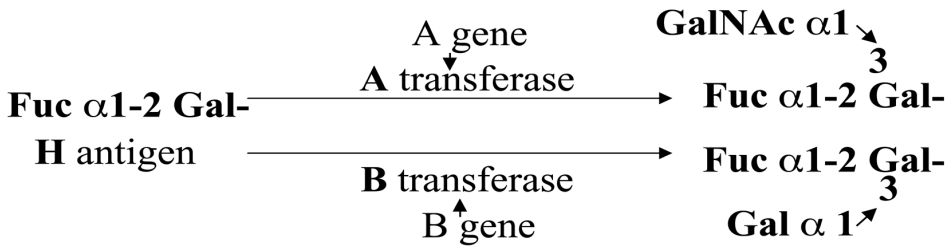


Fig. 1. ABO System. Discovery of the ABO blood groups, antigen-antibody hypothesis of RBC agglutination, chemical nature of A and B antigens and their biosynthetic pathways are shown.

importance of those sugars for the blood group specificity. A more direct approach was taken to purify A, B, and H determinants from ovarian cysts, which secrete soluble forms of these antigens in abundance. The cyst fluid was acid-hydrolyzed and alkaline-degraded, and the released carbohydrate fragments were analyzed for binding activity toward anti-A and anti-B antibodies. These studies revealed that the immunodominant structures of those antigens are oligosaccharides.[5,6] The structures of the related Le^a, Le^b, and Le^x substances were also determined. Their structures are as follows:

A: GalNAcα1-3(Fucα1-2)Gal-

B: Galα1-3(Fucα1-2)Gal-

H: Fucα1-2Gal-

Le^a: Galβ1-3(Fucα1-4)GlcNAcβ1-

Le^b: Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ1-

Le^x: Galβ1-4(Fucα1-3)GlcNAcβ1-

The peripheral disaccharide core structures on which ABH determinants are synthesized were also characterized. Five types (Types 1–4 and 6) of such structures were identified.

Type 1: Galβ1-3GlcNAcβ-

Type 2: Galβ1-4GlcNAcβ-

Type 3: Galβ1-3GalNAcα-

Type 4: Galβ1-3GalNAcβ-

Type 6: Galβ1-4Glc

The ABH antigens occur on glycoproteins, on glycolipids, and as free oligosaccharides. The structures of membrane-bound ABH antigens on erythrocytes were later characterized and found to contain the same immunodominant structures found in the soluble forms of ABH substances.

A and B glycosyltransferases catalyze the final step of biosynthesis of A and B oligosaccharide antigens.

Once the structures of A, B, H, and related antigens were determined, the biosynthetic pathways of those antigens were proposed in due course. Because these structures are oligosaccharides and cannot be the primary gene products of proteins, it was assumed that they were synthesized by the actions of enzymes encoded by the genes. Watkins and Morgan,[7,8] and separately Ceppellini,[9] proposed the following hypothesis. Functional *A* and *B* alleles at the *ABO* locus encode A and B glycosyltransferases that catalyze the addition of an *N*-acetyl-D-galactosamine and D-galactose by an α1-3 glycosidic linkage to synthesize the A and B structures, respectively. This hypothesis was later supported by the experimental data that correlated enzyme activities of those transferases and blood group phenotypes.[10,11] A transferase activity was observed in the tissues and sera that exhibited A antigens whereas B transferase activity was detected in the tissues and sera that exhibited B antigens.

A and *B* genes at the *ABO* genetic locus encode A and B glycosyltransferases.

The ABO polymorphism was one of the first genetic traits in humans that were shown to be inherited. To explain the mode of inheritance, Bernstein proposed the one gene locus–three allelic model (Fig. 1). He assumed that the *A, B,* and *O* genes are alleles at the same *ABO* genetic locus. He also assumed that the *A* and *B* alleles are co-dominant against the recessive *O* allele. Based on the combinations, six genotypes resulting in four phenotypes were postulated. In addition to the three major alleles, additional alleles specifying subgroups have later been integrated into the scheme. Regarding the mode of inheritance of ABO, two interesting phenomena have been reported that are called *cis*-AB and B(A). The expression of A and B antigens is specified by two separate *A* and *B* genes (one derived from the mother and the other derived from the father) in a common AB phenotype (*trans*-AB). However, in rare cases of AB, the expression of both A and (usually weak) B antigens is inherited by a single gene derived from either one of the parents, and this phenomenon was named *cis*-AB.[12,13] A similar phenomenon named B(A) was reported when weak A reactivity was demonstrated using certain monoclonal anti-A reagents with the cases that had been previously diagnosed to be type B.[14] Although the mode of inheritance was not demonstrated in those cases, it was assumed that small amounts of A antigens, in addition to larger amounts of B antigens, were produced by a special B transferase. This is quite likely because even the regular B transferase is able to transfer GalNAc to the acceptor substrate in vitro, although inefficiently, as previously demonstrated by Greenwell et al.[15]

The central dogma seemed correct that *A* and *B* genes encode A and B transferases, which synthesize A and B antigens, respectively, because the correlation

was established between the ABO phenotypes and the presence or absence of A and B transferase activity. However, conclusive proof had to wait for the cloning of the *ABO* genes.

A and *B* genes encode A and B transferases of different sugar specificity because of nucleotide substitutions resulting in amino acid substitutions. *O* genes are inactive because they cannot produce functional enzymes.

Isolation of blood group A and B transferases has been reported from several different sources including human plasma (A transferase[16–18] and B transferase[19]) and porcine submaxillary glands (A transferase[20,21]). Although tens to hundreds of thousands-fold concentrations of enzyme activity were attained, the fractions were not homogeneous. A protein that appeared to be the soluble form of human A transferase was purified to homogeneity from lung,[22] gastric mucosa,[23] and plasma.[24] The protein purified from lung by Clausen et al.[22] was a glycoprotein with a molecular weight of ~ 40kDa but it lost its enzyme activity during the last step of purification through reverse-column chromatography. The protein and the trypsinized peptides were later subjected to partial amino acid sequence determination. The N-terminal 13 amino acid sequence of the protein was identical to the sequence of a protein purified from gastric mucosa by Navaratnam et al.[23]

In collaboration with Clausen and Hakomori, Yamamoto[1] cloned cDNA encoding this protein that seemed to be the soluble form of A transferase. The partial amino acid sequence of a peptide was reverse-translated into two degenerate oligo primer sequences. PCR was performed to amplify genomic DNA and cDNA fragments that were hybridized with a radiolabeled oligo probe toward the internal sequence. Using this cloned fragment as a radiolabeled probe, MKN45 human stomach cancer cell line cDNA library was screened. This cell line was chosen because the cells expressed high activity of A transferase and large amounts of A antigens. Several cDNA clones hybridized with the probe were obtained. One of the cDNA clones, FY59-5, apparently contained an entire coding sequence. The cDNA predicted that it encoded a type II transmembrane protein consisting of 353 amino acids. Later it was found that alternative splicing had occurred and that this cDNA clone was missing three nucleotides and one amino acid at the splicing junction. Therefore, it is now presumed that A[1] and B transferases consist of 354 amino acids.

Two options for demonstrating the identity of the purified protein to be the soluble form of A transferase were conceived: either to express the cloned cDNA and detect the enzymatic activity/reaction products or to correlate different nucleotide sequences to alleles. Although the eukaryotic expression system had been established and was available, the correlation approach was taken because of the absence of information on the *ABO* genotype of the MKN45 cell line. It was believed that no activity or products would be detected if the genotype was *AO* and the cDNA represented the *O* allele. There were additional concerns that the cDNA might not be the mature form of mRNA and even more critically that the purified protein was not A transferase. In order to obtain a clear-cut answer, Yamamoto constructed 2 cDNA libraries with RNAs from SW48 and SW948 human colon cancer cell lines, which were derived from individuals with AB and O blood groups, respectively, and screened those libraries. If the purified protein was a real A transferase, it was assumed that cDNA clones from the SW48 cDNA library could be categorized into two groups representing *A* and *B* alleles based on the different nucleotide and amino acid sequences. It was also assumed that the nucleotide sequences of the cDNA clones, if any, obtained from the SW948 cDNA library should have nucleotide sequences that are different from either group of cDNA clones from the SW48 cDNA library. Results that confirmed both of those assumptions were obtained.[25] Two groups of cDNA clones that had differences in seven nucleotides resulting in four amino acid substitutions were recognized from the blood group AB cDNA library. The cDNA clones from the blood group O cDNA library were almost identical to the sequence of one group of the cDNA clones from the AB cDNA library, except that one nucleotide was missing at the N-terminal side of the coding sequence. The difference in the amino acid sequence explained the difference in specificity between the A and B transferases. The single nucleotide deletion, which is located close to the amino terminal side of the coding region, changed the frame of codons and produced truncated proteins rather than large functional enzymes, which explained the nonfunctionality of *O* alleles.

Yamamoto screened two additional cDNA libraries he constructed with RNAs from colon carcinoma cell

lines SW1417 from a blood group B individual and COLO205 with an O phenotype (the blood group of the patient was unknown). He categorized cDNA clones based on the differences in nucleotide sequence. The cDNA clones from the blood group B cDNA library were divided into two groups: one identical to one of the two groups from the SW48 blood group AB cDNA library and the other identical to the cDNA clones from the SW948 blood group O cDNA library. The cDNA clones from the COLO205 had the same single nucleotide deletion that was observed in the cDNA clones from the SW948 cDNA library in addition to several nucleotide substitutions. These results not only confirmed the identity of the purified protein to be A transferase, but also deciphered the molecular genetic basis of the ABO system. The central dogma of ABO was proved to be correct. The *A* and *B* alleles at the *ABO* genetic locus encode functional A and B transferases that have different amino acid sequences, resulting in the different donor nucleotide-sugar specificity between those enzymes. The *O* alleles were inactive because they were unable to produce functional transferases. Furthermore, the results of the SW1417 cDNA clones clearly demonstrated that ABO genotyping is possible. Yamamoto knew that the SW1417 cell line was derived from a blood group B individual but the *ABO* genotype (*BB* or *BO*) of that individual was unknown. However, the experiments showed that the patient's genotype was *BO,* having one *B* and one *O* allele each. Both the SW948 and COLO205 cDNA clones contained the common single nucleotide deletion, however, they differed in several nucleotide sequences. The results raised the question that there might be other mechanisms to nullify the gene functions in *O* alleles. Because the cloning experiments were done with cancer cell lines, it was necessary to examine the genomic DNA of normal cells to determine whether the same differences in nucleotide sequence would be found. Taking advantage of the presence of RFLP among the *ABO* alleles he identified, Yamamoto analyzed the nucleotide sequence polymorphism using genomic DNA prepared from blood samples with predetermined blood types and demonstrated that the differences he observed are common in the general population. It was concluded that (1) amino acid substitutions between A and B transferases (arginine, glycine, leucine, glycine in A transferase and glycine, serine, methionine, and alanine in B transferase at codons 176, 235, 266, and 268) should be responsible for different donor nucleotide-sugar specificity of these two enzymes and (2) the *O* allele is nonfunctional because the gene cannot produce functional glycosyltransferase enzymes. These are the molecular genetic basis of the ABO polymorphism (Table 2).

Table 2. Molecular genetics of the *ABO* genes

- **cDNA cloning of A transferase gene, cloning of B and O allelic cDNAs, and elucidation of molecular genetic basis of the ABO system**
  4 amino acid substitutions between A and B transferases (**R, G, L, G** in **A transferase** and **G, S, M, A** in **B transferase** at codons **176, 235, 266, 268**)
  261delG single nucleotide deletion in ***O*** **allele** coding region resulting in codon frameshift and producing a truncated non-functional protein
- **ABO genotyping**
  Restriction Fragment Length Polymorphism (RFLP)
  Allele-specific PCR amplification
  Discrimination of *AA* and *AO* genotypes and *BB* and *BO* genotypes
- **Proof of the central dogma of ABO**
  A/B **gene ⇒ A/B transferase ⇒ A/B antigen**
- **Identification of mutations in subgroup alleles (A^2, A^x, B^3), *cis-AB* and $B^{(A)}$ alleles, and another type of *O* allele**
  94 alleles deposited in the ABO database (October 2003)
  (http://www.bioc.aecom.yu.edu/bgmut/index.htm)

Mutations resulting in amino acid substitutions and alternative splicing can explain some of the ABO subgroup phenotypes.

The *ABO* gene was previously mapped on chromosome 9 at q34.[26] The gene organization was determined by Yamamoto et al.[27] and by Bennett et al.[28] in 1995. The *ABO* gene spans over 18 kilobases, and is comprised of 7 coding exons. The majority of the soluble form of the enzyme is encoded in the last two coding exons, 6 and 7. Because many of the cDNA clones contained unspliced introns, the partial nucleotide sequences in introns neighboring exons 6 and 7 were available prior to the elucidation of the entire gene organization. Making use of this information on the intron sequences, Yamamoto and collaborators determined the partial nucleotide and deduced amino acid sequences in those two exons for some of the subgroup alleles. They amplified DNA fragments containing exon 6 and 7 sequences, cloned into a plasmid vector, and determined the nucleotide sequences. Because of the absence of cell lines with known ABO subgroup phenotypes, genomic DNA from blood samples of subgroups was used.

The A^2 allele was characterized by the presence of a single nucleotide deletion close to the C-terminal of the coding sequence, resulting in the possession of an

additional 21 amino acid residues at the end.[29] Yamamoto et al,[30] in collaboration with Harris, Judd, and Davenport, observed heterogeneity among A^3 and B^3 alleles because not all of the genomic DNA clones possessed the same mutations. A single nucleotide substitution resulting in an amino acid substitution was observed in an A^x allele.[31] Yamamoto et al.[32] examined *cis-AB* and *B(A)* alleles of hereditary interest and found that these alleles encode chimeric enzymes of A and B transferases. In the course of characterization of subgroup alleles, Yamamoto et al.[33] identified another type of *O* allele that lacked the single nucleotide deletion but contained nucleotide substitutions resulting in an amino acid substitution at codon 268 that is critical for nucleotide-sugar recognition. These alleles are listed in Figure 2.

Since this discovery, the nucleotide and deduced amino acid sequences of additional subgroup alleles have been reported. The number of alleles deposited in the ABO System section of the Blood Group Antigen Gene Mutation (BGAGM) Database (http://www.bioc.aecom.yu.edu/bgmut/index.htm) has accrued to more than 90. The BGAGM Database, which was established and has been maintained by Blumenfeld, covers reported alleles of 24 different blood group systems, including ABO and Rh, and has provided useful information on blood group polymorphism.[34] In addition to the A^1, A^2, A^3, A^x, *B*, B^3, *O*, *cis-AB,* and *B(A)* alleles, the nucleotide and deduced amino acid sequences of A^{el}, A^w, B^{el}, and B^w alleles have also been added to the database. Olsson et al,[35–40,42–44] Hansen,[41] Ogasawara et al,[45–48] Roubinet et al,[49,50] Seltsam et al,[51,52] Yu et al,[53–55] Sun et al,[56] and Yip et al.[57–60] and their colleagues contributed greatly to this addition of other subgroup alleles (see the review by Olsson and Chester[44]). As opposed to the entire coding sequences of the three major alleles determined by cDNA cloning, most, if not all, of the sequences determined from those subgroup alleles were partial sequences of genomic DNA. Furthermore, for most alleles it remains to be experimentally demonstrated whether the identified mutations are responsible for the altered activity of subgroup transferases. A majority of the mutations are nucleotide substitutions resulting in amino acid substitutions or a single nucleotide deletion/insertion and correlate well with the presence of specific subgroup alleles. In addition to mutations in the coding sequences, mutations in intron sequences were also identified. Being located at the splicing junctions,

Exon Number	**6**			**7**												
Nucleotide Position	261	297	467	526	646	657	681	703	717	796	802	803	829	871	930	1054	1060
A alleles																	
A101	G	A	C	C	T	C	G	G	C	C	G	G	G	G	G	C	C
A102	*	*	**T**	*	*	*	*	*	*	*	*	*	*	*	*	*	*
A201	*	*	**T**	*	*	*	*	*	*	*	*	*	*	*	*	*	Δ
A301	*	*	*	*	*	*	*	*	*	*	*	*	*	**A**	*	*	*
Ax01	*	*	*	*	**A**	*	*	*	*	*	*	*	*	*	*	*	*
cis-AB01	*	*	**T**	*	*	*	*	*	*	*	*	**C**	*	*	*	*	*
B alleles																	
B101	*	G	*	**G**	*	T	*	**A**	*	**A**	*	**C**	*	*	A	*	*
B301	*	G	*	**G**	*	T	*	**A**	*	**A**	*	**C**	*	*	A	**T**	*
B(A)01	*	G	*	**G**	*	*	*	*	*	**A**	*	**C**	*	*	A	*	*
O alleles																	
O01	Δ	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
O02	Δ	G	*	*	A	*	A	*	T	*	*	*	A	*	*	*	*
O03	*	G	*	**G**	*	*	*	*	*	*	**A**	*	*	*	*	*	*
Possible Amino Acid Change	Frameshift	No change	P156L	R176G	F216I	No change	No change	G235S	No change	L266M	G268R	G268A	V277M	D291N	No change	R352W/G	Frameshift

Fig. 2. **Representative alleles at the *ABO* locus.** Nucleotide and deduced amino acid sequences are compared among a dozen *ABO* alleles whose sequences were determined by Yamamoto and colleagues. The *cis-AB* and *B(A)* alleles are included in the *A* and *B* alleles, respectively, because of higher relative sequence homology. The nucleotide substitutions that result in amino acid substitutions are shown in bold type. ∅= deletion of nucleotide.

some of these mutations seem to affect the maturation of mRNA and be responsible for weaker expression of the A/B antigen.[55,56]

Not all of the amino acid substitutions between A and B transferases are necessary to cause them to have different sugar specificity. The enzymes with strong A and B transferase activity were created by genetic engineering.

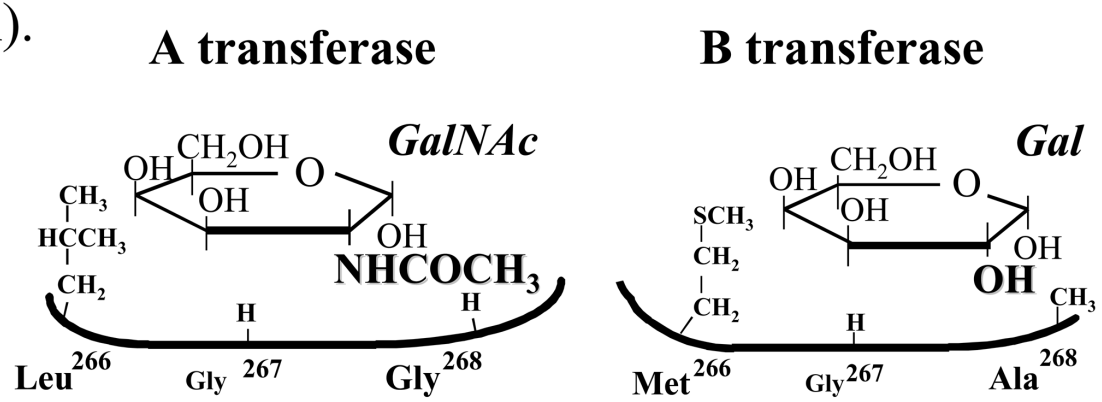
The identification of gene mutations and the functionality of the mutations are different. Seven nucleotide substitutions resulting in four amino acid substitutions were identified between *A* (A^1) and *B* alleles by the cloning study.[25] The three nucleotide substitutions that did not result in amino acid substitutions were silent and not involved in the difference in specificity between the A and B transferases. However, it was unclear whether all four amino acid substitutions were necessary for a difference in specificity. One, two, or three substitutions could have been sufficient. To answer this question, Yamamoto and Hakomori constructed A and B transferase cDNA expression constructs and transfected them to HeLa cancer cells of the uterus, which express type H antigens on the cell surface.[61] They detected the appearance of A and B antigens on the surface of cells transfected with A and B transferase cDNA expression constructs, respectively. They also constructed a total of 14 A-B transferase chimeric constructs with all possible combinations of the four amino acid substitutions between A and B transferases. The results showed the codon at 176 did not affect the specificity of the enzyme (Table 3). The codon at 235 was influential and those at 266 and 268 were crucial for the difference in specificity of the A and B transferases. The chimeric constructs that possess methionine of B at 266 and glycine of A at 268 were shown to express enzymes with strong A and B transferase activity, which was the first demonstration that the specificity of glycosyltransferase could be modified by genetic engineering. Yamamoto continued functional analysis with mutations specific to A^2, A^3, and B^3 alleles using DNA transfection assays. He has shown that the single nucleotide deletion in the A^2 allele and the single missense mutations in the A^3 and B^3 alleles that he identified were functionally important mutations that decrease the activity of the enzymes coded by those rare alleles.[62]

Table 3. Enzymology and evolution of the A and B transferases

- **Identification of amino acid substitutions responsible for different nucleotide-sugar specificity between A and B transferases**
  - Codon 176 (R in A transferase and G in B transferase); not important
  - Codon 235 (G in A transferase and S in B transferase); influential
  - Codons 266&268 (L&G in A transferase and M&A in B transferase); crucial
- **Successful modification of nucleotide-sugar substrate specificity by genetic engineering**
  - Several A-B transferase chimeras with both A and B transferase activity
- **Structural basis of different donor nucleotide-sugar specificity and activity using 40 in vitro mutagenized amino acid substitution constructs at codon 268**
  - Size and charge of the side group are crucial for transferase activity and nucleotide-sugar substrate specificity.
  - Not only codon 268 but also codon 266 is important.
- **Animal *ABO* genes**
  - Homologous sequences in chicken and mammals
  - Primates: Conserved amino acid substitutions corresponding to codons 266 and 268 of human transferases, depending on the ABO genotype (L and G in the ***A*** gene and M and A in the ***B*** gene, respectively)
  - Mouse: Genomic organization is similar to human gene.
    - Prevalent murine gene is functional *cis-AB* gene.
  - Pig: *O* allele deficiency in porcine AO system is due to a major deletion, and therefore, *O* gene activation in porcine organs/tissues xenotransplanted into humans is unlikely.
- ***ABO* gene evolution**
  - Construction of evolutionary trees of the *ABO* genes and *ABO* gene family

Yamamoto further studied the effects of the amino acid residue at codon 268 on the specificity and activity of the enzyme (Fig. 3A).[63] He constructed 20 each of cDNA expression constructs with A and B transferase backbones that possess any one of 20 amino acid residues at that position. DNA from those constructs was transfected into HeLa cells and the cell surface expression of A and B antigens was examined. The same DNA was also transfected into COS1 SV40-transformed African Green monkey kidney cells, and the enzymatic activity of A and B transferases in cellular extracts was analyzed. The results of the experiments showed that the size and charge of the side group of amino acid residue at codon 268 is crucial for the specificity and activity of the enzyme (Fig. 3B). The side groups of 20 amino acids are shown in Figure 3C. For example, at codon 268, A transferase possesses glycine that is the amino acid residue with the smallest side group of hydrogen atom (-H). When this glycine was replaced by alanine with a larger methyl group ($-CH_3$), the protein acquired the ability to transfer not only a GalNAc but also a galactose. Probably, the

A).



B). *A transferase constructs*

A.A. at Codon 268	HeLa cells FACS Analysis		COS1 cells Enzymatic Assay		Conclusion	
	A	B	A	B	A	B
Ala	78	18	16	1.6	++++	++
Arg	0	0	0	0	-	-
Asn	0	16	0	0	-	+
Asp	0	0	0	0	-	-
Cys	52	50	0	0	+++	++
Gln	0	0	0	0	-	-
Glu	0	0	0	0	-	-
Gly	100	0	100	0	+++++	-
His	33	0	0	0	+	-
Ile	0	0	0	0	-	-
Leu	0	0	0	0	-	-
Lys	0	0	0	0	-	-
Met	0	0	0	0	-	-
Phe	9	0	0	0	+	-
Pro	0	0	0	0	-	-
Ser	78	86	0.4	0.7	+++	+++
Thr	0	10	0	0	-	+
Trp	0	0	0	0	-	-
Tyr	0	0	0	0	-	-
Val	0	0	0	0	-	-
No DNA	0	0	0	0	-	-

B transferase constructs

A.A. at Codon 268	HeLa cells FACS Analysis		COS1 cells Enzymatic Assay		Conclusion	
	A	B	A	B	A	B
Ala	0	100	0.3	100	+*	+++++
Arg	0	0	0	0	-	-
Asn	0	91	0	0.7	-	++++
Asp	0	64	0	0	-	+++
Cys	0	86	0	1.8	-	+++++
Gln	0	45	0	0	-	++
Glu	0	0	0	0	-	-
Gly	85	95	12	25	++++	+++++
His	0	40	0	0	-	+++
Ile	0	0	0	0	-	-
Leu	0	55	0	0	-	+++
Lys	0	0	0	0	-	-
Met	0	8	0	0	-	+
Phe	0	34	0	0	-	++
Pro	0	68	0	0	-	+++
Ser	0	91	0	15	-	+++++
Thr	0	55	0	0	-	++++
Trp	0	0	0	0	-	-
Tyr	0	0	0	0	-	-
Val	0	55	0	0	-	+++
No DNA	0	0	0	0	-	-

C).

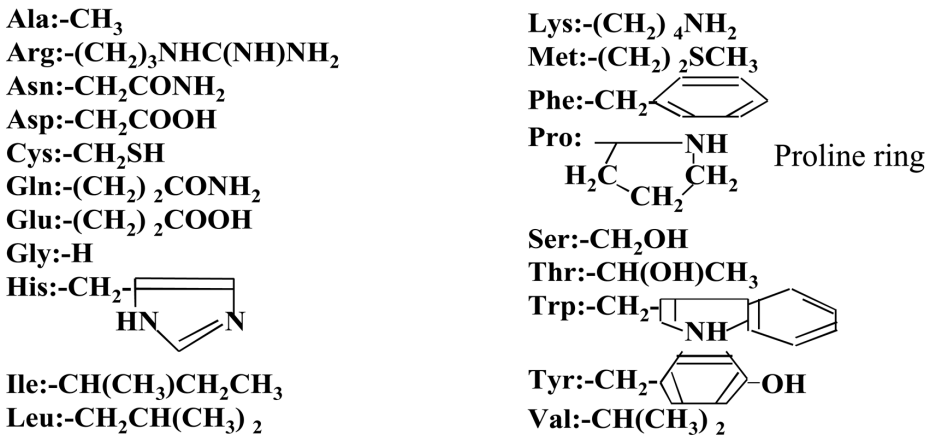


Fig. 3. **Specificity of the A and B transferases.** **(A).** The regions of the A and B transferases around codons 266 and 268 are schematically shown in combination with the sugar portion of the appropriate nucleotide-sugars. **(B).** DNA transfection experiments of the in vitro mutagenized A and B transferase constructs. The results of A and B antigen expression on the surface of the transfected HeLa cells and the results of the enzymatic assays of the A and B transferases in the extracts of the transfected COS1 cells are shown. **(C).** The side groups of 20 amino acids with the chemical formulas are shown. The nitrogen atom of the amino group is incorporated into a ring in proline, and therefore, the structure shown for proline is not the structure of the side group.

methyl group compensated for the smaller size of hydroxyl group (-OH) of galactose than the *N*-acetyl group (-$NHCOCH_3$) of GalNAc at carbon 2 of the sugar ring. When the glycine was replaced by serine (-CH_2OH) or cysteine (-CH_2SH), the next smaller amino acids, both A and B transferase activities were still observed. When it was replaced by the slightly larger threonine (-$CH(OH)CH_3$) or asparagine (-CH_2CONH_2), only B transferase activity was detected. The cavity for sugar interaction might become too small for the GalNAc entry. No other constructs expressed A or B transferase activity other than the histidine and phenylalanine constructs that exhibited weak A transferase activity, which had no easy explanation. Similarly, when the methyl group of alanine of the B transferase is replaced by glycine, the smaller size of hydrogen seems to permit the transfer of GalNAc in addition to galactose. When the alanine was replaced by amino acids with larger side groups, the activity diminished as the size increased. Because the results were different between constructs with the A transferase backbone and those with the B transferase backbone, the importance of other amino acid residues between the two enzymes was recognized, especially amino acid at codon 266.

Seto et al,[64,65] in collaboration with Palcic and Hindsgaul, expressed human A and B transferases and their recombinants in *E. coli* and performed more detailed enzymologic studies of those enzymes using a variety of substrates. They reached similar conclusions that the donor nucleotide-sugar specificity is attributed to the amino acids at codons 266 and 268. Patenaude et al,[66] in collaboration with Palcic, determined the three-dimensional structures of the human A and B transferases and claimed that only codons 266 and 268 of the critical amino acid residues were positioned to contact donor or acceptor substrates. In a more recent paper, however, the importance of the region around codon 235 for sugar specificity was acknowledged.[67] They reached this conclusion from additional crystal X ray diffraction studies of an enzyme coded by a novel *cis-AB* allele that possesses a single point mutation that replaced the conserved amino acid proline 234 with serine in the B transferase backbone.

ABO genes are found in other species of organisms.

ABH antigens were initially discovered in humans on RBCs. However, substances with the same or similar immunodominant structures were later found in a variety of living organisms, including plants and bacteria. Because human sera and plant lectins with broad specificity were used in the early studies, the chemical nature of many of those substances remains to be determined. After cloning the cDNA encoding human histo-blood group A transferase, Kominato et al.[68] examined the presence or absence of a homologous gene sequence by hybridizing human A transferase cDNA probe with genomic DNA prepared from other organisms. They observed weak hybridization with chicken DNA and strong hybridization with mouse DNA, however, no hybridization was detected with DNA from organisms that are located lower in the phylogenetic evolutionary tree: bacteria, yeast, nematode, clam, lobster, fly, sea urchin, and frog. They also examined genomic DNA from other species of mammals and observed hybridization with all the species examined: dog, cat, rabbit, cow, sheep, rat, hamster, and marmoset.

In the same paper,[68] they reported their findings on primate *ABO* genes. Bernstein's model of inheritance was known to apply to almost all primates. Depending on their genotypes, Old and New World monkeys express ABH substances in secretion.[69,70] Whereas four major phenotypes are known in humans, nonhuman primates seem to have fewer phenotypes, depending on the species. For example, only A and O phenotypes are reported in chimpanzees and all gorillas seem to have the B phenotype.

In anthropoid apes, chimpanzees and orangutans express the antigens on their RBCs, but gorillas do not have much expression of their B antigens. Furthermore, animals that do not produce the antigens possess antibodies in serum, similar to humans. Therefore, Landsteiner's Law also applies to primates. Kominato et al.[68] determined the partial nucleotide sequences of chimpanzee blood type *A* gene, gorilla *B* gene, orangutan *A* gene, macaque *A* gene, and baboon *A* and *B* genes. They found that the amino acid sequences corresponding to human codons 266 and 268 are conserved during evolution in these species (leucine and glycine in A and methionine and alanine in B), suggesting the importance of those two amino acid residues for different sugar specificity between A and B transferases. The results of this primate study of *A* and *B* alleles were confirmed by Martinko et al.[71] Kermarrec et al.[72] found that human and nonhuman primate *O* alleles are species-specific and result from independent silencing mutations, establishing the basis of the *O* allele deficiency in primates.[72]

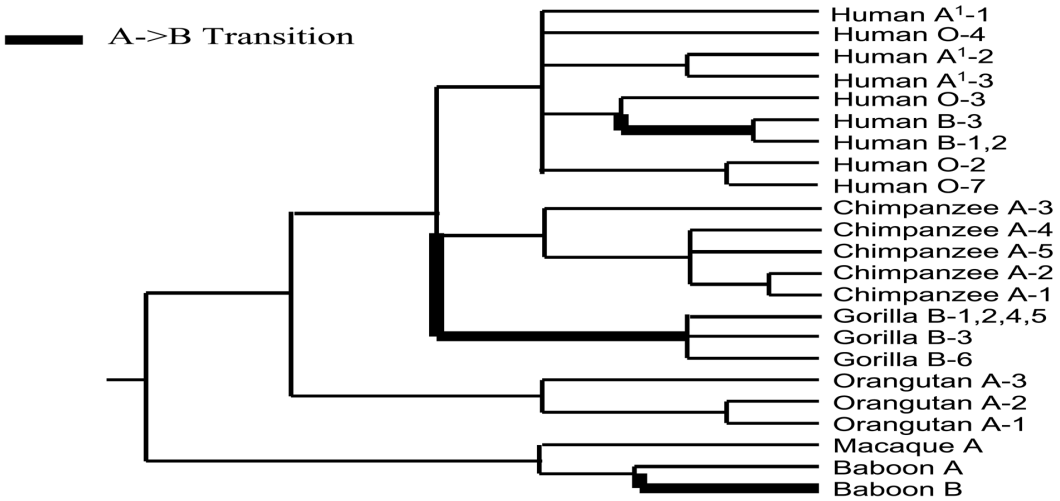
Recently, Yamamoto and Yamamoto elucidated the molecular genetic basis of the porcine AO blood system.[73] As opposed to human *O* alleles, the pig *O* allele was found to lack most of the structural gene encoding for the enzyme. Yamamoto et al.[74] studied the mouse gene and discovered that the mouse gene is a *cis-AB* gene that encodes an enzyme with both A and B transferase activity. They also showed that this gene is prevalent because all of the species and subspecies of mice they examined contained the same amino acid residues at positions critical for sugar specificity. The rat gene homologous to the human *ABO* gene was cloned by Cailleau-Thomas and Le Pendu[75] and separately by Iwamoto and Kobayashi.[76] The rat gene cloned from the BDIX strain of rat showed identical gene organization to the human gene and was mapped on the rat chromosome 3 at q11–12, a region homologous to human 9q34.[75] The cDNA encoded an enzyme with A transferase activity. Although the same enzyme exhibited weak B transferase activity in vitro, it was suggested that A and B antigens cannot be synthesized by alleles of the same gene in this rat inbred strain because of differential tissue expression of those antigens. Four cDNAs were cloned from a Wistar rat, three that showed A transferase activity and one that showed B transferase activity.[76] Strangely, however, the B antigen was not detected in any organs studied in wild Wistar rats despite the fact that the transcript from the ABO homologue with B transferase activity was ubiquitously present.

Evolution of *ABO* genes and related genes

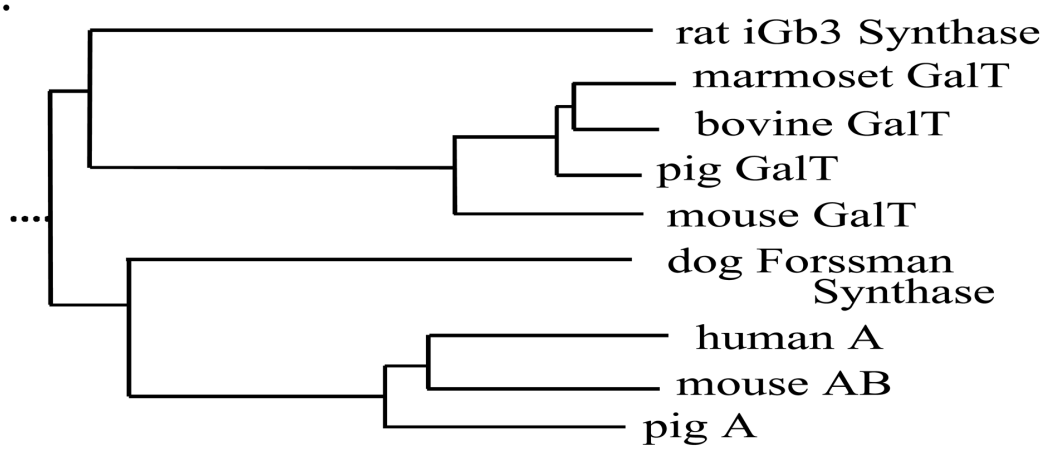
Based on the nucleotide and deduced amino acid sequences of the primate *ABO* genes, Martinko and Klein[71] claimed that the critical substitutions differentiating the *A* and *B* genes occurred before the divergence of the lineage leading to humans, chimpanzees, gorillas, and orangutans. Saitou and Yamamoto[77] constructed phylogenetic networks of human and nonhuman primate *ABO* alleles and observed at least three independent appearances of *B* alleles from the ancestral A form (Fig. 4A). They suggested that some kind of balancing selection might have been operating at the *ABO* locus. The view of convergent evolution over trans-species inheritance of ancestor alleles was supported by the study of primate *O* alleles by Kermarrec and Blancher.[72]

The *ABO* genes are polymorphic at the single genetic locus, *ABO,* so it is reasonable to assume that they are homologous. However, there are additional sequences in the human genome that are highly homologous to the *ABO* gene (Fig. 4B). These genes constitute the *ABO* gene family. The presence of genes from this family was initially recognized when the cDNA encoding human A transferase was cloned and sequenced. It became instantly evident[61] that the nucleotide and deduced amino acid sequences had significant homology with previously cloned genes of bovine[78] and murine[79] α1,3-galactosyltransferases. These enzymes catalyze the transfer of a galactose to synthesize the α-galactosyl epitope (Galα1-3Gal-). As opposed to A and B transferases, α1,3-galactosyltransferases utilize the acceptor substrates lacking a fucose linked to the galactose. Both the enzyme activity and the α-galactosyl epitope exist in most mammals except for humans, apes, and Old World monkeys.[80] These species instead possess the antibody against the α-galactosyl epitope. Presence of this antibody is the primary reason for acute rejection of pig organ xenotransplanted to humans.[81] The human gene encoding the α1,3-galactosyltransferase was shown to contain frameshift and nonsense mutations.[82,83] In addition to this nonfunctional α1,3-galactosyltransferase pseudogene, another homologous sequence was cloned from human genomic DNA and named hgt4.[84] This sequence was also shown to be a nonfunctional pseudogene. Two additional genes have been shown to have some homology with the *ABO* genes: Forssman glycolipid synthase *(FS)* gene[85] and *iGb3* synthase gene.[86] Forssman glycolipid synthase is UDP-GalNAc:globoside α1,3-*N*-acetyl-D-galactosaminyltransferase that synthesizes the Forssman glycolipid antigen (GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glc-Cer). iGb3 synthase is UDP-galactose: β-D-galactosyl-1,4-glucosylceramide α1,3-galactosyltransferase and directs the synthesis of isoglobo-glycosphingolipids (Galα1-3Galβ1-4Glcβ1-Cer) by transferring a galactose toward LacCer (Galβ1-4Glcβ1-Cer). The original cDNA clones of those glycosyltransferases were from dog and rat, respectively. A human FS cDNA was characterized, revealing an 86 percent similarity with the canine *FS* gene at the nucleotide and 83 percent of the predicted amino acid sequences.[87] However, no detectable FS enzyme activity was detected upon transfection of COS1 cells with the human FS cDNA, which explains the absence of the FS antigen expression in humans except in certain disease states, such as lung and gastrointestinal tumors.[88] As shown in Figure 4C, the amino acid residues corresponding to codons 266 and 268 of the human A and B transferases are important in

A).



B).



C).

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                                          *  *
Human A101          FTYERRPQSQAYIPKDEGDFYYLGGFFGG 272
Human B101          FTYERRPQSQAYIPKDEGDFYYMGAFFGG 272
Human O03           FTYERRPQSQAYIPKDEGDFYYLGRFFGG 272
Human cis-AB        FTYERRPQSQAYIPKDEGDFYYLGAFFGG 272
Mouse AB            FTYERRPQSQAYIPWDRGDFYYGGAFFGG 251
Pig A               FTYERRPLSQAYIPRDEGDFYYAGGFFGG 282
Dog Forssman        FPYERRHISTAFVAENEGDFYYGGAVFGG 267
Mouse GalT          FTYERRELSAAYIPFGEGDFYYHAAIFGG 312
Bovine GalT         FTYERRKESAAYIPFGEGDFYYHAAIFGG 286
Rat iGb3            LPYERDKRSAAALSLSEGDFYYMAAVFGG 259
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Fig. 4. **Evolution of the *ABO* and related genes. (A).** A phylogenetic tree of the *ABO* genes of primates. Three independent occurrences of A to B transitions are shown in bold lines. **(B).** A phylogenetic tree of the *ABO* family of genes. The murine *AB* and porcine *A* genes cloned by Yamamoto are clustered with the human *A* gene, indicating that they are ABO genetic equivalents. **(C).** Comparison of the deduced amino acid sequences of a variety of glycosyltransferases from the *ABO* gene family.

specifying the donor nucleotide-sugars of the other members of the *ABO* gene family of glycosyltransferases.

ABH antigen expression undergoes changes during development and differentiation, as well as carcinogenesis.

It has long been known that newborns express less (25–50%) of the A/B antigens on RBCs than do adults. ABH antigens are peripheral structures carried on a variety of branched and unbranched core structures. Because these antigens are produced by the enzymatic reactions, the presence or absence of the acceptor substrates affects the expression of the antigens. Smaller quantities of branched structures partially explain the lower expression of ABH antigens on fetal RBCs. Similarly, the concentrations of nucleotide-sugar substrates, such as UDP-GalNAc or UDP-galactose, also influence the enzymatic reactions. There is also competition among different glycosyltransferases for the same substrates. In addition to these classes and concentrations of acceptor substrates and donor nucleotide-sugar substrates available for the enzymatic reactions, the changes in expression of A/B antigens can also result from changes on levels upstream. For example, mutations in gene structure, transcription of A/B transferase mRNA, post-transcriptional modification of the mRNA, translation into A/B transferases, post-translational modifications of the enzymes, and translocation of enzymes into the Golgi apparatus all alter the A/B antigen expression (Table 4). Here, dynamic changes in the expression of ABH and related antigens are discussed.

Szulman performed initiatory immunohistochemical work and discovered the changes in the expression of ABH antigens at different stages of human development.[89,90] The changes in ABH antigen expression associated with cell migration and maturation from the basal germinal layer to the surface layer were reported by Oriol[91] and Orntoft.[92] Mandel et al.[93] reported a sequential appearance of precursor peripheral core determinants in the germinal layer, followed by an H antigen on the intermediate layers and later by A and B antigens on the more superficial layers of oral mucosa.

In addition to the physiological changes, ABH antigen expression undergoes changes in pathologic phenomena, most evident in carcinogenesis (see the review article by Hakomori).[94] A higher incidence of duodenal ulcer in group O individuals and of stomach cancer in group A individuals was reported from an etiological study.[95] Although different groups of individuals exhibit different degrees of susceptibility to diseases, the causality is still unclear. The most frequently observed change of the ABH antigen expression in cancer is the loss (or diminution) of expression. Loss of A/B antigens was initially reported in stomach cancer.[96] The reduction of those antigens was later found associated with the decrease in transferase activity.[97] On the contrary, in colon cancer the appearance of A/B antigens was reported.[98,99] In normal distal colon, the ABH antigen expression diminishes during development due to the reduced activity of α1,2-fucosyltransferase to synthesize the H antigen. It was suggested that the activation of the gene encoding this enzyme led to the synthesis of the H antigen that was transformed into A/B antigens by the A/B transferases already present in the cells. This provides an example of the very complex regulation of A/B antigen synthesis. Lee et al.[100] reported that the expression of A antigen in non-small-cell lung carcinomas in group A or AB patients is a favorable prognostic factor. Miyake et al.[101] reported that positive immunostaining by MIA-15-5 antibody, which defines H/Le^y: Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-)/Le^b antigens, among blood group A or AB patients with primary lung cancer is inversely correlated with survival. An interesting phenomenon named "incompatible" A antigen expression was discovered.[102–104] The phenomenon was named "incompatible" because they observed the expression of A antigens in tumors of group B and O patients that are not supposed to express functional A transferase. Early immunohistochemical studies used antibodies or lectins with broad specificity, and, therefore, cross-reactions were

Table 4. *ABO* gene expression

- **Regulatory mechanism of A/B antigen expression**
  Different levels of control
  Transcription, Post-transcription, Translation, Post-translation, Golgi localization
  Enzymatic reaction: substrate concentrations (both donor nucleotide-sugars and acceptor H structures)
- **Gene organization of the human *ABO* gene**
  Span over 18 kbp, 7 coding exons, CpG island in promoter
- **Transcriptional control**
  Promoter activity in the sequence just upstream of the transcription initiation sites
  Transcription factor Sp1 binding is required in both erythroid and epithelial cell lineages.
  An enhancer element is located further upstream.
  Transcription factor CBF/NF-Y can bind to the enhancer element and up-regulate gene expression in gastric cells.
  Repression of expression by CpG island methylation
  ABO gene transcription may be activated by demethylating agent 5-aza-dC.

suspected with antigens of similar structures such as Forssman antigen and Tn antigens (GalNAcα-Ser/Thr). However, later studies confirmed the presence of chemically defined A structure in the tumors from blood group O patients[105–107] and the presence of A transferase activity.[108,109] For example, David and Clausen examined the expression of A transferase in 31 cases of gastric tumors of phenotype O by immunohistology on frozen sections using monoclonal antibodies for the transferases. They found three positive cases of which they confirmed the activity using the extracts from the specimens. The A antigen was also identified immunohistologically in these three cases as well as in five other cases. However, the chemical presence of A antigen was not confirmed by thin-layer chromatography immunostaining analysis of glycolipid extracts from the three positive cases. David and Clausen speculated that incompatible A antigen expression is a result of transferase expression derived from the *O* gene by an undetermined mechanism.

In an effort to understand the molecular mechanisms of loss of A/B antigen expression, Meldgaard et al.[110] examined the *ABO* gene and its gene expression in two immortalized human urothelial cell lines. They found an intact *A* gene and A transferase mRNA, but no activity of A transferase or expression of A antigen on the cell surface. They later investigated the loss of heterozygosity in bladder tumors that were negative for A/B antigens and concluded that the loss of A/B antigen expression was not due to the loss of the transferase-producing functional allele or to the down-regulation of *ABO* gene transcription.[111] Instead, they implicated a potential mechanism of post-translational control to explain the disappearance of A/B antigens in the bladder tumors.

Kominato, in collaboration with Yamamoto, took a different approach and examined the promoter region of the *ABO* gene, which controls the expression of the gene.[112] They first mapped and characterized the promoter region by making nested deletion constructs and examined the promoter activity of the genomic DNA. In the promoter region they identified a CpG island that is abundant in the CpG dinucleotide sequence. Because CpG island methylation has often been associated with inactivation of transcription,[113,114] they proceeded to investigate the methylation status of the CpG island of the *ABO* gene.[115] DNA methylation was found to be inversely correlated with gene expression in the carcinoma cell lines they examined. They also showed constitutive transcription of the transiently transfected reporter plasmid containing the ABO gene promoter regions in either expressor or nonexpressor cells and that this transcription was diminished by pretreatment of the plasmid with *HhaI* methylase. Together with the fact that the treatment of nonexpressor gastric cancer cell line MKN28 cells with 5-aza-2′-deoxycytidine, an inhibitor of DNA methylation, could have reactivated transcription, though inefficiently, those results supported their contention that DNA methylation may play an important role in repressing the expression of the *ABO* gene. Iwamoto et al.[116] analyzed promoter methylation using genomic DNA from A antigen positive and negative clones of SW480 colon carcinoma cell line cells. They observed the ABO gene promoter methylated in A antigen negative clones, but unmethylated in A antigen positive clones. Whether DNA methylation plays a negative regulatory role in tumors, in addition to established cell lines, remains to be examined.

Why does the ABO polymorphism exist? What is the advantage?

Triggering the classical complement cascade, mammalian C-type retroviruses are inactivated by human serum.[117] However, HIV,[118] human T-cell leukemia virus,[119] and retroviruses that are produced in human cells[120] are resistant to inactivation by the human complement. Because humans do not have a functional α1,3-galactosyltransferase gene, but instead contain the natural antibody against the α-galactosyl epitope in sera, retrovirus inactivation mediated by anti-α-galactosyl epitope natural antibody was proposed.[121,122]

As opposed to this inhibition of interspecies infection, a similar role of natural anti-A and anti-B antibodies against intraspecies infection has been proposed and examined. For example, HIV viruses prepared from cultures of mixed PBMNCs from donors of blood group A were neutralized by the monoclonal antibody against A epitope, whereas the viruses prepared from donors of blood group B or O were not.[123] Recently, Preece et al.[124] addressed the same question using the measles viruses: whether viral particles can become glycosylated as determined by the glycosylation status of the producer cell and as a result be affected by human serum containing specific natural antibodies. They prepared virus in HeLa cells transfected with cDNA encoding, either human A transferase, B transferase, an inactive truncated O protein, or a porcine α1,3-galactosyltransferase. The

viruses carried the same ABO structures as the cells: A antigen if produced in type A cells and B antigen if produced in type B cells. Only H antigen was detected on the virus produced from type O cells, whereas reduced amounts of H antigen appeared on the viral particles prepared from type A and B cells. In addition, the α-galactosyl epitope was transferred onto measles viruses only when grown in human cells expressing this structure. When those viruses were treated with human preimmune sera, viral particles with type A, B, and α-galactosyl epitopes were partially neutralized in a complement-dependent manner. However, type O or α-galactosyl epitope-negative viral particles were not neutralized. They speculated that specific natural antibodies mediated the neutralization because synthetic A and Galα1-3Gal oligosaccharides specifically blocked the inhibition.

Inoue, Matsumoto, and Yamamoto performed similar experiments using amphotropic murine leukemia virus (MLV). However, they observed significant loss of infectivity of the viruses after treatment with sera irrespective of the ABO blood groups of sera as well as the ABO phenotypes of the producer cells (unpublished result). Because viruses were prepared from HeLa cell transfectants of human origin, the antibody against α-galactosyl epitope may not be responsible for this inactivation. Although what caused the drop in infectivity in those experiments remains to be determined, seeking the biological function of the ABO polymorphism in immunologic surveillance seems to be reasonable as these polymorphic oligosaccharides are exposed to, and potentially involved in communication with, the outer world.

Acknowledgments

I thank Mr. Lloyd Slivka for editorial assistance. I would also like to thank our colleagues and collaborators for their support of the work described in part of this review article. Additional reading is recommended for thorough information on early work on serology and biochemistry of the ABH antigens that is not covered in detail in this review. The chapters on ABO in the books by Daniels[125] and Schenkel-Brunner,[126] and the articles by Watkins,[5,6] are highly recommended.

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