2 ABO, H, and Lewis Systems

Part 1: History and introduction

Described in this chapter are three blood group systems, ABO, H, and Lewis (Table 2.1), although Lewis is really an 'adopted' blood group system because the antigens are not intrinsic to the red cells, but introduced into the membrane from the plasma. These three systems are genetically discrete, but are discussed in the same chapter because they are phenotypically and biochemically closely related. A complex interaction of genes at several loci controls the expression of ABO, H, Lewis, and other related antigens on red cells and in secretions.

The science of immunohaematology came into existence in 1900 when Landsteiner [1] reported that, 'The serum of healthy humans not only has an agglutinating effect on animal blood corpuscles, but also on human blood corpuscles from different individuals'. The following year Landsteiner [2] showed that by mixing together sera and red cells from different people three groups, A, B, and C (later called O), could be recognised. In group A, the serum agglutinated group B, but not A or C cells; in group B, the serum agglutinated A, but not B or C cells; and in group C (O), the cells were not agglutinated by any serum, and the serum appeared to contain a mixture of two agglutinins capable of agglutinating A and B cells. Decastello and Stürli [3] added a fourth group (AB), in which the cells are agglutinated by sera of all other groups and the serum contains neither agglutinin. Healthy adults always have A or B agglutinins in their serum if they lack the corresponding agglutinogen from their red cells (Table 2.2).

Epstein and Ottenberg [4] suggested that blood groups may be inherited and in 1910 von Dungern and Hirschfeld [5] confirmed that the inheritance of the A and B antigens obeyed Mendel's laws, with the presence of A or B being dominant over their absence. Bernstein [6,7] showed that only three alleles at one locus were necessary to explain ABO inheritance (Table 2.2).

Some group A people produce an antibody that agglutinates the red cells of most other A individuals. Thus A was subdivided into A_1 and A_2, and the three allele theory of Bernstein was extended to four alleles: A^1, A^2, *B* and *O* [8] (Section 2.4). Many rare subgroups of A and B have now been identified (Sections 2.7 and 2.8).

The structure and biosynthesis of the ABO, H, and Lewis antigens is well understood, thanks mainly to

Human Blood Groups, Third Edition. Geoff Daniels.

Table 2.1 Numerical notation for the ABO, Lewis, and H systems, and for Le^c and Le^d.

ABO (system 001)		Lewis (system 007)		H (system 018)		Collection 210	
ABO1	A	LE1	Le^a	H1	H	210001	Le^c
ABO2	B	LE2	Le^b			210002	Le^d
ABO3	A,B	LE3	Le^{ab}				
ABO4	A1	LE4	Le^{bH}				
		LE5	Ale^b				
		LE6	BLe^b				

Obsolete: ABO5, previously H.

Table 2.2 The ABO system at its simplest level.

ABO group	Antigens on red cells	Antibodies in serum	Genotype
O	None	Anti-A,B	*O/O*
A	A	Anti-B	*A/A* or *A/O*
B	B	Anti-A	*B/B* or *B/O*
AB	A and B	None	*A/B*

the pioneering work in the 1950s of Morgan and Watkins [9,10] and of Kabat [11]. A and B red cell antigens are carbohydrate determinants of glycoproteins and glycolipids and are distinguished by the nature of an immunodominant terminal monosaccharide: *N*-acetylgalactosamine (GalNAc) in group A and galactose (Gal) in group B. The *A* and *B* genes encode glycosyltransferases that catalyse the transfer of the appropriate immunodominant sugar from a nucleotide donor to an acceptor substrate, the H antigen. The *O* allele produces no active transferase (Sections 2.2 and 2.3). The sequences of the *A* and *B* alleles demonstrate that A- and B-glycosyltransferases (GTA and GTB) differ by four amino acid residues; the most common *O* allele contains a nucleotide deletion and encodes a truncated protein.

There are a multitude of *ABO* alleles, many of which affect phenotype, and at least two different terminologies. In this chapter the original terminology (e.g. A^1, A^2, O^1) will be used, with the dbRBC terminology often provided in parentheses.

H antigen is synthesised by a fucosyltransferase produced by *FUT1*, a gene independent of *ABO*. Very rare individuals lacking *FUT1* have no H antigen on their red cells and, consequently, are unable to produce A or B antigens, even when the enzyme products of the *A* or *B* genes are present (Section 2.12).

H antigen is present in body secretions of about 80% of Caucasians. The presence of H in secretions is governed by *FUT2*, another fucosyltransferase that is closely linked to *FUT1*. Individuals who secrete H also secrete A or B antigens if they have the appropriate *ABO* alleles. Non-secretors of H secrete neither A nor B, even when those antigens are expressed on their red cells (Section 2.6).

The first two examples of anti-Lewis, later to be called anti-Le^a, were described by Mourant [12] in 1946. These antibodies agglutinated the red cells of about 25% of English people. Andresen [13] found an antibody, later to become anti-Le^b, that defined a determinant only present on Le(a–) cells of adults. Six percent of group O adults lacked both antigens. Although Le^a and Le^b are not synthesised by red cells, but are acquired from the plasma, they are considered blood group antigens because they were first recognised on red cells. The terminology Le^a and Le^b is misleading as these antigens are not the products of alleles.

The Lewis gene (*FUT3*) encodes a fucosyltransferase that catalyses the addition of a fucose residue to H antigen in secretions to produce Le^b antigen or, if no H is present (non-secretors), to the precursor of H to produce Le^a. Consequently, as these structures are acquired from the plasma by the red cell membrane, red cells of most H secretors are Le(a–b+) and those of most H non-secretors are Le(a+b–). The Lewis-transferase can also convert A to ALe^b and B to BLe^b. About 6% of white people and 25% of black people are homozygous for a silent gene at the

FUT3 locus and, as they do not produce the Lewis enzyme, have Le(a–b–) red cells and lack Lewis substances in their secretions (Sections 2.3 and 2.15). In East Asia the red cell phenotype Le(a+b+) is common, caused by a weak secretor allele (Section 2.6.3).

The antigens Le^c and Le^d represent precursors of the Lewis antigens and are present in increased quantity in the plasma of Le(a–b–) individuals. Le^c is detected on the red cells of Le(a–b–) non-secretors of H and Le^d is detected on the red cells of Le(a–b–) secretors of H. Le^x and Le^y antigens, isomers of Le^a and Le^b, are not present in substantial quantities on red cells (Section 2.18.2).

ABH and Lewis antigens are often referred to as histo-blood group antigens [14] because they are ubiquitous structures occurring on the surface of endothelial cells and most epithelial cells. The precise nature of the histo-blood group antigens expressed varies between tissues within the same individual because of the intricacy of the gene interactions involved (Section 2.19).

ABO is on chromosome 9; *FUT1*, *FUT2*, and *FUT3* are on chromosome 19 (Sections 2.3.1, 2.3.2.4, and 2.3.5).

Part 2: Biochemistry, inheritance, and biosynthesis of the ABH and Lewis antigens

2.2 Structure of ABH, Lewis, and related antigens

ABH and Lewis antigens are carbohydrate structures. These oligosaccharide chains are generally conjugated with polypeptides to form glycoproteins or with ceramide to form glycosphingolipids. Oligosaccharides are synthesised in a stepwise fashion, the addition of each monosaccharide being catalysed by a specific glycosyltransferase. The oligosaccharide moieties responsible for expression of ABH, Lewis, and related antigens are shown in Table 2.3 and abbreviations for monosaccharides are given in Table 2.4. The biosynthesis of these structures is described in Section 2.3 and represented diagrammatically in Figure 2.1. There is a vast literature on the biochemistry of these blood group antigens and only some of the relevant references can be given in this chapter. The following reviews are recommended: [10,14–27].

2.2.1 Glycoconjugates expressing ABH and Lewis antigens

Two major classes of carbohydrate chains on glycoproteins express ABH antigens:

1 *N*-glycans, highly branched structures attached to the amide nitrogen of asparagine through GlcNAc; and
2 *O*-glycans, simple or complex structures attached to the hydroxyl oxygen of serine or threonine through GalNAc.

Glycosphingolipids consist of carbohydrate chains attached to ceramide. They are classified as lacto-series, globo-series, or ganglio-series according to the nature of the carbohydrate chain. Glycosphingolipid-borne ABH and Lewis antigens are present predominantly on glycolipids of the lacto-series, although ABH antigens have also been detected on globo-series and ganglio-series glycolipids. The carbohydrate chains of most ABH-bearing glycoproteins and of lacto-series glycolipids are based on a poly-*N*-acetyllactosamine structure; that is, they are extended by repeating Galβ1→4GlcNAcβ1→3 disaccharides (see Table 2.5 for examples).

On red cells, most ABH antigens are on the single, highly branched, poly-*N*-acetyllactosaminyl *N*-glycans of the anion exchange protein, band 3, and the glucose transport protein, band 4.5 [28]. There are about 1 million monomers of band 3 protein and half a million monomers of band 4.5 protein per red cell [29]. The other major red cell glycoprotein, glycophorin A, carries very low levels of ABH activity on both *O*- and *N*-glycans (Sections 3.2.1 and 3.2.2) and ABH determinants have also been detected on the Rh-associated glycoprotein [30]. Lewis antigens on red cells are not expressed on glycoproteins; they are not intrinsic to red cells, but are acquired from the plasma.

Glycolipids play a minor role in red cell ABH expression compared with glycoproteins. Red cell glycosphingolipids of the poly-*N*-acetyllactosaminyl type that express ABH antigens may have relatively simple linear or branched carbohydrate chains [15] (Table 2.5) or may be highly complex, branched structures called polyglycosylceramides, with up to 60 carbohydrate residues per molecule [31].

All the early work establishing the structures of the ABH and Lewis determinants was carried out on body secretions, especially the pathological fluid from human ovarian cysts, an abundant source of soluble A, B, and H substances [32]. ABH and Lewis antigens in secretions are glycoproteins; oligosaccharide chains attached to mucin by *O*-glycosidic linkage to serine or threonine (for reviews see [9,10]). These macromolecules have molecular weights varying from 2×10^5 to several millions. In milk and urine, free oligosaccharides with ABH and Lewis activity are also found [33,34]. ABH and Lewis determinants are present in plasma on glycosphingolipids, some

Table 2.3 Structures of A, B, H, Lewis, and related antigens (for abbreviations see Table 2.4).

Type 1		*Type 2*	
Precursor (Le^c)	Galβ1→3GlcNAcβ1→R †	**Precursor**	Galβ1→4GlcNAcβ1→R
H (Le^d)	Galβ1→3GlcNAcβ1→R † 2 ↑ Fucα1	**H** (CD173)	Galβ1→4GlcNAcβ1→R * 2 ↑ Fucα1
A	GalNAcα1→3Galβ1→3GlcNAcβ1→R † 2 ↑ Fucα1	**A**	GalNAcα1→3Galβ1→4GlcNAcβ1→R * 2 ↑ Fucα1
B	Galα1→3Galβ1→3GlcNAcβ1→R † 2 ↑ Fucα1	**B**	Galα1→3Galβ1→4GlcNAcβ1→R * 2 ↑ Fucα1
Le^a	Galβ1→3GlcNAcβ1→R † 4 ↑ Fucα1	**Le^x**	Galβ1→4GlcNAcβ1→R 3 ↑ Fucα1
Le^b	Galβ1→3GlcNAcβ1→R † 2 4 ↑ ↑ Fucα1 Fucα1	**Le^y**	Galβ1→4GlcNAcβ1→R 2 3 ↑ ↑ Fucα1 Fucα1
ALe^b	GalNAcα1→3Galβ1→3GlcNAcβ1→R † 2 4 ↑ ↑ Fucα1 Fucα1	**ALe^y**	GalNAcα1→3Galβ1→4GlcNAcβ1→R 2 3 ↑ ↑ Fucα1 Fucα1
BLe^b	Galα1→3Galβ1→3GlcNAcβ1→R † 2 4 ↑ ↑ Fucα1 Fucα1	**BLe^y**	Galα1→3Galβ1→4GlcNAcβ1→R 2 3 ↑ ↑ Fucα1 Fucα1
sialyl-Le^a	Galβ1→3GlcNAcβ1→R 3 4 ↑ ↑ NeuAcα2 Fucα1	**sialyl-Le^x**	Galβ1→4GlcNAcβ1→R 3 3 ↑ ↑ NeuAcα2 Fucα1

of which may become incorporated into the red cell membrane (Section 2.15.4).

2.2.2 Carbohydrate determinants

Expression of H, A, and B antigens is dependent on the presence of specific monosaccharides attached to various precursor disaccharides at the non-reducing end of a carbohydrate chain. There are at least five precursor disaccharides, also called peripheral core structures (reviewed in [14,18,21,23]):

Type 1	Galβ1→3GlcNAcβ1→R
Type 2	Galβ1→4GlcNAcβ1→R
Type 3	Galβ1→3GalNAcα1→R
Type 4	Galβ1→3GalNAcβ1→R
Type 6	Galβ1→4Glcβ1→R.

(Type 5 has only been chemically synthesised.)

H-active structures have Fuc α-linked to C-2 of the terminal Gal [35,36]; A- and B-active structures have GalNAc and Gal, respectively, attached in α-linkage to

Table 2.3 (*Continued*)

Type 3: O-linked mucin type

Precursor (T antigen) Galβ1→3GalNAcα1→O-Ser/Thr

H
Galβ1→3GalNAcα1→O-Ser/Thr
2
↑
Fucα1

A
GalNAcα1→3Galβ1→3GalNAcα1→O-Ser/Thr
2
↑
Fucα1

B
Galα1→3Galβ1→3GalNAcα1→O-Ser/Thr
2
↑
Fucα1

Type 3: repetitive type

H
Galβ1→3GalNAcα1→3Galβ1→4GlcNAcβ1→R *
2 2
↑ ↑
Fucα1 Fucα1

A
GalNAcα1→3Galβ1→3GalNAcα1→3Galβ1→4GlcNAcβ1→R *
2 2
↑ ↑
Fucα1 Fucα1

Type 4: globo-series

Globo-H
Galβ1→3GalNAcβ1→R
2
↑
Fucα1

Globo-A
GalNAcα1→3Galβ1→3GalNAcβ1→R
2
↑
Fucα1

*Intrinsic to red cells and detected in significant quantity on red cells of individuals of appropriate genotype.
†Adsorbed onto red cells from plasma in individuals of appropriate genotype.

Table 2.4 Some abbreviations for monosaccharides and the structures they are linked to.

Gal	D-galactose	Cer	Ceramide
GalNAc	*N*-acetyl-D-galactosamine	Asp	Asparagine
GlcNAc	*N*-acetyl-D-glucosamine	Ser	Serine
Fuc	L-Fucose	Thr	Threonine
NeuAc	Sialic acid (*N*-acetylneuraminic acid)		
Man	Mannose		
Glc	Glucose	R	Remainder of molecule

C-3 of this α1→2 fucosylated Gal residue (Table 2.3). Although Fuc does not represent the whole H determinant, it is the H immunodominant sugar because its loss results in loss of H activity. Likewise GalNAc and Gal are the A and B immunodominant sugars, respectively.

Le^a and Le^b antigens are expressed when Fuc is attached to the GlcNAc residue of the Type 1 precursor and Type 1 H, respectively [37–40]. Le^x and Le^y are the Type 2 isomers of Le^a and Le^b [36,39,41,42]. Fuc is linked α1→4 to the GlcNAc residue of a Type 1 chain in Le^a and Le^b and α1→3 to the GlcNAc of a Type 2 chain in Le^x and Le^y. Le^x and Le^y are not present in significant quantities on red cells [43]. The monofucosylated Le^a and Le^x

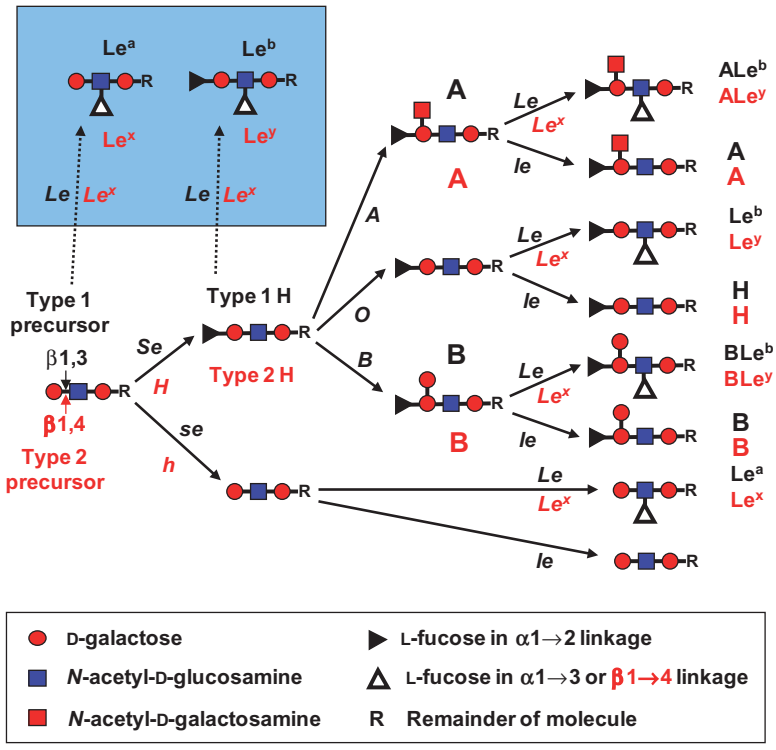

Figure 2.1 Diagram representing the biosynthetic pathways of ABH, Lewis, Le^x, and Le^y antigens derived from Type 1 and Type 2 core chains. Genes controlling steps in the pathway are shown in *italics* and the gene products are listed in Table 2.6. Type 1 and Type 2 precursors differ in the nature of the linkage between the non-reducing terminal Gal and GlcNAc: β1→3 in Type 1 and β1→4 in Type 2. Type 1 and Type 2 structures and the genes acting on them are shown in black and red, respectively. Dashed lines show how Le^a (Le^x) and Le^b (Le^y), produced from the precursor and H structures respectively, are not substrates for the H, Se, or ABO transferases and remain unconverted.

structures may be sialylated at the C-3 of Gal [44–46] (Table 2.3).

Type 1 ABH and Lewis structures are present in secretions, plasma, and endodermally derived tissues [21]. They are not synthesised by red cells, but are incorporated into the red cell membrane from the plasma [47]. Lewis antigens (Le^a and Le^b) are only present on Type 1 structures. Elongated carbohydrate chains with Type 1 peripheral structures are generally extended by repeating poly-*N*-acetyllactosamine disaccharides with the Type 2 (β1→4) linkage [48] (Table 2.5). Extended Type 1 structures with Le^a and Le^b activity have been detected in plasma, particularly in persons with Le(a+b+) red cells [49,50].

Antigens on Type 2 chains represent the major ABH-active oligosaccharides on red cells and are also detected in secretions and various ectodermally or mesodermally derived tissues [15,21]. Type 2 structures in secretions are probably more often difucosylated (Le^y, ALe^y, BLe^y) than monofucosylated (H, A, B) [51,52].

There are two forms of Type 3 ABH antigens, the *O*-linked mucin type and the repetitive A-associated type. In the *O*-linked mucin type the precursor exists as a disaccharide linked directly, by *O*-glycosidic bond, to a serine or threonine residue of mucin [53]. This precursor represents the T cryptantigen (see Section 3.17.2), but is not usually expressed because it is masked by substitution with sialic acid residues or other sugars. Type 3 ABH antigens of the *O*-linked mucin type are not found on red cells [54]. Repetitive Type 3 chains are present on red cell glycolipids and secreted mucins from group A individuals. They are restricted to group A because they are biosynthesised by the addition of Gal in β1→3 linkage to the terminal GalNAc of an A-active Type 2 chain followed by

Table 2.5 Examples of H-active glycoconjugates with Type 2 precursor chains (for abbreviations see Table 2.4).

Glycosphingolipid (simple linear)

Fucα1→2Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→Cer

Glycosphingolipid (branched)

Fucα1→2Galβ1→4GlcNAcβ1→3(Galβ1→4GlcNAcβ1→3)$_n$Galβ1→4Glcβ1→Cer
6
↑
Fucα1→2Galβ1→4GlcNAcβ1

N-*linked glycoprotein*

Fucα1→2Galβ1→4GlcNAcβ1
↓
6
Fucα1→2Galβ1→4GlcNAcβ1→3(Galβ1→4GlcNAcβ)$_n$1→2Man
Man-GlcNAc-GlcNAc-Asn
Fucα1→2Galβ1→4GlcNAcβ1→3(Galβ1→4GlcNAcβ)$_n$1→2Man
6
↑
Fucα1→2Galβ1→4GlcNAcβ1

O-*linked glycoprotein (complex mucin type)*

Fucα1→2Galβ1→4GlcNAcβ1
↓
6
Fucα1→2Galβ1→4GlcNAcβ1→3(Galβ1→4GlcNAcβ1→3)$_n$GalNAcα1→Ser/Thr
6
↑
Fucα1→2Galβ1→4GlcNAcβ1

n, 0–5 or more.

the fucosylation of that Gal to form Type 3 H [43,54–56] (Figure 2.2). Repetitive Type 3 chains are only present on group A cells because they are produced by the addition of Gal to the terminal GalNAc of a Type 2 A chain.

Type 4 ABH structures are only located on glycolipids. Type 4 precursor chain of the globo-series results from the addition of terminal Gal to globoside [57] (P antigen, see Chapter 4). Type 4 globo-H and globo-A have been detected in small quantities on red cells [57,58], but are more abundant in kidney [59]; Type 4 globo-B has only been found, in minute quantities, in kidney [60]. Kidney from a group A person with the p phenotype, which prevents extension of the globo-series structures, lacked Type 4 A [61] (see Chapter 4).

Type 6 chains have been found as free oligosaccharides in milk and urine [33,34].

The internal carbohydrate chains express I and i antigens. In fetal cells linear chains predominate and i antigen is expressed, whereas in adult glycoproteins and glycolipids there is branching of the inner core chains and I antigen is expressed (see Chapter 25).

2.3 Biosynthesis, inheritance, and molecular genetics

The carbohydrate antigens of the ABO, H, and Lewis blood group systems are not the primary products of the genes governing their expression. Carbohydrate chains are built up by the sequential addition of monosaccharides, each extension of the chain being catalysed by a specific glycosyltransferase. These enzymes catalyse the

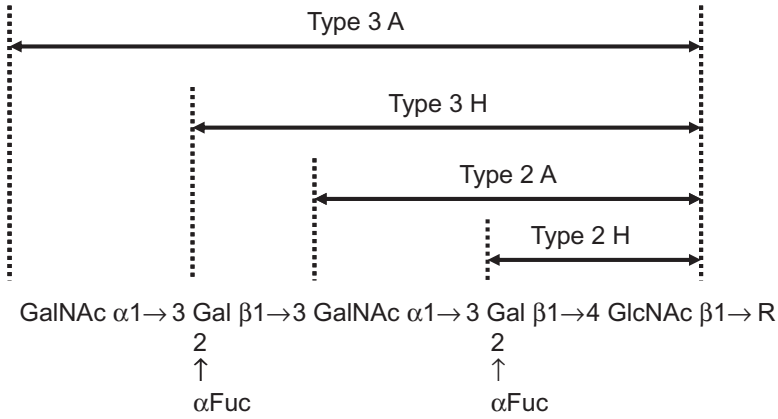

Figure 2.2 Diagram showing how a repetitive Type 3 A chain is built up from a Type 2 H chain. From right to left, Type 2 H is converted to Type 2 A in group A people. Type 2 A may be converted to Type 3 H. Type 3 H is then further converted to Type 3 A.

Table 2.6 Some ABH-related blood group genes and the glycosyltransferases they produce.

Locus	Allele	Transferase	
FUT1 (*H*)	*H*	α1,2-L-fucosyltransferase	EC 2.4.1.69
	h	None	
FUT2 (*SE*)	*Se*	α1,2-L-fucosyltransferase	EC 2.4.1.69
	se	None	
ABO	*A*	α1,3-*N*-acetyl-D-galactosaminyltransferase	EC 2.4.1.40
	B	α1,3-D-galactosyltransferase	EC 2.4.1.37
	O	None	
FUT3 (*LE*)	*Le*	α1,3/4-L-fucosyltransferase	EC 2.4.1.65
	le	None	

transfer of a monosaccharide from its nucleotide donor and its attachment, in a specific glycosidic linkage, to its acceptor substrate. Glycosyltransferases represent the primary products of the *ABO*, *FUT1* (H), *FUT2* (secretor), and *FUT3* (Lewis) genes (Table 2.6).

At least 100 glycosyltransferases are required for synthesis of the known human carbohydrates. The genes producing most of them have been identified and sequenced, including those for the ABO, H, and Lewis blood groups, and for secretion of H. The gene products are trans-membrane proteins of the Golgi apparatus. They share a common domain structure comprising a short N-terminal cytoplasmic tail, a 16–20 amino acid membrane-spanning domain, and an extended stem region followed by a large C-terminal catalytic domain. Soluble glycosyltransferases present in secretions may result from the release of membrane-bound enzymes by endogenous proteases or they may lack the membrane-spanning domain as a result of mRNA translation-initiation at an alternative site (reviewed in [62,63]).

The regulatory mechanisms required to assure that carbohydrate chains with the appropriate sequences are produced are complex. They involve the presence or absence of certain enzymes according to the genes expressed in various tissues and at different stages of development, and according to the genotype of the individual. Competition between different transferases for the same donor or acceptor substrate is also important in determining the carbohydrate chain produced (reviewed in [16]).

2.3.1 H antigen

H antigen is produced when an α1,2-L-fucosyltransferase catalyses the transfer of Fuc from a guanosine diphosphate (GDP)-L-fucose donor to the C-2 position of the terminal Gal of one of the precursor structures shown in Section 2.2.2 (Table 2.3, Figure 2.1). Two α1,2-L-fucosyltransferases, produced by *FUT1* (*H*) and *FUT2* (*SE*), catalyse the biosynthesis of H-active structures in different tissues. H-transferase, the product of *FUT1*, is active in tissues of endodermal and mesodermal origin, and synthesises red cell H antigen; secretor-transferase, the product of *FUT2*, is active in tissues of ectodermal origin, and is responsible for soluble H antigen in secretions (reviewed in [63]). *FUT1* has a higher affinity for Type 2 acceptor substrate than Type 1, whereas *FUT2* shows a preference for Type 1 acceptor substrate [64–67]. *FUT1* consists of four exons and *FUT2* of two exons, but in both genes only one exon (exon 4 in *FUT1*, exon 2 in *FUT2*) encodes the protein product [68,69].

FUT1 and *FUT2* share about 70% sequence identity and are 35 kb apart at chromosome 19q13.33 [70,71]. A pseudogene, *SEC1*, located within about 50 kb of *FUT2*, shares over 80% sequence identity with *FUT2*, but contains translation termination codons. *FUT1*, *FUT2*, and *SEC1* probably arose by gene duplication and are part of a linkage group that also includes the genes for the Lutheran (*BCAM*) and LW (*ICAM4*) blood groups (Section 6.2.4).

2.3.1.1 Red cells

A gene-transfer method was used to isolate *FUT1* [72–74]. Human genomic DNA was transfected into cultured mouse cells, which have all of the apparatus necessary to produce H-active carbohydrate chains apart from the *H*-gene-specified α1,2-fucosyltransferase. Transfected cells expressing H antigen were isolated with H-specific monoclonal antibodies and the human DNA in those cells used to produce secondary transfectants in mouse cells. Again cells producing H antigen were isolated immunologically. With an *Eco*RI restriction fragment common to all secondary transfectants expressing H as a probe, a mammalian cDNA library was screened; the *H* gene was isolated, cloned, sequenced, and expressed in cultured monkey (COS-1) cells [72,73]. The expressed enzyme was an α1,2-L-fucosyltransferase with an apparent K_m very similar to that of H-transferase and different from the putative *Se* gene product (see Section 2.3.1.2).

Stable transfection of Chinese hamster ovary (CHO) cells with human *FUT1* cDNA revealed that H-transferase does not indiscriminately act on all glycans, but favours glycoproteins containing polylactosamine sequences [75]. This explains why ABH expression is restricted to relatively few red cell surface glycoproteins.

Most people have H antigen on their red cells. Rare alleles at the *FUT1* locus produce little or no active transferase and individuals homozygous for these alleles have little or no H on their red cells (see Section 2.12).

2.3.1.2 Secretions

Almost everybody expresses H antigen on their red cells, but only about 80% of Caucasians have H antigen in their body secretions. These people are called ABH secretors because, if they have an *A* and/or *B* gene, they also secrete A and/or B antigens. The remaining 20% are called ABH non-secretors as they do not secrete H, A, or B, regardless of *ABO* genotype. In people of European and African origin, ABH secretor status appears to be controlled by a pair of alleles, *Se* and *se*, at the secretor locus (*FUT2*). *Se*, the gene responsible for H secretion, is dominant over *se* [76] (see Section 2.6).

The very different conformations of Type 1 (Galβ1→3GlcNAc) and Type 2 (Galβ1→4GlcNAc) disaccharides in two-dimensional models led Lemieux [77] to suggest the probable existence of two distinct fucosyltransferases, one specific for a Type 1 chain and the other for a Type 2 chain. It was well established that red cells produce only Type 2 H structures, whereas secretions of ABH secretors contain both Type 1 H and Type 2 H. Oriol and his colleagues [78,79] proposed that the *H* gene codes for an α1,2-fucosyltransferase specific for Type 2 substrate and is present in haemopoietic tissues, and that the *Se* gene codes for an α1,2-fucosyltransferase that utilises both Type 1 and Type 2 substrates and is present in secretory glands. Identification of two human α1,2-fucosyltransferases with slightly different properties and subsequent cloning of two α1,2-fucosyltransferase genes has confirmed the concept of two structural genes.

Le Pendu *et al.* [64] compared α1,2-fucosyltransferase from the serum of non-secretors with that from the serum of rare ABH secretors who lack H from their red cells (para-Bombay phenotype, see Section 2.12.3). The former transferase mostly originates from haemopoietic tissues and is the product of *FUT1*; the latter is believed to be the *FUT2* product [64,80]. Fucosyltransferases from these two sources differed from each other in various physicochemical characteristics such as K_m for GDP-fucose and sensitivity to heat inactivation. The transferase present in the serum of the non-secretors (*FUT1* product) favoured Type 2 acceptors, whereas that in

serum from the secretors with H deficient red cells (*FUT2* product) showed a definite preference for Type 1 substrate. Other, similar studies produced comparable results [65,66] and two α1,2-fucosyltransferases with different K_m values and electrophoretic mobilities were purified from pooled human serum [81].

In 1995, Rouquier *et al.* [70] exploited the close homology between the two α1,2-fucosyltransferase genes to clone *FUT2* from a human chromosome 19 cosmid library by cross-hybridisation with *FUT1* cDNA. *FUT2* encodes a 332 amino acid polypeptide, with substantial sequence homology to the product of *FUT1*, plus an isoform with 11 extra residues at the N-terminus [71]. The expressed product had α1,2-fucosyltransferase activity with a pH optimum and K_m similar to that ascribed to the secretor-transferase.

The common non-secretor allele of *FUT2* in people of European and African origin (se^{428}), with frequencies of 43–52% and 22–47%, respectively, contains a 428G>A nonsense mutation converting the codon for Trp143 to a translation stop codon, so no active enzyme is produced [71,82,83] (Table 2.7). This allele often also encodes a Gly247Ser substitution, but that change alone does not affect α1,2-fucosyltransferase activity [67,71].

The se^{428} allele is rare in Eastern Asia, but another *FUT2* allele (Se^{w385}), common in Eastern Asia and the South Pacific, encodes Ile129Phe in the stem region of the α1,2-fucosyltransferase [67,83,85–89]. This enzyme has identical substrate specificities to the normal *FUT2* product, but has at least a five-fold reduction in enzyme activity [67,86,87]. Se^{w385} has a gene frequency of 44% in Eastern Asia [83,86] and 40% in Samoa [94], but is very rare in Europeans and Africans [67,82]. Homozygosity for Se^{w385} (or heterozygosity for Se^{w385} and a non-secretor allele) results in reduced levels of secreted H and the Le(a+b+) red cell phenotype (Section 2.6.3). Se^{w385} also contains 357C > T, a synonymous change. In the Uygur of Urumqi (west of China) and in Bangladeshis both se^{428} and Se^{w385} are present with similar frequencies, suggesting admixed populations [94,97].

Many other inactive (non-secretor) alleles containing nonsense mutations have been found, some of which are listed in (Table 2.7) [83,84,89]. An allele with a single base deletion (778delC) was found in two of 101 black South Africans (Xhosa) [82].

Three alleles with deletions of exon 2 of *FUT2*, the whole of the coding region of the gene, were generated by three distinct *Alu–Alu* recombinations: se^{del} (10 kb deletion); se^{del2} (9.3 kb); se^{del3} (4 kb). Indian people with the rare Bombay phenotype have no H antigen on their red cells or in their secretions (Section 2.12.1). This phenotype results from homozygosity for an inactivating missense mutation in *FUT1* (Leu242Arg) together the se^{del} allele of *FUT2* [92,93]. The se^{del} allele linked with an active *FUT1* is relatively common in Bangladesh (7.4%) and in the Tamils of Sri Lanka [94,95]. Another *FUT2* deletion, se^{del2}, has a frequency of 10.4% in Samoans [94]. The se^{del3} allele was found in one Chinese [98].

Two inactive fusion genes are hybrids of *FUT2* and the pseudogene *SEC1*. One, se^{fus}, with a frequency of 5.5–7.9% in Japanese [96], consists of the 5′ region of *SEC1* and the 3′ region of *FUT2* and is presumably a product

Table 2.7 Some *FUT2* alleles responsible for ABH non-secretor phenotypes (*se*) or partial-secretor phenotype (Se^w).

Allele		Mutation	Amino acid substitution	Population	References
se^{302}		302C>T	Ile101Pro	Bangladeshi, Sri Lankan	[84]
Se^{w385}	*FUT2*01W.02*	385A>T	Ile129Phe	E. Asian, Polynesian, Filipino	[67,85–89]
se^{428}	*FUT2*01N.02*	428G>A	Trp143Stop	European, African	[71,82]
se^{571}	*FUT2*01N.04*	571C>T	Arg191Stop	E. Asian, Polynesian, Filipino, European	[71,82,86,89–91]
se^{688}	*FUT2*01N.09*	688_690del GTC	del230Val	Filipino	[91]
se^{778}	*FUT2*01N.11*	778delC	259fs275Stop	African	[82]
se^{849}	*FUT2*01N.12*	849G>A	Trp283Stop	Eastern Asian, Filipino	[89–91]
se^{del}	*FUT2*0N.01*	del exon 2		Indian, Bangladeshi, Sri Lankan	[92–95]
se^{del2}	*FUT2*0N.02*	del exon 2		Polynesians	[94]
se^{fus}	*FUT2*0N.03*	*SEC1–FUT2* fusion		Japanese	[86,96]

del, deletion; fs, reading frameshift.

of unequal crossing-over [86]. The other, a *SEC1–FUT2–SEC1* hybrid that was probably generated by gene conversion with the *FUT2* sequence derived from a se^{428} allele, has only been found in one person [99].

A single, multiplex PCR technique followed by RFLP digestion has been devised to detect many of the known *FUT2* mutations [100].

2.3.1.3 Other tissues

Control of expression of H antigen in various human tissues follows a general trend, summarised as follows: H antigens on tissues of ectodermal and mesodermal origin (e.g. primary sensory neurons, skin, vascular endothelium, and bone marrow) are Type 2 structures and produced by *FUT1*-specified α1,2-fucosyltransferase; those on tissues of endodermal origin (digestive and respiratory mucosae, salivary glands) are Type 1 and Type 2 structures and produced by the *FUT2*-specified enzyme [21]. There are, however, a number of exceptions to these rules (Section 2.19.3). Plasma α1,2-fucosyltransferase is predominantly haemopoietic in origin [101] and may originate from circulating red cells and platelets [102].

2.3.2 ABO antigens

2.3.2.1 ABO biosynthesis

H antigen, whether synthesised by the product of *FUT1* or *FUT2*, is the acceptor substrate of both *A* and *B* gene-specified glycosyltransferases (GTA and GTB) (Figure 2.1). GTA is an α1,3-*N*-acetyl-D-galactosaminyltransferase that transfers GalNAc from a uridine diphosphate (UDP)-GalNAc donor to the fucosylated Gal residue of the H antigen. GTB is an α1,3-D-galactosyltransferase that transfers Gal from UDP-Gal to the fucosylated Gal of H (Figure 2.3). *A* and *B* are alleles at the *ABO* locus; a third allele, *O*, does not produce an active enzyme and in *O* homozygotes H antigen remains unmodified. If no H structure is available, owing to the absence of H-transferase, A and B antigens cannot be produced despite the presence of GTA or GTB. This situation occurs in the secretions of ABH non-secretors and on red cells of the rare H-deficient (Bombay) phenotypes. The different species of GTA associated with A_1 and A_2 phenotypes are described in Section 2.4.1.

Anti-H reagents agglutinate group O cells far more readily than most A and B cells because H antigen activity is masked by GalNAc and Gal in A- and B-active structures.

A-, B-, and H-transferase activity has been demonstrated *in vitro*. GTA prepared from human gastric mucosa and other sources converts O or B cells to A or AB in the presence of UDP-GalNAc; likewise GTB from similar sources converts O cells to B cells in the presence of UDP-Gal [103–106]. Bombay phenotype cells, which lack the H-active substrate, could not be converted to B with GTB [104].

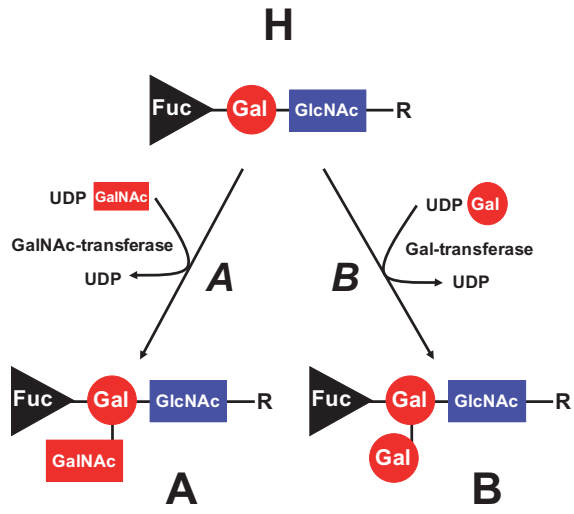

Figure 2.3 Pathways for biosynthesis of A and B antigens from their precursor, H.

2.3.2.2 Molecular genetics

GTA was purified to homogeneity from human lung and gastric tissues, and partial amino acid sequences were obtained [107,108]. Degenerate synthetic oligodeoxynucleotides based on the GTA partial amino acid sequence were employed by Yamamoto *et al.* [109] in the isolation and cloning of cDNA representing the *A* allele. The cDNA library was constructed from RNA isolated from a human gastric carcinoma cell line that expressed high levels of A antigen. The 1062 basepair (bp) sequence predicted a 353 amino acid protein with the three-domain structure characteristic of a glycosyltransferase. After the initial publication [109], it became apparent that the original clone from a gastric carcinoma contained a unique 3 basepair deletion [110]. The numbering of nucleotides and encoded amino acids used in this chapter and in most publications reflects the usual sequence of the gene. Based on the cDNA clone encoding GTA, *B* and *O* cDNA was also cloned and sequenced [111,112].

The coding region of *ABO* is organised into seven exons, spanning 18 kb. Exons 6 and 7 constitute 77% of the coding sequence [110,113] (Figure 2.4).

A and *B* alleles differ by seven nucleotides in exons 6 and 7, four of which encode amino acid substitutions (Figures 2.5 and 2.6). The most common *O* sequence is identical to that of A^1 apart from a deletion of nucleotide 261 in exon 6 causing a shift in the reading frame and generation of a premature translation stop signal at the codon for amino acid residue 116. This allele encodes a truncated protein with no catalytic domain (Figure 2.6) and may produce a mRNA transcript of reduced stability [114]. Cloned *A* and *B* cDNA transfected into recipient cells expressing H antigen resulted in A and B phenotypes that could be detected immunologically. The common *A* sequence in Caucasians (A^1 or *A101*) is often referred to as the 'consensus sequence' and is used as a reference for the sequences of all other *ABO* alleles. About 80% of A^1 alleles (*A102*) in Japanese and 93% in Chinese Han differ from A^1 in Europeans (*A101*) by 467C>T encoding Pro-156Leu [115–117]. This has no apparent affect on the phenotype. The A^2 (*A201*) allele has a single nucleotide deletion in the codon before the translation stop codon of A^1, resulting in disruption of that stop codon and a GTA product with an extra 21 amino acids at the C-terminus [118] (see Section 2.4.1).

Figure 2.4 Genomic organisation of the *ABO* gene, showing the seven coding exons and the number of amino acids encoded by each exon.

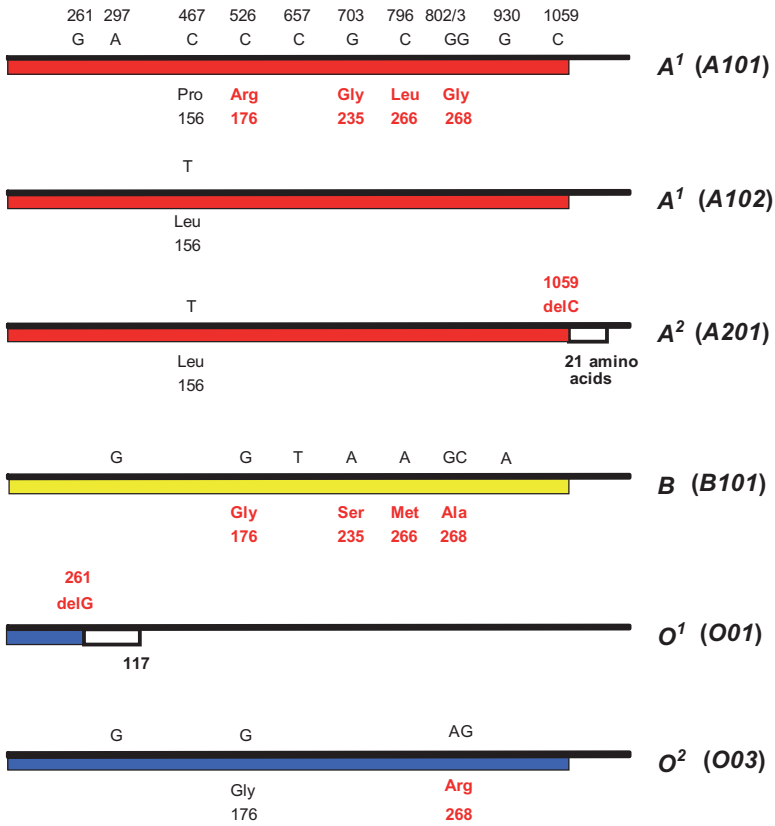

Figure 2.5 Diagram representing cDNA (black line) and protein products (coloured box) of six common *ABO* alleles, showing how they differ from the A^1 (*A101*) cDNA and its product. Seven nucleotide changes distinguish *A* and *B* alleles and result in four amino acid differences between GTA and GTB. A^1 (*A101*) and A^1 (*A102*) are the common A^1 alleles in Caucasians and East Asians, respectively. Single base deletions in A^2 and O^1 result in reading frameshifts and introduction and abolition of stop codons in O^1 and A^2, respectively. Amino acid substitution at position 268 is responsible for inactivation of the O^2 product.

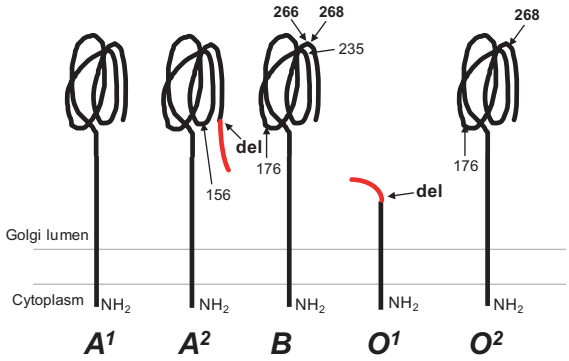

Figure 2.6 Diagrammatic representation of the products of five *ABO* alleles located in the membrane of the Golgi apparatus (modified from Clausen *et al.* [20], Copyright 1994, with permission from Elsevier), showing the positions of amino acids that differ from those of GTA_1 and the positions relating to the nucleotide deletions (del) in the A^2, O^1 alleles. Regions shown in red are the extra 21 amino acids in GTA_2 and the sequence encoded between the nucleotide deletion and the stop codon in the product of O^2.

The *O* allele described by Yamamoto *et al.* [111,112], with an A^1 sequence disrupted by a single base deletion, 261delG, is now named O^1 (*O01*). Another very common *O* allele, O^{1v} (O^1-variant, *O02*), has 261delG of O^1, preventing the production of any active transferase, but contains at least nine other nucleotide differences from O^1 and A^1 [119]. O^1 and O^{1v} are by far the most common *O* alleles and are present in all populations tested. The proportions of *O* alleles with 261delG that are O^{1v} are as follows: Swedes, 42% [119]; Australians, 42% [120]; Kuwaitis, 45% [121]; black Brazilians, 31% [122]; native Brazilians, 91% [122]; Japanese, 49–55% [115,116]; and Chinese, 40% [117,123]. Many rare variants of O^1, differing by a few point mutations, have been described [24]. A much less common *O* allele than O^1 and O^{1v}, O^2 (*O03*), lacks 261delG, but has nucleotide differences from A^1 exon 7 that encode Arg176Gly (identical to that of GTB) and Gly268Arg [124,125] (Figures 2.5 and 2.6). The substitution at position 268 introduces a charged arginine residue, completely blocking the donor GalNAc-binding site of GTA, whilst leaving the acceptor binding site unaffected [126]. *In vitro* expression of an A^1 cDNA construct with the Gly268Arg substitution introduced by site-directed mutagenesis resulted in no GTA activity or A antigen expression [127]. Between 2 and 6% of *O* alleles in white donors from Europe, Australia, and the United States are O^2 [120,125,128–131]; O^2 is not found in Japanese or Chinese [115,116,123]. Any *ABO* mutation that prevents production of an active transferase will be an *O* allele and numerous unique or very rare *O* alleles have been found, some having 261delG, others containing nonsense, frameshift, or enzyme-inactivating missense mutations [132–135]. In addition, hybrid genes containing 261delG will be *O* alleles. The debate on whether O^2 and other apparently inactivated *A* alleles produce any A antigen is discussed in Section 2.7.8.

Yamamoto and Hakomori [112] constructed *A-B* cDNA chimeras representing all 16 possible combinations of the four amino acid substitutions distinguishing *A* and *B* cDNA. Transfection experiments, in a group O human cell line, demonstrated that the third (266) and fourth (268) amino acid substitutions (Figure 2.5) are the most important in determining the specificity of the transferase. An enzyme with Met266 and Gly268 had dual GTA and GTB activity. *In vitro* mutagenesis experiments, in which cDNA constructs encoding every possible amino acid residue at position 268 were expressed, led to the conclusion that the side chain of the amino acid residue at position 268 is responsible for determining both activity and donor-substrate specificity of the transferase product [127] (and see Section 2.3.2.3).

The *ABO* gene contains a CpG island that extends from the immediate 5′ flanking region, through the first exon, and into the first intron. Methylation of this CpG island may play an important role in regulation of *ABO* expression in different tissues [136]. The most commonly used transcription site appears to be 12–38 bases upstream of the translation initiation codon in exon 1, but an alternative first exon (exon 1a) and transcription start site, utilised by both erythroid and epithelial lineages, is present at the 5′ end of the CpG island [137,138]. Exon 1a does not contain an ATG codon, but translation may be initiated from an alternative site in the transmembrane domain [138]. The promoter region binds the ubiquitous transcription factors Sp1 or Sp1-like [139] and transcription from the proximal promoter is partially dependent on an upstream N box [140]. In addition there is an erythroid-specific enhancer element within intron 1, 5.6–6.1 kb from the translation initiation site, containing two binding sites for the GATA-1 haemopoietic transcription factor [141]. Deletion of this intron 1 site results in the rare B_m phenotype, with almost no red cell B antigens expression, yet normal B antigen content in saliva (Section 2.8.3). Transcription regulation of *ABO* may also be dependent on a minisatellite, 3.8 kb upstream of the start of the translated sequence, that contains a CBF/NF-Y transcription factor-binding motif [142]. This

minisatellite usually consists of four copies of a 43 bp repeat sequence in A^2, B, O^1, and O^{1v} alleles, but only one copy in A^1 and O^2 alleles [143–145]. Transient transfection assays in a gastric cancer cell line suggested that the transcriptional activity of the *A* enhancer was substantially less than that of the *B* enhancer [144,146]. Strangely, transcripts from A^1 and A^2 alleles were not detected in peripheral blood, in contrast to readily detectable transcripts from B, O^1, O^{1v}, and O^2, whereas erythroid cells cultured from bone marrow expressed higher levels of A^1 and A^2 transcripts than those from B, O^1, O^{1v}, and O^2 [147]. Some weak B phenotypes appear to have been caused by sequence variations in the CBF/NF-Y regulatory region [148], although any affect of the number of repeats on transcription levels in another weak B phenotype is disputed [149] (Section 2.8.5).

2.3.2.3 ABO fusion genes

Many complexities of the *ABO* genes have been encountered. Some unusual *ABO* genes affect activity of the gene products and may result in subgroups of A and B (Sections 2.7 and 2.8). Numerous genes have been identified that appear to be hybrids, comprising partly of sequences characteristic of one *ABO* allele and partly of sequences characteristic of another. These fusion genes have probably arisen by meiotic crossing-over; in most the recombination has occurred within intron 6. Chester and Olsson [24] remark that the presence of *Chi* or *Chi*-like sequences near the 3′ end of intron 6, sequences associated with recombination hot-spots in *Escherichia coli*. Hybrid genes with exon 6 derived from O^1 or O^{1v} have 261delG and are inactive, regardless of the origin of exon 7. Hybrid genes with exon 6 derived from *A* or *B* are generally active, with the origin of exon 7 determining specificity. Exon 7 with A^1 or O^1 origin gives rise to A_1 activity; exon 7 with O^{1v} origin results in weakened A activity (A_2 or A_x).

Suzuki *et al.* [150] described a paternity case in which the mother was group B, the child group A, and the putative father group O; an apparent first order exclusion of paternity. Sequencing of the *ABO* genes showed that the child had an *ABO* gene in which exon 6 (and, presumably exons 1–5) had the sequence of a *B* allele and exon 7 the sequence of an O^1 allele. This hybrid gene had probably arisen in the germline of the mother as a result of crossing-over during meiosis. This B-O^1 gene would encode an enzyme with GTA activity because O^1 and A^1 have an identical sequence in exon 7, the region encoding the catalytic site; the absence of 261delG in exon 6 of *B* origin enables translation of this active enzyme. The child, therefore, had group A red cells, despite neither parent having an *A* gene. Such genetic events may be considered to be rare, yet similar recombinant alleles were estimated to occur with a frequency of about 1% in the Japanese population [150].

2.3.2.4 Linkage and evolution

ABO is closely linked to a gene for nail-patella syndrome (*LMX1B*), a dominantly inherited disorder characterised by dystrophic nails and deformed patellae and elbow joints, and the gene for adenylate kinase 1 (*AK1*). *ABO* location on the long arm of chromosome 9 was confirmed by *in situ* hybridisation [113] and the gene is now localised to 9q34.2.

The *ABO* genes have been well conserved during evolution [151,152,153]. *ABO* is part of the GT6 glycosyltransferases gene family, which is represented in all vertebrates [154]. Six GT6 genes other than *ABO* are present in humans, but all are pseudogenes and include *GBTG1*, the usually inactive Forssman-synthetase gene on chromosome 9q34.2 (but see Section 4.7), and the *ABO* pseudogene on chromosome 19 [111,155]. A minimum of 95% homology in nucleotide and deduced amino acid sequences was detected in the *ABO* genes of primates [151]. The critical substitutions differentiating the *A* and *B* genes occurred before the divergence of the lineages leading to humans, chimpanzees, gorillas, and orangutans [142]. The common human *O* mutation, 261delG, probably appeared once in human evolution, in the more ancient O^{1v} allele, with O^1 arising from recombination between O^{1v} and A^1 [132]. Kitano *et al.* [156] estimate that human *O* alleles appeared about 2 million years ago. From a phylogenetic network analysis involving the six most common *ABO* alleles, they propose that the original *A* allele became extinct in the human lineage and was resurrected less than 300 000 years ago as A^1 (*A101*) by recombination between *B* (exons 1–6) and O^1 (exon 7) [156].

2.3.2.5 Structure of the A- and B-transferases (GTA and GTB)

GTA and GTB, GalNAc-transferase and Gal-transferase, respectively, are called retaining enzymes because they do not alter the configuration of the nucleotide donor. They differ by four amino acids and are the two most homologous, naturally occurring glycosyltransferases that transfer different naturally occurring donors [157]. Models derived from the crystal structures of their catalytic

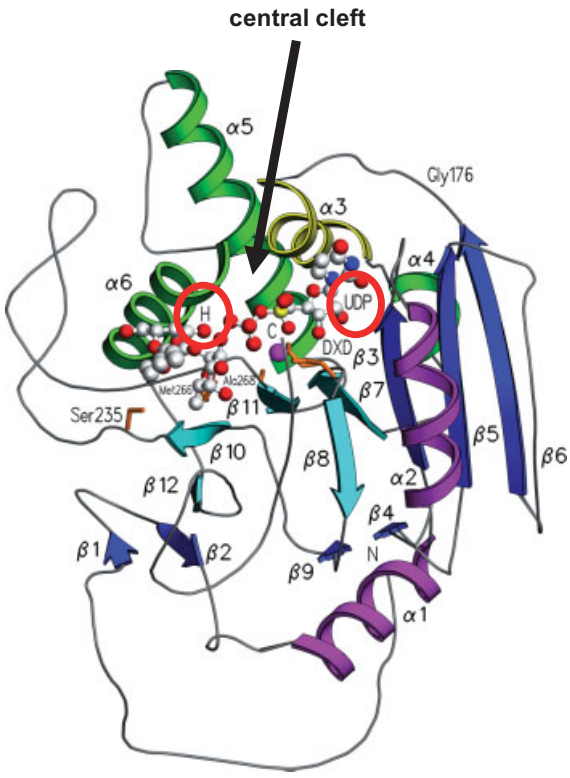

Figure 2.7 Structural model of GTB showing the two domains separated by a large central cleft, UDP-Gal and H antigen. Modified from Patenaude *et al.* [158] and provided by Dr Stephen Evans, University of Victoria, Canada.

domains, and of the enzymes in complex with the H-antigen disaccharide and UDP, reveal two domains separated by a cleft ~13 Å wide and containing the active site and all four amino acids that differ in GTA and GTB [158] (Figure 2.7). Of the four critical amino acids only residues 266 and 268 are positioned to contact donor and acceptor substrates and only Leu/Met266, which is most important for selection between donor sugars, is positioned to contact the characteristic acetamido/hydroxyl groups and so distinguishes UDP-GalNAc from UDP-Gal. The larger acetamido group in UDP-GalNAc is accommodated by the smaller Leu266 in GTA and the smaller hydroxyl group on UDP-Gal is accommodated by the larger Met266 in GTB. A fold near the active site, which is disordered in the unliganded state, undergoes conformational change and becomes ordered to cover the active site on binding of substrate [157,158].

2.3.2.6 Predicting ABO phenotype from DNA testing

Numerous methods have been developed for determining *ABO* genotype from genomic DNA and predicting ABO phenotype. Most tests involve two reactions: one determining the presence or absence of 261delG in exon 6; the other detecting the sequence for *A* (and possibly for A^1 and A^2), *B*, and O^2 in exon 7. Owing to the distance between the exon 6 and 7 critical sequences, haplotypes are rarely distinguished. Consequently, phenotypes are predicted on the basis that 261delG in exon 6 is usually associated with the *A* sequence in exon 7. To take an example, the presence and absence of 261delG in exon 6 together with an *A* and *B* sequence in exon 7 would be interpreted as group B phenotype, as the presence of 261delG would be assumed to be linked to the *A* sequence, representing an *O* allele, and the absence of 261delG would be assumed to be linked to the *B* sequence, representing a *B* allele. In most cases this interpretation would be correct, but errors could occur with A or B variants, non-deletion *O* alleles other than O^2, and hybrid alleles where 261delG is linked to a *B* sequence. Although errors arising from the presence of such confounding alleles would be relatively rare, any level of inaccuracy is unacceptable in ABO typing for transfusion purposes.

One method involving multiple PCR amplifications with allele-specific primers, some of which span from the 261-deletion site in exon 6 to various positions in exon 7 avoids most potential errors, including those arising from hybrid alleles [159]. A strategy that could be automated and could be accurate, involves sequence-based typing on physically separated haplotypes [160].

2.3.3 Lewis antigens

In 1948 Grubb [161] made the observation that people with Le(a+) red cells were mostly non-secretors of ABH. Subsequently the following general rule has been established for red cell Lewis phenotypes in adults:

Le(a+b–) red cells come from ABH non-secretors;
Le(a–b+) red cells come from ABH secretors;
Le(a–b–) red cells come from ABH secretors or non-secretors;
Le(a+b+) red cells come from ABH weak secretors.

Clearly there is an interaction between *FUT3*, the gene responsible for Le^a and Le^b on red cells, and the Secretor gene (*FUT2*). The Lewis and Secretor loci were shown by family studies to be genetically independent [162], although they are both on chromosome 19.

The Lewis-related antigens, Le^c and Le^d, are described in Section 2.18.2.

2.3.3.1 Lewis biosynthesis

The Lewis (*Le*) gene product is an α1,4-L-fucosyltransferase [163,164], which catalyses the transfer of L-fucose (Fuc) from GDP-Fuc to the GlcNAc of Type 1 acceptor substrates; to Type 1 precursor to form Le^a; to Type 1 H to form Le^b; to Type 1 A to form ALe^b; and to Type 1 B to form BLe^b. A pattern of interactions between *FUT3*, *FUT2*, and *ABO* determine whether Le^a or Le^b, or both, or neither, are present in secretions, plasma, and on red cells (Figure 2.1).

At the simplest level, two alleles at the *FUT3* locus can be considered: *Le*, which encodes an α1,4-fucosyltransferase, and *le*, which is apparently silent. People homozygous for *le* secrete neither Le^a nor Le^b and have the Le(a–b–) red cell phenotype, regardless of their ABH and secretor phenotypes.

In ABH non-secretors (*se/se*), no α1,2-fucosyltransferase is present in secretions to convert Type 1 precursor to Type 1 H. Consequently, the Type 1 precursor is available as an acceptor substrate for the Le-transferase, resulting in production of the monofucosylated Le^a antigen; so the secretions contain Le^a and the red cells are Le(a+b–). People with an *Se* allele produce Type 1 H, which can then be converted by the Le-transferase to the difucosylated Le^b antigen. If they also have an *A* or *B* gene, much of the Type 1 H will be converted to A or B structures and so the Le-transferase will produce ALe^b or BLe^b. Although Le-transferase can utilise either Type 1 precursor or Type 1 H acceptor substrates to produce Le^a and Le^b respectively, Le^a is a very poor substrate for the *Se* gene specified α1,2-fucosyltransferase. Consequently, there is competition between these two enzymes for substrate [165,166]. If any Le^a is produced from Type 1 'precursor' by the Le-transferase it cannot be converted further to Le^b by Se-transferase, so secretions of a person with *Le* and *Se* genes contain Le^a and Le^b, although very little Le^a is detected in the plasma or on the red cells. Similarly, Le^b is not an acceptor substrate for the A and B transferases, and secretions of an individual with *Le*, *Se* and *A* genes contain Le^a, Le^b, and ALe^b (Figure 2.1). The product of the weak secretor gene (Se^w), common in Eastern Asia and Pacific regions, competes with the Le-transferase less effectively than that of an *Se* allele, resulting in substantially greater production of Le^a than present in secretors. People homozygous for Se^w, or heterozygous Se^w/se, have Le^a and Le^b in their plasma and secretions and Le(a+b+) red cells [67,85,88,167,168].

Le-transferase has the exceptional ability to catalyse two distinct glycosidic linkages. In addition to α1,4-fucosyltransferase activity, it has some α1,3-fucosyltransferase activity and is often referred to as an α1,3/4-L-fucosyltransferase [169–172], although it is almost 100 times more efficient on Type 1 H than Type 2 H acceptors [173].

2.3.3.2 Molecular genetics

Kukowska-Latallo *et al.* [172] employed a gene transfer technique (like that described in Section 2.3.1.1 for isolation of *FUT1*) to clone and sequence cDNA encoding *Le* gene-specified α1,3/4-fucosyltransferase. The gene contains an intronless coding region that encodes a 361-amino acid protein with the three-domain structure typical of glycosyltransferases. There is a high level of sequence identity with some of the α1,2- and α1,3-fucosyltransferase genes.

The genetic basis for the Le(a–b–) red cell phenotype is heterogeneous, but is always associated with one or more missense mutations within the region of *FUT3* encoding the catalytic domain of the Lewis-transferase (Table 2.8). No Lewis nonsense mutation has been found. Transfection experiments with cDNA or chimeric *FUT3* constructs showed that Trp68Arg, Gly170Ser, and Ile356Lys caused complete or almost complete inactivation of α1,3/4-fucosyltransferase activity [175–177]. The enzyme is not inactivated by Thr105Met, which is associated with Trp68Arg [175]. The mutation encoding Leu20Arg is common in Lewis-negative alleles (Table 2.8). This substitution occurs within the transmembrane domain of the enzyme and does not affect catalytic activity [176,178,179], but may affect anchoring of the enzyme in the Golgi membrane [176]. Leu20Arg in the absence of any other Lewis mutation is relatively common in Indonesians and people homozygous for this allele have Le(a–b–) red cells, but secrete Lewis antigens [176].

In Caucasian populations, $le^{202,314}$ and $le^{59,1067}$ are the two most frequent Lewis-negative alleles [174,180,181], whereas $le^{59,508}$ is the most frequent in black Africans and in Eastern Asia (including $le^{59,508,980}$ in Africans) [87,174, 177,181,182] (Table 2.8).

The positions of the inactivating mutations in *FUT3* suggest that the catalytic domain of the Lewis-transferase includes the region from amino acid residues 68 to 356. Expression of *FUT3* constructs that produce truncated proteins demonstrated that a protein consisting of amino acids 62 to 361 is enzymatically active, but shorter forms were inactive [183].

Table 2.8 Some *FUT3* alleles, the encoded amino acid substitutions, and their frequencies in three populations (data from [174]). Synonymous substitutions are not shown.

Symbol	Amino acids															Allele frequencies		
	5	16	20	68	105	149	151	162	170	191	223	270	325	327	356	Caucasian	Ghanaian	Mongolian
*Le**	Gly	Cys	Thr	Trp	Thr	Leu	Arg	Asn	Gly	Glu	Gly	Val	Thr	Arg	Ile	0.71	0.44	0.61
$le^{13,484,667}$	Ser	–	–	–	–	–	–	Asp	–	–	Arg	–	–	–	–	0	0.10	0
$le^{13,484,667,808}$	Ser	–	–	–	–	–	–	Asp	–	–	Arg	Met	–	–	–	0	0.04	0
$le^{13,484,667,974}$	Ser	–	–	–	–	–	–	Asp	–	–	Arg	–	Met	–	–	0	0.03	0
$le^{47,202,314}$	–	Ser	–	Arg	Met	–	–	–	–	–	–	–	–	–	–	0.03	0	0
$le^{59,508}$	–	–	Arg	–	–	–	–	–	Ser	–	–	–	–	–	–	0.02	0.22	0.24
$le^{59,508,980}$	–	–	Arg	–	–	–	–	–	Ser	–	–	–	–	Gln	–	0	0.02	0
$le^{59,202,1067}$	–	–	Arg	Arg	–	–	–	–	–	–	–	–	–	–	Lys	0.01	0	0
$le^{59,445}$	–	–	Arg	–	–	Met	–	–	–	–	–	–	–	–	–	0.01	0	0
$le^{59,508}$	–	–	Arg	–	–	–	–	–	Ser		–	–	–	–	–	0	0	0.05
$le^{59,571,1067}$	–	–	Arg	–	–	–	–	–	–	Lys	–	–	–	–	Lys	0	0	0.01
$le^{59,1067}$	–	–	Arg	–	–	–	–	–	–	–	–	–	–	–	Lys	0.04	0.01	0.03
$le^{202,314}$	–	–	–	Arg	Met	–	–	–	–	–	–	–	–	–	–	0.17	0.07	0.06
$le^{202,314,451}$	–	–	–	Arg	Met	–	Gly	–	–	–	–	–	–	–	–	0	0.02	0
Other *le*																0.01	0.05	0

*Includes all functional alleles.

α1,4-fucosyltransferase activity has been identified in a number of tissues and secretions: kidney, gastric mucosa, submaxilliary glands, ovarian cyst linings, saliva, milk (see [10]). α1,4-fucosyltransferase activity has not been detected in serum, red cells, lymphocytes, granulocytes, or platelets [170,184–186], suggesting that there is no haemopoietic origin for this enzyme. High levels of *FUT3* transcripts are present in colon, stomach, small intestine, lung, and kidney; lesser amounts are present in salivary gland, bladder, uterus, and liver [187].

2.3.4 Le^x, Le^y, and sialyl-Le^x

Le^x (CD15) and Le^y represent the Type 2 isomers of Le^a and Le^b, respectively (Table 2.3). An α1,3-L-fucosyltransferase catalyses the transfer of Fuc from a nucleotide donor to C-3 of the subterminal GlcNAc of Type 2 precursor, Type 2 H, Type 2 A, or Type 2 B to produce Le^x, Le^y, ALe^y, and BLe^y, respectively (Figure 2.1). In analogy with the Lewis structures, Le^x is not converted to Le^y by H-transferase or Se-transferase, and Le^y is not converted to ALe^y or BLe^y by GTA or GTB. (The antigen described here as Le^x differs from the original Le^x antigen, called Le^{abx} in this chapter, see Section 2.18.1.)

Fucosylation of a 2,3-sialylated acceptor produces sialyl-Le^x (sialyl-CD15) [188,189] (Table 2.3), a ligand for the selectin family of cell adhesion proteins [190,191] (Section 2.18.3).

2.3.5 Other fucosyltransferase genes

In addition to *FUT3*, four other genes encoding enzymes with α1,3/4-fucosyltransferase activity, *FUT4–FUT7* and *FUT9*, plus two others with α1,3-fucosyltransferase activity, *FUT10* and *FUT11*, have been identified [192]. *FUT3*, *FUT5*, and *FUT6* have about 90% sequence homology and form a cluster on chromosome 19p13.3 (*pter–FUT6–FUT3–FUT5–cen*) [193], as part of a linkage group including *FUT1*, *FUT2*, *ICAM4* (*LW*), and *BCAM* (*LU*) (Section 6.2.4). The *FUT6–FUT3–FUT5* cluster, and possibly the other fucosyltransferase genes, probably arose by successive duplications followed by translocations and divergent evolution from a single ancestral gene. *FUT8*, which encodes an α1,6-fucosyltransferase, may represent the ancestral gene [173].

Nine percent of Indonesians from Java have α1,3-fucosyltransferase deficiency as a result of inactivating mutation in *FUT6*. Ninety-five percent of these individuals have Le(a–b–) red cells, indicating linkage disequilibrium between *FUT3* and *FUT6* [194].

Part 3: ABO, H, and secretor

2.4 A_1 and A_2

The existence of subgroups of A, with red cells of one subgroup demonstrating weaker expression of A antigen than those of the other, was first recognised by von Dungern and Hirszfield [195] in 1911. Landsteiner and Levine [196] named the two major subgroups A_1 and A_2.

The usual way of interpreting the A_1 and A_2 subgroups is as follows:

Group	Antigens	Anti-A (group B serum)	
		Anti-A	Anti-A_1
A_1	A A_1	+	+
A_2	A	+	–

Sera from group B individuals appear to contain two antibody components, anti-A and -A_1. A_1 cells react with both components, whereas A_2 cells react only with anti-A. Adsorption of some group B sera with A_2 cells removes anti-A leaving behind anti-A_1 [195]; continued adsorption of group B serum with A_2 cells, however, eventually removes all antibody [197]. Regrettably, the term anti-A has two meanings: the antiserum that reacts with A and AB cells and one of the two antibody components present in group B serum. In this chapter, the precise meaning of 'anti-A' should be apparent from its context.

Anti-A_1 is present in the serum of some A_2 and A_2B people [198,199]. By agglutination of A_1 cells at room temperature, anti-A_1 was found in the serum of 1–2% of A_2 and 22–26% of A_2B individuals [200,201]. More sensitive techniques revealed anti-A_1 in higher proportions of A_2 and A_2B donors [202,203].

The best and most widely used anti-A_1 reagent is *Dolichos biflorus* lectin [204]. Raw extract of *Dolichos* seeds agglutinates A_1 and A_2 red cells, but at a suitable dilution the lectin will easily distinguish A_1 and A_1B from A_2 and A_2B. Red cells from group A babies usually react only weakly with *Dolichos* lectin and may not be agglutinated at all by human anti-A_1. It should be remembered that *Dolichos* lectin also agglutinates rare red cells with a very

Table 2.9 A_1A_2BO genotypes and serologically determined phenotypes.

Genotype	Phenotype
A^1/A^1, A^1/A^2, A^1/O	A_1
A^2/A^2, A^2/O	A_2
B/B, B/O	B
A^1/B	A_1B
A^2/B	A_2B
O/O	O

strong Sd^a antigen and Tn polyagglutinable red cells, regardless of ABO group (Chapters 31 and 33).

A_2 red cells have substantially higher expression of H antigen than A_1 cells.

When determined by serological means, the A^1 allele appears dominant over A^2 and the genotypes A^1/A^1 and A^1/A^2 cannot be discriminated by blood grouping techniques (Table 2.9).

2.4.1 A_1- and A_2-transferases (GTA_1 and GTA_2) and the genes that produce them

A-transferase (GTA) isolated from sera or gastric mucosa of A_1 individuals is more effective at converting group O red cells to A-active cells than that from A_2 people [205–208]. When A_2 enzyme is used, the reaction is much slower and under normal conditions O cells are only converted to A_2 phenotype. After extended incubation with A_2 enzyme, however, O cells may be agglutinated weakly by A_1-specific reagents [208]. A_1 enzyme can convert A_2 cells to A_1 phenotype [206,207]. GalNAc-transferases from A_1 and A_2 sources have the same specificity for low molecular weight acceptors and both synthesise the same A determinant [10]. Yet at pH 5.5, activity of GTA from A_1 serum (GTA_1), with low molecular weight substrate, is 5–10 times higher than that from A_2 serum (GTA_2) [209].

Serum GTA_1 and GTA_2 have different pH optima: 5.6 for GTA_1 and between 7 and 8 for GTA_2 [210]. Sera from heterozygous A^1/A^2 individuals can be distinguished from sera from A^1/A^1 or A^1/O people by pH optima and by isoelectric point [211]. At pH 7.2, GTA_2, the less efficient enzyme, has a K_m value about 10 times higher than that for GTA_1 [210]. *In vitro* conversion of O cells to A activity by GTA generally requires the presence of Mn^{2+} ions. If Mn^{2+} is substituted by Mg^{2+}, GTA_1 remains active, but GTA_2 does not [210].

The A^2 allele (*A201*) in people of European origin contains a deletion of one of the three cytosines at positions 1059–1061 (CCC to CC). This deletion is in the codon before the translation stop codon and causes a reading frameshift and loss of the stop codon, resulting in a gene product with an extra 21 amino acid residues at its C-terminus [118] (Figures 2.5 and 2.6). The A^2 allele also contains 467C>T, Pro156Leu, which is common in A^1 (*A102*) in East Asia and has no effect on enzyme activity. An *A* allele with 1016delC, but without 467C>T (*A206*), has been found in Chinese [123].

In East Asia, where A_2 phenotype is rare, the A^2 allele with 1016delC is also rare. The two most common alleles responsible for A_2 in Japan do not have 1016delC, but have different missense mutations within codon 352, only three codons before the normal stop codon: 1054C>T, Arg352Trp (*A202*) and 1054C>G, Arg352Gly (*A203*) [212,213]. A B-O^{1v} hybrid allele (*A204*), also quite common in Japan, gives rise to an A_1 phenotype when paired with *O*, but an A_2B phenotype when paired with *B*, presumably because of competition for a common acceptor between the A-active hybrid transferase and GTB [213]. This allele is responsible for an imbalance in A_2 and A_2B phenotype frequencies in Japan. The most common A^2 allele among A_2B donors in Taiwan contains 467C>T, Pro156Leu and 1009A>G, Arg337Gly (*A205*) [213,214].

Other alleles responsible for an A_2 phenotype are listed in dbRBC [215]. Over 20 alleles containing 1016delC with additional missense mutations were responsible for a variety of phenotypes, ranging from very weak to nearly A_2, with the majority displaying A_x-like characteristics [216]. A weak A phenotype known as A_{bantu}, found in about 4% of black South Africans [217], results from a hybrid of the common A^2 allele with 1016delC and an O^1-like allele (O^{1bantu}), with a cross-over region near exon 5 (*Abantu01*) [218]. Another similar hybrid allele, with exons 1–5 derived from a variant O^{1v} allele and exons 6 and 7 from A^2 (*A201*), also found in people of African origin, gave rise to an A antigen weaker than that of A_2 phenotype [219].

2.4.2 A_1 and A_2 determinants differ quantitatively and qualitatively

After the A_1 and A_2 subgroups were first described there was controversy over whether A_1 and A_2 cells differ purely in the number of A determinants or whether these antigens actually show structural differences. Numbers of antigen sites per red cell, estimated from a variety of techniques, can be summarised as follows: A_1, $8–12 \times 10^5$; A_2, $1–4 \times 10^5$; A_1B, $5–9 \times 10^5$; A_2B, 1×10^5 [220–226]. Repeated adsorption of anti-A_1 from group B serum with A_2 cells will remove all antibody, suggesting only a quantitative difference [197,227], but A_2 and A_2B individuals often make anti-A_1, suggesting that A_2 cells lack a determinant present on A_1 cells [198,199].

The majority of red cells from A_2 individuals showed faint fluorescence with fluorescent *Dolichos* lectin, while a few cells demonstrated very strong fluorescence; conversely, in a population of A_1 cells, most had strong reactivity while around 10% exhibited only faint fluorescence [228]. This may explain the 'mixed field' appearance of agglutination usually observed with anti-A_1 reagents.

The precise biochemical background to A_1 and A_2 is controversial, but it appears that A_1 red cells have both repetitive Type 3 A and Type 4 A glycolipids (Section 2.2.2), whereas A_2 red cells either lack both Type 3 A and Type 4 A or have Type 3A, but lack Type 4 A glycolipids [43,55,56,58,229–231]. Svensson *et al.* [231] detected abundant Type 3 A glycolipids in A_2 red cell membranes and, therefore, considered that the major difference between A_1 and A_2 phenotypes is the dominance of Type 4 A glycolipids in the A_1 phenotype, which are essentially absent in A_2. It is probable, therefore, that GTA_2 is unable to utilise Type 4 H as an acceptor substrate, possibly as a result of the extension of GTA_2 compared with GTA_1 (Figure 2.6). It is probable that anti-A_1 is specific for, or at least shows a preference for, Type 4 A structures. *Dolichos* lectin, however, detects GalNAc and, when present in sufficient concentration, agglutinates A_2 cells, so its use as a reagent for subtyping group A cells probably depends more on the quantitative than the qualitative difference between A_1 and A_2 phenotypes.

2.4.3 A_{int}

Landsteiner and Levine [196] recognised that the red cells of some group A individuals could not be defined as either A_1 or A_2, but fell into an intermediate category. A_{int} does not represent a true intermediate, however, as the level of H is as high as that found in A_2 and may be higher [217,232–234].

A_{int} is more common in black than white people. Of group A African Americans, 8.5% were found to be A_{int} compared with about 1% of group A white Americans [235]. Of group A black South Africans, 13.7% were A_{int} [217].

A unique form of GTA in A_{int} sera was detected, which differed from GTA_2 in having a high affinity for UDP-GalNAc and from GTA_1 in having a low affinity for 2′-fucosyllactose, a soluble analogue for membrane-bound H-substance [236]. One *A* mutation in an $A_{int}B$ individual is listed in dbRBC: 923A>G, Lys308Arg [215].

2.5 ABO phenotype and gene frequencies

Millions of people have been ABO grouped and the frequencies of the four phenotypes, A, B, AB, and O, differ substantially throughout the world, and often show marked variations even within quite small countries. In 1976, Mourant *et al.* [237] published the results of ABO tests on nearly 15 million people from populations of virtually every country in the world. As an example of ABO frequencies in Britain, a study of unrelated individuals from the South of England is shown in Table 2.10.

Populations with a high frequency of *O* (gene frequency greater than 0.7, i.e. 70%) are found in North and South America, and in parts of Africa and Australia, but not in most of Europe or Asia. Some native people of South and Central America are virtually all group O and probably were entirely so before the European invasion. The frequency of *A* is quite high (0.25–0.55) in Europe, especially in Scandinavia and parts of Central Europe. High *A* frequency is also found in the Aborigines of South Australia (up to 0.45) and in certain Native American tribes where the frequency reaches 0.35. A^2 is found mainly in Europe and Africa, but is either very rare or absent from indigenous populations throughout the rest of the world. The frequency of A^2 in Lapland reaches 0.37, but elsewhere in Europe it does not exceed 0.1. *B*, almost absent from Native Americans and most Australian Aborigines, probably was absent before the arrival of Europeans. High frequencies of *B* are found in Central Asia (0.2–0.3). In Europe, *B* frequency diminishes from about 0.15 in the east to less than 0.05 in the Netherlands, France, Spain, and Portugal (data compiled from [237]). For a diagrammatic representation of some examples of ABO phenotypes in different populations, see Figure 2.8.

Table 2.10 A_1A_2BO phenotype, gene, and genotype frequencies in the South of England [238].

Phenotype			Gene		Genotype	
	No.	Frequency		Calculated frequency		Calculated frequency
O	1503	0.4345	*O*	0.6602	*O/O*	0.4349
A_1	1204	0.3481	A^1	0.2090	A^1/A^1	0.0437
					A^1/O	0.2760
					A^1/A^2	0.0291
A_2	342	0.0989	A^2	0.0696	A^2/A^2	0.0048
					A^2/O	0.0919
B	297	0.0859	*B*	0.0612	*B/B*	0.0037
					B/O	0.0808
A_1B	91	0.0263			A^1/B	0.0256
A_2B	22	0.0063			A^2/B	0.0085
Total	**3459**	**1.0000**		**1.0000**		**1.0000**

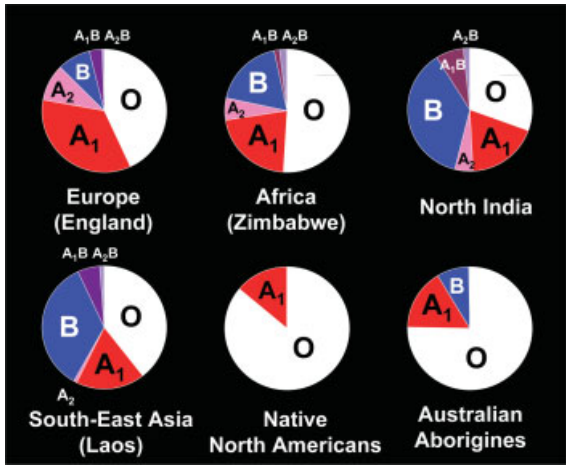

Figure 2.8 Diagram showing the distribution of ABO phenotypes in six selected populations.

Some gene frequencies determined by molecular methods are provided in Table 2.11. The frequencies for English donors correlate remarkably well with those calculated from serological data (Table 2.10), considering changes in the ethnicity of the donor populations over 60 years.

2.6 Secretion of ABO and H antigens

By 1926 it was apparent that A and B antigens were not confined to red cells, but were present in soluble form in seminal fluid and saliva [240]. In 1930, Putkonen [241] noted that a proportion of A, B, and AB individuals lacked A or B antigens from their body fluids. The ability to secrete A, B, and 'O' was found to be inherited in a Mendelian manner, genetically independent of ABO [76]. The locus controlling ABH secretion was called Secretor (*Se*, and subsequently *FUT2*): the ability to secrete (*Se*) is dominant over non-secretor (*se*). Although some other blood group antigens are also present in secretions, the terms 'secretor' and 'non-secretor' refer only to ABH secretion.

In secretor individuals of the appropriate ABO group, ABH antigens are detected in the secretions of the goblet cells and mucous glands of the gastrointestinal tract (saliva, gastric juice, bile, meconium), genitourinary tract (spermatic fluid, vaginal secretions, ovarian cyst fluid, urine), and respiratory tract, as well as in milk, sweat, tears, and amniotic fluid [32,242]. Secreted ABH antigens are mostly carried on mucins, glycoproteins of high molecular weight, but are also present in milk and urine as free oligosaccharides [10,33,34]. Secreted ABH

Table 2.11 *ABO* allele frequencies determined by PCR-based analyses of genomic DNA.

Population	No. of alleles tested	Alleles					References
		A^1	A^2	B	O^1	O^2	
Europeans	600	0.215	0.062	0.112	0.583	0.028	[129]
English	172	0.198	0.075	0.105	0.605	0.017	[130]
White Americans	240	0.188	0.017	0.108	0.671	0.017	[131]
Kuwaitis	166	0.136	0.030	0.166	0.660	0.009	[121]
Chinese (Han)*	417	0.213	0.002	0.209	0.572	0	[117]
Japanese	208	0.288	0	0.178	0.534	0	[239]

O^1 includes all alleles with 261delG.
*plus single examples of *cisAB06* and *O06* 87–88insGG.

antigens are expressed on Type 1, Type 2, and Type 3 structures [10,14,39,53].

Se and *se* are alleles of the endodermal α1,2-fucosyltransferase gene, *FUT2*. The symbol *se* represents numerous alleles containing inactivating mutations (Section 2.3.1.2 and Table 2.7). *Se* and *se* determine the presence or absence of H in secretions. A- and B-transferases are not under the control of the secretor gene, but are unable to catalyse the production of A and B substances in body fluids of non-secretors owing to lack of H, their acceptor substrate (Section 2.3.2). The study of dispermic chimeras has shown that in order to secrete A, an *A* gene and an *Se* gene must be expressed in the same cell, and the corresponding situation applies to cells that secrete B [243,244].

The simplest method for determining secretor status is by inhibition of haemagglutination. Saliva (previously boiled) is added to selected and appropriately diluted anti-A, -B, and -H (usually *Ulex europaeus* lectin), and inhibition determined by the failure of these mixtures to agglutinate A_2, B, and O cells, respectively.

2.6.1 Frequencies

In most European populations the frequency of secretors is about 80% [237]. Table 2.12 shows the results of secretor tests, with deduced gene and genotype frequencies, on over a thousand people from Liverpool. The frequency of the *Se* gene does not differ greatly from 0.5 in most ethnic groups, although in Australian Aborigines, Inuits, some Native Americans, and some Melanesians, the frequency approaches 1.0 [237]. In India there is more variation with a high frequency of *Se* in the North (up to 0.75) and low frequency in the South (0.22).

2.6.2 Quantitative aspects

A study of sibling pairs indicated that individual quantitative variation of salivary A, B, or H is, at least in part, inherited, and inherited in a polygenic manner [245]. The primitive salivary glands of a human fetus produce secretion rich in ABH antigens from the gestational age of about nine weeks [246] and ABH antigens are well developed in neonatal saliva [247,248]. A variety of techniques, mostly employing human anti-A or *Dolichos biflorus* lectin, has provided substantial evidence that A_1 saliva contains more A antigen than A_2 saliva [165,249–251]. Saliva from AB secretors contains less A and B than saliva from group A secretors and group B secretors, respectively [249–251], the result of competition between GTA and GTB for a common substrate.

Small quantities of H, A, and B substances can be detected in the saliva of most non-secretors [166, 252–254]. H production in non-secretor saliva is probably catalysed by the *FUT1* gene-specified α1,2-fucosyltransferase and not the *FUT2* gene product. Low levels of α1,2-fucosyltransferase in submaxillary gland preparations from non-secretors showed the Type 2 acceptor preference typical of *FUT1* gene-specified transferase [66].

2.6.3 Se^w

A weak secretor gene (Se^w or Se^{w385}), containing a missense mutation encoding Ile129Phe, is responsible for the Le(a+b+) red cell phenotype common in East Asia, Polynesia, and the Philippines [67,85–89] (Table 2.7). An α1,2-fucosyltransferase that is less efficient than the normal *Se* gene product competes less effectively with the Lewis-transferase for the Type 1 precursor substrate.

Table 2.12 Phenotype, gene, and genotype frequencies for secretor status of a random selection of people from Liverpool.

Phenotype			Gene		Genotype	
	No.	Frequency		Calculated frequency		Calculated frequency
Secretors	864	0.7728	*Se*	0.5233	*Se/Se*	0.2739
					Se/se	0.4989
Non-secretors	254	0.2272	*se*	0.4767	*se/se*	0.2272

Consequently, a greater quantity of the substrate is converted to Le^a so that less is available to be converted to Type 1 H and, subsequently, to Le^b (Section 2.3.3.1).

2.6.4 A, B, and H in plasma

A, B, and H are found in the plasma of secretors and non-secretors, although greater quantities are present in the former [79,255–257]. With anti-Type 1 H (serum from goats immunised with human saliva and adsorbed with immunoadsorbents coated with Type 2 H trisaccharide) and anti-Type 2 H lectin (*Ulex europaeus*), Le Pendu *et al.* [79] showed that plasma from ABH secretors contains Type 1 H and Type 2 H, but plasma from non-secretors contains only Type 2 H. They estimated that all of the Type 1 H and about one-third of the Type 2 H in plasma is controlled by the secretor system (*FUT2*), whereas most of the Type 2 H is independent of secretor and is presumably of haemopoietic origin. Plasma ABH substances are carried on glycosphingolipids and glycoproteins [258]. Their quantity is greatly affected by Lewis phenotype: Le(a–b–) ABH secretors have substantially more ABH determinants in their plasma than do Le(a–b+) ABH secretors [79,257–259].

Over a period of about two weeks, group O transfused red cells adsorb A and B antigens from the plasma of an AB recipient and become agglutinable with anti-A, -B, and -A,B [260]. A and B antigens adsorbed from plasma onto O red cells, *in vitro*, are glycosphingolipids and contain Type 1 chains [257].

2.7 Subgroups of A

In addition to the common phenotypes A_1 and A_2, numerous phenotypes with weak expression of A on the red cells have been found and a multitude of names have been adopted. Most of these phenotypes can be fitted into the following categories: A_3, A_x, A_{end}, A_m, A_y, and A_{el}. The serological characteristics of these phenotypes are shown in Table 2.13. All have normal or enhanced expression of H. Most result from inheritance of a rare allele at the *ABO* locus, usually involving missense mutations in exons 6 or 7, although other mechanisms do occur. Some alleles are listed in Table 2.14. The abnormal A phenotypes are only apparent when the variant gene is paired with *O* or *B*, not with A^1 or A^2. A_y probably results from germline mutation or from homozygosity for a rare gene at a locus independent of *ABO*. A_{end}, A_m, A_y, and A_{el} red cells are not agglutinated by most anti-A and are disclosed in routine testing because they resemble group O or B red cells, but no anti-A is present in the serum. A_x cells are agglutinated by group O (anti-A,B) serum and some monoclonal anti-A. In A subgroups the A antigen is more easily detected if the cells are protease treated. In many cases weak A subgroups have been found and associated with *ABO* mutations although serological analyses have been insufficient for categorising the phenotype, usually because of unavailability of secreted material. The subgroups are usually categorised as A_w (for weak). Olsson *et al.* [261] considered that, 'The relevance of categorical subgroup classification based on serological phenomena alone is becoming questionable; so use of the more general terms A_{weak} and B_{weak} is not unfounded'. The serological terms will continue to be used here, as they are still used in laboratories where detailed serological analyses are carried out.

Measurements of relative agglutinability, obtained by counting the number of cells agglutinated by anti-A in a cell counter, and site density, determined with radiolabelled rabbit IgG anti-A [223,224], are shown in Table 2.15. These quantitative techniques reveal substantial

Table 2.13 Serological and transferase characteristics of weak A subgroups.

	Reactions of cells with		Antibodies in serum			
Name	Anti-A	Anti-A,B	Anti-A	Anti-A_1	Antigens in saliva of secretors	A-transferase in serum
A_3	mf	mf	No	Sometimes	A H	Sometimes
A_{end}	mf	mf	No	Sometimes	H	No
A_x	–*/w	+	–/+	Usually	(A_x) H	Rarely
A_m	–*/w	–/+	No	No	A H	Yes
A_y	–*	–	No	No	A H	Trace
A_{el}	–*	–	Some	Yes	H	No

Red cells of none of the subgroups reacted with anti-A_1; all reacted with anti-H.
*Anti-A may be adsorbed onto and eluted from these cells.
(A_x), may require inhibition of agglutination of A_x cells for detection.
mf, mixed field agglutination; w, very weak agglutination.

Table 2.14 Some *ABO* alleles associated with weak A expression.

Phenotype	Allele	Nucleotide changes†	Amino acid changes†	References
A_3		None	None	[261,262]
A_3	*A301*	871G>A	Asp291Asn	[263]
A_{end} A_{finn}		IVS6+4A>G	Alternative splicing	[264]
A_{end} A_{bantu}	*Abantu01*	O^{1bantu}–A^2 hybrid	exon 4 del; Pro156Leu; 354fs+21aa	[218]
A_x	*Ax01*	646T>A	Phe216Ile	[212]
A_x	*Ax02*; B/O^2–O^{1v}	297A>G, 646T>A, 681G>A, 771C>T, 829G>A	Phe216Ile, Val277Met	[261,265]
A_x	*Ax03*; A^1–O^{1v}	646T>A, 681G>A, 771C>T, 829G>A	Phe216Ile, Val277Met	[261,265]
A_m	*Am01*	467C>T*, 761C>T	Pro156Leu*, Ala254Val	[266]
A_m	*Am02*	664G>A	Val222Met	[267]
A_{el}	*Ael01*	804insG	Phe269fs	[212,261,268]
A_{el}	*Ael02*	467C>T, 646T>A, 681G>A	Pro156Leu*, Phe216Ile	[212]
A_{el}	*Ael03*	804delG	Phe269fs	[269]
A_{el}	*Ael04*	IVS6+5G>A	No full transcript	[270]
A_{el}	*Ael05*	467C>T, 767T>C	Pro156Leu*, Ile256Thr	[271]
A_{el}	*Ael06*	425T>C, 467C>T	Met142Thr, Pro156Leu*	[272]
A_{el}	*Ael07*	A^1–O^{1v} hybrid	Pro156Leu*, Val277Met	[273]

fs, reading frameshift; aa, amino acids.
†Changes from the A^1 (*A101*) consensus sequence.
*Common in East Asian A^1 alleles (*A102*).

Table 2.15 Relative agglutinability with anti-A and A site density per red cell [223].

Phenotype	No. of subjects	Agglutinability	Antigen site density (A sites per red cell x 10^5)
A_1	4	100	10.5 (7.95–14.56)
A_2	10	96 ± 2	2.21 (1.29–3.53)
A_3	11	63 ± 10	0.35 (0.07–1.0)
A_{end}	7	10 ± 5	0.035 (0.011–0.044)
A_x	9	33 ±10	0.048 (0.014–0.10)
A_m, A_y	4	0	0.012 (0.001–0.019)
A_{el}	4	0	0.007 (0.001–0.014)

individual variation within a subgroup, but do determine a hierarchy in respect to red cell A antigen expression.

The serologically defined subgroups of A do not represent single genetic entities. In some cases A phenotype can differ according to whether the *trans* allele is *O* or *B*, either as a result of competition for substrate or allelic enhancement (Section 2.10).

2.7.1 A_3

The least rare of the weak A phenotypes is A_3. The frequency has been estimated as 1 per 1000 group A Danes [274], 9 in 150 000 French donors (0.0136% of group A) [275], and 2 in about 180 000 Canadians [276]. The main serological feature of A_3 phenotype is a characteristic 'mixed field agglutination' when red cells are incubated with anti-A and with most anti-A,B [277]. That is, small agglutinates are seen surrounded by a mass of unagglutinated, 'free' cells. On occasion A_3 serum contains anti-A_1. Group A substance is detected in the saliva of secretors.

Unlike A_{mos} described in Section 2.9, A_3 does not appear to be a mosaic of A_2 and O cells: in the A_3 phenotype anti-A can be eluted from the population of cells that was not agglutinated by it [276] and some anti-A,B agglutinate the whole population of cells [250]. Flow cytometry revealed a characteristic pattern of two main populations of cells either expressing A at a normal level for group A red cells or at a low level, similar to that of A_x cells, with a small number of cells expressing A antigen ranging between the two main populations [262]. It has been estimated that only 3–4% of the cells have sufficient sites to permit agglutination with anti-A [278].

Serum A-transferase was detected at low level in some A_3 individuals, with pH optima of either 6 or 7, typical of A_1 or A_2 enzymes, respectively [279–281]. Under optimum conditions the A_1 type of enzyme in the first category can convert O cells to A active cells, which do not display the characteristic A_3 agglutination pattern when incubated with anti-A and are agglutinated more strongly than are O cells converted with A_2 serum [279,281]. Surprisingly, in view of the high level of H antigen on the red cells, H-transferase levels in A_3 sera are generally considerably lower than H-transferase levels in A_1 or A_2 sera [281].

Sequencing of all seven exons of *ABO*, including intronic splice site sequences, plus the enhancer region, revealed no deviation from the consensus A^1 sequence in numerous individuals of Nordic origin with typical A_3 phenotype and A^1/O^1 or A^1/O^{1v} genotype [261,262]. Sequencing of exons 6 and 7 of the *A* gene from two A_3B individuals revealed an A^1 sequence with a single base change encoding Asp291Asn [263], but this was not a typical A_3 phenotype as shown by flow cytometry [262]. Eleven alleles encoding A_3 are listed in dbRBC, representing missense mutations in A^1 (*A101*), A^1 (*A102*), and A^2 alleles [215], though not all are necessarily associated with the characteristic mixed-field phenotype and so are not genuinely A_3.

2.7.2 A_{end} (A_{finnr}, A_{bantu})

A_{end} was the name given to a phenotype that resembles weak A_3 cells; A_{end} red cells give very weak 'mixed field' agglutination with some anti-A and -A,B [282]. The saliva of A_{end} secretors, however, contains H, but no A.

Anti-A_1 is present in some A_{end} sera. No GTA was detected in sera or red cell membranes of A_{end} individuals [279,280].

Two examples of A_{end} were found in testing 150 000 French donors (0.003% of group A) [275].

An A variant, which differs from A_{end} in only minor details, was found in Finns and named A_{finn} [283]. The frequency of A_{finn} in Finnish blood donors was estimated at about 1 in 6000 [283], but may be as high as 1 in 1000 in parts of southern Finland [284]. A_{finn} red cells display a characteristic pattern by flow cytometry, with most of the cells expressing no A antigen, while 2–4% expressed variable amounts of A [262]. A_{finn} individuals have an A^1 allele with a>g in the 5′ donor splice site of intron 6 [262,264]. Although skipping of exon 6 would introduce a reading frameshift and no active enzyme product, the mutation is not in the invariable splice site sequence, so a minor fraction of the RNA could be spliced normally.

A_{bantu} is another variation of A_{end}, found in about 4% of group A black South Africans [217], and in up to 8% of Bushmen and Hottentots [285]. Anti-A agglutinate A_{bantu} red cells more strongly than A_{end} cells. A_{bantu} results from a hybrid of the common A^2 allele and an O^1-like allele (O^{1bantu}), with a cross-over region near exon 5 (*Abantu01*) [218].This includes a deletion of a nucleotide in the 5′ intron 4 splice site, leading to the loss of 16 amino acids from the stem region of GTA, but does not include 261delG as exon 6 is derived from A^2.

2.7.3 A_x

The major serological characteristics of A_x phenotype [286] are:

1 the red cells are not agglutinated by most anti-A (group B) sera, yet are agglutinated by the majority of anti-A,B (group O) sera; no mixed field pattern is observed;

2 the serum usually contains anti-A_1 and occasionally an antibody that agglutinates A_1 and A_2 cells [287];

3 in addition to H substance, the saliva of A_x secretors contains a trace of A, which is best detected when A_x cells are used as indicator cells for inhibition of anti-A [288].

A_x phenotype is heterogeneous. Some other symbols (e.g. A_4, A_5, A_6, A_z, and A_o) have been used to describe subgroups of A that differ from the original A_x by only fine serological details.

Most sera from group B donors do not agglutinate A_x cells, although sera from group B volunteers immunized with A substance usually do [289]. Monoclonal anti-A reagents have been produced that are effective at detecting A_x cells, although, under certain conditions, these antibodies may also agglutinate some group B cells [290–292] (Section 2.11). Anti-A can be readily adsorbed onto and eluted from A_x cells.

In two separate studies, the frequency of A_x in France has been estimated as 1 in 77 000 (0.003% of group A) [275] and as 1 in 40 000 [281].

GTA cannot usually be detected in A_x serum or red cell membranes [212,279–281]. H-transferase activity in A_x sera is low [281].

The molecular genetics of A_x reflects the heterogeneity of the serological phenotypes. The most common A^x allele (*Ax01*) has the A^1 consensus sequence with 646T>A encoding Phe216Ile [212,261,293]. Twenty-one A^x alleles are listed in dbRBC; six of them (*Ax01–Ax06*) encode Phe216Ile [215]. Exon 7 of O^{1v} has the sequence encoding the amino acids important for A-specificity, but, like the typical A^x allele, also encodes Ile216. Consequently, hybrid genes in which exon 7 is derived from O^{1v} and exon 6 is derived from *A*, *B*, or O^2 alleles, and so lacks 261delG, produce an active GTA containing Phe216Ile, responsible for an A_x phenotype [261,265]. Three different cross-over regions in intron 6 were detected [261]. GTA containing Phe216Ile alone appears to produce more A antigen than GTA containing Phe216Ile plus Val-277Met (see Table 2.14) [262]. Several other amino acid substitutions have accounted for A_x phenotype [215] and one A_x New Zealander had an A^1-like allele encoding a nonsense mutation (Trp332Stop), which predicts the loss of 23 amino acids from the C-terminus of the GTA [261]. Of 10 alleles containing 1061delC, characteristic of A^2, plus other missense mutations, most displayed A_x-like patterns by flow cytometry [216].

The effects of allelic enhancement on A^x alleles are described in Section 2.10.2.

A very weak GTA, with higher activity at pH 8 than at pH 6 (A_2 type), was detected in the A_x mother of a baby who was A_2 at birth, but became A_x within 2 years [281,294].

2.7.4 A_m

A_m red cells are not agglutinated, or are agglutinated only very weakly, by anti-A and -A,B. Anti-A can be adsorbed onto and eluted from A_m cells. Saliva of A_m secretors, however, contains normal quantities of A and H substances. A_m serum does not usually contain anti-A_1.

A_m is inherited as a rare allele at the *ABO* locus [295–300]. The name A_m was originally coined for a new weak-A phenotype assumed to arise from homozygosity for a recessive regulator gene at a locus independent of *ABO* [301], but this phenotype is now called A_y and is discussed below.

One example of A_m was found in 150 000 French donors (0.0015% of group A) [275] and in 400 000 Chinese in Taiwan [302].

In most A_m samples the serum GTA had a pH optimum of 6 and the kinetic properties of GTA_1, whilst in serum from one A_m person the enzyme had a pH optimum of 7 and resembled GTA_2 [279,280,298,303]. In all cases enzyme activity was between 30 and 50% of that found in A_1 or A_2 sera and probably originated from tissues other than the haemopoietic tissue [304].

In a Japanese family the A_m father and child had an *A* gene that differed from A^1 (*A102*) by 761C>T, Ala254Val (*Am01*) [266], whereas A_m individuals from three generations of a family from Taiwan had an *A* gene that differed from A^1 by 664G>A, Val222Met (*Am02*) [267]. Whether these mutations severely reduce A expression on red cells, while permitting normal A expression in secretions, is not known. In the Taiwanese case GTA activity was virtually undetected in the serum and expression of cDNA encoding Val222Met (A^m) produced GTA with reduced activity compared with A^1 cDNA [267]. Furthermore, activity from the GTA expressed from A^m cDNA did not demonstrate different preferences for Type 1 and Type 2 H substrates, eliminating this as an explanation for normal A antigen expression in saliva, but not on red cells. An analogous phenotype, B_m, results from a deletion of an erythroid-specific regulator in intron 1 of *ABO* [141] (Section 2.8.3). It would be very valuable to test for similar mutations in A^m.

2.7.5 A_y

A_y phenotype is similar to A_m, but the most significant and definitive way in which A_y and A_m differ is by their mode of inheritance. A_y does not result from a rare allele at the *ABO* locus, but probably arises from a germline mutation of an *A* gene. Weiner *et al.* [301] reported two families: one A_y (then called A_m) propositus had a group O parent and A_1 and O siblings; the other was A_yB and had A_1B and B parents. Other similar families have been described since [305–307], yet none of the A_y propositi had an A_y sibling. In one family the A_y son of A^1/O and B parents had an A_yB son who, in turn, had an A_y son [308].

A_y differs from A_m phenotypically in the following ways: substantially less anti-A is eluted from A_y cells than from A_m cells incubated with the same serum; A_y secretor saliva contains considerably less A substance than A_m saliva; and A_y serum contains only a trace of GTA, whereas A_m serum contains readily detectable enzyme [279,280,303,309].

2.7.6 A_{el}

Under usual conditions A_{el} cells are not agglutinated by anti-A or -A,B, although they do bind these antibodies, as demonstrated by adsorption and elution [282,310–312]. Very low levels of A antigen were detected on A_{el} cells by flow cytometry [262] and immunogold electron microscopy [313,314]. Saliva from A_{el} secretors contains H, but no A substance. Serum from A_{el} individuals usually contains anti-A_1 and may also contain an antibody that agglutinates A_2 cells. No GTA has been detected in A_{el} serum or red cell membranes [212,279–281]. Serum H-transferase is weaker than that found in A_1 or A_2 serum [281]. No example of A_{el} was found in testing 150 000 French blood donors [275], but five were found among 400 000 Chinese from Taiwan [302]. As a result of allelic enhancement (Section 2.10.2), $A_{el}B$ cells may be weakly agglutinated by some monoclonal anti-A and may resemble B(A) phenotype (Section 2.11.1) [315].

The usual form of A^{el} (*Ael01*) has the A^1 consensus sequence except for a single G insert in a string of seven guanosines at nucleotides 798–804 [261,268]. This insert creates a reading frameshift, altering the amino acid sequence after Gly268 and abolishing the translation stop codon, so that the gene product is 37 amino acids longer than GTA_1 and 16 amino acids longer than GTA_2. A single nucleotide deletion at the same position was responsible for an A_{el} phenotype in an African (*Ael03*) [269]. The frameshift caused by a G insert at G798–804 in an otherwise normal A^2 allele was corrected by the 1059delC characteristic of A^2, but despite encoding a product of normal length this allele, O^3 or *O08*, was associated with no expression of A [145]. A Japanese individual with A_{el} red cells had an *A* (*A102*) allele encoding Phe216Ile (*Ael02*) [212]. Some other mutations associated with an A_{el} phenotype include other missense mutations in exon 7 [271,272], an intron 6 splice site mutation [270], and an A^1–O^{1v} hybrid with a recombination site within exon 7 in a blood group chimera with $A_{el}B_{el}$ phenotype [273] (Table 2.14). A mutation,1A>G, Met1Val, in the translation start codon of an A^2 allele was responsible for a phenotype referred to as $A_{el/weak}$ [216]; 2T>C, Met1Thr produced an A_3-like phenotype [316]. It must be presumed that these alleles produced weakly active, truncated GTA by initiation of translation at alternative start sites upstream of the usual initiator.

2.7.7 A_w

Many abnormal A antigens are referred to as A_w because the phenotype did not easily fit into any existing classification, often because sufficient serological testing was not

Table 2.16 Typical serological and transferase characteristics of weak B subgroups.

Name	Reactions of cells with			Anti-B in serum	Antigens in saliva of secretors	B-transferase in	
	Anti-B	Anti-A,B	Anti-H			Serum	Red cell membrane
B_3	mf	mf	+	No	B H	Yes	No
B_x	w	w	+	Yes	(B_x) H	No	No
B_m	–*/w	–/w	+	No	B H	Yes	Trace
B_{el}	–*	–	+	Sometimes	H	No	No

*Anti-B may be adsorbed onto and eluted from these cells.
(B_x), may require inhibition of agglutination of B_x cells for detection.
mf, mixed field agglutination; w, very weak agglutination.

possible. The A^w alleles commonly have single missense mutations in exon 6 or 7 of A^1 or A^2 alleles, though mutations have been found elsewhere in the gene. Many are listed in the dbRBC web site [215] or in several publications [159,216,261,262,317].

2.7.8 Do non-deletional *O* alleles produce any A antigen?

Most *O* alleles contain a single nucleotide deletion (261delG), preventing the production of any active transferase, but the allele known as O^2 or *O03*, lacks 261delG, but has two nucleotide differences from the A^1 exon 7 sequence that encode Arg176Gly (identical to that of GTB) and Gly268Arg [124,125] (Figures 2.5 and 2.6) (Section 2.3.2.2). Although initially considered an *O* allele, with Arg268 inactivating any potential GTA activity, there is now evidence supporting very low levels of GTA activity, though this is controversial (reviewed in [134]). The evidence is as follows: (1) minute quantities of anti-A adsorbed onto and eluted from red cells of individuals with O^2 [133,318]; (2) absence or reduced levels of anti-A and -A_1 in their plasma [133,318–320], which may cause typing problems on donors [319]; and (3) expression of A antigen, detected by adsorption and elution, on HeLa cells transfected with plasmids containing O^2 constructs [318]. Yazer *et al.* [320], however, were unable to adsorb and elute anti-A from O^2 red cells or to detect any A antigen on their surface by a very sensitive flow cytometry test.

An allele similar to O^2, encoding Gly268Arg and an additional Thr163Met substitution, was named *Aw08* as it also appeared to produce low levels of A antigen [318,321]. Some other rare non-deletional *O* alleles may also be responsible for production of marginal levels of A antigen [133,134].

2.8 Subgroups of B

Weak variants of B are very rare. They appear to be much rarer than weak A subgroups, although this probably reflects the relatively low frequency of the *B* gene in many populations. In Japan, for example, where the incidence of B is about half that of A, the frequencies of B_x and B_m are considerably higher than those of A_x and A_m [322].

Weak B subgroups have proved difficult to classify. Salmon [323] concluded that the best system for classifying B variants was by a loose analogy with the A variants: B_3, B_x, B_m, and B_{el}, plus B_w for those that do not fit any of the other four categories (Table 2.16), although others have suggested that the serological classification of B variants is no longer sustainable [261,262]. Some alleles encoding weak B antigens are listed in Table 2.17.

2.8.1 B_3

B_3 phenotype [323,330] is characterised by mixed field haemagglutination with anti-B and -A,B, by absence of anti-B in the serum, and by normal B antigen in the saliva (in secretors). B_3 was found in approximately one in 10 000 group B French donors [331] and in Chinese donors, one in 900 group B and one in 1800 A_1B [332].

GTB was detected in sera from B_3 individuals, but not in B_3 red cell membranes [333].

Eight B^3 alleles encoding single amino acid substitutions in GTB are listed in dbRBC [215]. One allele encoding Phe216Ile (*B302*) appears to represent a *B* allele with

Table 2.17 Some *ABO* alleles associated with weak B expression.

Phenotype	Allele	Nucleotide changes	Amino acid changes from *B* sequence	References
B_3	*B301*	1054C>T	Arg353Trp	[263]
B_3	*B302*	*B–O^{1v}–B* hybrid	Phe216Ile	[135]
B_3	*B303*	IVS3+5G>A	exon 3 del; del17–35	[324]
B_3	*B304*	247G>T	Asp83Tyr	[324]
B_3	*B305*	425T>C	Met142Thr	[325]
B_x	*Bx01*	871G>A	Asp291Asn	[212]
B_x	*Bx02*	905A>G	Asp302Gly	[326]
B_x	*Bx03*	541T>C	Trp181Arg	[326]
B_m	*B^m*	Intron 1 5.8 kb del		[141]
B_{el}	*Bel01*	641T>G	Met214Arg	[212]
B_{el}	*Bel02*	669G>T	Glu223Asp	[212]
B_{el}	*Bel03*	502C>T	Arg168Trp	[327,328]
B_{el}	*Bel04*	467C>T; 646T>A; 681G>A; 771C>T; 829G>A	Pro156Leu, Phe216Ile, Val277Met	[329]

a small region of exon 7 exchanged for that of O^{1v} [135]. A *B* gene (*B303*) containing a splice site mutation causing an in-frame skipping of exon 3 encodes a protein product lacking amino acids 17–35 [324].

2.8.2 B_x

B_x represents a heterogeneous group, but typical B_x red cells are weakly agglutinated by anti-B and -A,B [322,323]. The serum contains weak anti-B and the saliva of B_x secretors contains some B substance, which is often only detected by inhibition of agglutination of B_x cells by anti-B. GTB was not detected in serum or red cell membranes of B_x individuals [212,333].

Ten B^x alleles are listed in dbRBC, containing missense mutations in exon 7, though many more alleles labelled as B^w could possibly be classed as B^x [215].

2.8.3 B_m

B_m cells are not agglutinated by anti-B or -A,B; the B antigen is only detected by sensitive techniques such as adsorption and elution of anti-B [334,335]. The saliva of B_m secretors contains about as much B substance as that of a normal B secretor. Characteristically, sera from B_m individuals do not contain anti-B. In Japan B_m is relatively common for a B subgroup, with B_m and AB_m having a total frequency of 0.0244% [141].

Only very little GTB activity could be detected in B_m red cell membranes [336]. B_m sera demonstrated less than half of the GTB activity of B sera [105,335,337,338] and B_m saliva had normal GTB activity compared with that of B secretor saliva [105]. In families with B_m and A_1B_m members much higher levels of GTB activity were apparent in the A_1B_m sera than in the B_m sera, presumably a result of allelic enhancement [335,336] (Section 2.10.2).

A deletion of a 5.8 kb sequence in intron 1 of a *B* allele, which encompassed an erythroid-specific promoter site (Section 2.3.2.2) was present in 110 of 111 Japanese with B_m or AB_m phenotypes [141]. This deletion is predicted to ablate GTB production in haemopoietic tissue, but not in other tissues. The molecular basis for B_m in the other individual was not reported.

Homozygosity for a recessive gene that suppresses B in haemopoietic tissues has been proposed to explain abnormal inheritance of B_m-like phenotypes in a few families [339–341]. Red cells of the son of O and A_2B parents resembled B_m phenotype and should probably be called B_y, in analogy with A_y (Section 2.7.5). His serum contained normal H-transferase, but only about 70% of the normal level of GTB [341], the amount expected if all of it was non-haemopoietic in origin [304].

2.8.4 B_{el}

B_{el} red cells are not agglutinated by anti-B or -A,B [342,343]. They do bind anti-B, which can be detected in eluates. B is not present in the saliva of B_{el} secretors; anti-B may be present in the serum.

No GTB was detected in B_{el} sera or red cell membranes [212,333,343]. In a family with B_{el} and A_1B_{el} members, the A_1B_{el} red cells were weakly agglutinated by some anti-B

[343]. In another family, B_{el} was enhanced to B_3 in an *A/B* heterozygote [344] (see Section 2.10.2).

Eight B^{el} alleles are listed in dbRBC, five of them containing single missense mutations in exon 7, though many more alleles labelled as B^w could possibly be classed as B^{el} [215]. One B^{el} allele (*Bel04*) encodes three amino acid differences from normal GTB (Table 2.17), and probably represents multiple interallelic exchanges between *B* and O^{1v} [329]. Met214Arg encoded by *Bel01* is adjacent to the ^{211}Asp–Val–Asp^{213} motif that captures Mn^{2+} and is essential for enzyme activity [158]. (All transferases of this type contain an Asp–X–Asp motif.) Recombinant mutant GTB containing Met214Arg had a 1200-fold decrease in k_{cat} (the catalytic rate of an enzyme) compared with the normal GTB [345].

2.8.5 Other subgroups of B

Numerous other *ABO* alleles responsible for variant B phenotypes have been described, mostly as B^w [159, 215,261,317,346]. A structural analysis, based on the crystal structure of GTB, demonstrated that the mutations in variant *B* alleles are likely to disrupt molecular bonds important for enzyme function [346].

Four cases of B_w phenotype appeared to result from aberrant CBF/NF-Y motif sequences in the regulatory region upstream of exon 1 (Section 2.3.2.2) [148]. Two had *B* alleles with normal coding sequences, but two alleles had reduced numbers of 43-bp repeat units and in a third a novel CBF/NF-Y motif was present. The fourth was AB_w and whereas the *B* allele appeared normal, the *A* allele had an increased number of the enhancer elements, suggesting that enhanced GTA production resulted in weakened B antigen as a result of competition between GTA and GTB for acceptor substrate. A hybrid gene (*Bw26*) with exons 1–3 from O^2 and exons 4–7 from *B* differed from normal *B* by encoding Arg18Leu and having only one CBF/NF-Y repeat, compared with the four usually present in *B* [149]. Transcript levels from this gene were, however, about normal for a *B* allele, and Thuresson *et al.* [149] considered that the weak B expression resulted from the Leu18Arg substitution rather than the number of enhancer elements.

Immunofluorescence microscopy showed that GTB derived from a *B* allele is located almost entirely in the Golgi apparatus, whereas GTB from a B^w allele (*Bw21*) encoding Gly230Arg was evenly distributed throughout the cytoplasm [347]. This suggests that structural changes in ABO glycosyltransferases arising from amino acid substitutions may cause defective trafficking of the enzyme to the Golgi.

An inherited variant B antigen called B_v was characterised by the failure of B_v red cells to react with human anti-B reagents that had been adsorbed with rabbit red cells [348,349]. B_v red cells and secretions appear to lack normal human B antigen, but contain a B-like determinant, possibly the non-fucosylated B-like antigen on rabbit red cells (the 'Galili antigen' [350]). Sera of B_v individuals contain a form of anti-B; no GTB activity could be detected. Among 567 210 Hong Kong Chinese blood donors, 46 examples of B_v and eight examples of AB_v were found [349]. Of 18 Hong Kong Chinese with B_v red cells, 17 had a *B* allele containing 695T>C, Leu232Pro (*Bw11* [346]) and one had a *B* allele containing 721C>T, Arg241Trp (*Bw03* [261]); no mutation was detected in the coding region of the *B* gene of an AB_v individual [351].

2.9 A_{mos} and B_{mos}

In 1975 Marsh *et al.* [352] applied the names A_{mos} and B_{mos} to remarkably similar variants of A and B. In A_{mos}, agglutination tests with anti-A and -A,B revealed two separable populations of cells, one A_2, the other O. A_{mos} sera contained no anti-A, and the saliva contained H and possibly a trace of A. In addition to two A_{mos} families, B_{mos}, A_1B_{mos}, and $A_{mos}B$ phenotypes were described [352]. The inherited A+O and B+O mosaics previously reported in Japan probably represent earlier examples of A_{mos} and B_{mos} [353–355].

A_{mos} is inherited, apparently at the *ABO* locus; a characteristic that distinguishes it from most other forms of red cell mosaicism. All A_{mos} members within a family have about the same proportion of A and O cells, although these proportions vary substantially between different families. A_{mos} differs from A_3 serologically as the cells left unagglutinated with anti-A do not adsorb anti-A. The level of serum GTB activity in the B_{mos} members of a family was only about 7–20% of that of normal B controls [356].

2.10 *A* and *B* gene interaction

2.10.1 Allelic competition

It is well established that A antigen is weaker on A_2B cells than on A_2 cells and that A_1 is weaker on A_1B than A_1 cells; the effect of two different glycosyltransferases competing for the same acceptor substrate. Although generally less obvious, B is often weaker in A_1B than in B [357–360].

In some cases A^1/B genotype may be expressed as an A_2B phenotype [233,344,359,361–363] and A^2/B may be expressed as A_3B or A_wB phenotype [219,361,364]. In black populations the A_2B:A_1B ratio is often significantly higher than would be expected from the A_2:A_1 ratio [344,362]. In a study of 5000 African Americans, 80% of group A individuals were A_1 and 20% A_2, whereas 53% of the group AB individuals were A_1B and 47% were A_2B [362]. Similar discrepancies have been observed in white people [233,344], Chinese [332], and Japanese [213,363]. This imbalance in Japanese is due, at least in part, to an *ABO* allele (*A204*: Arg176Gly, Gly235Ser, Val277Met) that is expressed as A_1 in A^1/O genotype, but as A_2 in A^2/B genotype [213].

Sera from some A_2B black people contain GTA_1 and no GTA_2, plus GTB with activity considerably higher than that found in most group B sera [344,365]. Elevated GTB activity together with a GTA_1 was found in the sera of 50% of the A_2B African Americans [362]. This superactive GTB utilises the lion's share of available H sites, so that the GTA_1 cannot produce sufficient A antigen to provide the high site density required for A_1 status.

2.10.2 Allelic enhancement

'Le renforcement allélique' is a gene interaction, the reverse of allelic competition described in the previous section [288,366]. It is an enhancement of expression of weak *A* or *B* alleles in *A/B* heterozygotes. For example, A_x may be inherited from an A_2B parent [288,366–368] because the presence of a *B* allele enhances the expression of A^x to that expected of A^2. The effect of allelic enhancement is clearly visualised by flow cytometry [262]. In one family the A_x cells had 11 200 A sites per red cell, whereas the A_2B cells, with the same *A* allele, had 96 000 A sites [366]. Weak GTA activity was detected in the sera of people with an A_2B phenotype (genotype A^x/B) resulting from allelic enhancement; no enzyme was found in the serum of their A_x siblings who have the same *A* gene [366]. In a Taiwanese family, an A^x (*Ax11*) allele was expressed as A_x in an A^x/O^1 individual, but as A_3B in her A^x/B father [369]. Other families have shown that a *B* gene responsible for B_x phenotype was expressed more strongly in A_1B members [370,371]. In addition to *A* and *B*, the non-deletional O^2 allele has also been responsible for allelic enhancement. A^x alleles (*Ax03* and *Ax04*) behaved as *O* when paired with O^1 or O^{1v}, but as A^x when paired with *B* or O^2 [262,372].

The molecular mechanism for allelic enhancement is not known. One possible explanation is that ABO glycosyltransferases can form dimers [126] and heterodimer formation between a defective enzyme and a different full-length protein – an *A*, *B*, or O^2 product – can lead to functional rescue of the defective transferase [262,372].

2.11 Overlapping specificities of A- and B-transferases (GTA and GTB)

The glycosyltransferase products of the *A* and *B* alleles differ in their donor substrate specificity, although they share a common acceptor substrate. GTA and GTB, however, are not precise in their choice of donor substrate and there is a small degree of overlap in their specificity [373–376].

Under the appropriate conditions, enzyme from group B serum can catalyse the transfer of GalNAc from UDP-GalNAc to 2′-fucosyllactose (a low molecular weight analogue of H) to form an A-active structure. Concentrated GTB could even make group O cells strongly agglutinable with anti-A. If equivalent quantities of UDP-Gal and UDP-GalNAc were present only B activity could be detected; in the presence of UDP-Gal, thrice the quantity of UDP-GalNAc was required to produce A activity. As would be expected, when there is competition for substrate, GTB is far more efficient at catalysing the transfer of Gal than of GalNAc [373,375,376]. Likewise, GTA can, under appropriate conditions, catalyse the synthesis of B-active structures [108,374].

2.11.1 B(A) and A(B)

The observation in several laboratories that highly potent monoclonal anti-A reagents capable of agglutinating A_x red cells also weakly agglutinated some group B cells led to the recognition that the phenomenon described above, the ability of GTB to produce A determinants *in vitro*, may also be observed *in vivo*. Red cells from 25 of 3458 group B donors were reactive with one example of monoclonal anti-A [377]. The reaction of these B(A) cells with some anti-A could be inhibited by group A secretor saliva [377] and the A-activity removed by treatment of the cells with α-*N*-acetylgalactosaminidase, but not α-galactosidase [378]. People with B(A) red cells were mostly black and were shown to have highly active serum GTB, in some cases five to six times more active than that from most other group B individuals [377]. In B(A), the hyperactive α1,3-galactosyltransferase catalyses the transfer of sufficient GalNAc to its acceptor substrate to permit agglutination by certain anti-A.

Molecular genetic analyses have revealed at least six alleles responsible for B(A) phenotype, mostly in China

Table 2.18 Enzymes with dual A- and B-transferase activity: amino acid substitutions at the four positions (176, 235, 266, 268) characteristic of A and B and at positions 214 and 234.

Phenotype*	Alleles	Amino acids						Shorthand	References
		176	214	234	**235**	**266**	**268**		
A_1	*A101* or *A102*	Arg	Met	Pro	Gly	Leu	Gly	AAAA	
B	*B*	Gly	Met	Pro	Ser	Met	Ala	BBBB	
B(A)	*B(A)01 & B(A)03*	Gly	Met	Pro	**Gly**	Met	Ala	BABB	[269,293]
B(A)	*B(A)02*	Gly	Met	**Ala**	Ser	Met	Ala	BBBB	[379]
B(A)	*B(A)04*	Gly	**Val**	Pro	Ser	Met	Ala	BBBB	[380]
B(A)	*B(A)05*	Gly	**Thr**	Pro	Ser	Met	Ala	BBBB	[380]
cisAB A_2B_3	*cis-AB01*	Arg	Met	Pro	Gly	Leu	**Ala**	AAAB	[381]
cisAB A_2B_w	*cis-AB02*	Gly	Met	Pro	Ser	**Leu**	Ala	BBAB	[382]
cisAB A_2B	*cis-AB03*	Gly	Met	**Ser**	Ser	Met	Ala	BBBB	[383]
cisAB A_2B	*cis-AB04*	Arg	Met	Pro	Gly	**Met**	Gly	AABA	[384]
cisAB AB or B(A)	*cis-AB05* or *B(A)06*	Gly	Met	Pro	Ser	Met	**Gly**	BBBA	[215,385,386]
cisAB A_2B		Gly	Met	Pro	Ser	**Val**	Ala	BB^B	[387]

*Typical phenotype when paired with an *O* allele.
B(A)01 & B(A)03 differ only by synonymous changes.

and Japan (Table 2.18), a situation not concordant with the theory of a hyperactive GTB producing some A antigen. Three are *B* alleles apart from encoding an amino acid substitution at one of the four positions that differentiate GTA and GTB: *B(A)01* and *B(A)03* giving the BABB sequence and *B(A)06* (which is also named *cis-AB05*) BBBA [269,293,385,386]. The other three alleles encode substitutions at other positions, with *B(A)04* (Val214) and *B(A)05* (Thr214) encoding different amino acids substituting Met214, which is adjacent to the Asp–Val–Asp motif [345,379,380,385] (see Section 2.8.4). With all of these *B(A)* alleles, B(A) phenotype is more likely to have resulted from a shift in substrate specificity of the enzyme rather than greatly enhanced GTB activity.

One monoclonal anti-B brought about agglutination of 1.42% of group A red cell samples, all A_1, and this was considered to represent A(B) phenotype; B activity resulting from GTA_1 activity [388]. A(B) was not associated with elevated GTA activity, but A_1(B) cells did have elevated levels of H antigen and plasma H-transferase activity.

2.11.2 cisAB

In 1964, Seyfried *et al.* [389] described a Polish family in which inheritance of the ABO groups did not fit the single locus concept for ABO genetics. A and B appeared to have been inherited together in this family: an A_2B woman with a group O husband and a group O mother had two A_2B children. Numerous other similar families have been encountered since. Yamaguchi *et al.* [390] proposed the term cisAB for this phenotype. Fourteen cisAB samples were found from over a million Japanese blood donors, 0.012% of the 112710 group AB bloods tested [391].

2.11.2.1 Serological characteristics

Although the main feature of cisAB is its unusual mode of inheritance, the cisAB phenotype almost always differs from 'transAB' serologically. Serological characteristics differ from family to family, but remain consistent within a family [392,393]. Whereas B(A) red cells have normal B antigen expression and only trace levels of A, cisAB cells are readily agglutinable by both anti-A and -B.

1 The A antigen of cisAB is usually referred to as A_2, but cisAB cells generally express more A than A_2B and less than A_1B [392,394–396]. The genotype *cisAB/B* may be expressed as A_xB or A_3B [380,387]. The phenotype cisA_1B is not unknown [397,398].

2 B is almost always expressed weakly, often being described as B_3 [391]. The B antigen of cisAB may not

always be atypical; an A_2B mother of two group O children had an apparently normal B antigen [399].

3 An unusually high level of H, about the level found on A_2 cells, which is higher than that of normal A_2B cells [392].

4 Sera from cisAB people almost always contain weak anti-B. This antibody apparently recognises part of the B antigen lacking from cisAB cells [397,400]. Serum from the *cisAB/O* woman with normal B antigen did not contain anti-B [399].

5 Salivas from cisAB secretors contain normal quantities of A and large quantities of H, plus a very little B that is only detectable by inhibition of agglutination of cisAB cells by anti-B [392,393].

2.11.2.2 Molecular genetics and transferase activities

Although there have been suggestions that the cisAB phenomenon could result from the presence of both *A* and *B* alleles on the same chromosome, producing separate transferases [401], it is now clear that the usual cause is a mutated *A* or *B* gene producing an enzyme capable of transferring significant quantities of both GalNAc and Gal to the H acceptor substrate.

All of the *cisAB* genes listed in Table 2.18 represent *A* or *B* alleles containing single nucleotide changes encoding single amino acid substitutions, mostly at positions 266 or 268, the two most important residues for distinguishing between GTA and GTB activity. Two alleles (*cis-AB01* AAAB and *cis-AB04* AABA) are basically *A* alleles encoding the *B* amino acid at one of those positions and two (*cis-AB05* BBBA and *cis-AB02* BBBA) are *B* alleles encoding the *A* amino acid at one of those positions. The *cis-AB05* allele has also been named *B(A)06* [215], demonstrating a blurring of the distinctions between cisAB and B(A) phenotypes, though from their brief descriptions [385,386] cisAB appears most appropriate. Another *cisAB* allele is a *B* allele encoding a Met266Val [387]. One allele (*cis-AB03*) encodes Pro234Ser, which is not in any of the four discriminating positions [383,402]. The specificity reversal has been explained by the breakdown of van der Waals contacts that occur between Pro234 and Met266 of GTB, enabling the aliphatic tail of Met266 to adopt a configuration that opens the enzyme pocket to accommodate the larger GalNAc [403]. *B(A)02* encodes GTB with Ala234 [379].

The phenotype cisAB is most commonly found in East Asia and the most common allele is *cis-AB01* [403]. GTA and GTB activities of *cis-AB01*-transferase are 29 and 27% of normal GTA and GTB activities, respectively [403]. The alleles *cis-AB02*, *cis-AB04*, *cis-AB05* were also found in East Asians; *cis-AB03* and *cis-AB05* have been found in Caucasians.

2.12 H-deficient phenotypes

The H-deficient phenotypes are those rare phenotypes in which the red cells are totally or partially deficient in H antigen. H may or may not be present in secretions; that is, individuals with H-deficient red cell phenotypes may be ABH secretors or non-secretors. The various H-deficient phenotypes are summarised in Table 2.19.

2.12.1 Genetics of red cell H-deficient phenotypes

In 1955, Watkins and Morgan [404] suggested that H expression may be controlled by a gene at a locus independent from *ABO*, and that the Bombay phenotype could arise from homozygosity for a rare allele, *h*, at that locus. As described in Section 2.3.1, *FUT1* controls α1,2-fucosyltransferase activity in haemopoietic tissue and, consequently, H antigen expression on red cells, whereas *FUT2* controls α1,2-fucosyltransferase activity in secretory tissue. Red cell H-deficient phenotypes, whether in secretors or non-secretors, result from homozygosity or compound heterozygosity for mutations in *FUT1* that totally or partially inactivate the H-transferase in red cell progenitors. The ISBT database [405] lists 44 *FUT1* alleles. Two of them reflect an Ala/Val12 polymorphism, which is in the transmembrane domain and probably has no effect on H-transferase activity. Twenty-three of the alleles are responsible for weakened H-transferase activity; 21 of these encode amino acid substitutions, but one encodes a single nucleotide deletion (990delG) responsible for a stop signal at codon 336, near the C-terminus [406] and another a triplet insertion. The remaining 19 alleles produce inactive enzymes: 10 of these encoding amino acid substitutions and nine encoding early termination of translation either through nonsense mutation (7) or dinucleotide deletion (2). The dbRBC [215] lists 50 *FUT1* alleles. Some key references are [92,406–412].

Expression of the mutant alleles by transfection of cultured cells has shown that some alleles give rise to no α1,2-fucosyltransferase activity and some produce low levels of enzyme activity [92,93,406,407,409]. This explains the different levels of H expression found in H-deficient phenotypes.

Red cells of a Swiss non-secretor reacted with one anti-H, despite being a compound heterozygote for two *FUT1*

Table 2.19 H-deficient phenotypes.

		Antigens							Glycosyltransferases					
		Red cells*			Secretions				Serum			Red cells		
Type	Notation	A	B	H	A	B	H	Antibodies	A	B	H	A	B	H
H-deficient, non-secretor (Bombay)	O_h	–	–	–	–	–	–	anti-H	–	–	–	–	–	–
	O_h^A	–	–	–	–	–	–	anti-H	+	–	–	+	–	–
	O_h^B	–	–	–	–	–	–	anti-H	–	+	–	–	+	–
H-partially deficient, non-secretor	O_h †	–	–	–/w	–	–	–	anti-H	–	–	–/+	–	–	–
	A_h	+/w	–	–/w	–	–	–	anti-H	+	–	–/+	+	–	–
	B_h	–	+/w	–/w	–	–	–	anti-H	–	+	–/+	–	+	–
H-deficient, secretor (para-Bombay)	O_h^O-secretor	–	–	–/w	–	–	+	anti-HI	–	–	–/+	–	–	–
	O_h^A-secretor	+/w	–	–/w	+	–	+	anti-HI	+	–	–/+	+	–	–
	O_h^B-secretor	–	+/w	–/w	–	+	+	anti-HI	–	+	–/+	–	+	–
H_m (dominant)	OH_m	–	–	w	–	–	+	none	–	–	+	–	–	+
	AH_m	w	–	w	+	–	+	none	+	–	+	+	–	+
LADII		–	–	–	–	–	–	none	–	+	+	nt	nt	nt

*Tested by direct agglutination.
†only distinguished from 'atypical' O_h-non-secretor by family studies.
w, weak expression of antigen; nt, not tested.

alleles encoding premature stop codons (421A>G, Trp-140Stop and 826C>T, Gln276Stop) [411]. A possible explanation is that recombination between the two alleles could result in *FUT1* containing neither stop codon, a speculation previously put forward to explain weak A expression in an individual with compound heterozygosity for two different *O* alleles [173]

The structural loci *FUT1* and *FUT2* are very closely linked. In most cases, *FUT1* mutant alleles are associated with the same *FUT2* allele, even in unrelated individuals [93,406]. In six red cell H-deficient Japanese, nine 695G>A *FUT1* alleles were linked to *FUT2* Se^{w385}, whereas one 695G>A *FUT1* allele was linked to *FUT2 Se*; the other two *FUT1* alleles contained 721T>C [406].

Two families are described in which recombination between *FUT1* and *FUT2* may have occurred. One family contains red cell H-deficient, secretor and non-secretor members [413]. In the other family it can be inferred that a father has passed inactive *FUT1* and *FUT2* alleles to his five Bombay phenotype children, active *FUT1* and *FUT2* alleles to his four group B, secretor children, and active *FUT1* but inactive *FUT2* to his group B, non-secretor daughter [78,414].

2.12.2 Red cell H-deficient, non-secretor; the Bombay phenotype

In 1952, Bhende *et al.* [415] described the abnormal blood groups of three men from Mumbai whose red cells were group O, but H negative. All had anti-H in their serum. This rare phenotype later became known as the Bombay or O_h phenotype. Many other examples have been found since through the presence of anti-H in the serum.

2.12.2.1 Serological characteristics and genetics

O_h red cells are not agglutinated by anti-H, -A, -B, or -A,B. No H, A, or B antigens can be detected, by adsorption and elution techniques, on red cells with the 'typical O_h' phenotype [416]. Red cells of phenotypes that have been called 'atypical O_h', however, do bind anti-H, which can be detected in an eluate [417,418]. It may also be possible to adsorb and elute anti-A and/or anti-B from these cells [417,419,420].

As with most ABH non-secretors, O_h red cells are usually Le(a+b–), but may be Le(a–b–). O_h red cells never express Le^b.

No H, A, or B antigens are present in O_h saliva, which may contain Le^a, but never Le^b.

The serum of O_h individuals always contains anti-H, -A, and -B.

When describing Bombay phenotypes the appropriate superscript may be added to the O_h notation when the *ABO* genotype is determined by family study, by glycosyltransferase analysis, or by molecular genetical tests: O_h^O, O_h^A, O_h^B, O_h^{AB}.

The typical Bombay phenotype in people originating from India results from homozygosity for *FUT1* 725T>G, Leu242Arg, together with homozygosity for a deletion of *FUT2* [92,93].

Heterozygosity for an inactive *FUT1* allele may result in reduced H expression [421] (see Section 2.12.5.1).

Unlike Rh_{null} cells, there is no evidence to suggest that Bombay phenotype cells are haematologically abnormal. Autologous ^{51}Cr-labelled O_h red cells survive normally [422,423].

2.12.2.2 Glycosyltransferases

H-transferase has not been detected in the serum or red cell membranes of O_h individuals [101,184,424]. O_h sera and red cells contain GTA and GTB when *A* and *B* genes are present [101,205]. These enzymes are unable to act in the absence of their acceptor substrate (H antigen) and neither A nor B structure is produced. O_h red cells that have been made H-active *in vitro*, in the presence of H-transferase, can be converted to A- or B-active cells by GTA or GTB [425]. In families, sera from heterozygous *H/h* members have about half the H-transferase activity of serum from *H/H* homozygotes [426].

2.12.2.3 Frequency and distribution

The Bombay phenotype is very rare, but appears to be less rare in India than elsewhere with an O_h frequency of about 1 in 7600 Indians in Bombay, an *h* gene frequency of 0.0115 [427]. A rich source of two types of H-deficiency phenotype exists in Réunion Island in the Indian Ocean: typical O_h in the Tamil Indian population and partial red cell H-deficiency, non-secretor in the population of European origin [428]. O_h has also been found in other ethnic groups, including people of European origin [407,411,419,420], where the 'atypical Oh' phenotype may predominate, African Americans [429], and in Japanese, where homozygosity for Se^{w385} suggests that they are actually weak secretors [406].

2.12.3 Red cell H-partially deficient, non-secretor

Levine *et al.* [430] used the notation A_h to describe a phenotype in a non-secretor Czech woman whose red cells lacked H, but were weakly agglutinated by anti-A. The equivalent B phenotype, B_h, was also found in a Czech [431]. AB_h has also been described [428,432]. A_h, B_h, and AB_h have mainly been reported in people of European origin [428,430–434]. The term para-Bombay has been used for these red cell H-partially deficient, non-secretor phenotypes, but is better reserved for H-deficient and -partially deficient secretors.

2.12.3.1 Serological characteristics and genetics

The strength of A expression on red cells of some A_h individuals resembles weak A_2 [430,433], whereas those of others are more like A_x, being agglutinated by only a minority of anti-A sera [434–436]. Likewise, B_h red cells have weak B antigen [431,437]. Little or no H antigen is detected on these cells. No H, A, or B antigen is present in the saliva and, like red cells of most non-secretors, A_h and B_h cells are usually Le(a+b–), but may be Le(a–b–) [426,432]. The serum contains anti-H. A_h serum contains anti-B, but no anti-A, although anti-A_1 is usually present [430,435,436]; in B_h, anti-A is always present and anti-B may also be detected [431,437].

Many examples of A_h, B_h, and AB_h, as well as O_h, have been identified in the people of French origin on the small island of Réunion, off the East Coast of Africa [428,432]. O_h in this population arises from the same *FUT1* genotype as the A_h, B_h, and AB_h phenotypes, because they are present in the same families and have the same *FUT1* mutation, 349C>T encoding His117Tyr in the stem region of the enzyme (together with the European non-secretor allele, se^{428}, in *FUT2*) [93]. This 'Réunion O_h phenotype' can be distinguished from Bombay phenotype by the quantity of H on the cells [428]. Purified *Ulex europaeus* lectin agglutinated papain-treated Réunion phenotype cells, but not Bombay phenotype cells, and high-titred H antibodies found in the sera of Bombay phenotype Indians agglutinated Réunion phenotype cells. These same sera agglutinated red cells from some O_h Europeans [428]. The term O_h is ambiguous. It can represent homozygosity for an *h* allele that produces no active α1,2-fucosyltransferase (Bombay phenotype), or homozygosity for an *h* allele that produces weakly active α1,2-fucosyltransferase in people with no *A* or *B* gene and, therefore, no weak expression of A or B antigen. There is a series of weak *FUT1* alleles resulting in different degrees of red cell H deficiency.

2.12.3.2 Glycosyltransferases

Trace quantities of H-transferase activity were detected in sera from red cell H-partially deficient, non-secretor

individuals from Réunion Island [426], although no H-transferase activity could be detected in sera or red cell membranes from A_h or B_h individuals [101,184,424]. As with O_h^A and O_h^B, A_h and B_h sera contain *A* and *B* gene-specified glycosyltransferases, respectively [101,424].

Red cell H-partially deficient phenotypes arise from homozygosity for a mutant gene at the *FUT1* locus, which produces only a very weakly active H-transferase. Consequently, the small amount of H produced is completely converted to A or B. Mulet *et al*. [438] demonstrated that H is the precursor of B on the cells of a B_h individual. B_h (B+ H–) red cells treated with α-galactosidase extract of *Trichomonas foetus* lost their B antigen and became H-active. These B– H+ cells could then be converted to A_h (A+ H–) by A-transferase. If the α-galactosidase-treated B_h red cells (B– H+) were treated with H-degrading α-fucosidase from *T. foetus*, they could no longer be converted to A activity.

2.12.4 Red cell H-deficient, secretor

Red cells of people with another type of H-deficiency have little or no H, A, and B red cell antigens, yet they are ABH secretors, with secretions containing normal quantities of H, A, and B. In the first family showing that people lacking H from their red cells could secrete H, two brothers, whose red cells lacked H and bound anti-A, but were not agglutinated by it, secreted A and H; a third brother, with group O, H-negative red cells, secreted H alone [439]. A secretor of B and H with H-deficient red cells was subsequently found [440]. The terms O_h-secretor, A_h-secretor, and B_h-secretor are recommended to describe the phenotypes (Table 2.19).

2.12.4.1 Serological characteristics and genetics

Red cells of O_h-secretors are not agglutinated by most H antibodies, but they may be agglutinated weakly by the potent anti-H in some O_h sera and by other strong anti-H reagents [441]. Adsorption and elution of anti-H may or may not reveal H antigen on red cells of O_h-secretors [80,440].

O_h-secretor red cells are not usually agglutinated by anti-A or -B, but some O_h^A-secretor cells behave like A_x cells and are agglutinated by anti-A,B and very potent anti-A [439,442]. Sometimes the A antigen can only be detected by adsorption and elution of anti-A. A similar variation exists with B antigen strength in O_h^B-secretors [80,440,442,443].

Like those of most secretors, O_h-secretor red cells are usually Le(a–b+), but may be Le(a–b–). The Le(a+b+) phenotype, common in the Far East, was not found in 25 Taiwanese O_h-secretors [408], but was found in two of 51 Hong Kong Chinese O_h-secretors, about half the normal incidence [441].

H substance is present in saliva, in approximately normal quantities for an O secretor [80,439–444]. A and B substances are detected in normal quantities in the secretions when *A* and *B* genes are present.

The serum almost always contains an H-like antibody, which is generally weak and reacts only at low temperature. This antibody, called anti-HI, is not inhibited by secretor saliva and does not react with group O cord cells. Two thirds of O_h-secretors from Hong Kong had anti-HI or anti-H active at 37°C [441].

Numerous *FUT1* mutations have been found to be responsible for red cell H-deficiency in secretors [215, 405], most of them from East Asia. Probably the two most common in China contain dinucleotide deletions, 547delAG and 880delTT [408,412], whereas in Japan the two most common alleles appear to be 990delG and 721T>C, Tyr242His [406].

2.12.4.2 Glycosyltransferases

Originally no H-transferase was detected in O_h-secretor sera or red cell membranes, although the appropriate A- and B-transferases were present [424,445]. In four O_h-secretor sera H-transferase activity representing about 5–10% of that found in sera of people with normal H phenotypes indicated that this enzyme derived from secretory tissues [80].

2.12.4.3 Frequency and distribution

Most H-deficient secretors have been found in Eastern Asia – Chinese [408,412,441,446] and Japanese [406, 409,440] – but also very rarely in other ethnic groups including people of European origin [80,411,443,447], from the Middle East [80,411], and a Native American [448]. Some estimated frequencies: one in 5000 Thais [442], one in 8000 Taiwanese [449], and one in 15 620 Hong Kong Chinese [441]. Among 324 Lahu Chinese, a nomadic ethnic minority in China, seven (2.2%) were H-deficient secretors. All were either homozygous or heterozygous for one or both of two *FUT1* alleles: 328G>A, Ala110Thr and 658C>T, Arg220Cys [450].

2.12.5 Other H-deficient phenotypes

2.12.5.1 H_m

The primary characteristic of the H_m phenotype (Table 2.19) is its dominant mode of inheritance, the rare phenotype appearing in several generations of the same family [424,447,451,452]. H_m red cells are weakly

agglutinated by anti-H, but the H deficiency is not as dramatic as in Bombay or para-Bombay phenotypes. The saliva contains normal quantities of H and H-transferase is present in serum and red cell membranes. AH_m cells show depression of the A antigen. In one family the proposito was A_2, but had very little H on his cells; the GTA was of the A_1 type, but presumably insufficient H was available for A_1 antigen expression [424,445].

Two individuals with slightly weakened A or B antigens had H– red cells [421], a phenotype resembling H_m. Each had one *FUT1* allele with the consensus sequence and one inactive allele with either 684G>A (Met228Ile) or 694T>C (Trp232Pro). It is possible that the H_m phenotype simply results from heterozygosity for an inactive *FUT1* allele.

2.12.5.2 Leucocyte adhesion deficiency type II (LADII)

LADII (also known as congenital disorder of glycosylation type IIc, CDGIIc) is a generalised fucosylation defect associated with recurrent infections, short stature, mental retardation, and a distinctive facial appearance, but also with H-deficient (Bombay phenotype) red cells, ABH non-secretion, and Le(a–b–) red cell phenotype (reviewed in [453]). Transferase assays on one patient revealed normal levels of H- and Le-transferase activity in his serum and saliva, respectively [454]. The leucocyte adhesion defect results from a deficiency of sialyl-Le^x, a fucosylated ligand for selectins (Section 2.18.3). Treatment with oral L-fucose reverses most of the symptoms of LADII in some patients, although it has no effect on the red cell Bombay phenotype [455].

LADII results from homozygosity for mutations in the gene (*SLC35C1*) that encodes the GDP-fucose transporter, responsible for transfer of GDP-fucose, the donor substrate for various fucosyltransferases, from the cytosol to the lumen of Golgi apparatus where *N*-glycans are fucosylated. Six patients are reported: four Arab children [456] with *SLC35C1* 923C>G, Thr308Arg [457]; a boy of Turkish origin [458] with 439C>T, Arg147Cys [457,459]; a girl of Pakistani origin with 969G>A, Leu322stop [460]; and a patient of Brazilian origin with 588delG introducing premature termination of translation [461].

2.12.6 I and i expression in H-deficient phenotypes

The I and i antigen structures represent carbohydrate chains that are precursors of H, A, B, Le^a, and Le^b, so it is not surprising that I and i expression is elevated in H-deficient red cells (see Chapter 25). This effect has been demonstrated on O_h cells and on red cells of some H-deficient, secretors by agglutination titrations with anti-I and -i. Measurement of percentage agglutination by an electronic cell counter demonstrated significantly higher agglutination by anti-I with O_h, A_h, and B_h cells from non-secretors (90.2%), compared with control cells (73.5%) [462].

2.13 Acquired alterations of A, B, and H antigens on red cells

Since the ABO blood groups were shown to be inherited characters, numerous rare variants have been recognised, many of which have been described in this chapter. Most of these variants are inherited, resulting from mutant genes at the *ABO*, *FUT1*, and occasionally other loci. Some ABO anomalies, however, are acquired, generally as a result of infection or malignancy, or the effect of laboratory intervention.

2.13.1 Acquired B

Over a period of four years, Cameron *et al.* [463] identified seven patients with some kind of red cell B antigen, but with apparently normal anti-B in their sera. The anti-B did not react with the patients' own red cells. Cameron *et al.* [463] gradually came to appreciate that this B-like antigen was an acquired character, probably associated with disease. All seven patients were A_1, the secretors secreted A and H, but no B, and four had group O children and therefore had an A^1/O genotype.

Most individuals with acquired B are ill, although examples of acquired B in healthy subjects are recorded [464–466]. An estimated 64% of reported cases had diseases of the digestive tract, most of those being carcinoma of the colon [467]. In a survey of 200 patients (106 group O, 94 group A) with gastrointestinal disease, 10 cases of acquired B were found, all in group A patients [468].

Sera from patients with acquired B antigen contain GTA, but no GTB [469]. No *B* allele was present in the genome of patients with acquired B [261,470,471].

2.13.1.1 Serological characteristics

Acquired B is only found on group A cells. These are nearly always A_1, although A expression may be depressed [467]. A few examples of A_2 with acquired B have been found: in one case the cells became A_1 as B expression diminished [472]; in another the patient had serum GTA_2 [473]; and in two cases the patients had A^2/O genotypes [261]. One example of acquired B was associated with weak expression of A and H antigens [474].

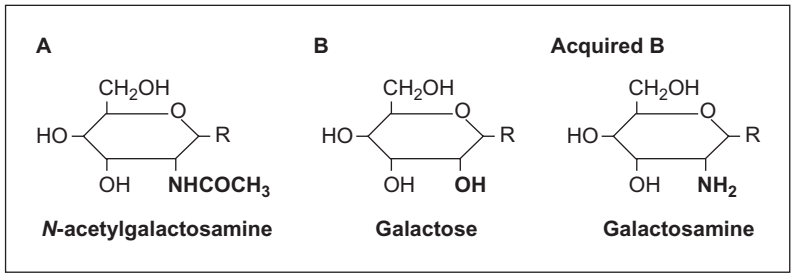

Figure 2.9 Terminal immunodominant sugars for A, B, and acquired B, demonstrating the similarity between Gal and galactosamine (deacetylated GalNAc). R, remainder of molecule.

Acquired B antigen is usually weak, but varies in different individuals and with time. Often a proportion of cells remain unagglutinated with anti-B. Sera from A_2 donors are better at detecting acquired B than are sera from A_1 donors [469]. Some group A and O sera contain a specific anti-acquired B, which does not react with normal B cells and can be separated from anti-B by adsorption and elution [465,475]. Anti-acquired B was produced by immunising a rabbit with acquired B red cells [465].

Some monoclonal anti-B react with acquired B cells [475–478] and monoclonal anti-acquired B has been produced by immunising mice with acquired B red cells [479,480]. A blood-grouping reagent containing a monoclonal anti-B clone (ES4) that strongly agglutinates acquired B cells, greatly increased the rate of detection of this phenotype [478]. A group A patient with acquired B was grouped as AB with reagents containing ES4 and suffered a fatal haemolytic reaction following transfusion with four group AB units [481]. The patient's weak anti-B was not detected by abbreviated compatibility testing. ES4 is no longer used as an anti-B reagent.

Serum from acquired B individuals contains anti-B, which does not react with acquired B cells. Saliva of acquired B secretors contains A and H, but no B.

Acquired B red cells are often polyagglutinable (see below).

2.13.1.2 Cause of acquired B

Evidence suggesting that acquired B might result from enzyme action on red cells included the conversion of group A cells to acquired B activity, *in vitro*, by bacterial filtrates [482] or by sera from individuals with acquired B [466,483,484]. This led Gerbal *et al.* [474] to hypothesise that bacterial deacetylases convert GalNAc, the A immunodominant sugar, to galactosamine, which is similar enough to Gal, the B immunodominant sugar, to react with some anti-B (Figure 2.9). A wealth of evidence, summarised below, confirms that deacetylation of GalNAc is the most common cause of acquired B [467–469,481,485].

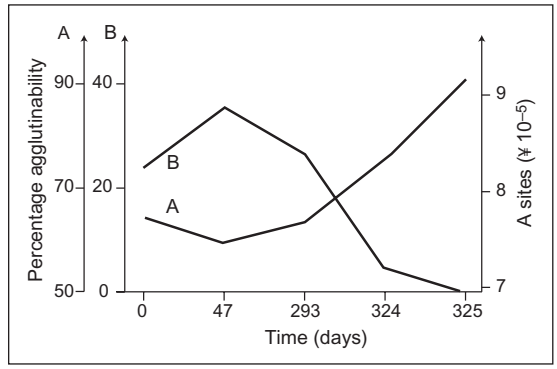

Figure 2.10 Graph demonstrating the inverse relation between A and acquired B expression, as measured by percentage agglutination and number of A sites, in a patient studied over several months. As acquired B expression increased, A antigen expression decreased, and vice versa. Adapted from [469].

1 Only group A cells acquire B antigen.

2 The strength of A antigen expression on acquired B cells is inversely related to the strength of the acquired B antigen [469] (Figure 2.10). Two populations of red cells from a patient with acquired B were separated with *Dolichos biflorus* lectin: the A_1 cells agglutinated by the lectin had only weak acquired B, whereas the remaining A_2 cells had strong acquired B expression [467].

3 Deacetylases have been isolated from the bacteria *Clostridium tertium* A and *Escherichia coli* K12 [486,487]. Acquired B cells could be created, *in vitro*, by treating A_1 cells with culture filtrate from *C. tertium* or from one of six strains of *E. coli* [467,469]. Group O cells were not converted to B activity.

4 Chemical acetylation of acquired B cells with acetic anhydride destroyed the B activity and raised the A activity back to that of normal A_1 cells [485].
5 A-trisaccharide [GalNAcα1→3(Fucα1→2)Gal] that has been chemically deacetylated inhibits the reaction of anti-B with acquired B, but not with normal B cells [488]. B-trisaccharide [Galα1→3(Fucα1→2)Gal], in which the hydroxyl group of carbon-2 of the α-Gal residue has been substituted by an amino group (see Figure 2.9), had the same effect [489]. Agglutination of acquired B cells with anti-B is dispersed by the addition of galactosamine [490].
6 Suspension of acquired B red cells in an acid medium (pH 6) reduces reactivity with anti-B [469], presumably because the NH_2 group of the galactosamine residue is converted to NH_3^+.

2.13.1.3 Polyagglutination

Acquired B red cells are usually polyagglutinable [467,491]; they are agglutinated, at least weakly, by most AB sera. This polyagglutination evolves in parallel with the acquired B phenomenon, but disappears before B activity during recovery; it is not apparent at pH 4.5 or below and it disappears after chemical acetylation of the cells [467,476,485]. Agglutination of acquired B cells by AB serum is inhibited by deacetylated A-trisaccharide, by amino-substituted B-trisaccharide, and by galactosamine [475,488,489].

It is possible that there are antibodies present in most human sera specific for the deacetylated A antigen that are responsible for the acquired B phenomenon. As polyagglutinable cells can be produced by deacetylation with *C. tertium* filtrate of O cells, as well as A cells [467], a different antigen from acquired B is probably involved, possibly involving glucosamine produced by deacetylation of GlcNAc [492].

Acquired B is a unique type of polyagglutination. Acquired B cells react with AB sera from which anti-T, -Tk, -Tn, -Cad, and anti-CDA II have been removed by adsorption [485,493] (see Chapter 33). However, other cryptantigens, responsible for other forms of polyagglutination, are often revealed on acquired B cells, especially Tk, but also T and Th [466,475,483,494,495].

2.13.2 Alterations in leukaemia patients

2.13.2.1 Serology

The association of weak A expression with acute myeloid leukaemia (AML), first recognised in 1957 [496], is well documented (reviewed in [497,498]). In some cases all red cells show weakness of A, whereas in others two populations of red cells are clearly apparent [499–502]. Two populations of red cells from a patient with acute monoblastic leukaemia were separated [499]. Initially only 2% were agglutinated with anti-A, but in remission the proportion of agglutinable cells rose to 65% before falling again shortly before death. In another patient 26% of the red cells were group AB, 12% A, 42% B, and 20% O [503]. Presumably the patient was genetically AB; 62% of his cells had lost their A antigen and 32% their B antigen. Leukaemia-associated changes in B and H antigens are also recorded [502–504]. Between 17% and 37% of patients with leukaemia have significantly lower A, B, or H antigenic expression compared with healthy controls [504–507]. By flow cytometry, 55% of A, B or AB patients with myeloid malignancies had decreased expression of A or B compared with healthy controls of the same ABO genotype; 21% of group O patients had reduced H [508]. In almost all cases the changes represent a loss or diminution of antigen strength and not the expression of a new red cell antigen, although one case is reported of a group O (O^{Iv}/O^{Iv}) patient with myelodysplastic syndrome acquiring an A antigen [261].

Although modifications of ABH antigens are usually associated with acute leukaemia, they are also often manifested before diagnosis of malignancy and therefore indicate preleukaemic states [497]. Loss of an ABH antigen in a patient with a haematological disorder is generally prognostic of AML [509]. For example, a 4-year-old girl whose red cells gave mixed field agglutination with anti-A initially had no sign of haematological disease, but was diagnosed with AML 18 months later [510].

Acute leukaemia has, on occasion, been associated with loss or weakening of Lewis antigens [499,509,511].

2.13.2.2 Transferases and epigenetics

Depression of A or B antigens in AML and in preleukaemic states is generally associated with a severe reduction in red cell GTA or GTB activity, but little or no reduction in red cell H-transferase activity [510,512,513]. In patients with separable populations of red cells, GTA or GTB activity was greatly reduced in the membranes of those cells that had lost their A or B antigens, but were normal in those that had not [512]. During clinical remission, the A antigen of one of the patients returned to normal, as did membrane GTA activity [512]. Thus, the loss of A or B expression in AML results from a defect or deficiency of the *A* or *B* gene products and not a defect in enzyme substrates.

About 58% of AML patients with ABH antigen loss, detected either by conventional serology or by flow

cytometry, had corresponding loss of expression of mRNA, suggesting gene inactivation, and 73% of these patients had hypermethylation of the *ABO* promoter [514]. Furthermore, *ABO* transcripts were re-expressed in leukaemic cell lines after treatment with demethylating agents. Consequently, at least one cause of silencing of the *ABO* gene in AML appears to be epigenetic modification of the *ABO* promoter.

Other mechanisms may also be involved. Gene deletion would be a rare cause as no loss of heterozygosity was detected in 28 AML patients with ABH antigen loss [514]. In a patient with erythroleukaemia, however, about 50% of the red cells had lost their A antigen and those cells also showed a very low level of adenylate kinase-1 an enzyme encoded by a gene close to *ABO* on chromosome 9 [515]. This was presumed to result from a chromosome lesion in the part of the chromosome containing both *ABO* and *AK1*. The *ABL1* oncogene maps between *ABO* and *AK1* on chromosome 9q34 and is at the breakpoint of the Philadelphia-chromosome, a leukaemia-specific reciprocal translocation involving paternal chromosome 9 and maternal chromosome 22 [516]. In four informative cases of A or B antigen loss during AML, the allele that was lost could only have been maternally derived [517]. The significance of this is unclear, but the results suggest that imprinting affects other loci on chromosome 9q34 other than *ABL1*.

Serum H-transferase activity is generally reduced in patients with AML [512,518–520], but increased in those with chronic granulocytic leukaemia [520,521].

2.13.3 Other acquired changes in ABO antigens

Acquired loss of A from a proportion of the red cells occasionally occurs in healthy, elderly individuals [522, 523]. Weakened A expression may also occur in pregnancy, and this is often most obvious in women with A^2/B genotype [261,262].

A premature baby (26 weeks) was typed as group B at birth, but became A_2B by day 73, presumably an effect of the prematurity [524]. A healthy child who was A_2 at birth, but later became A_x, is described in Section 2.7.3 [294].

Following transplantation of a liver from a group AB donor, a proportion of the red cells of a group O child became transiently group AB, as did group O transfused red cells [525]. The cause of this phenomenon is unknown, but could have been caused by hepatic transferase activity. Weak A activity on red cells of group A recipients of group O bone marrow transplants may result from adsorption of A substance from the plasma of the recipient [526].

2.13.4 *In vitro* enzymatic degradation of A, B, and H antigens

The exoglycosidases α-*N*-acetylgalactosaminidase (A-zyme) and α-galactosidase (B-zyme) cleave the A and B immunodominant monosaccharides GalNAc and Gal from A- and B-active oligosaccharides, to destroy A and B blood group activity, reveal H, and produce enzyme-converted group O (ECO) red cells (reviewed in [527–529]). In times of blood insufficiency there is often a particular shortage of group O. Large-scale production of ECO cells from A, B, and AB, RhD-negative cells would be a boost to the blood supply and would assist in providing suitable blood for patients with antibodies to very common antigens, patients with multiple antibodies to polymorphic antigens, and transfusion-dependent patients who should receive matched blood to help prevent them from making multiple antibodies.

Although α-galactosidase from green coffee beans (*Coffea canephora*) appeared to convert group B red cells to group O, the enzyme was not very efficient and required low pH for optimum activity. Phase I and phase II clinical trials, on healthy volunteers and patients, respectively, have shown that group B red cells treated with either native or recombinant α-galactosidase from coffee beans are safe and efficacious when transfused to group O or A subjects, once, in multiple-unit volumes, or on more than one occasion [530,531]. Early attempts at converting A red cells to O with α-*N*-acetylgalactosaminidases from chicken liver or from the bacteria *Ruminococcus torques* IX-70 and *Clostridium perfringens* were less successful. Although A_2 cells could be converted, A_1 cells remained stubbornly A-active, possibly because of the complex repetitive Type 3 A structures present on glycolipids of A_1, but not A_2, red cells (Section 2.4.2).

In 2007, Liu *et al.* [532] went fishing through 2500 fungal and bacterial isolates for more suitable enzymes. The upshot was the production of novel recombinant glycosidases, derived from *Elizabethkingia meningosepticum* and *Bacteroides fragilis*. The enzymes derived from these bacteria had high efficiency and substrate specificity for cleavage of the A and B immunodominant monosaccharides, respectively, under reaction conditions suitable for maintenance of red cell integrity and functions, and with properties that facilitate enzyme removal from the converted red cells by routine washing methods. Consequently, following a 60-minute incubation time with the

appropriate enzyme, whole units (200 ml) of A_1, A_2, B, and A_1B red cells expressed neither A nor B antigens as determined by licensed blood grouping reagents. The results of clinical trials with ECO red cells are still to be published [529].

An α-fucosidase isolated from *Aspergillus niger* abolished H activity on group O cells [533].

2.13.5 Modification of antigen expression by polyethylene glycol (PEG)

Another methodology investigated as a way to convert group A or B red cells to artificial group O cells is the use of PEG to mask the A and B determinants (stealth red cells). PEG is a non-ionic polyether that exists in many configurations. Pegylation of red cell surface glycoproteins could provide a coating of PEG molecules and water attracted by the PEG, which blocks access of antibodies. Coating of red cells with modified high molecular weight PEG molecules of different chain lengths has led to the production of red cells with substantially reduced surface antigen expression (reviewed in [534]). Unfortunately, PEG is immunogenic and antibodies to PEG shorten survival of PEG-treated red cells in rabbits and of pegylated proteins in humans [534]. Consequently, it is unlikely that PEG-modified 'universal' red cells will become part of transfusion practice.

2.14 ABH antibodies and lectins

2.14.1 Anti-A and -B

Anti-A and -B are almost always present in sera of people who lack the corresponding antigen from their red cells (Table 2.2). With the exception of newborn infants, deviations from this rule are extremely rare; only about 1 in 12 000 adults lack expected anti-A or -B [535]. Missing agglutinins may indicate a weak subgroup of A or B, a twin or tetragametic chimera, hypogammaglobulinaemia, or old age, though very rarely are missing agglutinins with no apparent explanation encountered.

Antibodies detected in the serum of neonates are usually IgG and maternal in origin [536], but may, on occasion, be IgM and produced by the fetus [537]. Maternal ABO haemagglutinins, which have the potential to cause fatal HTRs, were detected in 6.4% of neonates, but were cleared within the first month of life [538]. Generally, ABO agglutinins are first detected at an age of about 3 months and continue to increase in titre, reaching adult levels between 5 and 10 years [537,539].

Levels of A and B antibodies appear to be influenced mainly by environmental factors, genetics playing no more than a minor role [540,541]. Anti-A and -B are often referred to as naturally occurring, probably appearing in infants as a result of immunisation by A and B substances present in the environment. Springer *et al.* [542,543] found that chickens, which normally develop an antibody to human group B red cells within a few months of hatching, fail to do so if kept in a germ-free environment. Feeding human infants with killed bacteria (*Escherichia coli* O_{86}) stimulated increased anti-B activity [544]. Changes in diet were considered responsible for a decrease in anti-A and -B titres in Japanese donors over a period of 15 years [545].

Changes in the characteristics of anti-A or -B occur as a result of further immunisation by pregnancy or by artificial means, such as incompatible transfusion of red cells or other blood products. Typical changes, serologically detectable, are increase in titre and avidity of agglutinin, increase in haemolytic activity, and greater activity at 37°C. Such 'immune' sera are generally difficult to inhibit with saliva or with A or B substances.

Anti-A and -B molecules may be IgM, IgG or IgA; some sera may contain all three classes [537,546]. Anti-A and -B of non-stimulated individuals are predominantly IgM, although IgG and IgA may be present [547,548]. During a programme of immunising donors with human A or B glycoproteins, with the purpose of producing potent blood grouping reagents, some IgG anti-A or -B was detected in all donors prior to immunisation [549]. These donors, who had been selected for high titre antibodies, all showed an increase in IgG after stimulation; IgA anti-A and -B, which could not be detected in any of the sera pre-immunisation, was present in all sera post-immunisation. Table 2.20 shows some of the characteristics of IgM, IgG, and IgA ABO antibodies. IgG1 and IgG2 anti-A and/or -B were present in most sera from mothers of group A or B children; almost 40% of the sera also contained IgG3 and/or IgG4 anti-A/B [550]. IgG2 usually had a higher titre than antibodies of the other subclasses. A quantitative analysis of sera from 235 healthy blood donors showed IgG1 and IgG2 anti-A/B predominant, with IgG3 and IgG4 playing only a minor role [546].

ABO antibodies may be found in various body fluids including saliva, milk, cervical secretions, tears, and the contents of cysts [551–556]. They are primarily IgA [554] and generally most active in fluids from group O individuals.

Anti-A_1 is described in Section 2.4.

Table 2.20 Some characteristics of IgM, IgG, and IgA anti-A and -B (compiled mostly from [537]).

Characteristic	IgM	IgG	IgA
Present in sera of			
non-immunised donors	Yes	Sometimes	Rarely
immunised donors	Yes	Usually	Usually
Agglutinates red cells	Yes	Yes	Yes
Agglutination enhanced in serum medium	No	Yes	
Haemolytic	Yes	Yes	No
Binds complement	Yes	Yes	No
Titre increased in antiglobulin test	No	Yes	Yes
Inhibited by secretor saliva or purified glycoprotein	Yes, easily	Poorly	Yes, less easily than IgM
Thermal optimum	4°C	4-37°C	
Activity destroyed by 2-ME or DTT	Yes	No	Partially
Activity destroyed by heating to 56°C	Yes	No	No
Present in colostrum	Sometimes	No	Yes

2-ME, 2-mercaptoethanol; DTT, dithiothreitol.

An interesting antibody described in 1953 remains unique [557]. In a saline medium this antibody agglutinated only group A Rh D+ cells; A_1 D+ cells gave stronger reactions than A_2 D+ cells. O D+ and A D– cells were not agglutinated. When the reactivity of the antibody was enhanced by addition of albumin it behaved as anti-D. The antibody could be completely adsorbed by O D+ cells, but it was not adsorbed by A_1 D– cells. Perhaps this D-like antibody recognised conformational changes in the Rh complex occurring with the presence or absence of an A determinant on the Rh-associated glycoproteins (see Section 5.5.6).

2.14.2 Anti-A,B of group O serum

Sera from group O people do not simply contain two separable antibodies, anti-A and -B, but a cross-reacting antibody called anti-A,B; an antibody that detects a structure common to both A and B determinants [11,558–561]. If an eluate is made from group A cells incubated in group O serum the antibody in the eluate will agglutinate A and B cells [198]. The same effect is observed if group B cells are used. There is, however, no such effect if artificial anti-A+B is made by mixing group A and group B sera [562,563].

The cross-reactivity of group O sera is often asymmetrical; some group O sera eluted from A cells react with B cells, yet when eluted from B cells will not react with A cells [563]. In group O people immunised with A cells or A substance the cross-reacting antibody usually shows a preference for A cells [549,564]; that is, it has a higher binding constant for A cells than for B cells [565]. The reverse is true in group O individuals immunised with B antigen.

Anti-A,B are mostly IgG, but may be IgM or IgA [537].

2.14.3 Clinical significance of ABO antibodies

Transfusion of ABO major incompatible red cells (e.g. A to O, B to O, A to B, B to A), where antibody in the recipient will destroy the transfused red cells, will almost always result in symptoms of an HTR and may cause disseminated intravascular coagulation, renal failure, and death. Of 36 Americans who received more than 50 ml of incompatible blood, 23 (64%) manifested signs or symptoms related to the incompatible transfusion and 6 (17%) died [566]. (See [537] for details on transfusion reactions.)

In transfusion practice group O is often considered the 'universal donor' and transfusion of O blood to an A or B recipient considered a compatible transfusion. The presence of ABO antibodies in minor incompatible (e.g. O to A, O to B) whole blood transfusion, however, may lead to destruction of the recipient's red cells and an HTR (see [537]), though this can be avoided by screening for donors with high levels of anti-A or -B. There is increasing evidence that infusion of relatively large quantities of ABO incompatible plasma, as frequently occurs when

transfusing ABO incompatible platelets, could cause impaired cellular immune function, infection, and multiorgan failure by a mechanism unrelated to haemolysis [567]. This might result from tissue damage caused by the presence of ABO antibodies in the transfused plasma or the presence of circulating immune complexes comprising soluble ABO substances in the transfused plasma and the recipient's antibodies. Furthermore, in a large cohort of plasma recipients, mortality was significantly higher in those who received ABO compatible, non-identical plasma, than in those who received ABO identical plasma [568].

Anti-A_1 is rarely clinically significant and most examples are not active above 25°C. There are, however, a few reports of HTRs caused by anti-A_1 [569–572, 854].

When it occurs, HDFN caused by ABO antibodies is usually in A_1 or B babies of group O mothers. Very rarely, group B babies of A_2 mothers may be affected. About 15% of pregnancies in women of European origin involve a group O mother with a group A or B fetus, yet ABO HDFN requiring clinical intervention is rare, though minor symptoms involving a small degree of red cell destruction may be relatively common. Hydrops caused by ABO HDFN is exceedingly rare, but very occasionally exchange transfusion for the prevention of kernicterus is indicated. Severe ABO HDFN is uncommon, despite the presence of IgG ABO antibodies in the serum of most group O women, because of the relatively low density of A and B antigens on fetal red cells and the presence of soluble A and B substances in the fetal plasma, which neutralise maternal antibodies. The complement deficiency of fetal plasma may also play a part in the rarity of ABO HDFN as IgG anti-A that haemolyses red cells in the presence of complement will not lyse cord cells if neonatal serum is used as the source of complement [573]. Also see Section 25.8.

2.14.4 ABO autoantibodies

ABO autoantibodies are rare. In one English blood centre, only six of 4668 patients with autoantibodies studied over 32 years had autoantibodies with ABO specificity [574]. Some apparent autoanti-A and -B do not react with group A or B cord cells and so their true specificity is anti-AI or -BI (Section 25.7.6). Several examples of autoanti-A and -B have caused AIHA [574], one resulting in fatal haemolysis and kidney failure [575]. One autoanti-B was associated with acrocyanosis, but no AIHA [576]. Autoanti-A_1 has been reported, but not implicated in AIHA [574].

2.14.5 ABO and transplantation

ABO antigens are expressed throughout the body (Section 2.19) and so represent histocompatibility antigens that are very pertinent to transplantation. Anti-A and -B can cause hyperacute rejection of incompatible kidney, liver, and heart, yet major ABO incompatible (ABOi) kidney transplantation has now become common practice, reducing the burden on the donor pool and facilitating use of living kidney donors. To achieve a successful ABOi transplant, ABO antibody levels in the recipient are reduced by pre-, peri-, and post-operative plasma exchange, either with plasma of donor type, which will contain soluble A or B antigens, or with autologous plasma with ABO antibodies removed by passing the plasma through a column containing A- or B-active oligosaccharides on an insoluble matrix. The patient is treated with intravenous immunoglobulin (IVIg) and with anti-CD20 (Rituximab) to inhibit B-cell activity (reviewed in [566,577–580]). Eventually the antibodies levels return from their low levels, but the graft usually continues to function well as a result of accommodation, an acquired resistance of an organ to immune-mediated damage, the mechanism of which is still uncertain [579]. Owing to the extremely low level of expression of A antigen on non-erythroid tissues of A_2 donors, A_2 grafts can be treated as group O for transplantation. Infants do not produce ABO antibodies during the first months of life, so ABOi heart and lung transplants can be carried out without applying the special procedures required for ABOi transplantation in adults [581,582].

Anti-A and -B can usually be disregarded for tissue transplants, including cornea, skin, and bone [583].

About 40–50% of haemopoietic progenitor cell (HPC) transplants are ABOi. Whether major ABOi has any deleterious effect remains controversial. Complications of major ABOi are haemolysis of residual red cells in the graft, delayed erythroid engraftment, resulting in extended dependency on transfusion, and pure red cell aplasia (reviewed in [577,584–587]). The risks of delayed engraftment and pure red cell aplasia are increased in non-myeloablative procedures.

Passenger lymphocyte syndrome is a form of graft versus host disease in which lymphocytes of donor origin are transferred to the recipient of a minor ABOi solid organ or HPC transplant, and leads to haemolysis of the patient's own red cells (reviewed in [577,587,588]). In solid organ transplantation the risk is higher in lung and heart/lung transplants, with greater levels of lymphoid tissue engrafted, than in liver and kidney transplants. Typically the ABO antibodies are IgG, appear 7–10 days

after transplantation, and last for about one month. They are occasionally responsible for severe haemolysis and have caused acute renal failure and death. Passenger lymphocyte syndrome is more commonly a complication of minor ABOi HPC transplants, especially following non-myeloablative conditioning. Haemolysis may be severe and even fatal.

2.14.6 Monoclonal antibodies

Three years after Köhler and Milstein [589] described their method for *in vitro* production of monoclonal antibodies from hybridomas of murine myeloma cells and lymphocytes from immunised mice, Barnstable *et al.* [590] reported the first monoclonal blood group antibody, anti-A. This antibody resulted from immunising a mouse with human tonsil lymphocyte preparations.

Numerous other monoclonal anti-A and -B followed: some produced deliberately by immunising mice with group A or B red cells or purified substance [360,476, 477,591–595]; others accidentally by immunising with other cells or with biomolecules expressing A. Some monoclonal anti-A bind preferentially to the A-terminal trisaccharide whereas others detect an epitope involving the oligosaccharide backbone. The former type of anti-A are more effective at agglutinating A_2B red cells with weak A expression and are more suitable for use as reagents [596,597]. Monoclonal antibodies behaving as anti-A_1 have also been reported [55,593,598].

Human monoclonal anti-A and -A_1 have been generated by Epstein-Barr virus (EBV) transformation of lymphocytes obtained either from hyperimmunised plasmapheresis donors [599] or from splenic tissue after *in vitro* stimulation with group A red cells [600].

Monoclonals that react with both A and B cells (anti-A,B) have been produced after immunising mice with A substance, group A red cells, or AB red cells [290,593, 594,601]. Some of these antibodies react more strongly with A cells than with B cells [290,601].

A Fab-phage was isolated from a human IgG1 phage-display library derived from splenocytes from a group O donor by panning with group B red cells. The 'antibody' agglutinated B, but not A or O red cells, but displayed interaction with A and B epitopes by inhibition techniques [602]. Other anti-A and -B scFv fragments are reported [603].

Details of numerous ABO monoclonal antibodies submitted to four international workshops are described in the workshop reports [604–607].

2.14.7 Anti-H

H antibodies detect the precursor of A and B antigens. They characteristically agglutinate group O and A_2 cells more strongly than A_1 and B cells.

Typically, H antibodies are inhibited by secretor saliva and react with group O cord cells, although often less strongly than with O adult cells. Morgan and Watkins [608] distinguished anti-H, which is inhibited by secretor saliva, from 'anti-O', which is not. The latter specificity is now generally called anti-HI (Section 2.14.8).

Antibodies specific for Type 1 H (Table 2.3) are often referred to as anti-Le^d or -Le^{dH} [259,609] (Section 2.18.2.1).

2.14.7.1 Anti-H in Bombay sera

Anti-H is generally present in the sera of people with H-deficient, non-secretor (Bombay, O_h, A_h, and B_h) phenotypes. These anti-H vary greatly in strength, ability to agglutinate cord cells, degree of inhibition by O saliva, and IgG content. Sera with the greater IgG content show least difference in strength between O cord and O adult cells and are least readily inhibited by saliva. O_h sera contain both anti-Type 1 H and -Type 2 H [610,611]. Réunion phenotype individuals (red cell H-partially deficient, non-secretors), however, produce a large quantity of anti-Type 1 H, but only little anti-Type 2 H, presumably because a small quantity of Type 2 H antigen is present on their red cells [611].

Anti-H in sera of red cell H-deficient, non-secretors has the potential to cause HTRs [437,612] and only H-deficient red cells are suitable for transfusion. Only 2% of group O cells injected into an O_h patient survived 24 hours [613]. In an A_h patient, 67% of A_1 cells were destroyed within 1 hour of injection, despite being only weakly agglutinated at 37°C by the serum of the patient [614]. Anti-H in an O_h mother caused severe HDFN [615], but all of 16 babies of O_h phenotype mothers in South Africa were either mildly affected or unaffected by HDFN and no exchange transfusions were required [612].

2.14.7.2 Other sources of human anti-H

Anti-H in the serum of people who do not have H-deficient red cell phenotypes, usually found in ABH non-secretors, are generally weak and only reactive at low temperatures [616]. An exceptionally potent anti-H from an A_1 Le(a–b+) person (Toml) was inhibited by secretor saliva, including the patient's own saliva, reacted with cord cells, and did not react with O_h cells [617]. Mono-

clonal IgM autoanti-H in a patient with lymphoma was responsible for fatal AIHA [618].

2.14.7.3 Monoclonal anti-H

Numerous mouse monoclonal H antibodies have been produced following immunisation by a variety of immunogens [52,229,230,604–607,619,620]. Unlike human anti-H, murine monoclonal anti-H are often not inhibited by secretor salivas, or at least are inhibited by only a minority of secretor salivas [619]. In one set of 11 H-like monoclonal antibodies, all reacted with either monofucosyl Type 2 H or difucosyl-Le^y, or with both structures; none reacted with the Type 1 structures [52]. Those antibodies reactive with only Type 2 H reacted with red cells, but not with salivary substances, presumably because of a predominance of difucosylated structures in saliva; those specific for the difucosylated Le^y structure did react with salivary structures, but not with red cells; and those reactive with both monofucosylated and difucosylated structures reacted with red cells and with saliva. Mollicone *et al.* [620] subdivided 28 monoclonal anti-Type 2 H into seven categories, based on their cross-reactivities with synthetic oligosaccharides.

2.14.7.4 Anti-H from other sources

Anti-H has been made in animals (including chickens, cattle, buffalo, goats, and sheep [621]) by immunising with O red cells or with purified H substance, and adsorbing with O_h cells. H-specific lectins are described in Section 2.14.9.4.

2.14.8 Anti-HI and -Hi

Anti-HI (or -IH) agglutinate red cells carrying both H and I [622]; they do not agglutinate, or agglutinate only very weakly, H-deficient cells (non-secretor or secretor) or I-deficient cells (cord and adult i cells). Anti-HI are usually weak antibodies reacting only at low temperatures.

In line with the observation of Sanger [616] that anti-H is only made by ABH non-secretors, the H-like agglutinin found in the serum of H-deficient secretors is generally anti-HI. Anti-HI in the serum of H-deficient secretors is not generally considered clinically significant. Although it has been responsible for rapid destruction of small quantities of radiolabelled group O red cells, it was predicted that transfusion of whole units of blood would result in near normal survival [623].

Autoanti-HI are generally benign, but anti-HI of broad thermal range have caused acute HTRs in group A_1 patients following transfusion of A_2 red cells [624–626] and in a group B woman following transfusion of 100 ml of group O red cells [627].

An autoagglutinin in the serum of an A_1 woman, which behaved like anti-H but was not inhibited by secretor saliva and reacted exceptionally strongly with group A_2 adult i cells, was called anti-Hi [628]. Three more examples have been reported since [629].

2.14.9 Lectins

The name lectin originally described plant extracts capable of agglutinating red cells [630], before Goldstein *et al.* [631] broadened this definition to, 'A sugar-binding protein or glycoprotein of non-immune origin, which agglutinates cells and/or precipitates glycoconjugates'. Thus, the vast array of haemagglutinating substances found in plant (mostly seed) extracts and in some animals such as snails, fish, and snakes can all be termed lectins.

The agglutinating activity of lectins is inhibited by simple sugars, usually monosaccharides. It is assumed that these sugars represent the binding site for the lectin on the cell surface. Through the use of lectins, Morgan and Watkins [632] obtained some of the early information on the nature of the A, B, and H antigens. Some plant extracts contain more than one lectin.

The variety of lectins with A, B, or H specificity are too numerous to itemise here. Most are seed extracts, predominantly from plants of the family Leguminosae, although many other sources exist. Lectins with anti-A, -B, and -H specificity are found in the fruiting bodies of many fungi [633]. A few lectins are listed in Table 2.21. For reviews see [634–637].

2.14.9.1 Anti-A

Renkonen [638] found anti-A activity in the seeds of *Vicia cracca*, the first blood group lectin to be recognised. Group A specificity has been found since in many seeds including *Dolichos biflorus*, an extremely useful blood grouping reagent because it agglutinates A_1 cells far more readily than A_2 cells and so, when appropriately diluted, distinguishes A_1 and A_1B from A_2 and A_2B [204]. *Dolichos* lectin is specific for terminal GalNAc [639] and so will also agglutinate Tn+ and Sd(a++) cells (see Chapters 31 and 33). *Dolichos* lectin probably differentiates A_1 and A_2 red cells on the basis of quantitative rather than structural differences [230].

The eggs and albumin glands of several species of snails, mostly of the family Helicidae, contain anti-A (GalNAc) activity and have often been used in automated

Table 2.21 Some lectins with A, B, A,B, or H activity.

Species	Source	Blood group activity	Monosaccharide specificity	Comments
Dolichos biflorus	Seed	Anti-A_1	GalNAc	Anti-Tn, -Cad also
Phaseolus limensis	Seed (lima bean)	Anti-A		
Phaseolus lunatus	Seed (lima bean)	Anti-A	GalNAc	
Helix pomatia	Snail	Anti-A	GalNAc	
Helix hortensis	Snail	Anti-A	GalNAc, NeuAc	
Fomes fometarius	Tree fungus	Anti-B		Anti-P^k also
Ptilota plumosa	Seaweed	Anti-B		
Salmo salar	Salmon roe	Anti-B	Gal	Anti-P also
Sophora japonica	Seed	Anti-A,B		Reacts strongly with En(a–) cells. Anti-B strongest.
Phlomis fructosa	Seed (Jerusalem sage)	Anti-A,B	GalNAc, Gal	
Bandeiraea simplicifolia	Seed	Anti-A,B	GalNAc, Gal	BSI, one of 3 lectins. Anti-B strongest
Ulex europaeus	Seed (gorse)	Anti-H	I Fuc II GlcNAc*	Lectin most commonly used for detecting H secretion
Lotus tetragonolobus	Seed	Anti-H (-HI)	Fuc	
Anguilla anguilla	Eel serum	Anti-H (-HI)	Fuc	
Cystisus sessifolius	Seed	Anti-H	GlcNAc*	
Laburnum alpinum	Seed	Anti-H	GlcNAc*	

*Probably requires terminal Fuc residue.

ABO grouping [635,640,641]. Lectins from the albumin glands of *Helix pomatia* are a heterogeneous mixture of polypeptides encoded by several separate genes [642].

2.14.9.2 Anti-B

B-specific lectins are less abundant than A-specific lectins. They are found, together with anti-H, in the arils (seed coats) of various species of *Evonymus* [643], in the fungus *Fomes fomentarius* [644], and in the seaweed *Ptilota plumosa*. Anti-B activity is also found in the roe of various species of fish, especially those of the salmon and herring families [645–647]. These lectins are D-galactose-specific and may also show some P, P1, and P^k specificity owing to the galactosyl determinants common to these antigens [647,648].

2.14.9.3 Anti-A,B

Several seed extracts agglutinate A and B cells but not O cells. In some cases this may be due to one lectin cross-reacting with both A and B structures. BSI, one of at least three lectins in *Bandeiraea simplicifolia* seeds, comprises five isolectins made up of different proportions of two subunits. Both subunits have a high affinity for Gal but one of the subunits also binds strongly to GalNAc [649]. A and B activity of *Phlomis fructicosa* lectin was inhibited by GalNAc, whereas Gal only inhibited B activity [650]. If separate A- and B-specific molecules are found in these lectins, the notation anti-A,B is inappropriate, anti-A+B being more suitable.

2.14.9.4 Anti-H

Lectins in the seeds of common gorse (*Ulex europaeus*) behave as anti-H [651] and this is the most widely used and probably best reagent for identifying secretor status from salivas of group O individuals. At least two lectins are present in *U. europaeus* seed extracts [652]: *Ulex* I is inhibited by L-fucose; *Ulex* II is not inhibited by L-fucose, but is inhibited by di-*N*-acetylchitobiose, a sugar with a GlcNAc residue [653]. Both *Ulex* I and *Ulex* II are H-specific and both fail to react with group O red cells treated with α-L-fucosidase [654]. It seems likely that *Ulex* II reacts with subterminal GlcNAc in the H structure, but only in the presence of terminal L-fucose.

Other H-specific lectins fall into two classes [632,655]: **1** those, like *Ulex* I, that are inhibited by L-fucose, for example *Lotus tetragonolobus* seeds and eel serum;

Table 2.22 Interaction of Lewis and secretor genes and the resulting red cell and secreted phenotypes (in group O individuals).

Genotype			Antigens in secretions	
Lewis	Secretor	Red cell phenotype	Le^a	Le^b
Le/Le or *Le/le*	*Se/Se* or *Se/se*	Le(a–b+)	+	+
Le/Le or *Le/le*	Se^w/Se^w or Se^w/se	Le(a+b+)	+	+
Le/Le or *Le/le*	*se/se*	Le(a+b–)	+	–
le/le	Any	Le(a–b–)	–	–

2 those, like *Ulex* II, that are inhibited by GlcNAc derivatives, for example seeds of *Cystisus sessilifolius* and *Laburnum alpinum*.

Ulex I is more readily inhibited by oligosaccharides with Type 2 chains, including those with difucosyl structures, than those with Type 1 chains [609,610,656,657]. *Lotus tetragonolobus* lectin is strongly specific for Type 2 chains and does not react with Type 1 chains [658], which explains why it is not a useful reagent for inhibition tests. *U. europaeus* seed extract has a similar reaction strength with either I-positive or I-negative (adult i) cells, but *L. tetragonolobus* lectin and eel serum lectin behave like anti-HI, with little activity for adult i or cord cells [610,659]. *Cystisus sessilifolius* and *Laburnum alpinum* lectins occupy an intermediate position, reacting with adult i cells less strongly than with I-positive cells [659].

Part 4: Lewis system

2.15 Le^a and Le^b antigens and phenotypes

The structure and biosynthesis of the Lewis antigens are described in Part 2 of this chapter. The details discussed here are mainly related to the serological expression of Lewis antigens, although some structural matters are addressed.

2.15.1 Red cells

A general rule applies to red cell Lewis phenotypes of European and African people. Adults with an *Le* gene are Le(a–b+) or Le(a+b–); if they are secretors of ABH their red cells are Le(a–b+); if non-secretors they are Le(a+b–). People homozygous for *le* have Le(a–b–) red cells (Table 2.22). There are, as might be expected, exceptions to this rule. Many anti-Le^b, often referred to as anti-Le^{bH}, fail to agglutinate A_1 Le(a–b+) cells (see Section 2.17.2.1) and A_1 Le(a–b+) cells may be falsely typed as Le(a–b–). Red cells from fetuses, cord samples, and neonates are generally Le(a–b–). Infants may be transiently Le(a+b+) before becoming Le(a+b–). Lewis-positive women may become transiently Le(a–b–) during pregnancy (Section 2.15.6).

Flow cytometry appears to be a more reliable method for determining Lewis phenotypes of red cells than conventional serological techniques. The following results were obtained with commercial anti-Le^b reagents on red cells of Europeans genotyped for *FUT2* and *FUT3*: A_1 Le(a–b+), 71% positive; B Le(a–b+), 95%; O and A_2 Le(a–b+), 99%; Le(a–b–) and Le(a+b–), <10% [660].

The red cell phenotype Le(a+b+), with both Le^a and Le^b strongly expressed, is very rare in European and African adults, but is common in East and Southeast Asia, and in the Pacific region [167,168,237,661,662]. The explanation for this is the presence of a weak *Se* (*FUT2*) gene and the rarity of *se* (explained in Section 2.6.3). Some anti-Le^b reagents do not detect the Le^b of Le(a+b+) red cells by some techniques, which may have led to failure to recognise Le(a+b+) in some studies [22].

Some Le^a expression may be detected on the red cells of O or A_2 Le(a–b+) adults with selected anti-Le^a if sensitive enough techniques are used, such as an IAT with enzyme-treated cells [537]. Le(a–b+) red cells were destroyed, *in vivo*, in a patient with potent anti-Le^a [663].

2.15.2 Secretions

Lewis antigens are easily detected in human saliva by haemagglutination inhibition. Anti-Le^a is inhibited by saliva of individuals with Le(a+b–) or Le(a–b+) red cells,

the former inhibiting more strongly than the latter [161,664]. Le^b is present in saliva of individuals with Le(a–b+) red cells [665]. Le^a and Le^b is present in saliva of people with Le(a+b+) red cells [167,662] (Table 2.22).

Lewis antigens have also been detected in, and isolated from, human milk [37], gastrointestinal juices [666,667], urine [666,668,669], seminal fluid [666,670], ovarian cyst fluid [9,10], and amniotic fluid [671].

2.15.3 Plasma

Le^a is easily detected in plasma of individuals with Le(a+b–) red cells [664]. Unlike saliva, only a trace of Le^a may be detected in plasma of people with Le(a–b+) red cells [672]. Le^b is present in plasma of individuals with Le(a–b+) red cells [672–674].

The site of synthesis of plasma Lewis substances is unknown. Recipients of transplants of bone marrow [675,676], kidney [668], or liver grafts [677] maintain their own red cell Lewis phenotypes, even though Lewis antigens of donor origin are detected in the urine of kidney recipients and in the bile of liver recipients. In view of the very high levels of intestinal Le^a active glycolipids, Hanfland and Graham [609] suggested that plasma Lewis substances might originate from the intestine. Non-secretor patients with coeliac disease have reduced quantities of urinary Le^a antigen [669]. Evans *et al.* [669] proposed that Le^a in urine and plasma derives from large Le^a-active molecules in the small intestine, which are digested to smaller molecules and absorbed into the blood stream. Some of these small molecules are subsequently excreted *via* the kidney. In coeliac disease these molecules cannot be absorbed by the intestine, resulting in reduced levels of Le^a substance in the urine. Following regeneration of the intestinal mucosa, normal quantities of urinary Le^a are detected. All of eight patients with intestinal failure, seven of whom had resection of the ileum and 80% of the jejunum, had Le(a–b–) red cells, statistically significant from the 6% expected [678]. Furthermore, Le(a–b–) red cells of a patient with failure of the small intestine became Le(a–b+) following an intestinal transplant [679], further evidence that Lewis glycolipids in the plasma, and consequently on the red cells, are intestinally derived. Henry *et al.* [22] consider that other exocrine organs, such as liver, kidney, and pancreas, may contribute to Lewis-active plasma glycolipids, explaining the differences in plasma and intestinal glycolipid profiles.

Trace amounts of glycolipids with the Le^b structure have been identified in plasma from individuals with Le(a–b–) red cells, suggesting some α1,4-fucosyltransferase activity [680.681]. This probably occurs either because the Lewis-fucosyltransferase encoded by an *le* allele is not completely inactive or because one of the α1,3-fucosyltransferases, possibly the product of *FUT5*, has low-level α1,4-fucosyltransferase activity [681].

Enhanced quantity of sialylated-Le^a (sLe^a or CA 19-9) in plasma is associated with cancer of the pancreas and colon, and has been used to support diagnosis (see Section 2.19.4).

2.15.4 Uptake of Lewis antigens by red cells

Red cells do not synthesise carbohydrate chains with a Type 1 backbone and consequently cannot synthesise Lewis antigens. Plasma lipids exchange slowly but freely with lipids in the red cell membrane; the composition of phospholipids and fatty acids in red cell membranes resembles that in plasma [682]. Consequently, plasma glycosphingolipids bearing Lewis structures become incorporated into the red cell membrane [47].

Sneath and Sneath [672] first recognised that Le(a–b–) red cells acquire Le^a and Le^b during incubation in plasma from Le(a+b–) and Le(a–b+) individuals, respectively, and that Le(a+b–) and Le(a–b+) red cells lose their Lewis antigens during incubation in Le(a–b–) plasma. These experiments were carried out with one volume of packed red cells shaken continuously in 10 volumes of plasma at 35°C for at least 3 days, with the plasma being changed daily. Le^a antigen cannot be removed from Le(a+) red cells simply by repeated washing of the cells [663].

Group O red cells transfused into an A_1 Le(b+) recipient were not agglutinated by anti-A_1Le^b on the first and second days post-transfusion, but were weakly agglutinated on days four and five, and were strongly agglutinated on day six [683]. Two days after transfusion of Le(b+) cells to an Le(a–b–) patient, the transfused cells still reacted strongly with anti-Le^b, but after seven days the transfused cells gave a scarcely detectable reaction with anti-Le^b [684].

Glycosphingolipids isolated from plasma were extremely effective at converting Le(b–) red cells to Le(b+); only five minutes incubation at 37°C made Le(b–) cells agglutinable with anti-Le^b [47]. Group O Le(a–b–) red cells exposed to glycosphingolipid fractions prepared from plasma of A_1 Le(a–b+) donors were agglutinated by anti-A_1Le^b [257]. These glycosphingolipids, once adsorbed, could not be washed off with saline. About one

third of the total Le^b-active glycolipid in whole blood is associated with the red cells, the rest being in the plasma [685]. Lewis antigens in saliva are glycoproteins and cannot be adsorbed onto red cells [674].

Twin chimeras have permanent bone marrow grafts derived, *in utero*, from their twin. Their peripheral red cell population is often a mixture of their own cells and those of their twin, yet, unlike other blood groups, the chimera's own genes determine the Lewis phenotype of both populations of red cells, further proof that Lewis antigens are not haemopoietic in origin. In one chimeric twin, a secretor of H, all her red cells were Le(a–b+), despite half of her cells being derived from her non-secretor brother; all the red cells of her non-secretor brother were Le(a+b–), including those from his secretor sister [686].

A_1Le^b can only be produced by individuals with *Le*, *Se*, and A^1 genes. Group A_1 red cells produced by grafted tissue in a group O secretor host are not agglutinated by anti-A_1Le^b, even though they carry both A_1 and Le^b antigens, because the A_1 originates from the graft and the Le^b originates from the host [304,683,687]. One chimeric twin made anti-A_1Le^b even though 50% of her red cells were A_1 Le(b+) [683]. The antibody reacted with none of her own red cells, but did react with all of those of her chimeric twin brother, a secretor of A and H. Recipients of successful bone marrow transplants acquire the blood groups of the donor with the exception of Lewis, which remains that of the recipient [675,676].

2.15.5 Development of Lewis antigens

Le^a and Le^b cannot usually be detected on cord red cell samples by direct agglutination [13]. When more sensitive serological techniques are used, such as IAT or agglutination of enzyme-treated cells with potent antibodies, traces of Le^a and possibly Le^b may be detected on some cord or fetal red cells [663].

Lewis antigens start to appear on red cells soon after birth [688]. Le^a develops first; red cells of infants with an *Le* gene generally become Le(a+) during the first few months of life. In white people, 80% of infants are Le(a+) at 3 months of age, this number dropping to the adult level of 20% by 2 years [689–691]. During this period the red cell phenotype Le(a+b+) is not uncommon [691,692]. By 6 years of age the proportion of Le(b+) reaches the adult level [691].

Appearance of Le^a and Le^b in the plasma of infants correlates with the appearance of those antigens on the red cells [635] and cord red cells can be made Le(b+) by incubation in plasma from an Le(b+) adult [674]. At the source of production of plasma Lewis substances in infants with an *Se* allele, the Le-fucosyltransferase appears to become active before the *Se* (*FUT2*) specified fucosyltransferase, resulting in the appearance of Le^a in the plasma before Le^b.

In Chinese, 50% of cord samples were found to be Le(a–b+) and 50% Le(a–b–) [680]. The Le(a–b+) cells became Le(a+b+) before reverting to Le(a–b+); the Le(a–b–) cells either became Le(a+b+) and maintained that phenotype or remained Le(a–b–).

Lewis antigens in the saliva of neonates with an *Le* gene are the same as those detected in adult salivas: ABH secretors have Le^a and Le^b in their saliva; ABH non-secretors have only Le^a [692]. Lewis substances in amniotic fluid corresponded to the Lewis and secretor phenotypes of the fetus and were present as early as 15 weeks' gestation [671].

2.15.6 Lewis antigens during pregnancy

Agglutinability of red cells with anti-Le^a or -Le^b may be reduced during pregnancy [693–695] and pregnant women with a transient Le(a–b–) phenotype may produce Lewis antibodies. In one survey the frequency of the Le(a–b–) red cell phenotype was 11% in non-pregnant women, but 36% in women at time of delivery [695]. Women who were A_1 most often became Le(b–) during pregnancy. The change in Lewis phenotype occurred as early as the 24th week of the pregnancy and Le^b antigen was detectable again 6 weeks after delivery. The concentration of Le^b glycolipid in plasma decreased only slightly during pregnancy, so, unlike the situation in infants, changes in Le^b red cell expression during pregnancy do not appear to result directly from the quantity of Le^b in the plasma. Hammar *et al.* [695] suggest that, as a result of the increased concentration of plasma lipoprotein that occurs during pregnancy, more Lewis determinants become attached to plasma lipoproteins and consequently less are available to become bound to the red cell surface. Levels of Le^b-active oligosaccharides secreted in the urine of Le(a–b+) women increase during pregnancy and lactation [694].

2.16 Antigen, phenotype, and gene frequencies

2.16.1 Red cells

Based on 1796 tests on red cells, the frequency of Le^a in England was 22.38%. This frequency is very similar to

Table 2.23 Frequencies of Lewis phenotypes in three populations.

Phenotype	Population		
	Europeans & white Americans [696–698]	African Americans [673,697]	Hong Kong Chinese [699]
Le(a–b+)	70–72%	52–55%	62%
Le(a+b–)	19–22%	19–23%	0%
Le(a+b+)	0%	0%	27%
Le(a–b–)	4–11%	22–29%	11%

that found in other European countries [237] and in white Americans [696,697]. Table 2.23 shows phenotype frequencies for Europeans and white Americans obtained from tests with anti-Le^a and -Le^b on O and A_2 cells. The frequency of the *Le* gene varies from 0.67–0.80 in white populations.

Two studies on African Americans revealed higher frequencies for the Le(a–b–) phenotype (Table 2.23) [673,697], giving a frequency of 0.46–0.53 for *Le*. The incidence of Le(a–b–) was as high as 35% in West Africans (*Le* = 0.59), with 15% Le(a+) (only O and A_2 tested [700]).

The Le(a+b+) red cell phenotype, rare in European and African people, is relatively common in East Asia, Southeast Asia, the Pacific region, and Australasia [237]. Le(a+b+) has an incidence of 27% in Hong Kong Chinese [699] (Table 2.23), between 16% and 25% in Taiwan Chinese [662], between 10% and 40% of Polynesians [701], and 10% in Australian Aborigines [702]. Le(a+b–) is rare or absent in Chinese [662,699,703].

Frequencies of *FUT3* alleles derived from DNA analysis are shown in Table 2.8.

2.16.2 Secretions

Frequency of the *Le* gene can be determined by inhibition tests, using saliva to inhibit agglutination of Le(a+b–) red cells by anti-Le^a. Salivas of individuals with an *Le* gene contain Le^a; those of *le/le* individuals do not. From saliva tests the following estimates of *Le* gene frequency have been obtained: 0.82 in English; 0.69 in Swedes; about 0.45 in Africans; 0.70 in Australian Aborigines; and 0.36–0.57 in native South Americans [237]. These are similar frequencies to those determined by red cell testing.

2.17 Lewis antibodies

2.17.1 Anti-Le^a

2.17.1.1 Human anti-Le^a

Anti-Le^a is not uncommon; haemagglutination tests in Denmark and France revealed anti-Le^a in about one in every 300 sera [704]. Agglutinating alloanti-Le^a is only found in people with Le(a–b–) red cells, possibly only those who are ABH secretors [673], and less often in group O individuals than in people of other ABO groups [704]. An exception to this rule appears to occur in Taiwan, where 10 Le(a–b+) patients with anti-Le^a were found; possibly the result of a different molecular background of some Le(a–b+) phenotypes in that population [705].

Le^a antibodies are usually 'naturally occurring' and predominantly IgM. Very rarely is anti-Le^a purely IgG [537,706]. Most potent anti-Le^a sera contain an IgG component detectable by radioimmunoassay or enzyme-linked immunoadsorbent assay [706–708], but seldom detectable by an antiglobulin test with anti-IgG [537].

Anti-Le^a usually cross-react with Le^b oligosaccharide and will sometimes react with the Lewis disaccharide Fucα1→4GlcNAc or with Le^x, the Type 2 isomer of Le^a (Table 2.3) [709]. IgM anti-Le^a have broader specificity for synthetic oligosaccharides than IgG anti-Le^a [710].

Despite the common occurrence of anti-Le^a, human antibodies potent enough to be useful as reagents are rare. As most sera containing anti-Le^a either contain weak anti-Le^b or cross-react with Le^b, methods of enhancing reactivity may lead to 'false positive' reactions with Le(a–b+) cells. Agglutination of red cells with anti-Le^a is generally strongest at low temperatures, often not occurring at all at 37°C. Anti-Le^a usually fix complement [711,712]; a two-stage complement-addition antiglobulin test is often a successful way of detecting anti-Le^a. Some Le^a antibodies lyse Le(a+) cells in the presence of complement. Another method for increasing the strength of reaction with anti-Le^a is to use protease-treated cells.

Some anti-Le^a are lymphocytotoxic [713,714].

2.17.1.2 Monoclonal anti-Le^a

Numerous murine monoclonal anti-Le^a have been produced [604–607]; some make excellent blood grouping reagents [715] and commercially produced monoclonal anti-Le^a are generally used.

Of the four monoclonal anti-Le^a described by Young *et al.* [716], one detected the Le^a trisaccharide Galβ1→3(Fucα1→4)GlcNAc, another the tetrasaccharide Galβ1

→3(Fucα1→4)GlcNAcβ1→3Gal, and two were directed mainly towards the disaccharide Fucα1→4GlcNAc. All monoclonal anti-Le^a cross-react with the Type 1 precursor Galβ1→3GlcNAc (Le^c) [717]. Some monoclonal anti-Le^a cross-react with the T disaccharide Galβ1→3GalNAc and agglutinate Le(a–) T-activated red cells [718] (see Chapter 33).

2.17.2 Anti-Le^b

2.17.2.1 Human anti-Le^b

The original anti-Le^b described by Andresen [13] only reacted specifically with group O Le(b+) or A_2 Le(b+) red cells, whereas the anti-Le^b reported by Brendemoen [665] reacted with all Le(b+) red cells. The two types of Le^b antibodies were named anti-Le^{bH} and anti-Le^{bL} by Ceppellini *et al.* [719].

Anti-Le^{bH}, which cross-reacts with H, can only be used reliably to type O or A_2 cells as it often fails to agglutinate A_1 or B, Le(a–b+) cells. Anti-Le^{bH} is inhibited by saliva from all ABH secretors regardless of whether their red cells are Le(a–b+) or Le(a–b–).

Anti-Le^{bL} agglutinates Le(b+) red cells regardless of ABO group. It is only inhibited by salivas of ABH secretors who also secrete Le^a; that is, not those who have Le(a–b–) red cells. Only anti-Le^{bL} are suitable as anti-Le^b blood grouping reagents.

Like anti-Le^a, anti-Le^b are found most often in people with Le(a–b–) red cells; unlike anti-Le^a, these people are generally non-secretors of ABH [673]. There are reports, however, of group A_1 or A_1B Le(a+b–) people who have made anti-Le^b of the anti-Le^{bH} type [665,720]. Compared with anti-Le^a, anti-Le^b is a relatively infrequent antibody. Tests on the sera of 72 000 Parisians revealed 259 anti-Le^a, 29 anti-Le^{bH}, and only 2 anti-Le^{bL}.

Anti-Le^b show many of the serological characteristics described for anti-Le^a above. Anti-Le^b are generally IgM. Of 14 anti-Le^b sera tested with synthetic oligosaccharides, four cross-reacted with Le^a trisaccharide and seven cross-reacted with Le^y tetrasaccharide (the Type 2 isomer of Le^b), four of these also reacting with H trisaccharide [709] (see Table 2.3).

Some anti-Le^b are lymphocytotoxic [775,721].

2.17.2.2 Monoclonal anti-Le^b

Many monoclonal anti-Le^b have been produced, some anti-Le^{bL}, some anti-Le^{bH}, and some giving intermediate reactions (anti-Le^{bH,L}) [604–607]. All anti-Le^{bL} and -Le^{bH,L} cross-react with Le^a; all anti-Le^{bH} and anti-Le^{bH,L} cross-react with Type 1 H (Le^d) and Le^y [717]. Despite these cross-reactivities, when the appropriate methods are adopted some monoclonal anti-Le^b make excellent blood grouping reagents.

2.17.3 Anti-ALe^b

Anti-A_1Le^b, an antibody that reacts only with the red cells of A_1 Lewis-positive secretors [A_1 Le(a–b+)], was first found in the serum of a group A_1B Le(a–b–) man [722]. Other examples have been described since [683,723]. Anti-ALe^b may be lymphocytotoxic [714]. Monoclonal anti-ALe^b may or may not cross-react with ALe^y, the Type 2 isomer [724,725].

Anti-BLe^b has not been reported.

2.17.4 Clinical significance of Lewis antibodies

Despite being relatively common antibodies, only a few examples of HTRs have been attributed to anti-Le^a [726–729], and even less to anti-Le^b [730–733]. This is probably because most Lewis antibodies are not active at 37°C, because Lewis antigens in the donor's plasma neutralise the recipient's Lewis antibodies, and because Le^a or Le^b antigens elute from red cells transfused into an Le(a–b–) recipient.

Lewis antibodies that are only active *in vitro* at temperatures below 37°C do not cause increased clearance of antigen-positive transfused red cells *in vivo* [734]. Consequently, patients with Lewis antibodies may be transfused with red cells that are compatible by IAT at 37°C.

Lewis antibodies do not cause serious HDFN, presumably because Lewis antigens are present in fetal secretions, but generally not on fetal red cells.

2.17.5 Lewis antibodies and renal transplantation

Kidney cells, particularly cells of the distal tubule epithelium and the endothelium of arteries, glomerula capillaries, and veins, biosynthesise and express Lewis antigens. Consequently, Lewis antibodies are histocompatibility antibodies in renal transplantation [668,735,736]. Two-year graft survival rates were found to be significantly lower in Le(a–b–) recipients than in Le-positive recipients [735] and in several retrospective studies [737–739] and a prospective study [740] Lewis-identical donor-recipient pairs were shown to have better graft survival than Lewis-incompatible pairs. Several cases are reported in which Lewis antibodies, anti-Le^a and -Le^b, were implicated as the possible cause of renal transplant rejection [741,742]. Anti-Le^a was detected by a highly sensitive kinetic enzyme-linked immunosorbent assay in all of eight Le(a–b–) patients with failed renal grafts, but not

in the single Le(a–b–) recipient of an Le(a–b–) graft [743]. Most of these antibodies could not be detected by haemagglutination.

In contrast, in two other prospective studies and one retrospective study [744–746], no significant difference in survival between Lewis-matched and Lewis-mismatched donor-recipient pairs was detected, although Le(a–b–) patients appeared to be at higher risk of graft failure when receiving HLA mismatched kidneys. These data led to the recommendation that Lewis phenotype should not be a basis for selection of kidneys for engraftment [745].

Anti-Le^a, presumably of graft origin, has been blamed for renal failure in bone marrow transplant recipients [676,747]. Autoanti-Le^{bH} in an Le(a–b+) patient who had received two kidney transplants which had both been rapidly rejected, agglutinated the patient's own red cells and was inhibited by his own saliva [741,748].

2.18 Other antigens associated with Lewis

2.18.1 Le^{abx}, the antigen originally called Le^x

An antigen named Le^x by Andresen and Jordal [691,749,750], present on Le(a+b–) and Le(a–b+) cells, but not Le(a–b–) cells, differs from inseparable anti-Le^{ab} because it reacts with 90% of umbilical cord red cell samples, despite cord red cells being Le(a–b–). These 90% are from infants with an *Le* gene, soon to develop red cell Le^a or Le^b [751]. As the symbol Le^x is commonly used for CD15, the monofucosyl Type 2 isomer of the Le^a trisaccharide (Section 2.18.3), the antigen originally called Le^x will now be referred to as Le^{abx}.

Anti-Le^{abx} reacts equally strongly with A_1, A_2, and O, Le(abx+) cells [749,750]. Anti-Le^{abx} is inhibited by saliva, amniotic fluid, and serum from Lewis-positive individuals [751].

Le^{abx} antibodies appear to be heterogeneous: some detect the Lewis disaccharide Fucα1→4GlcNAc and some detect larger determinants common to Le^a and Le^b [709,752,753]. These determinants must be more accessible on cord cells to anti-Le^{abx} than to anti-Le^a.

2.18.2 Le^c and Le^d

Le^c and Le^d are antigens on Le(a–b–) red cells from ABH non-secretor adults and ABH secretor adults, respectively. The symbols Le^c and Le^d are somewhat inappropriate as neither structure is produced by a Lewis gene-specified transferase, and Le^d is now known to be Type 1 H.

Like Le^a and Le^b, Le^c and Le^d are not intrinsic to the red cell but are incorporated into the red cell membrane from the plasma [754].

The phenotype Le(a–b–c–d–) is extremely rare and is associated with a deficiency of an α1,3-fucosyltransferase [755].

2.18.2.1 Le^d or Type 1 H (210002)

As a result of immunising a goat with saliva from an Le(a–b+) person and adsorbing the serum with Le(a+b–) cells, Potapov [756] identified two antibodies: anti-Le^b and a new antibody that agglutinated Le(a–b–) red cells from ABH secretors, but not those from non-secretors. He called this second antibody anti-Le^d, leaving anti-Le^c for the predicted antibody that would react with Le(a–b–) cells from non-secretors. Potapov proposed the following four Lewis phenotypes and corresponding genotypes:

Le(a+b–c–d–)	*Le/Le* or *Le/le*	*se/se*
Le(a–b+c–d–)	*Le/Le* or *Le/le*	*Se/Se* or *Se/se*
Le(a–b–c+d–)	*le/le*	*se/se*
Le(a–b–c–d+)	*le/le*	*Se/Se* or *Se/se*

Two years later human anti-Le^c was identified [757].

Le^d and Type 1 H are synonymous. Anti-Le^d reacts with oligosaccharides containing the Type 1 H determinant, but not the Type 2 H determinant (Table 2.3). Type 1 H active glycosphingolipids isolated from plasma of O Le(a–b–) secretors inhibited goat anti-Le^d [609]. Anti-Le^d, produced by immunising rabbits with synthetic Type 1 H oligosaccharide, reacted with tissues of Le(a–b–) secretors [758].

Type 1 H in the plasma of Le(a–b–) ABH secretors does not get converted to Le^b and so plenty is available to become adsorbed onto the red cell in detectable levels, giving rise to the Le(a–b–c–d+) red cell phenotype. Le^d in group A and B individuals is carried on Type 1 A (ALe^d) and Type 1 B (BLe^d), respectively (see Table 2.3).

Monoclonal anti-Type 1 A (anti-ALe^d) has been used in a biochemical analysis of red cell glycolipids [759]. Lymphocytotoxic murine monoclonal anti-A_1Le^d did not agglutinate red cells, but lysed A_1 Le(d+) red cells after addition of rabbit complement [760].

Antibodies that react with red cells of O and A_2 Le(a–b–) secretors, but not with those of A_1 Le(a–b–) secretors have been called anti-Le^{dH} [259].

2.18.2.2 Le^c (210001)

The symbol anti-Le^c was initially used for a weak antibody, made by injecting a rabbit with Le(a–b–) secretor saliva, that reacted with Le(a–b–) cells from both secretors and non-secretors [761,762] and so may have represented anti-Le^c+Le^d in the later notation [756]. Anti-Le^c of the type predicted by Potapov [756], an antibody that only reacts with red cells of Le(a–b–) non-secretors, was found during the fourth pregnancy of an O Le(a–b+) woman, a secretor of H, Le^a, and Le^b [757]. A second example of human anti-Le^c was found in a group A Le(a–b+) man who had never been transfused [763]. Other examples of anti-Le^c have subsequently been raised in goats by injection of Le(a–b–) non-secretor saliva and adsorption of the immune serum with Le(c–) red cells [259,764,765].

The structure of Le^c is less clearly defined than that of Le^d. Graham *et al.* [259] suggested that Le^c could represent the non-fucosylated Type 1 chain Galβ1→3GlcNAc, the precursor of Le^a and Type 1 H, unmodified by Le- or Se-fucosyltransferases. Further evidence that the Le^c determinant contains this disaccharide was provided when antibodies with anti-Le^c specificity were raised in rabbits immunised with Galβ1→3GlcNAc [765]. Furthermore, antibodies raised in goats immunised with Le(a–b–) non-secretor salivas and affinity-purified with Galβ1→3GlcNAc adsorbents behaved as anti-Le^c. Hanfland *et al.* [766] found that the glycosphingolipid fractions isolated from O Le(a–b–) non-secretor plasma that were most effective at inhibiting anti-Le^c of either human or goat origin carried a branched oligosaccharide chain, one branch containing Type 1 precursor Galβ1→3GlcNAc, the other a monofucosylated Type 2 structure. They concluded that unsubstituted Type 1 chain alone does not represent Le^c, although it does play a part. Further evidence that a monofucosylated Type 2 structure is an essential part of the Le^c determinant is provided by the very rare Le(a–b–c–d–) phenotype, which has only been found in two individuals, both with α1,3-fucosyltransferase-deficiency [755].

It is likely that anti-Le^c represents a heterogeneous group of antibodies that determine different structures abundant in the plasma of individuals who lack both *Le* and *Se* genes and which are incorporated into the red cell membrane.

2.18.3 Le^x, Le^y, and sialyl-Le^x

Le^x (CD15) and Le^y, the Type 2 isomers of Le^a and Le^b, are not really blood group antigens as they are not detectable on red cells [43]. Their structure and biosynthesis have been described in Part 2 of this chapter, and they are discussed briefly here because of their biochemical relationship to ABH and Lewis antigens (Table 2.3). Like Le^b and Le^d, the presence or absence of Le^y in secretions is principally dependent on alleles at the *FUT2* locus.

Human alloantibodies to the Le^x and Le^y determinants have not been found, although some anti-Le^a cross-react with Le^x and some anti-Le^b cross-react with Le^y [709]. Murine monoclonal antibodies to the Le^x, Le^y, and ALe^y determinants have been described [42,51,724,759,767].

Sialyl-Le^x (sLe^x, CD15s), the sialylated derivative of Le^x antigen, and, to a lesser extent, sialyl-Le^a, function as ligands for the lectin-like N-terminal domains of selectins, a family of cell adhesion molecules on endothelium (E-selectin), leucocytes (L-selectin), and platelets (P-selectin) (reviewed in [19,190,191]). Attachment of selectins to their carbohydrate ligands is important in the binding of leucocytes and platelets to endothelium, particularly at the region of inflammation.

Part 5: Tissue distribution, disease associations, and functional aspects

2.19 Expression of ABH and Lewis antigens on other blood cells and in other tissues

2.19.1 Leucocytes

A, B, and H antigens may be detected on lymphocytes, but they are acquired from the plasma and their expression is, therefore, under the control of *FUT2* [675, 768–771]. Cytotoxic anti-A react with lymphocytes from A secretors, but not with lymphocytes from A non-secretors; the strongest reactors are lymphocytes from A Le(a–b–) secretors [769]. The same antibodies reacted with group O lymphocytes exposed to a glycosphingolipid fraction prepared from plasma of A Le(a–b–) secretors. Lymphocytes cultured in serum from A or B individuals acquired A or B antigens [714]. Lymphocytes do not contain α1,2-fucosyltransferase [102,772]. Like red cells, lymphocytes adsorb Lewis antigens from the plasma [714].

Granulocytes and monocytes do not express ABH or Lewis antigens regardless of secretor phenotype [771, 773,774]. No α1,2-fucosyltransferase activity was detected in granulocytes or monocytes [102,186]. Granulocytes, monocytes, and lymphocytes do, however, have α1,3-fucosyltransferase activity [102] and express Le^x and sialyl-Le^x on their surface; determinants that function as

ligands for selectins, a family of cell adhesion molecules [190,191].

2.19.2 Platelets

ABH antigens are present on platelets and megakaryocytes [772,775–777]. Type 2 structures are endogenous and under control of *FUT1*; Type 1 ABH structures are adsorbed onto platelets from the plasma and are dependent on secretor phenotype [772]. Platelet ABH antigens are expressed on glycolipids and on the platelet glycoproteins, which include GPIIb/IIIa, GPIb/IX, GPIa/IIa, GPIc, GPIV, GPV, CD31 (PECAM), and CD109 [778]. Platelets from A_2 people carry almost no A antigen and can be considered group O for platelet transfusion (but with no anti-A) [779–781]. Platelets contain H-, A-, and B-transferases [185], and release of α1,2-fucosyltransferase during clotting may represent a major source of this enzyme in plasma [102,520]. Around 4–7% of A_1 and B Japanese and white Americans are high expressors (HPX) of platelet A and B antigens, with a 2- to 3-fold increase of antigen expression [782,783]. Flow cytometry demonstrates two subclasses of HPX: type 1 have platelets with high expression and platelets with moderate to low expression of ABO antigens, whereas in type 2 all the platelets have high ABO expression [783]. Although HPX appears to be inherited, the molecular background remains unknown.

ABO plays an important part in safe and efficacious platelet transfusion (reviewed in [778,784–786]). Major ABO incompatibility does not preclude platelet transfusion, as it does for red cells, and is often ignored. There is, however, ample evidence for some degree of platelet refractoriness, following ABO major incompatible platelet transfusions, particularly in children and in patients requiring long-term support. Minor ABO incompatibility, usually involving transfusion of group O platelets to a non-group O patient, may cause haemolysis of the patient's red cells, sometimes resulting in an acute HTR, which on rare occasions has proven fatal. One way to avoid this is to screen donors for high-titre anti-A and -B, and this is done in some countries, particularly in Europe and Japan [778]. In addition, there is evidence that infusion of relatively large quantities of ABO incompatible plasma could cause impaired cellular immune function, infection, and multiorgan failure [567] (see Section 2.14.3).

ABH antigen expression on platelets may be enhanced by platelet activation and apheresis and prolonged storage of A_1 platelets has led to higher levels of A antigen on the cell surface [780].

Anti-B appeared to be responsible for neonatal alloimmune thrombocytopenia in two group B babies with type 2 HPX platelets and a group O mother with high-titre IgG anti-B [787].

Le^a, assimilated into the membrane from the plasma, can be detected on platelets [788].

2.19.3 Other tissues

ABH and Lewis antigens are widely distributed in the human body and are often called histo-blood group antigens (reviewed in [14,17,21,789]). A scheme devised by Oriol and his colleagues [21] to summarise the genetic control of ABH and related antigens in various tissues is shown in Figure 2.11. Although a few exceptions exist, the following general rules prevail.

1 In tissues derived from ectoderm and mesoderm, ABH antigens are under the control of *FUT1* (H). Type 2 ABH structures are expressed, often together with Le^x and/or Le^y antigens. In addition to haemopoietic tissue, these tissues include skin, primary sensory neurons, vascular endothelium, and renal glomeruli and convoluted tubules.

2 In tissues of endodermal origin – such as digestive and respiratory mucosae and renal urinary epithelium – ABH, Lewis, and Lewis-related structures may be Type 1 or Type 2 and are primarily controlled by *FUT2* (Secretor) and *FUT3* (Lewis).

Exceptions to these rules include the following: some deep areas of digestive mucosa where, despite being of endodermal origin, histo-blood group antigens are independent of *FUT2* and *FUT3*; parts of the mammary and sweat glands (tissues of ectodermal origin), where ABH and Lewis antigens are governed by *FUT2* and *FUT3*.

Ravn and Dabelsteen [789] have devised an alternative scheme, in which it is the process of differentiation of epithelial cells, rather than embryonic origin, that determines whether H antigens are controlled by *FUT1* or *FUT2*.

Le^a and Le^b have been detected, immunochemically and by structural analyses, in small intestine and colon of individuals with Le(a–b–) red cells and whose saliva contains no Le-transferase [790–792]. See Section 2.15.3 for a possible explanation.

2.19.4 Tumours

ABH antigens are often absent from glycoproteins and glycolipids of malignant tissue of the gastrointestinal tract, oral cavity, uterine cervix, lung, prostate, breast and bladder, despite being present in the surrounding epithelium. In certain types of carcinoma loss of ABH

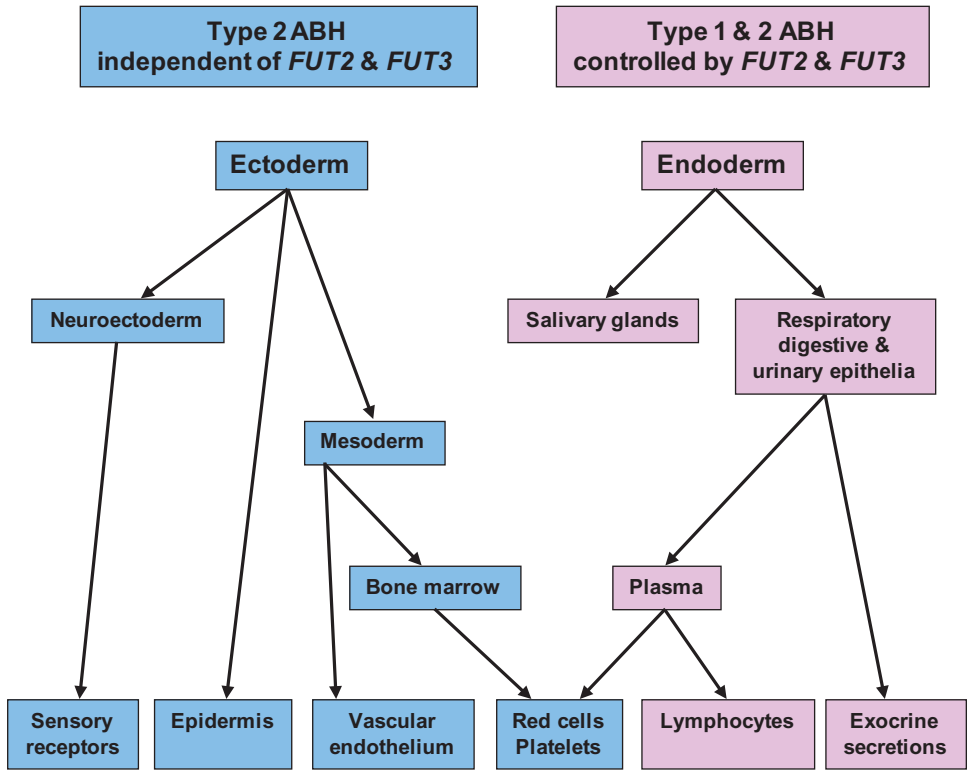

Figure 2.11 Scheme devised by Oriol [21] to demonstrate the genetic control of ABH and related antigens in human tissues derived from ectoderm, mesoderm, and endoderm. Some exceptions to this model are described in the text.

antigens precedes formation of distant metastases and hence is associated with poor prognosis [18,793]. Loss of A or B antigens from tumour cells may increase their motility and, consequently, their ability to form metastases. In addition, this absence of A or B antigens generally arises from disappearance of A- or B-transferase activity and results in an accumulation of H, Le^b, Le^y, sialyl-Le^a or sialyl Le^x. Sialyl-Le^a and sialyl Le^x are ligands for selectins, and their presence promotes the metastatic process by facilitating interaction with distant organs. ABO antigen loss results from downregulated transcription of *ABO*, and no A or B mRNA can be detected in high-grade tumours. At least two different mechanisms may be involved, possibly occurring in tumours derived from different sources:

1 loss of heterozygosity (allelic loss) involving deletions within chromosome 9q34, which contain the *ABO* locus in addition to tumour suppressor genes; and

2 hypermethylation of the CpG island of the *ABO* promoter region, which down-regulates transcription [794–796].

See Section 2.13.2 for discussion on ABH antigen loss in leukaemia patients.

Type 1 and Type 2 ABH structures and Le^b, Le^x, and Le^y antigens are present on fetal distal colon; they are not present in healthy adult distal colon, but are often re-expressed in adults in carcinoma of the distal colon [797]. Malignancy in the distal colon is associated with increased activity of α1,2-fucosyltransferase of H and Se types, indicating that α1,2-fucosyltransferases control, at least partially, oncodevelopmental expression of ABH and related antigens in colon [798].

Tumour-associated carbohydrate antigens, which include T, Tn, sialyl-Tn (Section 33.2.3), Le^x, sialyl-Le^x, sialyl-Le^a, Le^y, and Globo (Type 4) H, have been considered potential targets for the production of vaccines as agents of immunotherapy for cancer [799].

Up-regulation of glycosyltransferases in tumours may result in increased levels of certain carbohydrate structures in the plasma [800]. The quantity of circulating sialylated-Le^a (sLe^a), otherwise known as the CA19-9 antigen, is widely used as a marker to support diagnosis

of colorectal, pancreatic, and gastric cancer, and as an aid to prognosis after potential curative surgery [44,801–803]. CA19-9 levels vary according to Lewis and secretor genotypes, complicating cancer diagnosis; Le(a–b–) (*le/le*) individuals have no circulating CA19-9 [802]. A marker for cancerous regions of the colon of Le(a–b–) patients is sialyl-Le^c (DU-PAN-2), a precursor of sialyl-Le^a [802].

Another phenomenon associated with malignancy is the incompatible A antigen, occasionally expressed on tumours of group O or B people [804]. About 10% of colonic tumours from group O patients, shown to be homozygous for the O^1 allele, express A antigen and contain A-transferase activity [805,806]. The molecular basis of this O to A conversion is not known, but that alternative splicing of *ABO* RNA, resulting in loss of exons 5 and 6, would introduce no frameshift or translation-termination codons [110]. The putative gene product would be a truncated glycosyltransferase lacking the G261 nucleotide deletion, characteristic of the O^1 allele, and with a potential for A-transferase activity (see Section 2.3.2.2). The higher incidence of gastric and ovarian adenocarcinomas in group A people [807] could result from suppression of development of tumours bearing an A antigen by the anti-A naturally present in group O and B people.

2.20 Associations with disease

Some examples of associations between ABO group and disease have already been mentioned, notably HDFN (Section 2.14.3), leukaemia (Section 2.13.2), cancer (Section 2.19.4), acquired B resulting from bacterial infection (Section 2.13.1), and leucocyte adhesion deficiency type II (Section 2.12.5.2). A multitude of other associations between ABO and diseases have been reported, mostly based on observed ABO phenotype frequency discrepancies between patients with the disease and the healthy population (reviewed in [23,807,808]). Susceptibility to infection by numerous pathogenic micro-organisms is associated with ABO phenotype or secretor status [23].

2.20.1 Bacterial infections

Group O individuals are more susceptible than non-O to the severe effects of infection with enterotoxigenic *Escherichia coli* [809], responsible for millions of cases of gastrointestinal infections annually, and for some strains of the cholera bacterium, *Vibrio cholerae* [810]. This effect could result from a binding preference of bacteria-derived heat labile toxins for A- or B-active structures over H antigen [811].

Helicobacter pylori is a gram-negative bacterium present in over 50% of the world's population and associated with development of chronic gastritis, gastric and duodenal ulcers, and adenocarcinoma, though most infected individuals show no clinical symptoms [812,813]. In 1993, Borén *et al.* [814] provided evidence that Le^b mediates attachment of certain strains of *H. pylori* to gastric mucosa cells. Bacterial binding to gastric mucosa cells was abolished by the presence of Le^b-active soluble glycoproteins and by Le^b-specific monoclonal antibodies. Gastric mucosa cells expressing Le^b bound *H. pylori* P466, whereas cells lacking Le^b did not. Type 1 H also mediated *H. pylori* attachment, but to a much lesser degree. Le^a, ALe^b, and BLe^b did not bind the bacteria in this South American population, which could explain why group O individuals have an increased probability of developing gastric and duodenal ulcers [815]. Subsequent studies, however, have shown that other strains of *H. pylori* from around the world are less specific for Le^b in group O and will also bind ALe^b or BLe^b [816–818].

H. pylori expresses protein adhesins that confer close adherence to the gastric epithelium. These include blood group antigen-binding adhesin (BabA), which binds Le^b (and ALe^b and BLe^b) on mucous cells [819], and sialic acid-binding adhesin (SabA), which binds sialyl-Le^x glycolipids, rarely expressed in normal gastric mucosa [820]. Carbohydrate chains of lipopolysaccharides of the outer cell wall in most strains of *H. pylori* express Le^x, sialyl-Le^x, Le^y, Type 1 H, Le^a, Le^b, and A [821]. These antigens undergo phase variation, the random, reversible high-frequency switching of phenotype. In contrast to previous suggestions, molecular mimicry between *H. pylori* and host glycoconjugates does not appear to play a significant role in host evasion or autoimmunity [813,821].

ABH non-secretors appear to be more susceptible than secretors to infections by *Haemophilus influenzae*, *Neisseria meningitides*, and *Streptococcus pneumoniae* and urinary tract infection by *E. coli* [817].

2.20.2 Viral infections

Norovirus (NoV), a member of the Caliciviridae family, is the commonest cause of acute epidemic gastroenteritis (winter vomiting disease), affecting millions of people worldwide (reviewed in [817,818,822,823]). Despite being highly contagious, ABH non-secretors are resistant to symptomatic infection with most NoV strains, as demonstrated by challenge studies with human volunteers

[824] and by analyses of institutionalised outbreaks [825]. In three sporadic outbreaks of acute gastroenteritis in Sweden, 49% of symptomatic individuals were homozygous secretors (*Se/Se*), 51% were heterozygous (Se/se^{428}), and none was a non-secretor (se^{428}/se^{428}), compared with a non-secretor rate of 29% in asymptomatic individuals and 20% in Swedish blood donors [825]. Furthermore, recombinant NoV capsid proteins bound to intestinal epithelia from secretor, but not from non-secretor, individuals [826], suggesting that their receptors are controlled by *FUT2*. In thin-layer chromatography, capsid proteins from the Norwalk NoV bound to glycoconjugates with Types 1 and 2 H and A, Le^b, ALe^b, Le^y, and ALe^y, but not to B or BLe^b structures [823,827].

Non-secretor (se^{428}/se^{428}) genotype also appears to confer some degree of resistance to HIV-1 infection [817].

Rotaviruses are the most common cause of severe diarrhoea in infants and young children. A cell-attachment protein (VP8*) of a human rotavirus recognised A antigen and virus infectivity of a human cell line was abrogated by anti-A and infectivity was enhanced in Chinese hamster cells when genetically modified to express A antigen [828].

2.20.3 Malaria

There is convincing evidence that a major selection pressure driving the evolution of the ABO polymorphism has been provided by *Plasmodium falciparum* malaria. The global distribution of ABO phenotypes shows that O is more common, relative to non-O, in those parts of the world where *P. falciparum* infection is endemic [829,830]. Published evidence for group O individuals being more susceptible to *P. falciparum* infection is contradictory, but literature reviews, meta-analyses and a genome-wide association study provide very strong evidence that group O is associated with milder disease and group A with more severe disease, particularly in children [829–833, 855].

P. falciparum-infected red cells express on their surface the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), 'sticky knobs' that are manufactured by the parasite and bind A and, possibly to a lesser extent, B antigens on uninfected red cells to produce clusters of infected and uninfected red cells called rosettes [832,834]. Rosettes still form with group O red cells, with the involvement of other receptors such as CR1 (see Section 20.7), but they are smaller and weaker than in non-O red cells. A and B antigens are also involved in sequestration, the adherence of infected red cells to the vascular endothelium [829,830,834]. Rosetting and sequestration protect the parasite by delaying the circulation of the infected cells towards their destruction in the spleen, but also play a key role in the severe and fatal cerebral malaria that kills so many children in affected areas of the world.

Since transfusion of A or B red cells to patients with severe malaria could promote rosetting, it has been proposed that it might be preferable to give O red cells to non-O patients with severe malaria requiring transfusion [834].

Although there are many other selective factors that have affected ABO evolution, including the bacterial and viral infections and thrombotic diseases described in this section, there can be little doubt that the ABO profile was shaped through blood group expression on red cells by the most virulent form of malaria, described as 'the strongest known force for evolutionary selection in the recent history of the human genome' [835].

2.20.4 Clotting

For well over half a century statistical associations between clotting anomalies and ABO phenotype have been recognised, with thrombotic disease more common in A than in O individuals and bleeding more common in O than in A [808]. The coagulation glycoproteins von Willebrand factor (VWF) and Factor VIII (FVIII) form a tight, non-covalently bound complex in the plasma. VWF has two major functions: to mediate the capture and interaction of platelets at sites of vascular damage and to stabilise plasma FVIII and carry it to sites of vascular injury. Plasma from non-O individuals contains about 25–30% more VWF than that from group O individuals, with $AB > A > B > O > O_h$ (Bombay) (reviewed [836, 837]). VWF quantities are also affected by ABO genotype, with A/O^1 and B/O^1 individuals having lower VWF plasma levels than *A/A* and *B/B* individuals, respectively. ABH-active oligosaccharides are located on the complex *N*-glycans of VWF.

Plasma VWF levels are regulated in part by the metalloprotease ADAMTS13, which cleaves VWF at Tyr1605–Met1606, facilitating clearance from the plasma. Reduced cleavage can lead to vascular occlusion, whereas excessive cleavage results in increased bleeding. Proteolysis was significantly faster for group O compared with non-O VWF. The cleavage site on VWF is flanked by *N*-glycans at Asn1515 and Asn1574. These *N*-glycans express ABH antigens. It is likely that the additional GalNAc and Gal residues on the A- and B-active oligosaccharides affect VWF conformation, reducing access of ADAMTS13 to the VWF cleavage site and reducing clearance of VWF from the plasma. The resultant higher levels of plasma

VWF associated with non-O account for increased prevalence of venous and arterial thrombosis in non-O individuals. Associations of plasma FVIII levels with ABO phenotype are probably secondary affects of VWF levels [836,837].

A systematic review and meta-analysis confirmed historical associations between some vascular disorders and non-O phenotype: myocardial infarction, angina, peripheral vascular disease, cerebral ischaemia of arterial origin, and venous thromboembolism (VTE) [838]. The importance of *ABO* as a risk factor for VTE and in coronary artery disease was further confirmed by genome-wide association studies [839,840]. All of these associations result from the effect of *ABO* on VWF.

2.20.5 Pancreatic cancer

Since the 1950s numerous statistical analyses have indicated that group A individuals are at a higher risk than group Os for a variety of forms of cancer [808], although a recent meta-analysis suggested that the association between ABO and cancer is limited to exocrine pancreas malignancy [841]. Pancreatic cancer is a common cause of cancer-related mortality in the developed world, owing mainly to its late diagnosis and poor response to therapy. In 2009, in two large, independent populations, ABO was statistically associated with the risk of pancreatic cancer, with the highest risk observed for group B, intermediate risks for A and AB, and the lowest risk for O [842]. An increased risk was observed for A_1 over O, but not A_2 over O [843]. In the same year a genome-wide association study revealed that a SNP in intron 1 of *ABO*, in complete linkage disequilibrium with the *O* allele, is associated with lower risk of pancreatic cancer [844]. No significant effect on risk was associated with secretor status [843]. Risch [845] proposes that ABO phenotype influences the behaviour of *H. pylori* (Section 2.20.1), which affects gastric and pancreatic secretory function, which, in turn, influences the pancreatic carcinogenicity of dietary- and smoking-related *N*-nitrosamine exposures, and hence risk of pancreatic cancer.

2.20.6 Fucosidosis

Fucosidosis is a rare lysosomal storage disease with an autosomally recessive mode of inheritance, often fatal within the first 5 years of life. It is characterised by an accumulation of fucosylated glycolipids and glycoproteins in neural and visceral tissues, as a result of an α-L-fucosidase deficiency resulting from inactivating mutations [846,847]. Fucosidosis patients may have enhanced expression of Lewis antigens on their red cells and in their saliva [848–850]. Two siblings with fucosidosis were both H secretors and yet had Le(a+b+) red cells, with very high levels of red cell and salivary Le^a and Le^b expression [849]. Both had normal H activity, suggesting that the deficient fucosidase is specific for the α1→4 linkage to GlcNAc found in Lewis active structures and not the α1→2 linkage to Gal of H-active structures (see Table 2.3). These results suggest that biosynthesis of fucosylated structures in healthy individuals depends on a balance of fucosyltransferase and fucosidase activities.

2.21 Functional aspects

The role played by sialyl-Le^x and, to a lesser extent, sialyl-Le^a as ligands for lectin-like cell adhesion molecules, selectins, has been mentioned in Sections 2.3.4 and 2.18.3. Otherwise, almost nothing is known about the functions of ABO and Lewis antigens.

The red cell membrane has about 10^6 molecules of band 3 (anion transporter), 7×10^5 molecules of the glucose transporter, and about 10^6 molecules of polyglycosylated lipids [851]. All these molecules, plus some others of lower abundance, carry ABH antigens. The ABH-active oligosaccharides contribute to the glycocalyx or cell coat, an extracellular matrix of carbohydrate that protects the cell from mechanical damage and attack by pathogenic micro-organisms [851,852].

Carbohydrate structures on cell surfaces are exploited by pathogenic micro-organisms to gain entry to the cell or to facilitate parasite survival within the infected cell. Carbohydrate polymorphisms have almost certainly arisen in an attempt to evade microbial infection and have subsequently been maintained over at least 13 million years by a variety of conflicting selective forces (Section 2.20) [853].

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