The ABO, H, P, I, and Lewis blood group antigens reside on structurally related carbohydrate molecules, and reflect the activity of genetically determined glycosyltransferase enzymes. Carbohydrate molecules carry sugars that may determine several antigens, providing an opportunity for interaction between genetic products in several systems. The antigens appear when transferases add individual sugars to sites on short chains of sugars (oligosaccharides) that often are part of other, much larger molecules. The added sugars are called immunodominant because they confer specific antigenic activity on the oligosaccharide chains.

Oligosaccharides are chains of sugars that can be attached to either protein (glycoprotein), sphingolipid (glycosphingolipid), or lipid (glycolipid) carrier molecules. When attached to proteins, oligosaccharides are linked either to the amide nitrogen of asparagine via an N-acetylglycosamine (GlcNAc) or to the hydroxyl oxygen of serine or threonine via an N-acetylgalactosamine (GalNAc). Glycoproteins are associated with the membranes of red cells and other cells. The body’s serous and mucous secretions contain soluble glycoproteins with blood group antigen activity. In glycosphingolipids, structurally similar oligosaccharides are attached via glucose (Glc) to ceramide residues. Glycosphingolipids form part of the membranes of red cells, of most endothelial cells, and some epithelial cells. Soluble forms are present in plasma as glycolipids, but are not secreted in body fluids.

The ABO System

A series of tests reported by Karl Landsteiner in 1900 led to the discovery of the ABO blood groups and to the development of routine blood grouping procedures. Landsteiner tested blood samples from himself and several colleagues by combining each serum specimen with a suspension of red cells from each person. Noting agglutination in some mixtures but not in others, he was able to classify the blood samples into one of three groups, now named A, B, and O.
Landsteiner recognized that the presence or absence of only two antigens, A and B, was sufficient to explain the three blood groups and he predicted that a fourth group should exist. AB was discovered in 1902 by his pupils von Decastello and Sturli. He also demonstrated that each person’s serum contained antibody against the antigen absent from that person’s red cells.

The first blood group system to be discovered, ABO remains the most significant for transfusion practice. It is the only system in which the reciprocal (or antithetical) antibodies (see Table 12-1) are consistently and predictably present in the sera of normal people who have had no exposure to human red cells. Because of these antibodies, transfusion of ABO-incompatible blood may cause severe intravascular hemolysis as well as the other manifestations of an acute hemolytic transfusion reaction (see Chapter 25). Testing to detect ABO incompatibility between a recipient and the donor is the foundation on which all pretransfusion testing is based.

Antigens of the ABO System

Biochemical and Genetic Considerations

Glycosphingolipids carrying A or B oligosaccharides are integral parts of the membranes of red cells, epithelial cells, and endothelial cells (Fig 12-1) and are also present in soluble form in plasma. Secreted body fluids such as saliva contain glycoprotein molecules that may, if the person possesses an Se gene, carry identical oligosaccharides. A and B oligosaccharides unattached to carrier protein or lipid molecules are also found in milk and urine.

Genes at three separate loci (ABO, Hh, and Sese) control the occurrence and the location of the A and B antigens. Three common alleles (A, B, and O) are located at the ABO locus on chromosome 9. The A and B genes encode glycosyltransferases that produce the A and B antigens, respectively. The O gene is considered to be nonfunctional because its protein product determines no detectable blood group antigen. The red cells of group O persons lack A and B, but carry an abundant amount of H antigen, the precursor material on which A and B antigens are built.

Family studies have shown that the genes at the remaining two loci, Hh and Sese (secretor), are on chromosome 19 and are closely linked. Each locus has two recognized alleles, of which one has no demonstrable product and is considered an amorph. The active allele at the H locus, H, produces a transferase enzyme that acts at the cellular level to form the antigen on which A or B are built. The amorph, h, is very rare.

The Se gene is directly responsible for the expression of H (and indirectly responsible for the expression of A and B)

Table 12-1. Routine ABO Grouping

<table>
<thead>
<tr>
<th>Reaction of Cells Tested With</th>
<th>Reaction of Serum Tested Against</th>
<th>Interpretation</th>
<th>Frequency (%) in US Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>Anti-B</td>
<td>B Cells</td>
<td>O Cells</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

+= agglutination; 0 = no agglutination
on the glycoproteins in epithelial secretions such as saliva. Eighty percent of the population are described as secretors; they have inherited the Se gene and their secreted glycoproteins express H, which can be converted to A and/or B if they also have the A or B gene. The amorph is called se.

The oligosaccharide chains to which the A or B immunodominant sugars are attached may exist as simple repeats of a few sugar molecules linked in linear fashion. They can also exist as more complex structures, with many sugar residues linked in branching chains. Differences between infants and adults in cellular A, B, and H activity may be related to the number of branched structures present on cellular membranes at different ages. The red cells of infants are thought to carry predominantly linear oligosaccharides, which have only one terminus to which the H (and subsequent A or B) sugars can be added. In contrast, the red cells of adults appear to carry a high proportion of branched oligosaccharides. Branching creates additional expressions of oligosaccharide for conversion to H and then to A and B antigens.

A and B genes do not produce antigens directly; their products are enzymes, called glycosyltransferases, that add specific sugars to oligosaccharide chains that have been converted to H by the fucosyl transferase produced by the H gene. H antigens are constructed on oligosaccha-

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Figure 12-1. Diagrammatic representation of some membrane glycoproteins and glycosphingolipids that carry blood group antigens.
ride chains that end in Type 1 or Type 2 linkages between the sugars β-D-galactose (Gal) and N-acetylglucosamine (GlcNAc) (see Fig 12-2). In Type 1 chains, the number 1 carbon of Gal is linked to the number 3 carbon of GlcNAc; in Type 2 chains, the number 4 carbon of GlcNAc is the acceptor for Gal. Oligosaccharides of Type 1, carrying A, B, and H activity, are widely distributed in the body. Glycolipids with Type 1 A, B, and H are present in plasma and on endodermally derived tissues such as the epithelial lining of the gut. Glycoproteins with Type 1 chains contribute A, B, and H activity to body fluids and secretions. Unattached Type 1 oligosaccharides can be found in milk and urine. In saliva, glycoproteins of Type 1 and Type 2 carry A, B, and H.

A, B, and H antigens on the red cell surface are formed on Type 2 chains present in highly branched oligosaccharides attached to integral proteins of the red cell membrane, notably bands 3 and 4. The number of potential A, B, and H sites on red cells is thought to be in excess of two million.

The H gene produces a transferase that adds fucose (Fuc) to the number 2 sugar of the galactose that constitutes the terminal sugar of Type 1 and Type 2 chains (see Fig 12-3). The A and B gene transferases can attach their immunodominant sugars to the number 3 sugar of the same galactose only if fucose is

**Figure 12-2.** Type 1 and 2 oligosaccharide chains differ only in the linkage between the GlcNAc and the terminal Gal.

**Figure 12-3.** Gal added to the subterminal Gal confers B activity; GalNAc added to the subterminal Gal confers A activity to the sugar. Unless the fucose moiety that determines H activity is attached to the number 2 carbon, galactose does not accept either sugar on the 3 carbon.
already attached, ie, the core chain has been converted to H. Attachment of the A- or B-defining sugar diminishes the H configuration in a reciprocal manner; the expression of A or B and of H are inversely proportional. Initially, it was believed that addition of A and B sugars to the terminal galactose halted chain growth, ie, that A or B antigens could not serve as acceptor substrates. The discovery of glycolipids in which two A structures appeared linked in tandem altered this view (see Fig 12-4).

**ABO Genes at the Molecular Level**

Yamamoto and Hakomori have shown that A and B genes differ from one another by seven single-base substitutions. This results in four possible amino acid substitutions (at positions 176, 235, 266 and 268) in the protein chains of the A and B transferases. Substitutions at positions 266 and 268 are most significant in determining sugar-nucleotide specificity, although substitution at position 235 also has some effect. The alleles of A and B that result in subgroups (phenotypes of A and B that express aberrant levels of antigen) may have additional mutations that result in enzymes with slightly different abilities to convert H antigen. A single nucleotide deletion was found in A2 genes close to the carboxyl terminus of the A transferase coding sequence. As a result of frame-shifting, the glycosyltransferase specified by A2 possesses an extra domain of 21 amino acids; this domain seems responsible for the low transferase activity and restricted substrate recognition of this enzyme.

The base sequence of the O gene appears to be similar to that of A except for a single base deletion at position 258 in the coding region close to the N-terminus of the resulting protein. This single deletion shifts the reading frame to produce a premature stop codon, resulting in a truncated protein with no transferase activity. Thus, of the three primary genes in the ABO system, two are functional and one determines a non-functional product.

Persons who lack the Se gene have Type I and Type 2 chains in their secretions that express no H, A, or B activity. It is thought that the H and Se genes each encode a fucosyltransferase. The enzyme produced by H acts primarily on Type 2 chains, which predominate on the red cell membranes; the enzyme produced by Se prefers (but does not limit its action to) Type 1 chains, and acts primarily in the secretory glands. Studies performed on the secretions of individuals with the rare O, phenotype support the concept that two types of H antigen exist. Individuals who lack both H and Se genes (genotype hh and sese) have no H and, therefore, no A or B antigens on their red cells or in their secretions. However, H, A, and B antigens are found in the secretions of some hh individuals who appear, through family studies, to possess at least one Se gene.

![Figure 12-4. Type 3 A antigen structure.](image-url)
Direct agglutination tests are used to detect A and B antigens on red blood cells. Reagent antibodies frequently produce weaker reactions with red cells from newborns than with red cells from adults. Although they can be detected on the red cells of 5-6 week-old embryos, A and B antigens are not fully developed at birth, presumably because the branching oligosaccharide structures develop gradually. By the time a person is 2-4 years old, A and B antigen expression is fully developed, and remains fairly constant throughout life.

Subgroups

Subgroups are ABO phenotypes that differ in the amount of antigen carried on red cells and, in secretors, present in the saliva (see Table 12-2). Subgroups are more commonly encountered and more significant for A than for B. The two principal subgroups of A are A₁ and A₂. The A₁ and A₂ genes determine different transferases, which mediate both quantitative and qualitative differences between red cells of the A₁ and A₂ phenotypes. Red cells from A₁ and A₂ persons both react strongly with reagent anti-A in direct agglutination tests. The serologic distinction between A₁ and A₂ cells derives from tests with reagent anti-A₁ prepared from group B human serum or with the lectin of Dolichos biflorus seeds. Under prescribed testing conditions, anti-A₁ reagents agglutinate A₁ but not A₂ red cells. Approximately 80% of group A or group AB individuals have red cells that are agglutinated by anti-A₁ and thus are classified as A₁ or A₁B. The remaining 20%, whose red cells are agglutinated by anti-A but not by anti-A₁, are called A₂ or A₂B.

Anti-A₁ occurs as an alloantibody in the serum of 1-8% of A₂ persons and 22-35% of A₂B persons. Anti-A₁ can cause discrepancies in ABO testing and

Table 12-2. Serologic Reactions Observed in Persons With A and B Phenotypes*

<table>
<thead>
<tr>
<th>Red Blood Cell Phenotype</th>
<th>Reaction of Cells With Known Antiserum to</th>
<th>Reaction of Serum Against Reagent Red Blood Cells</th>
<th>Saliva of Secretors Contains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A,B</td>
</tr>
<tr>
<td>A₁</td>
<td>4+</td>
<td>0</td>
<td>4+</td>
</tr>
<tr>
<td>A₁int</td>
<td>4+</td>
<td>0</td>
<td>4+</td>
</tr>
<tr>
<td>A₂</td>
<td>4+</td>
<td>0</td>
<td>4+</td>
</tr>
<tr>
<td>A₃</td>
<td>2⁺mf</td>
<td>0</td>
<td>2⁺mf</td>
</tr>
<tr>
<td>Aₐ</td>
<td>0/±</td>
<td>0</td>
<td>0/±</td>
</tr>
<tr>
<td>Aₓ</td>
<td>0/±</td>
<td>0</td>
<td>1+2+</td>
</tr>
<tr>
<td>Aₑl</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B₁</td>
<td>0</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>B₃</td>
<td>0</td>
<td>1⁺mf</td>
<td>2⁺mf</td>
</tr>
<tr>
<td>Bₐ</td>
<td>0</td>
<td>0</td>
<td>0/±</td>
</tr>
<tr>
<td>Bₓ</td>
<td>0</td>
<td>0/±</td>
<td>0/2+</td>
</tr>
</tbody>
</table>

1+ to 4+, agglutination of increasing strength; ±, weak agglutination; mf, mixed-field pattern of agglutination; 0, no agglutination.

*Modified from Beattie

†The occurrence of anti-A₁ is variable in these phenotypes. A₂ persons frequently have anti-A₁; A₃ persons usually do not, but a few A₃ individuals with anti-A₁ in the serum have been found.
incompatibility in crossmatches with A₁ or A₁B red cells. Anti-A₁ usually reacts better or only at temperatures well below 37 C, and is considered clinically insignificant unless there is reactivity at 37 C. Routine testing with anti-A₁ is unnecessary for donors or recipients. This information is useful only when working with blood samples that contain anti-A₁.

Subgroups weaker than A₂ occur infrequently and, in general, are characterized by decreasing numbers of A antigen sites on the red cells and a reciprocal increase in H antigen activity. Classification of weak A subgroups is generally based on the:

1. Degree of red cell agglutination by anti-A and anti-A₁.
2. Degree of red cell agglutination by anti-A,B.
3. Degree of red cell agglutination by anti-H (Ulex europaeus).
4. Presence or absence of anti-A₁ in the serum.
5. Presence of A and H substances in the saliva of secretors.

See Table 12-2 for the serologic characteristics of A phenotypes.

Red cells of the Aₓ, Aₑ₁, Aₑ₂, or A₃ subgroups are seen only rarely in transfusion practice. Aₓ red cells are characteristically not agglutinated by human anti-A from group B persons, but are agglutinated by anti-A,B from group O persons (see following section on antibodies). Aₓ red cells may react with some murine monoclonal anti-A reagents, depending on which monoclonal antibody is selected for the reagent. Aₑ₁ red cells are not agglutinated by anti-A or anti-A,B of any origin, and presence of A antigen is demonstrable only by adsorption and elution studies. Aₑ₂ red cells react more weakly than A₁ red cells with anti-A₁, yet more strongly with anti-H than A₂ red cells; Aₑ₂ phenotype is detected only if anti-A₁ testing is performed and quantified. A₃ red cells produce a characteristic mixed-field pattern of small agglutinates among many free red cells in tests with anti-A and anti-A,B. Weak subgroups of A such as Aₓ, Aₑ₂, and Aₑ₁ cannot reliably be identified on the basis of blood typing tests alone. Saliva studies, adsorption/elution studies, and family studies provide confirmatory information.

Subgroups of B are even less common than subgroups of A. Criteria for their differentiation resemble those for subgroups of A (see Table 12-2). Amino acid substitutions have been reported in some of the glycosyltransferases coded by the weak subgroup genes. Widespread use of monoclonal anti-A and anti-B reagents will lessen the detection of weak A or B subgroups because monoclonal antibodies are selected for reagent use based on their ability to agglutinate cells with weak or aberrant antigen expression.

Antibodies to A and B

Ordinarily, individuals possess antibodies directed toward the A or B antigen absent from their own red cells (see Table 12-1). This predictable complementary relationship permits ABO testing of serum as well as of red cells (see Methods 2.2 and 2.3). One hypothesis for the development of these antibodies is based on the fact that the configurations that confer A and B specificities on molecules of the red cell membrane also exist in other biologic entities, notably bacterial cell walls. Bacteria are widespread in the environment, and their presence in intestinal flora, dust, food, and other widely distributed agents ensures a constant exposure of all persons to A-like and B-like antigens. Immunocompetent persons react to the environmental antigens by producing antibodies to those that are absent from their own systems. Thus, anti-A is produced by group O and group B persons and anti-B is produced by group O and group A persons. Group
AB people, having both antigens, make neither antibody. This “environmental” explanation for emergence of anti-A and anti-B remains a hypothesis that has not been proved.

**Time of Appearance**

Anti-A and anti-B can generally be detected in serum after the first few months of life. Occasionally infants can be found who already produce these antibodies at the time of birth, but most antibodies present in cord blood are of maternal origin. Antibody production increases until reaching adult level at 5-10 years of age and declines later in life. Elderly people ordinarily have lower anti-A and anti-B levels than those seen in young adults. Anti-A and anti-B testing on serum from newborns or infants younger than 4-6 months cannot be considered valid because some or all of the infant’s antibodies are acquired by placental transfer of maternal IgG anti-A and anti-B.

**Reactivity of Anti-A and Anti-B**

IgM is the predominant immunoglobulin class of anti-A produced by group B individuals and anti-B produced by group A individuals, although small quantities of IgG molecules are also present. IgG is the dominant class of anti-A and anti-B of group O serum. Because IgG readily crosses the placenta and IgM does not, the infants of group O mothers are at higher risk for ABO hemolytic disease of the newborn (HDN) compared to those of group A or B mothers.

The distinguishing features of IgM and IgG anti-A and anti-B are given in Table 12-3. Both immunoglobulin classes preferentially agglutinate red cells at room temperature (20-24 C) or below, and efficiently activate complement at 37 C. The complement-mediated lytic capability of these antibodies becomes apparent if serum testing includes an incubation phase at 37 C. Some patients or donors are found whose serum causes hemolysis of ABO-incompatible red cells at temperatures below 37 C. Hemolysis due to ABO antibodies should be suspected when the supernatant fluid of the reverse grouping test is pink to red or when the cell button is absent or reduced in size. Hemolysis must be interpreted as a positive result. Because the hemolysis is complement-mediated, it will not occur if reagent red cells are suspended in solutions that contain EDTA or other agents that prevent complement activation, or if plasma is used for testing.

**Anti-A,B (Group O Serum)**

Serum from group O individuals contains an antibody designated as anti-A,B because it reacts with both A and B red cells and the anti-A and anti-B specificities cannot be separated by differential adsorption. When group O serum is incubated with group A or group B cells, eluates prepared from each adsorbing cell exhibit reactivity against both A and B test cells. Saliva from secretors of either A or B substance inhibits the ac-

| Table 12-3. Distinguishing Characteristics of IgM and IgG Anti-A and Anti-B |
|-----------------|----------|----------|
| Reactions enhanced | IgM yes | IgG yes |
| —with enzyme-treated rbc's | yes | yes |
| —by lowering temperatures | yes | no |
| Readily inhibited by | IgM yes | IgG no |
| soluble A or B antigens | yes | no |
| Inactivated by 2-ME or DTT | yes | no |
| Predominant in non-immunized group A and B donors | yes | no |
tivity of this antibody against both A or B red cells.

Group O serum has been used to prepare potent grouping reagents capable of agglutinating either A or B cells. Reagent preparations containing blended monoclonal antibodies also agglutinate A or B cells, and either form of anti-A,B readily distinguishes group O red cells from the other three groups. Anti-A,B containing blended monoclonal antibodies may react as well as, or better than, natural human anti-A,B with red cells of weak A phenotypes, depending on the monoclonal preparations selected.

**Anti-A1**

The anti-A of group B serum appears, from simple studies, to contain separable anti-A and anti-A1. Native group B serum agglutinates A1 and A2 red cells; after adsorption with A2 red cells, group B serum reacts only with A1 red cells. If further tests are performed, however, the differences in A antigen expression between A1 and A2 red cells appear to be quantitative rather than qualitative. Anti-A1 is sometimes found in the serum of persons with A2 or other subgroup A phenotypes.

A reliable anti-A1 reagent can be manufactured from the lectin of *Dolichos biflorus* (see Method 2.10). The plant extract will react with both A1 and A2 red cells, but the appropriately diluted reagent preparation will not agglutinate A2 cells and thus constitutes an anti-A1.

**Routine Testing for ABO**

Tests that use anti-A and anti-B to determine the presence or absence of the antigens are often described as direct or red cell tests. The use of reagent A1 and B red cells to detect anti-A and anti-B in serum is called serum testing. Routine tests on donors and patients must include both red cell and serum tests, each serving as a check on the other. To confirm the ABO type of donor units that have already been labeled, or to test blood of infants less than 4 months of age, ABO testing on red cells only is permissible. Procedures for ABO typing by slide, tube, and microplate tests are described in Methods 2.1, 2.2, and 2.3.

Some ABO red cell typing reagents are prepared from pools of sera from individuals who have been stimulated with A or B blood group substances to produce antibodies of high titer. Other ABO grouping reagents are manufactured from monoclonal antibodies derived from cultured cell lines. Both types of reagents agglutinate most antigen-positive red cells on direct contact, even without centrifugation. Slide testing cannot, however, be recommended because of the likelihood of spillage and contact with blood. Anti-A and anti-B in the serum of most patients and donors are usually too weak to agglutinate red cells without centrifugation, so serum tests should be performed by tube or microplate techniques, not on slides.

**B(A) Phenotype**

In 1984, Yates et al reported that a 10,000-fold concentrate of purified B gene-specified galactosyltransferase, isolated from the sera of group B individuals has the capacity, in vitro, to attach small amounts of A sugar to human milk fucosyllactose, a blood group H-like structure. High levels of galactosyltransferase associated with the B gene can attach the A-determining sugar, GalNAc, to a fucosylated substrate molecule, both in vitro and in vivo. Red cells of some group B individuals were agglutinated by an FDA-licensed anti-A reagent that contained a particular murine monoclonal antibody MHO4. These group B individuals had excessively high levels of B gene-specified galactosyl-
transferase, and the designation B(A) was given to this blood group phenotype.

Recognition of the B(A) phenotype is usually made with the discriminating monoclonal anti-A reagent, with which the B(A) red cells show varying reactivity. The majority of examples react weakly, and the agglutinates are fragile and easily dispersed, although some examples have reacted as strongly as 2+. Serum from these individuals agglutinates both A₁ and A₂ cells, so except for newborns and immunocompromised patients, serum testing should distinguish this phenomenon from the AB phenotype in which a subgroup of A is accompanied by anti-A₁. Studies of the transferase or the gene sequence should provide conclusive proof. The transferase for GalNAc should be present in the serum from the A₂B individual and absent in the B(A) sample. Compared with B transferase, the transferase determining B(A) exhibits a single amino acid substitution at the second of the four amino acid substitution sites (aa 235) that discriminate between A₁ and B transferases. The amino acid residue it possesses is glycine, which characterizes the A₁ transferase.

Nonroutine ABO Testing

Additional reagents may be used for ABO testing, especially anti-A,B for red cell tests and A₂ and O red cells for serum tests. Some workers use anti-A,B in routine tests in the belief that it is more effective than anti-A or anti-B in detecting weakly expressed antigens. This is not true. Of the weak subgroups undetected by anti-A or anti-B, only A₁ is detected by human anti-A,B, and then only if the serum and cells are incubated at room temperature for 10–60 minutes. Some monoclonal blends labeled anti-A,B react well with red cells of weak subgroups, on immediate-spin tests. If the manufacturer’s directions recommend using anti-A,B for the detection of weak subgroups, it means that its reactivity against A₄ red cells has been demonstrated. AABB [Standard] for Blood Banks and Transfusion Services does not require special techniques to detect weak subgroups because the absence of expected serum antibodies usually distinguishes these specimens from group O specimens (see Table 12-2).

Some commercially marketed sets of serum testing cells contain A₂ red cells in addition to A₁ and B red cells. The A₂ red cells are intended to facilitate the recognition of anti-A₁ in the serum of specimens exhibiting a subgroup of A. Because most A specimens do not contain anti-A₁, routine use of this reagent is inappropriate unless discrepancies between red cell and serum tests are encountered. A₂ cells for differential testing can be selected by testing donor or patient samples with anti-A₁, although having a readily available reagent may be more convenient.

Manufacturers of ABO reagents provide, with each reagent package, detailed instructions for use of the reagent. Testing details may vary from one manufacturer to another, and it is important to follow the directions supplied with the specific reagent in use.

Discrepancies Between Red Blood Cell and Serum Tests

Table 12-1 shows the results and interpretations of routine red cell and serum tests for ABO. A discrepancy exists when the results of red cell tests do not complement that of serum tests, and the expected two positives and two negatives are not observed. When a discrepancy is encountered, the discrepant results should be recorded, but interpretation must be delayed until the discrepancy is resolved. If the specimen is from a donor unit, the unit may not be released for
transfusion until the discrepancy is resolved. When the blood is from a potential recipient, it may be necessary to administer group O red cells of the appropriate Rh type before the investigation is completed. It is important to obtain a sufficient amount of the patient’s pretransfusion blood to complete any additional studies that may be required.

Red cell and serum test results may be discrepant because of intrinsic problems with red cells or serum, because of test-related problems, or because of technical errors. Discrepancies may be signaled either because negative results are obtained when positive results are expected, or positive results are found when tests should have been negative.

**False Negatives**

False-negative test results may occur because of failure to:
1. Add reagent or test serum to a tube.
2. Identify hemolysis as a positive reaction.
3. Use the appropriate ratio of serum (or reagent) to red cells.
4. Centrifuge tests sufficiently.
5. Incubate tests at temperatures of 20-24°C or below.
6. Interpret or record test results correctly.

**False Positives**

False-positive test results may occur because of:
1. Overcentrifugation of tubes.
2. Use of contaminated reagents, red cells, or saline.
3. Use of dirty glassware.
4. Incorrect interpretation or recording of test results.

**Specimen-Related Problems in Testing Red Cells**

Grouping tests on red cells may give unexpected results for many reasons.

1. A patient who has received red cell transfusions or a bone marrow transplant may have circulating red cells of more than one ABO group, and thus constitute a transfusion or transplantation chimera.
2. Red cells from individuals with variant A or B genes may carry poorly expressed antigens. Antigen expression may be weakened on the red cells of some persons with leukemia or other malignancies. Agglutination tests with reagent anti-A and anti-B may fail to give expected reactions.
3. Inherited or acquired abnormalities of the red cell membrane can lead to what is called a polyagglutinable state. The abnormal red cells can be unexpectedly agglutinated by reagent anti-A, anti-B, or both.
4. Abnormal concentrations of serum proteins, the presence in serum of macromolecules or, in cord blood samples, the presence of Wharton’s jelly may cause nonspecific aggregation of serum-suspended cells that simulates agglutination.
5. Exceptionally high concentrations of A or B blood group substances in the serum can combine with reagent antibodies and neutralize them to produce an unexpected negative reaction against serum- or plasma-suspended red cells.
6. Serum may contain antibodies to the dyes used to color anti-A and anti-B reagents. These antibodies can cause false-positive agglutination reactions if serum- or plasma-suspended red cells are used in testing.
7. A patient with potent cold-reactive autoagglutinins may have red cells so heavily coated with antibody that they agglutinate spontaneously in the presence of diluent, independent of the specificity of the reagent antibody.
Specimen-Related Problems in Testing Serum

Serum grouping tests are also subject to false results.

1. Small fibrin clots that may be mistaken for agglutinates may be seen in ABO tests if plasma or incompletely clotted serum is used.

2. Abnormal concentrations of proteins, altered serum protein ratios, or the presence of intravenous contrast materials of high molecular weight plasma expanders can cause nonspecific red cell aggregation that is difficult to distinguish from true agglutination.

3. Antibodies other than anti-A and anti-B in a test sample can agglutinate reagent A\textsubscript{1} or B red cells if they carry the corresponding antigen.

4. Antibodies to constituents of the diluents used to preserve reagent A\textsubscript{1} and B red cells can agglutinate the cells independent of ABO antigens and antibodies.

5. Patients who are immunodeficient due to disease or therapy may have such depressed immunoglobulin levels that there is little or no ABO agglutinin activity. Samples from elderly patients whose antibody levels have declined with age or from patients whose antibodies have been greatly diluted by plasma exchange procedures may also have unexpectedly weak agglutinins.

6. Negative or weak results are seen in serum tests from infants under 4-6 months of age. Serum from newborns is not usually tested, since antibodies present are generally passively transferred from the mother.

7. Very high-titer complement-binding anti-A and anti-B may cause so many C1 molecules to associate with the red cell surface that binding to the membrane antigens is sterically hindered and agglutination does not occur. This phenomenon has been reported in serum grouping tests using red cells suspended in diluents that lack EDTA.\textsuperscript{16}

8. If the patient has received a bone marrow transplant of a compatible but dissimilar ABO group, serum antibodies will not agree with red cell antigens. For example, a group A individual transplanted with group O marrow will have circulating group O red cells, but produce only anti-B in the serum.

9. Recent transfusion with plasma components containing ABO agglutinins may cause unexpected reactions.

Resolving ABO Discrepancies

The first step in resolving an apparent problem should be to repeat the tests on the same sample. If initial tests were performed on red cells suspended in serum or plasma, repeat testing should use a saline suspension of washed cells. If the discrepancy persists, the following procedures can be incorporated into the investigation.

1. Obtain a new blood specimen from the donor unit or patient and test the new sample. This should resolve discrepancies due to mislabeled or contaminated specimens.

2. Wash the test and reagent red cells several times to remove any serum or chemical constituents that may be causing unexpected positive reactions.

3. Test the red cells with anti-A,B, anti-A\textsubscript{1}, or anti-H as appropriate for the individual problem.

4. If anti-A\textsubscript{1} is suspected, test the serum against several examples of group A\textsubscript{2} red cells.

5. Review results of the antibody screening test against group O red cells to detect interfering effects.
from nonspecific cold-reactive alloantibodies.

6. Incubate tests for 30 minutes at room temperature to facilitate the detection of weak antigens or antibodies. Tests can be subjected to even lower temperatures, but parallel tests with group O and autologous cells should be observed to rule out interference by broadly reactive agglutinins, such as anti-I or anti-H, that react with the red cells of all adults.

**Resolving Discrepancies Due to Absence of Expected Antigens**

Red cells of most A or B people are strongly agglutinated (3–4+) by the corresponding reagent antibody, and the serum of these samples usually strongly agglutinates A1 or B reagent red cells (2–4+). The cause of a discrepancy can sometimes be inferred from the strength of reactions obtained in red cell or serum grouping tests. For example, serum that strongly agglutinates group B red cells but not A1 cells probably comes from a group A person, even though the red cells are not agglutinated by anti-A or anti-B. A or B antigens may be weakly expressed on cells from individuals who have inherited variant alleles, or with disease-related antigen depression. The following procedures can be used to enhance detection of weakly expressed antigens.

1. Incubate washed red cells with anti-A and anti-A,B for 30 minutes at room temperature to increase association of antibody with the scant amount of antigen. Incubating the test system at 4 C may further enhance antibody attachment, but tests performed at 4 C must be controlled with group O and autologous red cells to ensure that reactions observed are due to anti-A and anti-B and not to other cold-reactive agglutinins.

2. Treat the patient’s red cells with a proteolytic enzyme such as ficin, papain, or bromelain. Enzyme treatment increases the antigen-antibody reaction with anti-A or anti-B. In some instances, reactions between reagent antibody and red cells expressing antigens will become detectable at room temperature within 30 minutes if enzyme-treated red cells are employed. Enzyme-treated group O red cells must be tested in parallel as control for the specificity of the ABO reaction.

3. Incubate an aliquot of red cells at room temperature or at 4 C with human anti-A or anti-B (as appropriate) to adsorb antibody to the corresponding red cell antigens. Anti-A1 lectin or monoclonal reagents should not be used for adsorption/elution studies, and group O red cells should be subjected to parallel adsorption and elution to serve as a control. Following incubation, wash the red cells thoroughly and prepare an eluate. (See **Method 2.4**) Test the eluate against group A1, B, and O cells. If the red cells carried the A antigen, the eluate will agglutinate A1, but not B or O red cells. Eluates prepared from group B red cells will agglutinate only other B red cells. The eluate prepared from group O cells should be nonreactive. Activity in the control group O eluate invalidates results obtained with the patient’s red cells, and can mean either the anti-A or anti-B serum contained other antibodies, or that the adsorption/elution procedure was not performed correctly.

4. Test the saliva for the presence of H and A or B substances (see **Method 2.5**) for hemagglutination-inhibition
tests for salivary antigens). Saliva tests help resolve ABO discrepancies only if the person is a secretor, but this may not be known until after testing is completed.

Resolving Discrepancies Due to Unexpected Reactions with Anti-A and Anti-B

Red cell grouping tests sometimes give unexpected positive reactions. For example, reagent anti-A may weakly agglutinate red cells from a sample in which the serum gives reactions expected of a normal group B or O. Variant alleles at the ABO locus or problems unrelated to the actions of ABO genes may be responsible. The following paragraphs describe some events that can cause unexpected reactions in ABO grouping tests and the steps that can be taken to identify them.

Acquired B Phenotype. A serum containing strong anti-B and red cells agglutinated strongly by anti-A and weakly by anti-B suggests the acquired B state. The acquired B phenotype arises when microbial deacetylating enzymes modify the A antigen by altering the A-determining sugar (N-acetylgalactosamine) so that it resembles the B-determining galactose. Only A1 red cells exhibit acquired B activity in vivo. The acquired B antigen develops at the expense of A, so a concomitant decrease in the strength of A may be seen in the acquired B state. If sufficiently numerous, acquired B antigens cause the red cells to be agglutinated by human anti-B. Most red cells with acquired B antigens react weakly with anti-B, but occasional examples are agglutinated quite strongly. Behavior with monoclonal anti-B reagents varies with the particular clonal products used. Acquired B antigens have been observed with increased frequency in tests with certain FDA-licensed monoclonal anti-B blood grouping reagents. To confirm that group A1 red cells carry the acquired B structure:

1. Check the patient’s diagnosis. Acquired B antigens are usually associated with tissue conditions that allow colonic bacteria to enter the circulation, but acquired B antigens have been found on the red cells of apparently normal blood donors.

2. Test the patient’s serum against autologous red cells. The individual’s anti-B will not agglutinate his or her own red cells that carry the acquired B.

3. Test the red cells with monoclonal anti-B reagents for which the manufacturer’s instructions give a detailed description. Unlike human-source some monoclonal antibodies do not react with the acquired B phenotype; this information may be included in the package insert.

4. Test the red cells with human anti-B serum that has been acidified to pH 6.0. Acidified human anti-B no longer reacts with the acquired B antigen.

5. If the patient is a secretor, test saliva for the presence of A and B. Secretors whose red cells carry acquired B structures will have A, but not B, substance in their saliva.

6. Treat the red cells with acetic anhydride, which reacetylates the surface molecules and markedly diminishes the reactivity of acquired B red cells. Reactivity of normal group B antigens is not affected by acetic anhydride.

Acquired A-Like Antigens. ABO discrepancies can be seen in the condition known as Tn polyagglutination, in which defective synthesis of oligosaccharides normally present on sialoglycoprotein molecules leave abnormal antigenic structures (cryptantigens) exposed on the red cell surface. The residual sugar uncovered is N-acetylgalactosamine, the sugar that confers A specificity. A so-
matic mutation on hematopoietic stem cells results in a permanent population of cells described as Tn-activated. Group O or group B cells that have been Tn-activated behave as if they have acquired an A antigen, which reacts with human or monoclonal anti-A reagents. The A-like antigen of Tn-activated red cells can be differentiated from the A produced by the A-gene transferase if red cells are treated with proteolytic enzymes before testing. Proteolytic enzymes degrade the molecule that expresses the cryptantigen, abolishing the reactivity with anti-A.

Other forms of polyagglutination may interfere in ABO grouping tests. See Method 2.10 to classify polyagglutinable red cells using lectins.

Mixed-Field Agglutination. Occasional samples are encountered that contain two distinct, separable populations of red cells. Usually this reflects recent transfusion of group O red cells to a non-O recipient or receipt of a bone marrow transplant of an ABO group different from the patient's own. Red cell mixtures also occur in a condition called blood group chimerism, resulting either from intrauterine exchange of erythropoietic tissue by fraternal twins or from mosaicism arising through dispermy. In all such circumstances, ABO red cell tests may give a mixed-field pattern of agglutination. Mixed-field reactions due to transfusion last only for the life of the transfused red cells. After bone marrow transplantation, the mixed-field reaction usually disappears when the patient's own red cells are no longer produced. Persistent mixed-cell populations do occur in some bone marrow recipients. Mixed-field reactions that arise through blood group chimerism persist throughout the life of the individual.

Mixed-field agglutination is characteristic of the reaction between A$_3$ red cells and reagent anti-A. If the agglutinated red cells are removed and the remaining red cells again tested with anti-A, mixed-field agglutination occurs in the residual, previously nonagglutinated population.

Antibody-Coated Red Blood Cells. Red cells from infants with HDN, or from adults suffering from autoimmune or alloimmune conditions, may be so heavily coated with IgG antibody molecules that they agglutinate spontaneously in the presence of reagent diluents containing high protein concentrations. Usually this is at the 18-22% range found in some anti-D reagents, but sometimes the sensitized red cells also agglutinate in ABO reagents with protein concentrations of 6-12%. Gentle elution at 45 C can be used to remove much of this antibody from the red cells so they can be tested reliably with anti-A and anti-B.

Red cells from a specimen containing cold-reactive IgM autoagglutinins may agglutinate spontaneously in saline tests. The antibodies can usually be removed by incubating the cell suspension briefly at 37 C and then washing several times with saline warmed to 37 C. If the IgM-related agglutination is not dispersed by this technique, the red cells can be treated with the sulfhydryl compound dithiothreitol (DTT) (see Method 2.12).

Resolving Discrepancies Due to Unexpected Serum Reactions

The following paragraphs describe some events that can cause unexpected or erroneous serum test results, and the steps that can be taken to resolve them.

1. Immunodeficient patients may not produce detectable levels of anti-A and anti-B. These antibodies are absent from the serum of newborns and may be absent or very weak in serum from normal elderly persons.
2. Abnormally high concentrations of anti-A and anti-B have caused prozone reactions that lead to false-negative results. In these instances the ABO group can be inferred by red cell tests, by dilution of the serum, or by the use of EDTA (2.5%)-treated serum.

3. Anti-A_1 in the serum of individuals of A_2, A_2B, or other subgroups agglutinate A_1 reagent red cells. To demonstrate this as the cause of the discrepancy:
   a. Test the serum against several examples of group A_1, A_2, and O red cells, preferably three of each. Only if the antibody agglutinates all A_1 red cells and none of the A_2 or O red cell samples can it be called A_1.
   b. Use anti-A_1, either Dolichos biflorus lectin or absorbed human group B serum, to demonstrate that the individual’s cells belong to a non-A_1 subgroup.

   Most examples of anti-A_1 react only at temperatures below 30 C and are considered clinically insignificant. Some examples have reactivity at 37 C and should be considered clinically significant; only A_2 or O red cells should be used for transfusion.

4. Cold-reactive autoagglutinins, such as anti-I and anti-IH can, if sufficiently strong, agglutinate red cells of all adults, including autologous cells and reagent red cells. With few exceptions, agglutination caused by the cold-reactive autoagglutinin is weaker than that caused by anti-A and anti-B. The following can be done when such reactivity interferes to the point that interpretation of serum tests is difficult.
   a. Warm serum and reagent red cells to 37 C before mixing and testing. Read serum tests at 37 C and convert them to the antiglobulin phase if necessary. Weakly reactive examples of anti-A or anti-B may not be detected by this method, however, because 37 C is above the reactive optimum for these antibodies. In group A and B individuals, ABO antibodies are predominantly of the IgM class, which will not be detected in routine antiglobulin tests that employ anti-IgG reagents. Examples of anti-A and anti-B with little or no IgG component can go undetected under these conditions.
   b. Remove the cold-reactive autoagglutinin from the serum using a cold autoadsorption method as described in Method 6.1. The adsorbed serum can then be tested against A_1 and B reagent red cells.
   c. Treat the serum with DTT (see Method 4.3) and use the treated serum for reverse-grouping tests. Because DTT destroys the agglutinating activity of IgM antibodies, tests that use DTT-treated serum must be converted to the antiglobulin phase, in which IgG forms of the antibodies will be detected. Because many group A or B persons do not produce more than minute quantities of IgG anti-A or anti-B, negative tests should be interpreted with caution.

5. Unexpected alloantibodies that react at room temperature, such as anti-P_1 or anti-M, may agglutinate the red cells used in serum tests if they carry the corresponding antigen. Ordinarily, one or more of the reagent cells used in the antibody detection test will also be agglutinated at room temperature, but a rare serum may react with an antigen on the serum testing cells that is not present on cells used for antibody detection. Steps to determine the...
correct ABO type of sera containing other cold-reactive alloantibodies include:

a. Raising the temperature to 30-37°C before mixing the serum and cells. If the thermal optimum of the alloantibody is below the temperature at which anti-A and anti-B react, this may resolve the discrepancy.
b. Identify the alloantibody, as described in Chapter 17, and test the reagent A₁ and B cells to determine which, if either, carries the corresponding antigen. Obtain A₁ and B red cells that lack the antigen and use these for serum testing.
c. If the antibody detection test is negative, test the serum against several examples of A₁ and B red cells. The serum may contain an antibody directed against an antigen of low incidence, which will be absent from most randomly selected A₁ and B red cells.

6. Serum from patients with abnormal concentrations of serum proteins, or altered serum protein ratios or who have received plasma expanders of high molecular weight, can aggregate reagent red cells and mimic agglutination. Some of these samples cause aggregation of the type described as rouleaux. Rouleaux formation is easily recognized on microscopic examination if the red cells assume what has been described as a “stack of coins” formation. More often, the aggregates appear as irregularly shaped clumps that closely resemble antibody-mediated agglutinates. The results of serum tests can often be corrected by diluting the serum 1:3 in saline, to abolish its aggregating properties, or by using a saline replacement technique (see Method 3.4).

**The H System**

The H system has two genes, H and h, and one antigen, H, which serves as the precursor molecule on which A and B antigens are built. On group O red cells, there is no A or B, and the membrane expresses abundant H. The amount of H antigen is, in order of diminishing quantity, O >> A₂ >> A₁B >> A₁ >> A₂B. As for A and B configurations, H-like antigens are found in nature. Individuals of the rare Oₜ phenotype, whose red cells lack H, have anti-A and anti-B, and a potent and clinically significant anti-H in their serum. Occasionally, group A₁, A₁B, or (less commonly) B persons have so little unconverted H antigen on their red cells that they produce anti-H, but this form of anti-H is relatively weak, virtually always reacts at room temperature or below, and is not considered clinically significant.

**Oₜ Phenotype**

The term “Bombay” has been used for the phenotype in which red cells lack H, A, and B, because examples of such red cells were first discovered in Bombay, India. The symbol Oₜ has been selected to denote the phenotype because results of routine ABO typing tests mimic those of group O persons. Oₜ red cells are not agglutinated by anti-A, anti-B, or anti-A,B, and the serum contains strong anti-A and anti-B. The Oₜ phenotype becomes apparent when serum from the Oₜ individual is tested against group O red cells, and strong, immediate reactions occur. The anti-H of an Oₜ individual reacts over a thermal range of 4-37°C with all red cells except those of other Oₜ people. Oₜ patients must be transfused with only Oₜ blood because their non-red-cell-stimulated antibodies rapidly destroy cells with A, B, or H antigens. The Oₜ phenotype is demonstrated by the absence of reaction when cells are exposed
to the anti-H lectin of *Ulex europaeus*. If other examples of O\textsubscript{h} red cells are available, further confirmation can be obtained by demonstrating compatibility of the serum with O\textsubscript{h} red cells and no others.

**Para-Bombay Phenotypes, A\textsubscript{h}, B\textsubscript{h}, and AB\textsubscript{h}**

A\textsubscript{h}, B\textsubscript{h}, and AB\textsubscript{h} red cells lack serologically detectable H antigen but carry small amounts of A and/or B antigen, depending on the individual’s genes at the *ABO* locus. Tests with anti-A or anti-B give weak reactions, but the cells are nonreactive with anti-H lectin or with anti-H serum from O\textsubscript{h} persons. The para-Bombay phenotype is thought to reflect the presence of variant H genes that produce only minute amounts of H antigen, all of which undergoes conversion to A or B by the products of the A and B genes, respectively. The serum of A\textsubscript{h} and B\textsubscript{h} people contains anti-H in addition to the expected anti-A or anti-B.

### The Lewis System

The Lewis system has two genes, *Le* and *le*. The Lewis antigens are not intrinsic to red cells, but are expressed on glycosphingolipids that are adsorbed from plasma to the red cell membranes. Their presence in plasma (and consequently on red cells) depends on the product of the *Le* gene, a fucosyltransferase that adds Fuc in \(\alpha(1\rightarrow4)\) linkage to the subterminal GlcNAc of Type 1 oligosaccharides (see Fig 12-2). In Type 2 chains, the number four carbon is not available for fucose attachment so Type 2 chains never express Lewis system activity. The oligosaccharides present on molecules intrinsic to the red cell membrane are all Type 2.

### Genes and Antigens

Antigens of the Lewis system are constructed by addition of fucose moieties to the Type 1 oligosaccharide. The product of the *Le* gene attaches fucose in \(\alpha(1\rightarrow4)\) linkage to the subterminal GlcNAc; this configuration has *Le\textsuperscript{a}* activity. The *Se* gene determines a transferase that attaches a fucose in \(\alpha(1\rightarrow2)\) linkage to the terminal Gal, but only if the adjacent GlcNAc is already fucosylated. The configuration with two fucose moieties has *Le\textsuperscript{b}* activity. Thus *Le\textsuperscript{b}* reflects the presence of both the *Le* and *Se* genes; *Le* without *Se* results in *Le\textsuperscript{a}* activity; *Se* without *Le* has no consequences in terms of Lewis system activity.

Plasma glycosphingolipids are adsorbed to the red cell membrane; if Lewis activity is present, adsorption will confer Lewis reactivity on the circulating cells. *Le\textsuperscript{b}* is adsorbed preferentially over *Le\textsuperscript{a}* so the individual possessing both *Le* and *Se* genes will have red cells that express *Le\textsuperscript{b}* but not *Le\textsuperscript{a}* Red cells from persons with *Le* but not *Se* will express *Le\textsuperscript{a}* The individual homozygous for the amorphic allele *le* will have no Lewis reactivity on red cells or on Type 1 oligosaccharides in other body sites. Table 12-4 shows phenotypes of the Lewis system and their frequencies in the population. Red cells that type as *Le(a+b+) are only rarely found when human antisera are used in blood grouping. Such red cells are seen more frequently when more potent monoclonal anti-*Le\textsuperscript{a}* and anti-*Le\textsuperscript{b}* reagents are used.

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**Table 12-4. Phenotypes and Frequencies in the Lewis System**

<table>
<thead>
<tr>
<th>Reactions with Anti-</th>
<th>Adult Phenotype</th>
<th>Frequency %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Whites</td>
</tr>
<tr>
<td><em>Le\textsuperscript{a}+</em></td>
<td><em>Le(a+b–)</em></td>
<td>22</td>
</tr>
<tr>
<td>0 +</td>
<td>*Le(a–b+)</td>
<td>72</td>
</tr>
<tr>
<td>0 0</td>
<td>*Le(a–b–)</td>
<td>6</td>
</tr>
<tr>
<td>+ +</td>
<td>*Le(a+b+)</td>
<td>rare</td>
</tr>
</tbody>
</table>

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Lewis Antibodies

Lewis antibodies occur almost exclusively in the serum of Le(a–b–) individuals, usually without known red cell stimulus. Those people whose red cell phenotype is Le(a+b+) do not make anti-Le\textsuperscript{a} because small amounts of unconverted Le\textsuperscript{a} are present in their saliva and plasma. It is unusual to find anti-Le\textsuperscript{b} in the serum of a Le(a+b–) individual, but anti-Le\textsuperscript{b} may exist along with anti-Le\textsuperscript{a} in the serum of Le(a–b–) individuals. Lewis antibodies are predominantly IgM and do not cross the placenta. Because of this, and because Lewis antigens are poorly developed at birth, the antibodies have not been implicated in HDN. Lewis antibodies may bind complement, and fresh serum that contains anti-Le\textsuperscript{a} (or infrequently anti-Le\textsuperscript{b}) may hemolyze incompatible red cells in vitro. Hemolysis is more often seen with enzyme-treated red cells than with untreated red cells.

Serologic Characteristics

Most Lewis antibodies agglutinate saline-suspended red cells of the appropriate phenotype. The resulting agglutinates are often fragile and are easily dispersed if red cell buttons are not resuspended gently after centrifugation. Agglutination sometimes is seen after incubation at 37 C, but rarely of the strength seen in tests incubated at room temperature. Some examples of anti-Le\textsuperscript{a}, and less commonly anti-Le\textsuperscript{b}, can be detected in the antiglobulin phase of testing. Sometimes this reflects complement bound by the antibody; in other cases, antiglobulin reactivity results from an IgG component of the antibody.

Sera with anti-Le\textsuperscript{b} activity can be divided into two categories. The more common type reacts best with Le(b+) red cells of group O and A\text{2}; these antibodies have been called anti-Le\textsuperscript{bH}. Antibodies that react equally well with the Le\textsuperscript{b} antigen on red cells of all ABO phenotypes are called anti-Le\textsuperscript{bl}. Anti-Le\textsuperscript{bl}, but not anti-Le\textsuperscript{bH}, can be neutralized by saliva from group O persons who are secretors of H substance, but are Le(a–b–).

Table 12-5 lists the serologic behavior of the common Lewis system antibodies.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>In-Vitro Hemolysis</th>
<th>Saline 4 C</th>
<th>Saline 22 C</th>
<th>Albumin 37 C</th>
<th>AGT</th>
<th>Enzyme 37 C</th>
<th>AGT</th>
<th>Associated With HDN</th>
<th>HTR</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-Le\textsuperscript{a}</td>
<td>some</td>
<td>most</td>
<td>most</td>
<td>some</td>
<td>many</td>
<td>most</td>
<td>most</td>
<td>no</td>
<td>few</td>
</tr>
<tr>
<td>anti-Le\textsuperscript{b}</td>
<td>rare</td>
<td>most</td>
<td>most</td>
<td>few</td>
<td>some</td>
<td>some</td>
<td>some</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>anti-P\textsubscript{1}</td>
<td>occ.</td>
<td>most</td>
<td>some</td>
<td>occ.</td>
<td>rare</td>
<td>some</td>
<td>few</td>
<td>no</td>
<td>rare</td>
</tr>
<tr>
<td>anti-P</td>
<td>some</td>
<td>most</td>
<td>some</td>
<td>some</td>
<td>some</td>
<td>some</td>
<td>some</td>
<td>no</td>
<td>?</td>
</tr>
<tr>
<td>anti-P\textsubscript{1}+P\textsubscript{1}+P\textsubscript{k}</td>
<td>some</td>
<td>most</td>
<td>some</td>
<td>some</td>
<td>some</td>
<td>some</td>
<td>some</td>
<td>rare</td>
<td>?</td>
</tr>
</tbody>
</table>

Recognition of Other Antigens

Two other antibodies (anti-Le\textsuperscript{c} and -Le\textsuperscript{d}) have been given Lewis designations but the determinants with which they react are glycosphingolipids with Type 1 oligosaccharides from Le(a–b–) persons. Anti-Le\textsuperscript{c} reacts with unsubstituted Type 1 chains, on which neither the Gal nor the GlcNAc is linked to fucose. Anti-Le\textsuperscript{d} reacts with Type 1 chains from lele per-
sons who possess Se; the configuration is H antigen expressed on Type 1 chains.

Transfusion Practice

Lewis antigens readily adsorb to and elute from red cell membranes. Transfused red cells shed their Lewis antigens and assume the Lewis phenotype of the recipient within a few days of entering the circulation. Lewis antibodies in a recipient’s serum are readily neutralized by Lewis blood group substance in donor plasma. For these reasons, it is exceedingly rare for Lewis antibodies to cause hemolysis of transfused Le(a+) or Le(b+) red cells. However, antibodies that cause hemolysis in vitro or that give strong reactions in the antiglobulin phase of the crossmatch have been associated with posttransfusion hemolysis.

It is not considered necessary to type donor blood for the presence of Lewis antigens before transfusion or when crossmatching for recipients with Lewis antibodies. Reactions seen on crossmatches with anti-IgG reagents, with or without prewarming, provide a good index of transfusion safety.

Lewis Antigens in Children

Red cells from newborn infants usually react with neither human anti-Le\(^a\) nor anti-Le\(^b\) and are considered to be Le(a−b−). Some can be shown to carry small amounts of Le\(^a\) when tested with potent monoclonal or goat anti-Le\(^a\) reagents. Reliable Lewis grouping of young children may not be possible, as test reactions may not reflect the correct phenotype until approximately 6 years of age. Among children, the incidence of Le(a+) red cells is high and that of Le(b+) red cells low. The phenotype Le(a+b+) may be transiently observed in children whose phenotypes as adults will be Le(a−b+).

Cord red cells are agglutinated by an antibody called anti-Le\(^x\), which agglutinates adult red cells of Le(a+b−) and Le(a−b+), but not Le(a−b−) phenotypes. In serologic tests, this antibody behaves as if it contains an inseparable combination of anti-Le\(^a\) and -Le\(^b\). The determinant it defines has been called Le\(^x\), which is present on the majority of cord red cells and on adult red cells that express either Le\(^a\) or Le\(^b\). Anti-Le\(^x\) is not a more potent or more avid form of anti-Le\(^a\). The Le\(^x\) determinant is the Type 2 oligosaccharide to which Fuc is attached in \(\alpha(1\rightarrow3)\) linkage to GlcNAc; it represents a class of antigens that are developmentally regulated embryonic oligosaccharides. The sialylated form of Le\(^x\) appears to be a ligand for the adhesion receptor called endothelial leukocyte adhesion molecule 1 (ELAM-1) or E-selectin, and thus may play a role in certain metastatic cancers.

The I/i Antigens and Antibodies

The unexpected antibodies most frequently encountered in serologic tests performed at room temperature are anti-H and anti-I. The antigens reside in the subterminal portions of the oligosaccharides that eventually are converted to H, A, or B antigens and are found on membrane-associated glycoproteins and glycosphingolipids. Both Type 1 and Type 2 oligosaccharide chains include multiple units of Galβ(1→4)GlcNAcβ(1→3). Two of these disaccharide units linked in a straight chain oligosaccharide constitute the i antigen. On the red cells of adults many of these linear chains are modified by the addition of branched structures consisting of Galβ(1→4)GlcNAc linked in β(1→6) to a galactose residue internal to the repeating sequence. The branching configuration confers I specificity.
Different examples of anti-I appear to recognize different portions of the branched oligosaccharide chain.

Fetal red cells carry few branched oligosaccharides and, therefore, are rich in i and poor in I. During the first 2 years of life, I antigen gradually increases at the expense of i. The red cells of most adults are strongly reactive with anti-I and react weakly or not at all with anti-i. Rare adults exist whose red cells are I–negative. Their sera usually contain anti-I, but activity may be so weak as to require enzyme techniques for detection.

**Antibodies to I/i**

Anti-I characteristically agglutinates, at room temperature, red cells from nearly all adults, but does not react with red cells from cord blood or from I-negative adults. If tests are performed at 4 C, serum from many I-positive people can be shown to have autoanti-I. Anti-I is a common autoantibody, but it usually behaves as a cold-reactive agglutinin, acting within a narrow thermal range and, even at 4 C, at a titer no higher than 64. Anti-I assumes pathologic significance in cold agglutinin disease or mixed-type autoimmune hemolytic anemia, in which it behaves as a complement-binding antibody with a high titer and wide thermal range (see Chapter 16). Autoanti-i is less often implicated in symptomatic disease than anti-I. On rare occasions, anti-i may be seen as a relatively weak cold autoagglutinin reacting only at 4-10 C. Patients with infectious mononucleosis often have transient but potent anti-i.

**Complex Reactivity**

Some antibodies appear to recognize branched oligosaccharides that have been further modified by, for example, transferases for H and P1. Anti-IH occurs quite commonly in the serum of A1 individuals; it reacts strongly with red cells that have high levels of H as well as I. Thus, it reacts very little with adult group A1 cells or cord cells of any group, and very strongly with adult group O cells. Anti-IH should be suspected when serum from a group A patient causes direct agglutination of all cells used for testing.

**Table 12-6. Serologic Behavior of the I Blood Group Antibodies with Saline Red Cell Suspensions**

<table>
<thead>
<tr>
<th></th>
<th>Anti-I</th>
<th>Anti-i</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 C</td>
<td>Iadult</td>
<td>4+</td>
</tr>
<tr>
<td></td>
<td>Icord</td>
<td>0-2+</td>
</tr>
<tr>
<td></td>
<td>Iadult</td>
<td>0-1+</td>
</tr>
<tr>
<td>22 C</td>
<td>Iadult</td>
<td>2+</td>
</tr>
<tr>
<td></td>
<td>Icord</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Iadult</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 12-6 illustrates the serologic behavior of anti-I and anti-i at 4 C and 22 C. Reaction strengths should be considered relative; clear-cut differences in reactivity between the two are seen only with weaker examples of the antibodies. Titration studies may be needed to differentiate strong examples of the antibodies.

Serum containing anti-I or anti-i is sometimes reactive at the antiglobulin phase of testing. Such reactions rarely indicate antibody activity at 37 C; rather, the reaction is between anticomplement in a polyspecific antiglobulin reagent and complement components bound when serum and cells interact at lower temperatures. The complement remains bound to cell surfaces after the antibody dissociates during 37 C incubation. The problem can usually be avoided either by warming the serum and red cells to 37 C before combining them and then keeping them strictly at 37 C for all phases of testing, including centrifugation and washing, or by omitting any potentiator (if used) and using anti-IgG instead of polyspecific antihuman globulin.
antibody detection, but is compatible with all or most group A donor blood.

The P Blood Group and Related Antigens

The first antigen of the P blood group was discovered by Landsteiner and Levine in 1927, in a series of animal experiments that led also to the discovery of M and N. Originally called P, the name of the antigen was later changed to P1. The designation P has since been reassigned to an antigen present on almost all human red cells. The International Society for Blood Transfusion has recently made an effort to classify P antigens in a logical manner (see Table 12-7). The definitive antigen in this system is P1. The P, Pk, and Luke (LKE) antigens, formerly considered part of the P system, have been assigned to the globoside collection of antigens. Another antigen, p, has not yet been assigned to a system. Red cells lacking P1, but shown to possess P, are of the P2 phenotype. P1 is present on the red cells of approximately 80% of Whites and 94% of Blacks.

Biochemistry

The antigenic determinants of the blood group P system are chemically based on

<table>
<thead>
<tr>
<th>Reactions With Anti-</th>
<th>Phenotype Frequency %</th>
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<tbody>
<tr>
<td>P1</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td></td>
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<tr>
<td>Pk</td>
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<td>PP1Pk</td>
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<td>P1</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td></td>
</tr>
<tr>
<td>Pk</td>
<td></td>
</tr>
<tr>
<td>PP1Pk</td>
<td></td>
</tr>
</tbody>
</table>

Table 12-7. Phenotypes and Frequencies in the P Blood Group

*Usually negative, occasionally weakly positive.

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addition of GalNAc in $\beta(1\rightarrow3)$ linkage. Neither $P^k$ nor $P$ serve as substrate for $P_1$. Instead, Gal is added in $\alpha(1\rightarrow4)$ linkage to a different structure called paragloboside to form $P_1$. Paragloboside is the precursor on which cellular A, B, H, I, and i antigens are constructed. The $p$ antigen results from addition of NeuNAc to the terminal galactose in $\alpha(2\rightarrow3)$. The gene specifying the responsible transferase is thought to be independent of the $P$ system. The biochemical structures of these antigens are shown in Fig. 12-5.

Rare Phenotypes
Several rare phenotypes are associated with the P blood groups and these are shown in Table 12-7. The $P^k$ phenotype occurs when the $P^k$ antigen is not converted to $P$. Individuals whose red cells have $P^k$ instead of $P$ consistently make a strong alloreactive anti-P that reacts in tests with $P_1^+P^+$ ($P_1$ phenotype) and $P_1^–P^+$ ($P_2$ phenotype) red cells. The biphasic hemolysin of paroxysmal cold hemoglobinuria (PCH) is often, but not always, of this specificity. Unlike the IgM anti-P in the serum $P^k$ persons,

Figure 12-5. Biochemical structures of P antigens. 28
the autoreactive anti-P found in people with PCH is IgG.29

Very rare people lack P1, P, and Pk. Red cells from these individuals are said to be of the p phenotype. Characteristically, a potent hemolytic IgM antibody with anti-P1+P+Pk specificity is found in their serum. The antibody, formerly called anti-Tjα, has caused hemolytic transfusion reactions and, occasionally, HDN.29

There is a curious association between anti-P1+P+Pk and abortions occurring early in pregnancy in p women.30

Anti-P1

The serum of P2 persons commonly contains anti-P1. If sufficiently sensitive techniques are applied, it is likely that anti-P1 would be detected in the serum of virtually every P2 person. The antibody reacts optimally at 4 C but may occasionally be detected at 37 C. As anti-P1 is nearly always IgM, it does not cross the placenta and has not been reported to cause HDN, but has, rarely, been reported to cause hemolysis in vivo.29,31

The strength of the P1 antigen varies among different red cell samples, and antigen strength has been reported to diminish when red cells are stored. These characteristics sometimes create difficulties in identifying antibody specificity in serum with a positive antibody screen. An antibody that reacts weakly in room temperature testing can often be shown to have anti-P1 specificity by incubation at lower temperatures or by the use of enzyme-treated red cells. Hydatid cyst fluid or P1 substance derived from pigeon eggs inhibits the activity of anti-P1. Inhibition may be a useful aid to antibody identification, especially if it is present in a serum with antibodies of other specificities.

It has recently been shown that red cells of P1k and P2k phenotypes can be agglutinated by the meningitis-causing bacterium Streptococcus suis; the P antigen has been shown to serve as a receptor for parvovirus B19, which causes erythema infectiosum.33 Indeed, persons of p phenotype who lack globose are naturally resistant to infection with this pathogen. These recent findings implicate the P blood group system in the pathogenesis of these diseases.33

References

10. Mulet C, Cartron JP, Badet J, Salmon C. Activity of α-2-L-fucosyltransferase in human sera and red cell membranes. A study of common ABH blood donors, rare “Bombay” and

Suggested Reading


