

Immunohematology

JOURNAL OF BLOOD GROUP SEROLOGY AND EDUCATION

SPECIAL MILLENNIUM ISSUE

VOLUME 16, NUMBER 1, 2000



American Red Cross

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ABO blood group system: a review of molecular aspects

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Key Words: ABO blood group, genotyping, glycosyltransferase, molecular basis-ABO

Introduction

The ABO blood group system is the most clinically significant system in transfusion medicine. Antigens of the ABO system consist of an A or a B carbohydrate structure carried on the substrate H antigen. The A or B glycosyltransferase encoded at the *ABO* locus on chromosome 9 defines which specific carbohydrate is added to the end of the H substance oligosaccharide chains (GalNAc α 1-3 for A and Gal α 1-3 for B). The H antigen is defined by a fucose sugar attached to the C-2 position of a terminal galactose residue in an α 1-2 linkage on a type II precursor substrate chain (predominantly) via a fucosyltransferase encoded at a locus on chromosome 19. ABO phenotyping using modern blood banking reagents allows for serologic detection of these specific sugars on red blood cells (RBCs), determining an individual's ABO blood group. To a limited extent, polymorphisms in the ABO blood group system can be serologically detected in the form of subgroups. The degree and reactivity pattern of RBC agglutination with monoclonal reagents including anti-A, -B, -A,B and -H along with the presence or absence of A, B, and H substances in the saliva help to distinguish one subgroup from another.¹ The adsorption and elution method is capable of serologically detecting the presence of small amounts of ABO antigens carried on the RBC. However, quite often, unexplainable ABO phenotyping results cannot be resolved serologically. If this occurs in a patient, group O blood is usually transfused and the clinical care of the patient is not compromised. Due to recent advances in molecular biology, many of the ABO variations seen serologically can now be explained at the genetic level and have been shown to be a consequence of point mutations, deletions, and gene rearrangements that result in variant ABO glycosyltransferases with different specificity and activity.²⁻²² Despite this detailed knowledge, we still use basically the same approach to ABO typing as that described by Landsteiner 100 years ago.

Glycosyltransferases

Glycosyltransferases are enzymes that catalyze transglycosylation reactions between sugar-nucleotide donor and acceptor substrates. The A glycosyltransferase, α (1,3) *N*-acetylgalactosaminyltransferase, and the B glycosyltransferase, α (1,3) galactosaminyltransferase, use the same substrate, a fucose that defines the H antigen. The ability of the glycosyltransferases (which are proteins) to efficiently catalyze transglycosylation reactions (addition of a sugar) depends on their specific conformational structure that allows optimal binding to the substrate. The conformational or tertiary structure of a protein is defined by its amino acid sequence. The chemical properties of each amino acid, whether it is hydrophobic or hydrophilic, polar or nonpolar, greatly affect the conformational structure of a protein. An amino acid substitution within a protein has the ability to alter the specificity of the binding site, resulting in a less catalytically functional enzyme. Amino acid substitution, resulting from mutations, deletions, or gene recombination within exon 6 and 7 of the coding DNA of variant ABO glycosyltransferases, is responsible for less efficient transfer of the immunodominant sugar residues to the H substrate, which results in weak serologic reactions.

ABO Genes

The molecular genetic basis of the *ABO* genes has been revealed during the past decade. In 1990, the partial amino acid sequence was derived from a soluble form of A gene-defined transferase, UDP-GalNAc:Fuc1-2 Gal1-3 GalNAc from human lung tissue.¹⁶ Based on this partial amino acid sequence, cloning of the cDNA for the A transferase was made possible.¹⁷ Poly (A) RNA from human stomach cancer cell lines expressing high levels of A antigen were used to construct a cDNA library. The A transferase protein consists of three domains: a short N-terminal, a hydrophobic transmembrane, and a long C-terminal domain. The purified soluble form of the enzyme is catalytically active but was shown to lack the N-terminal and the hydrophobic domains, leaving the long C-terminal domain most likely responsible for cat-

alytic activity. Because exons 6 and 7 of the *ABO* gene are responsible for translation of the C-terminal domain of glycosyltransferases, researchers focused on these exons to discriminate *ABO* alleles and to clarify serologic problems seen at the phenotypic level. Cloning and sequencing cDNA of human colon adenocarcinoma cell lines of phenotypes AB, B, and O showed that there are seven nucleotide differences between A and B alleles at positions 297, 526, 657, 703, 796, 803, and 930, resulting in four amino acid changes at residues 176, 235, 266, and 268.¹⁸ Previous studies based on gene reconstruction experiments and studies of expression in DNA transfected HeLa cells show that the second, third, and the fourth amino acids are crucial in determining nucleotide-sugar specificity. The first amino acid substitution (Arg176Gly) was not found to be important in determining the sugar-nucleotide specificity (see Tables 1 and 2).¹⁹⁻²³ Clearly, the C-terminal region of the protein is important for efficiency and acceptor specificity of enzymatic function. Perhaps the best example of this would be the group O phenotype (see Table 3).³⁻⁷

Molecular Events that Silence A or B Gene (O Phenotype)

All the adenocarcinoma cell lines from group O individuals have a structural sequence identical to that of the original *A*¹ primordial gene described, with the exception of a nucleotide deletion in the coding region at nucleotide position 261, indicating that the lack of transferase activity in group O individuals is due to a shift in the reading frame that prematurely codes for a stop codon and translation of an entirely different protein devoid of any enzymatic function.¹⁸ In 1994, a mutant *O* allele in which the nucleotide deletion at position 261 was absent was detected and sequenced, but the B-associated 297A>G and 526C>G mutations were observed. The lack of A or B transferase activity was explained by the presence of a third mutation, 802G>A, resulting in a glycine-to-arginine substitution in the region of the glycosyltransferase thought to be involved in enzymatic substrate specificity (Table 3). This allele was named *O*^{2.4}

Table 1. A Alleles

Phenotype	Allele	Other Name	Exon 1-5 nt 0 to 239		Exon 6 nt 240 to 374										Exon 7 nt 375 to 1062												
			nt:	aa:	261	297	467	526	564	641	646	657	669	681	703	721	771	796	802	803	798-804	829	871	930	1009	1054	1059-1061
A ₁	ABO*A101	A ¹		G A	C	C	C	T	T	C	G	G	G	C	C	C	G	G	GGGGGGG	G	G	G	G	C	CCC		
					Pro	Arg		Met	Phe		Glu	Gly				Leu	Gly	Gly		Val	Asp			Arg			
A ₁	ABO*A102	A ¹			T																						
					Leu																						
A ₁	ABO*A103						T																				
					Leu																						
A ₁	ABO*A104			G																							
A ₂	ABO*A105	A ²			T																				CC [†]		
					Leu																				→		
A ₂	ABO*A106																								T		
																									Trp		
A ₂	ABO*A107																								G		
																									Gly		
?A ₂	ABO*A111				T																				G		
					Leu																				Gly		
A ₃		A ³																			A						
																					Asn						
A _X	ABO*A108	A ^X																									
A ₁₀	ABO*A109																										
A ₁₀₁	ABO*A110				T																						
					Leu																						
A ₂	ABO*R101			G		G				T																	
						Gly																					
Cis-AB	ABO*C101				T																						
					Leu																						
A		A ^{stomach}				G																					
						Gly																					

† Deletion of nt 1059(C) and then extra 21 aa.

An O^3 allele that does not contain the 261 nucleotide deletion was recently identified in one Swedish-Danish family. In a recent study to validate an ABO genotype screening method, one ABO genotype assignment did not correlate with the serologically obtained phenotype. The proband and his father consistently phenotyped as group O and had strong anti-A and anti-B in their serum. Adsorption and elution studies failed to detect the presence of A or B antigens on their RBCs. The proband and his father were initially genotyped as A^2O^1 . Suspecting a novel O allele, exons 6 and 7 of the *ABO* gene of the proband were sequenced after cloning into a bacterial vector. The ABO genotyping revealed the proband and his father to be a combination of the A^2 and A_{el} alleles, containing a triple mutation that totally inactivated the glycosyltransferase, resulting in a serologically group O phenotype.⁵

In the Brazilian population, previously unidentified alleles have been found that are believed to be the cause of recombination, hybrid alleles, and crossover events between O , A , and B alleles, resulting in a catalytically active enzyme and a serological phenotype of group O. Indeed, as more populations are genotyped for ABO, more alleles will be identified.⁶

Molecular Events that Cause Altered Activity of Glycosyltransferases⁷

The most common ABO subgroup identified is the A_2 phenotype, which is serologically identified by its ability to be agglutinated by anti-A but not by anti- A_1 . It has been a long standing debate as to whether the difference of the A antigen on A_1 and A_2 RBCs is a quantitative or qualitative one. In a study by Yamamoto et al.,⁸ it was found that genomic DNA from all eight of A_2 samples analyzed contained a single nucleotide deletion at position 1059, 1060, or 1061 (all of which are C) as compared to an A^1 allele. This single base deletion located at the end of the C-terminus changes the reading frame and results in a protein with 21 additional amino acids, leading to a 30- to 50-fold decrease in the A transferase activity. This finding reinforces the view that the C-terminal region of the protein is important for efficiency and acceptor specificity of enzymatic function.⁸

Another variant ABO phenotype seen serologically is the A_3 phenotype. This is serologically classified by a mixed-field pattern of reactivity with commercially available anti-A sera. Molecular analysis has shown that two different A_3B people have a single nucleotide substitution at 871 G>A as compared to A^1 alleles, resulting in

the amino acid substitution Asp291Asn. In the A_3 phenotype, one nucleotide substitution effects agglutination of approximately 60 percent of RBCs.⁹

A rare phenotype encountered in blood banking is the *cis-AB* phenotype. Two possible genetic mechanisms were proposed for this phenotype. The first was based on an unequal crossover event resulting in a gene with part of A and part of B . The second theory was based on a structural glycosyltransferase mutation in either the A or B gene, resulting in bifunctional activity. Molecular analysis showed that the *cis-AB* alleles are identical to A^1 alleles except for two nucleotide substitutions. One at nucleotide 467 (C>T) corresponds to the amino acid substitution of Pro156Leu, and the other at nucleotide 803 (G>C) corresponds to an amino acid change from glycine to alanine at position 268 as compared with the A^1 allele. This glycine-alanine substitution is located at the fourth position of the substitutions, which discriminates A_1 and B transferases. It is now believed that the *cis-AB* allele arose through a point mutation from A transferase during evolution of *ABO* genes rather than through the recombination between A and B alleles.¹⁰⁻¹²

H Gene

The human H gene encoding the H enzyme, an α 1,2 fucosyltransferase, was first cloned by the expression cloning method. Molecular analyses of various Bombay and para-Bombay individuals have identified deletions and missense mutations in the H allele that abolish or greatly reduce the enzymatic activity of the fucosyltransferase (Table 4).^{13-17,24,25} Each glycosyltransferase must have a well-defined substrate to transfer their immunodominant sugar. Therefore, a mutation existing within the H gene will result in a reduced amount of H antigen available on the RBC for the wild type A and B glycosyltransferase to effectively transfer their immunodominant sugar. In these cases, serologic reactions with anti-A or anti-B will be weakened due to a mutation within the H gene regardless of normal A and B alleles present.¹³⁻¹⁷

Perspective

The dramatic advances in molecular biology within the past decade have provided blood bankers with the knowledge of different *ABO* alleles and methods to detect them. Polymerase-chain reaction (PCR)-based techniques, such as restriction fragment-length polymorphism, allele-specific PCR, single-stranded conformation polymorphism, and denaturing gradient gel elec-

trophoresis, have been used for the identification of ABO genotypes. Blood bankers and forensic scientists are employing these techniques because they offer several advantages over routine serologic methods; genotyping does not require RBCs and only a small sample is needed for prenatal and forensic investigations.^{23,26}

In previous studies, *ABO* genotyping results were compared with the determined serologic phenotype.²³ A correlation of 98.7 percent of the individuals studied was achieved. However, caution must be exercised when attempting to predict an individual's phenotype from ABO genotyping results. The requirement that nucleotide substitutions are clearly related to specific phenotypes must be met. Genotyping methods for some rare blood groups whose allelic sequences are unknown are prone to be misinterpreted. The A^1 allele cannot be directly detected; its determination is always linked to exclusion of other known alleles.^{23,26}

Phenotype prediction also depends on the functional integrity of the allele. ABO genotyping often will identify heterozygotes with one "normal" *ABO* allele and one allele with a mutation. It is often difficult to predict the catalytic capabilities of the less efficient glycosyltransferase and, therefore, the phenotype. Recently, a superactive B transferase was identified in black and Japanese populations. This strong B transferase is two to five times more efficient at converting H substance to B antigens than a "normal" B transferase. It has been shown that an individual who inherits a normal A^1 allele (*ABO***A101*) and the allele encoding this strong B transferase will phenotype as A_2B as a result of competition between the A_1 transferase and the overpowering B transferase for converting the H antigen. An individual with an A_2B phenotype has the potential of having an anti- A_1 in their serum. ABO genotyping would predict this individual to be an AB phenotype without distinguishing between A_1B and A_2B .²⁷⁻²⁹

There are many factors affecting what is seen at the molecular level to what protein will be expressed on the surface of the RBC. If DNA sequencing of the entire *ABO* gene becomes a routine screening procedure, defects in gene control and expression still could indicate a genotype that is inconsistent with the serologically observed phenotype. The clinical implications of a misinterpreted ABO phenotype prevents genotyping from replacing serology in transfusion medicine. Serologic investigations supplemented with molecular biology have unraveled many blood banking mysteries and provide faster and safer transfusions for patient care.

Acknowledgments

We thank Robert Ratner for preparing this article.

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The Rh blood group system: the first 60 years of discovery

C. LOMAS-FRANCIS AND M.E. REID

Key Words: Rh blood group, RhD, RhCE, RhAG, Rh proteins, *RH* genes

Introduction

The Rh blood group system consists of at least 45 independent antigens and is considered the most polymorphic human blood group system. The great immunogenicity of the Rh antigens makes the system of considerable clinical importance in transfusion medicine: Rh antigens are involved in hemolytic transfusion reactions, hemolytic disease of the newborn (HDN), and autoimmune hemolytic anemia. The morphological and functional abnormalities of red blood cells (RBCs) deficient in Rh antigens (Rh_{null} and Rh_{mod} phenotypes) indicate that the Rh proteins are necessary for red cell membrane integrity.

From kill to overkill: 100 years of (perhaps too much) progress

P.D. ISSITT

Key Words: compatibility tests, antibody screening tests, antiglobulin tests, enzyme-treated red cells, polybrene, PEG, solid phase, gel methods, automation

Introduction

The 1900 report of Landsteiner,¹ describing discovery of the ABO groups, provided, by far, the most significant advance ever made in enhancing the safety of red cell blood transfusions. In the 100 years that followed, numerous other blood group systems were discovered and methods used to detect blood group antibodies in the sera of patients to be transfused became increasingly sensitive. Great emphasis has been placed on increasing that sensitivity. A general philosophy about any new method has been that if it detects more antibodies, it must be better. In the year 2000, such reasoning is no longer valid. Some recently devised methods detect antibodies that are of no clinical relevance to the patients in whom they are found. The use of oversensitive tests increases the cost of transfusion and, worse, may prevent or delay essential transfusion while a clinically meaningless *in vitro* finding is investigated. In the detection of blood group antibodies, more is not always better. The 1900 discovery provided a means by which killing the patient with ABO incompatible blood could be avoided. One hundred years later, we are sometimes guilty of overkill in our zealous attempts to detect blood group antibodies. This review documents the historical development of antibody screening and compatibility testing methods and identifies the point at which overkill became a reality.

Improving transfusion safety: the bookends of a century

W.C. SHERWOOD

Key Words: transfusion safety, transmissible disease, red cell modification, human blood alternatives

Introduction

The twentieth century was escorted in with the extraordinary finding of the ABO blood groups. Karl Landsteiner, in 1900, using blood samples collected from his co-workers described the agglutination and lysis of red cells when they were mixed with another person's serum. He sorted these findings to define the A, B, and O (zero) blood groups of "man."^{1,2} With similar technique and with Landsteiner's urging, Decastello and Sturli described the remaining blood group, AB, in 1902.³ These findings, which today seem simplistic and often lost in significance, provided the most important contribution to safety throughout the history of transfusion medicine. The 1930 Nobel Prize for Medicine awarded to Karl Landsteiner was among those most richly deserved.

Almost 100 years later, as if metered by a chronological design, the century closes with a frenetic push of blood microbial tests and microbial inactivation procedures that advanced transfusion safety another giant stride.

PEG-coated red blood cells— simplifying blood transfusion in the new millennium?

T.C. FISHER

Key Words: poly(ethylene glycol), PEG, blood groups, antigenicity, immunogenicity, alloimmunization, blood banking

Background

Blood group antigens and their significance for blood transfusion

Blood group antigens are polymorphisms in protein or carbohydrate molecules on the surface of red blood cells (RBCs), which may be recognized as foreign by a recipient's immune system after RBC transfusion. Over 200 different blood group antigens have been identified to date.¹

The ABO system antigens are of primary significance for blood transfusion, because antibodies to the A or B antigens occur naturally in most subjects. The accidental administration of ABO-incompatible blood usually results in rapid intravascular hemolysis of the transfused RBCs, with serious and sometimes fatal consequences. For this reason, every donated unit of blood is routinely ABO typed, and great care is taken to ensure that every unit of transfused blood is ABO compatible. Thus, the ABO antigens rarely cause any transfusion-related complications for the recipient. The practical significance of the ABO antigens relates more to the logistics of providing blood for transfusion, i.e., to cover the possible demand for each type, blood banks must collect and store more blood than is actually needed for transfusion. Thus, some units will remain unused and become outdated. Differences in the frequencies of A, B and O antigens between the recipient and donor pools also may produce relative shortages and surpluses of certain types, requiring the transfer of blood between different regions to equalize supply and demand.

Preview 2000: proposal for a new terminology to describe carbohydrate histo-blood group antigens/glycotopes within the ISBT terminology framework

S.M. HENRY AND J.J. MOULDS

Key Words: proposed ISBT terminology, glycotopes, carbohydrate blood group antigens

Introduction

In 1980, the International Society of Blood Transfusion (ISBT) formed a working party with the mandate to devise a genetically based terminology for red cell surface antigens.¹ After 20 years this group has assigned numbers to most systems and antigens. As of 1999, most carbohydrate systems have been assigned system numbers and many of the

structures commonly recognized by antibodies within the systems have been given (antigen) numbers (Table 1).²

Table 1. Summary of current (1998) terminology (major carbohydrate blood group antigens only)

System	Antigen number				
	001	002	003	004	005
006					
001 ABO	A	B	A,B	A ₁	
003 P	P ₁				
007 LE	Le ^a	Le ^b	Le ^{ab}	Le ^{bH}	ALe ^b
	BLe ^b				
018 H	H				
209 GLOB	P	P ^k	LKE		
210	Le ^c	Le ^d			

At present the ISBT working party does not have a terminology that can accurately describe all the different forms of carbohydrate antigens/glycotopes present on a red cell. Glycotope is the word now used to describe carbohydrate epitopes.³ Being able to describe antigens and glycotopes is important. Although the blood group glycotopes on the large glycoconjugate structures do not exceed several sug-

ars in size, the presence of a glycotope on different-sized structures influences their expression and probably biological characteristics, especially when present in the (red) cell membrane. For example, a blood group A glycotope present on a short backbone is generally less reactive on red cells than one that is present on an extended chain. Alternatively, a structure bearing two identical glycotopes can cross-link antibody (monogamous bivalency), which significantly changes the binding avidity.