Platelets express a variety of immunogenic markers on the cell surface. Some of these antigens are shared with other cell types as in the case of HLA antigens, which are shared with virtually all nucleated cells in the body, whereas others are observed to be essentially platelet specific. This chapter reviews the antigens expressed on platelets, the various patterns of alloimmunization to these antigens, and their impact on platelet transfusion responses. Strategies to treat and prevent alloimmunization are summarized.

Antigens on the platelet surface

Antigens shared with other tissues

HLA antigens

HLA class I, but not HLA class II, molecules are expressed on platelets. Class I A, B, and C antigens are all expressed on the platelet membrane, and in particular the A and B antigens are important targets for antibodies implicated in platelet transfusion refractoriness. In fact, platelets are the major source of HLA class I antigens present in blood. Human platelets are reported to express on average about 20,000 molecules of HLA class I per platelet. However, platelet expression of HLA antigens varies amongst individuals.

Although platelets ultimately derive from nucleated precursors (the megakaryocytes) and hence might be anticipated to acquire their HLA antigens through mechanisms in common with other nucleated cells, early studies demonstrated that, in vitro, platelets absorb and express soluble HLA antigens after incubation in plasma. These studies, as well as others purporting to show that platelet class I antigens could be stripped from the platelet membrane by chloroquine treatment, suggested that HLA absorbed from plasma contributed significantly to the total HLA class I present on platelets. However, it is now recognized that low pH treatment of platelets does not "strip" the HLA class I heavy chain from the cell membrane of platelets. Rather, this treatment of platelets actually disrupts the trimolecular complex of HLA class I heavy chain, peptide, and β2-microglobulin, destroying antigenic epitopes and preventing the binding of specific HLA antibodies.

It is now clear that most HLA class I molecules on platelets are integral membrane proteins persisting from the megakaryocyte stage of development. Evidence for this includes studies of phorbol ester stimulation of platelets that resulted in phosphorylation of their HLA molecules, and mRNA from platelets that is capable of producing small amounts of HLA class I, both of which are consistent with HLA molecules being an integral part of the platelet membrane. In addition, peptides presented by HLA-A2 molecules on human platelets have been isolated, sequenced, and shown to be identical to those commonly expressed by HLA-A2 on nucleated cells. One ubiquitously expressed peptide is derived from the megakaryocyte-platelet-specific glycoprotein (GP) IX. Detectable HLA class I surface molecules that were removed by either incubation at 37°C or treatment with pH 3.0 citrate could be almost completely restored after addition of exogenous β2-microglobulin and peptide ligand. This indicates (1) that platelets themselves are not able to load HLA molecules with endogenous peptides, but that this occurs during HLA protein synthesis at the megakaryocyte stage; and (2) although B2-microglobulin and peptide become unstable and dissociate from the platelet surface either when platelets are incubated at 37°C or when treated with low pH, the class I heavy chain remains embedded in the membrane. The above evidence suggests, then, that the vast majority of HLA class I molecules on platelets are intrinsic transmembrane proteins synthesized and acquired at the megakaryocyte stage before platelets become cytoplasmic fragments.

ABH blood group antigens

Platelets express low levels of the blood group antigens I, p, and ABO(H), but with the exception of ABH, antibodies against these antigens do not appear to have clinical relevance to transfused platelet recovery or survival. The A, B, and H antigens are expressed on the carbohydrate moieties attached to virtually all platelet glycoproteins. The endothelial cell adhesion molecule PECAM-1 (CD31) accounts for the majority of these antigens per platelet while GPIa/IIa expresses the highest levels of ABH per molecule. Several studies have demonstrated that levels of A and B antigens on platelets vary between individuals, with levels of A1 antigens between individuals calculated to range from 2100 to 16,000 molecules per platelet. Variability of expression also exists within an individual’s own platelet population, although not all groups have found this to be significant. The variable expression of ABH on an individual’s platelets, to the extent it occurs, may explain why early studies showed a rapid destruction of a subset of transfused ABO-incompatible platelets followed by near-normal survival of the remaining cells. Notably, individuals of the A2 subtype express no A antigens on their platelets and therefore these platelets can be successfully substituted for group O platelets for transfusion. About 4–7% of non–group O individuals express...
substance is present on platelets and detectable in varying quantities. Platelets and monocytes (Type I de II H chains con...

Studies by several groups using monoclonal probes speci...

it is believed that as in red cell development, A- and B-UDP sugars have been debated with con...

However, more recent studies show that a great majority of blood group A, B, and H antigens on platelets are not passively absorbed from the plasma, but are expressed on many of the integral platelet membrane glycoproteins, including GPIIb, GPIIIa, GPIV, GPV, PECAM-1, GPib/IX, GPIa/IIa, and CD109. It is now believed that as in red cell development, A- and B-UDP sugars are attached in the Golgi apparatus of the megakaryocyte to the Type II H precursor chains present on these platelet glycoproteins. Studies by several groups using monoclonal probes specific for Type II H chains confirm that this membrane-intrinsic form of H substance is present on platelets and detectable in varying quantities according to the ABO group of the individual (greatest in group O and A2 platelets; less in groups B, A1, and A1B; and least in O, [Bombay]),. It is now believed that a small amount of ABH on platelets is acquired by absorption of glycolipids expressing these antigens from the surrounding plasma, the majority, as for HLA, is intrinsically derived at the megakaryocyte stage.

Other antigens on platelets
Platelet GP IV or CD36 is expressed on various human cells, including platelets, monocytes and macrophages, capillary endothelium, erythroblasts, and adipocytes. Some apparently normal individuals lack CD36 on their platelets (Type II deficiency) or platelets and monocytes (Type I deficiency). CD36 deficiency is common in Asian (3–11%) and African (3–6%) populations but is extremely rare in European populations (0.1%). It is interesting that the most common gene mutations responsible for CD36 deficiency in those of Asian and African ethnicity are different—a C→T,78 point mutation in exon 4 (Asians) and T→G,264 stop mutation in exon 10 (Africans). The higher frequency of CD36 deficiency in these groups is thought to be related to their living in regions of the world where malaria is endemic. CD36 is a known receptor for red cells infected with Plasmodium falciparum; thus, it is believed that CD36 deficiency may afford partial resistance to malaria infection. However, one report suggests CD36 deficiency may actually be a risk factor for more severe forms of malaria infection.

CD109 is a 175-kD glycoprotein found on cultured endothelial cells, activated T cells, several tumor cell lines, and platelets.. Two alloantigens, human platelet antigen-15b (HPA-15b) (Gov) and HPA-15a (Gov), have been identified on platelet CD109, and unlike most platelet-specific alloantigens, both alleles are highly expressed: 77% (HPA-15a) and 65% (HPA-15b) in those of European ethnicity (Table 18.1). CD109 is a member of the α2-macroglobulin/complement gene family, and a single nucleotide polymorphism A→C,218 resulting in a Tyr,75Ser,703 change in the protein defines the HPA-15a/b alloantigens. The number of CD109 molecules expressed on platelets is small (<2000/platelet), and the HPA-15 antigens are labile in storage, making detection of HPA-15 antibodies difficult using currently available serologic methods, suggesting that alloimmunization to these antigens may be underreported. Results from several studies show that antibodies to HPA-15a or HPA-15b are uncommonly detected in sera collected from women who have given birth to infants affected with FNAIT, but are more frequently seen in sera from patients with PTP (2%) and platelet transfusion refractoriness (3–4%). This suggests that HPA-15 may be as immunogenic as the HPA-5 antigen system, which is second only to the highly immunogenic HPA-1a (P1a) platelet antigen system.

Platelet-specific antigens
Antibodies recognizing platelet-specific antigens have been discovered in three clinical situations: mothers who give birth to infants with FNAIT, patients who develop thrombocytopenia after blood transfusion (PTP), and patients who have received multiple transfusions. To date, 33 HPAs expressed on six different platelet glycoproteins—GP IIb (CD41), GP IIIa (CD61), GP Ibα—have been described. Each has been assigned a unique nomenclature and is thought to be expressed in A antigen expression—broad histogram. Platelets from a Type I high expresser, like the normal expresser, give a broad histogram but have overall higher levels (histogram shifted to the right) of A antigen. Type II high expressers have much higher levels and show a more homogeneous (narrow histogram at top) expression of A antigens per platelet.
Table 18.1 Human Platelet Alloantigens\textsuperscript{46,47,50}

<table>
<thead>
<tr>
<th>Alloantigens</th>
<th>Other Names</th>
<th>Phenotypic Frequency (Caucasian)</th>
<th>Glycoprotein Location/ Amino Acid Substitution</th>
<th>Encoding Gene/ Nucleotide Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPA-1a</td>
<td>HA, Zw</td>
<td>72% a/b 26% a/b &lt; 1% a/c</td>
<td>GPIIIa</td>
<td>ITGB3</td>
</tr>
<tr>
<td>HPA-1b</td>
<td></td>
<td>26% a/b 14% a/b 1% b/b</td>
<td>L33P</td>
<td>ITGB3</td>
</tr>
<tr>
<td>HPA-1c</td>
<td></td>
<td>2% b/b &lt; 1% a/c</td>
<td>L33V</td>
<td>ITGB3</td>
</tr>
<tr>
<td>HPA-2a</td>
<td>Ko, Sib</td>
<td>85% a/a 14% a/b 1% b/b</td>
<td>GPIII-alpha</td>
<td>GPIIIA</td>
</tr>
<tr>
<td>HPA-2b</td>
<td></td>
<td>14% a/b 1% b/b</td>
<td>T145M</td>
<td>C482T</td>
</tr>
<tr>
<td>HPA-3a</td>
<td>Bak, Lek</td>
<td>37% a/a 48% a/b 15% b/b</td>
<td>GPIib</td>
<td>ITGA28</td>
</tr>
<tr>
<td>HPA-3b</td>
<td></td>
<td>37% a/a 48% a/b 15% b/b</td>
<td>IB435</td>
<td>T2621G</td>
</tr>
<tr>
<td>HPA-4a</td>
<td>Pen, Yuk</td>
<td>&gt;99.9% a/a &lt;0.1% a/b &lt;0.1% b/b</td>
<td>GPIIIa</td>
<td>ITGB3</td>
</tr>
<tr>
<td>HPA-4b</td>
<td></td>
<td></td>
<td>R143Q</td>
<td>G506A</td>
</tr>
<tr>
<td>HPA-5a (Brb)</td>
<td>Br, Hc</td>
<td>80% a/a 19% a/b 1% b/b</td>
<td>GPIa</td>
<td>ITGA2</td>
</tr>
<tr>
<td>HPA-5b (Bra)</td>
<td></td>
<td>80% a/a 19% a/b 1% b/b</td>
<td>E505K</td>
<td>G1600A</td>
</tr>
<tr>
<td>HPA-6bw</td>
<td>Caa, Tu</td>
<td>&lt;1% a/b</td>
<td>GPIIIa</td>
<td>ITGB3</td>
</tr>
<tr>
<td>HPA-7bw</td>
<td>Mob</td>
<td>&lt;1% a/b</td>
<td>GPIIIa</td>
<td>ITGB3</td>
</tr>
<tr>
<td>HPA-8bw</td>
<td>Sra</td>
<td>&lt;0.1% b/b</td>
<td>GPIIIa</td>
<td>ITGB3</td>
</tr>
<tr>
<td>HPA-9bw</td>
<td>Maxa</td>
<td>&lt;1% a/b</td>
<td>GPIIIa</td>
<td>ITGB3</td>
</tr>
<tr>
<td>HPA-10bw</td>
<td>Laa</td>
<td>1% a/b</td>
<td>GPIIIa</td>
<td>ITGB3</td>
</tr>
<tr>
<td>HPA-11bw</td>
<td>Groa</td>
<td>&lt;0.5% b/b</td>
<td>GPIIIa</td>
<td>ITGB3</td>
</tr>
<tr>
<td>HPA-12bw</td>
<td>Iya</td>
<td>1% a/b</td>
<td>GPIIIa</td>
<td>ITGB3</td>
</tr>
<tr>
<td>HPA-13bw</td>
<td>Sita</td>
<td>&lt;1% a/b</td>
<td>GPIIIIb</td>
<td>ITGA2</td>
</tr>
<tr>
<td>HPA-14bw</td>
<td>Oea</td>
<td>1% a/b</td>
<td>GPIIIa</td>
<td>ITGB3</td>
</tr>
<tr>
<td>HPA-15a</td>
<td>Gov</td>
<td>35% a/a 42% a/b 23% b/b</td>
<td>GPIIIa</td>
<td>ITGB3</td>
</tr>
<tr>
<td>HPA-15b</td>
<td></td>
<td>35% a/a 42% a/b 23% b/b</td>
<td>GPIIIa</td>
<td>ITGB3</td>
</tr>
<tr>
<td>HPA-16bw</td>
<td>Duva</td>
<td>&lt;1% a/b</td>
<td>GPIIIa</td>
<td>ITGB3</td>
</tr>
<tr>
<td>HPA-17bw</td>
<td>Vaa</td>
<td>&lt;1% a/b</td>
<td>GPIIIa</td>
<td>ITGB3</td>
</tr>
<tr>
<td>HPA-18bw</td>
<td></td>
<td>&lt;1% a/b</td>
<td>GPIIIa</td>
<td>ITGB3</td>
</tr>
<tr>
<td>HPA-19bw</td>
<td>GQ16H</td>
<td>&lt;1% a/b</td>
<td>GPIIIa</td>
<td>ITGA2</td>
</tr>
<tr>
<td>HPA-20bw</td>
<td>K137Q</td>
<td>&lt;1% a/b</td>
<td>GPIIIa</td>
<td>ITGA2</td>
</tr>
<tr>
<td>HPA-21bw</td>
<td>T619M</td>
<td>&lt;1% a/b</td>
<td>GPIIIIa</td>
<td>ITGA2</td>
</tr>
<tr>
<td>HPA-22bw</td>
<td></td>
<td>&lt;1% a/b</td>
<td>GPIIIIa</td>
<td>ITGA2</td>
</tr>
<tr>
<td>HPA-23bw</td>
<td></td>
<td>&lt;1% a/b</td>
<td>GPIIIa</td>
<td>ITGA2</td>
</tr>
<tr>
<td>HPA-24bw</td>
<td></td>
<td>&lt;1% a/b</td>
<td>GPIIIa</td>
<td>ITGA2</td>
</tr>
<tr>
<td>HPA-25bw</td>
<td></td>
<td>&lt;1% a/b</td>
<td>GPIIIa</td>
<td>ITGA2</td>
</tr>
<tr>
<td>HPA-26bw</td>
<td></td>
<td>&lt;1% a/b</td>
<td>GPIIIa</td>
<td>ITGA2</td>
</tr>
<tr>
<td>HPA-27bw</td>
<td></td>
<td>&lt;1% a/b</td>
<td>GPIIIa</td>
<td>ITGA2</td>
</tr>
<tr>
<td>HPA-28bw</td>
<td></td>
<td>&lt;1% a/b</td>
<td>GPIIIa</td>
<td>ITGA2</td>
</tr>
</tbody>
</table>

* Phenotypic frequencies for the antigens shown are for the white population only. Significant differences in gene frequencies may be found in African and Asian populations.

(CD42b, GP1b(β) (CD42c), GPIa (CD49b), and CD109—have been described, including localization to platelet surface GPs, quantification of their density on the platelet surface, and determination of DNA polymorphisms in genes encoding for them (see Table 18.1)).\textsuperscript{46,47} For a current list, see http://www.ebi.ac.uk/ipd/hpa/table1.html and http://www.ebi.ac.uk/ipd/hpa/table2.html.\textsuperscript{46} Thirteen antigens are clustered into one triallelic\textsuperscript{48} (HPA-1) and five biallelic groups (HPA-2, HPA-3, HPA-4, HPA-5, and HPA-15). HPA for which antibodies against only one of the alleles have been detected are labeled with a “w” for workshop (e.g., HPA-8bw). To date, 20 such low-frequency single-allele HPAs have been discovered, essentially all involved in FNAIT cases.\textsuperscript{49}

Although the frequencies of HPA have been most extensively studied in Caucasian populations, it should be noted that they have been determined for other racial and ethnic groups as well and in some cases vary significantly from Caucasian frequencies. For example, HPA-1b is expressed on the platelets of approximately 15% of persons of European ancestry but of less than 1% of persons of Asian ancestry. (For more information regarding HPA frequencies in different populations, readers are directed to: http://www.ebi.ac.uk/ipd/hpa/freqs_1.html.)\textsuperscript{46}

Alloimmunization to platelet antigens

Immunization to HLA antigens

Pregnancy and transfusion account for the development of HLA alloimmunization, with exposure to leukocyte-containing blood components resulting in high rates of antibody formation in patients receiving multiple transfusions over time. Antibodies to class I and not class II HLA antigens can significantly affect the recovery and survival of transfused platelets because the former and not the latter are expressed on the platelet surface. Among the class I antigens, the HLA-A and -B markers are most important.\textsuperscript{1} HLA-C antigens are also present and reported to be expressed at approximately the same density as A antigens,\textsuperscript{51} but with rare exceptions,\textsuperscript{52} antibodies to HLA-C antigens do not appear to significantly affect transfused platelets.

The natural history of HLA sensitization in patients receiving platelet transfusions for hematologic diseases was studied in 1978,\textsuperscript{53} before the advent of widespread use of leukocyte-reduced blood components. By actuarial analysis, 60% of patients who were not positive for lymphocytotoxic antibodies at the beginning of the study were predicted to develop them as early as 10 days after primary exposure or four days after secondary exposure if they had been transfused or pregnant in the past. The number of transfusions was not related to the likelihood of immunization, an observation that was confirmed in a later study.\textsuperscript{54} Patients who were alloimmunized at the beginning of the study had the poorest responses to transfused platelets, those who did not develop HLA antibodies had the best responses, and those whose antibodies developed during the period of observation had intermediate responses.\textsuperscript{53} A later study of platelet preparation methods to reduce alloimmunization in newly diagnosed acute myelogenous leukemia (AML) patients found a similarly high HLA sensitization rate of 45% in the control group (131 patients) who received unmodified, pooled, whole blood–derived platelet transfusions over an eight-week period.\textsuperscript{55} Rates of alloimmunization in other studies involving hematology–oncology patients receiving non-leukocyte-reduced blood components range to up to 70% (Table 18.2).
The risk of HLA alloimmunization is influenced by several patient and blood component factors. Transfused patients who were exposed previously to allogeneic HLA via transfusion or pregnancy developed HLA antibodies sooner—and, in many studies, more often—than patients who were not exposed previously.55–58 In the Trial to Reduce Alloimmunization to Platelets (TRAP) study,55 62% of previously pregnant women with AML receiving untreated blood components (control product) developed lymphocytotoxic antibodies compared with 33% of those who had not been pregnant or transfused previously. There is general agreement that primary alloimmunization to HLA antigens is unlikely to occur before 3–4 weeks after the first transfusion in patients receiving multiple transfusions, and that HLA antibodies detected sooner than this most likely represent secondary immune responses in patients with remote histories of transfusion or pregnancy.

The underlying disease for which patients require platelet transfusion also influences the rate of HLA alloimmunization. In one study, patients undergoing induction chemotherapy for AML were more likely to become alloimmunized than were patients being treated for acute lymphoblastic leukemia (ALL).59 Although both groups of patients received similarly intensive chemotherapy and required roughly the same level of transfusion support, HLA antibodies developed in 44% of the AML group, compared with 18% of the patients with ALL (p = 0.00002). The authors postulated that the difference may be attributable to either an additional immunosuppressive effect of the high-dose corticosteroids given in ALL or to a decreased immune responsiveness in patients with ALL caused by their underlying disease. Others have corroborated this finding and have noted, moreover, that alloimmunization seems to occur sooner in patients with AML than in those with ALL.56,60

Except for prospective solid organ transplant recipients, there have been limited studies of HLA alloimmunization in patients without malignant hematologic-oncologic disorders who received transfusions of red blood cells (RBCs) or whole blood units, but interest in sensitization rates in such patients has increased, particularly with the advent of stem cell transplantation for hemoglobinopathies such as sickle cell disease. Sensitization to HLA can inhibit platelet transfusion responses (discussed further in this chapter), increasing the risk of thrombocytopenic hemorrhage in patients undergoing stem cell transplantation who require multiple platelet transfusions. One study of chronically transfused sickle cell disease patients determined that 85% of those with at least 50 past red cell transfusions had evidence of platelet-reactive antibodies, predominately anti-HLA, whereas 48% of those with fewer than 50 past exposures were sensitized. HLA antibodies were not detected in those patients who had no past exposure to transfusion.60 A second study of 60 thalassemia patients examined the development of HLA antibodies and found that 32 (53%) were positive for HLA antibodies at baseline and seven more became sensitized in the follow-up period of 1 year, for a total HLA sensitization rate of 65%.67 The type of blood components used for these two groups of patients may have contributed to these significant rates of alloimmunization.

Details about the type of RBC transfusions given in the first study were not provided in the report, but there did not appear to be any systematic attempts to give leukocyte-reduced units. Washed RBCs were routinely used in the second study, and these were estimated to have residual leukocyte content of >5 x 10⁶ per unit. Therefore, neither study used RBC units that were leukocyte reduced to the extent that HLA sensitization would be lessened (also discussed further in this chapter). A more recent report of β thalassemia patients from Middle Eastern countries coming to stem cell transplant also noted a high rate of HLA alloimmunization, presumably due to their being heavily transfused in the past.68

Still other investigators have sought to document the rates of HLA alloimmunization in nonhematologic conditions requiring shorter periods of transfusion support. In a study of 117 cardiac surgery patients who were exposed to a single episode of RBC transfusion, 18% became sensitized.69 In contrast, in a second study of 40 more seriously ill patients requiring placement of left ventricular assist devices as a bridge to cardiac transplant, 45% formed Class I HLA antibodies after a course of support that included a number of platelet as well as RBC transfusions. By actuarial analysis, 63% of those who received more than six transfusions of pooled whole blood–derived platelets were projected to become HLA immunized.70 This rate was strikingly similar to that of the patients with hematologic malignancies followed in the earlier study noted above who received non-leukoreduced blood.53

These studies documenting HLA alloimmunization in patients with non-hematologic-oncologic conditions suggest that, apart from patients with ALL, most patients who require repeated red cell and/or platelet transfusions for treatment of their hematologic-oncologic condition are at risk of becoming sensitized to HLA at a rate similar to that of patients who would be assumed to have normally functioning immune systems.

Both the number and type of platelet products also affect the sensitization rate. There is some disagreement in the literature regarding the importance of a dose–response relationship between platelet transfusions from different donors and the risk of alloimmunization. One study failed to note such a relationship in a group of patients with AML receiving induction therapy. However, most of the patients in this study were exposed to more than 20

### Table 18.2 Platelet Alloimmunization in Multitransfused Patients Receiving Non-Leukocyte-Reduced Blood Components

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of Patients</th>
<th>Anti-HLA</th>
<th>Anti-PSA</th>
<th>Loss/Decrease in Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seftel et al. (2004)</td>
<td>315</td>
<td>61/315 (19%)</td>
<td>11/311 (8%)</td>
<td>43/95 (38%)</td>
</tr>
<tr>
<td>TRAP (1997)</td>
<td>131</td>
<td>59/131 (45%)</td>
<td>11/131 (8%)</td>
<td>29/45 (64%)</td>
</tr>
<tr>
<td>Alias et al. (1993)</td>
<td>134</td>
<td>95/134 (71%)</td>
<td>45/130 (42%)</td>
<td>12/20 (59%)</td>
</tr>
<tr>
<td>Meenaghan et al.</td>
<td>106</td>
<td>37/106 (35%)</td>
<td>45/106 (42%)</td>
<td>30/55 (54%)</td>
</tr>
<tr>
<td>Godau et al. (1992)</td>
<td>50</td>
<td>13/50 (26%)</td>
<td>4/50 (8%)</td>
<td>29/45 (64%)</td>
</tr>
<tr>
<td>Pamphilon et al.</td>
<td>49</td>
<td>20/49 (41%)</td>
<td>11/49 (22%)</td>
<td>12/20 (59%)</td>
</tr>
<tr>
<td>Murphy et al. (1987)</td>
<td>154</td>
<td>55/154 (36%)</td>
<td>5/154 (3%)</td>
<td>30/55 (54%)</td>
</tr>
</tbody>
</table>

* Frequency of HLA antibody and platelet-specific antibody formation in studies of patients with hematologic and oncologic diagnoses requiring repeated platelet transfusion. The final column contains frequencies of antibody loss or decline in these studies.

1 HLA antibodies determined by lymphocytotoxicity testing.

2 Platelet-specific antibodies determined by a variety of methods.
different platelet donors. Indeed, animal data\textsuperscript{71} and human transfusion trials\textsuperscript{72} suggest that, with fewer donor exposures (i.e., less than 20), there is a dose–response relationship between the number of exposures and the rate of alloimmunization. Fewer donor exposures can be achieved using apheresis platelets, which provide adequate doses of platelets from a single donor rather than pooled platelet concentrates. Alloimmunization can be delayed and perhaps reduced using this type of platelet product.\textsuperscript{72}

Despite the established correlation between HLA antibodies and refractoriness to platelet transfusions (discussed in this chapter),\textsuperscript{73,74} HLA antibodies are frequently a transient complication in patients with hematologic-oncologic diagnoses requiring repeated platelet transfusions. Studies document that 17\% to 67\% of patients demonstrating these antibodies eventually lose them (Table 18.2).\textsuperscript{56,59,61,62,65,75,76} The loss of these antibodies may be related to discontinuance of the antigenic exposure after marrow recovery or to switching of platelet transfusion support to HLA-matched platelets; however, it can occur despite continued exposure to whole blood–derived platelet transfusions.\textsuperscript{59,65,75,77} One interesting report\textsuperscript{62} found that two-thirds of patients with decreasing anti-HLA reactivity despite continued exposure had developed anti-idiotypic antibodies that reacted with the V region of anti-HLA IgG. In 36\% of these patients, the serum actually inhibited binding of the patient’s own prior anti-HLA to appropriate lymphocyte targets. A history of pregnancy did not affect the ability to produce these anti-idiotypic antibodies. In contrast, those patients with persistently detectable anti-HLA did not develop anti-idiotypic reactivity. HLA antibodies that are detected before the onset of transfusion therapy (i.e., because of remote transfusion or pregnancy) tend to persist, whereas those antibodies that develop de novo during a transfusion support episode are more likely to be transient and to decrease in strength or disappear altogether despite continued exposure to allogeneic blood and platelets.\textsuperscript{65}

The major route of primary HLA immunization to transfused platelets involves the donor leukocytes, and not the platelets, per se. In vitro experiments showed that highly purified HLA-A2+ platelets could not induce allo-cytotoxicity in HLA-A2-negative peripheral blood mononuclear cells (PBMCs)—not even in the presence of helper HLA-A2-negative PBMCs.\textsuperscript{8} Studies in humans show that when leukocyte reduced platelets are transfused, primary immunization to HLA is very much delayed or does not occur at all,\textsuperscript{78–81} whereas unmodified platelet concentrates are associated with a rate of HLA immunization ranging from 19\% to 71\% (Table 18.2). These observations implicate the leukocytes in both platelet and red cell transfusions as the source of primary immunization.

Early animal studies suggested that the production of major histocompatibility complex (MHC) antibodies requires recognition of class II MHC molecules on donor antigen-presenting cells (APCs) by recipient T cells.\textsuperscript{82} Later work found that although the majority of MHC (or, in humans, HLA) sensitization in a platelet transfusion model was due to this direct pathway, by significant prestorage leukocyte reduction of platelets (i.e., to <10\(^3\) WBC/\(\mu\)L) the majority of such antibody production could be inhibited; but exhaustively leukoreduced SCID mouse platelets (<0.05 WBC/\(\mu\)L) with very low levels of MHC class II–bearing WBCs were even more immunogenic than platelets that had had class II MHC WBCs added back to them. The results suggested that donor MHC class II–positive APCs must have a dual function in modulating their sensitization to MHC class I–bearing platelets. At normal concentrations, they are the dominant pathway through which antigen is presented to the recipient’s immune system, whereas at levels lower than that required for this direct pathway, they actually inhibit the indirect pathway of recipient sensitization that requires recipient APCs presenting antigens to recipient T cells. Finally, removal of almost all donor APCs results in an uninhibited indirect pathway of alloimmunization and restored MHC antibody production.\textsuperscript{1}

**Immunization to platelet-specific antigens**

The importance of HPAs in the clinical syndromes FNAIT and PTP is undisputed; however, their relevance to platelet transfusion practice is controversial.

One kind of evidence implicating HPA or platelet-specific antibodies in the destruction of transfused platelets is the failure of some transfusions of HLA-matched platelet concentrates given to patients who are refractory to random whole blood–derived platelets.\textsuperscript{79} The poor responses to these platelet transfusions, despite HLA matching, indicate that other antigens, perhaps platelet-specific markers, are involved. The other kind of evidence involves the demonstration of platelet-reactive antibodies in the absence of anti-HLA activity. The conclusion drawn is that antibodies directed at platelet-specific antigens cause these reactions.\textsuperscript{77,79} In contrast to antibodies to HLA antigens, the majority of platelet-specific antibodies identified using these strategies do not seem to be associated with poor transfused platelet recovery or survival, however.

With the advent of Phase III platelet antibody assays that detect platelet glycoprotein (GP)-specific reactions, it is now possible to assay directly the sera from multitransfused patients for platelet-specific antibodies. Using one such method, the monoclonal antibody-specific immobilization of platelet antigens (MAIPA) assay, one group documented that among 252 patients with hematologic-oncologic diagnoses receiving platelet transfusions, 20 (8\%) developed platelet-specific antibodies with clear-cut specificities. The most common specificity in these patients was for HPA-5b in 10 of the 20; followed by HPA-1b in four; HPA-5a in two; and one each with HPA-2b, HPA-1a, HPA-1b plus HPA-5b, and HPA-1b plus HPA-2b.\textsuperscript{83} This study confirmed earlier findings in the TRAP study where 8\% of the 530 patients in the trial formed platelet-specific antibodies, the most common being anti-HPA-1b.\textsuperscript{55}

These two studies confirm the finding that many of the best-documented platelet-specific antibodies detected in patients who receive transfusions are directed against platelet antigens, the phenotypic frequencies of which are less than 30\% in the blood donor population.\textsuperscript{55,69,83} Therefore, it is difficult to attribute refractoriness to whole blood–derived and/or HLA-matched platelet transfusions to these antibodies alone. Indeed, the majority of refractory patients with platelet-specific antibodies also have HLA antibodies.\textsuperscript{84} Alloimmunization to high-frequency platelet-specific antigens would be expected to present a major challenge in finding compatible platelets to support a patient requiring multiple platelet transfusions. Fortunately, these cases are extremely rare.\textsuperscript{69,85,86}

Although platelet-specific antibodies directed at defined platelet alloantigens (HPAs) can result in transfusion failures,\textsuperscript{87–89} most platelet glycoprotein reactivity lacking definite specificity does not seem to influence transfusion responses.\textsuperscript{55,64,90}

There have been several reports of platelet transfusion refractoriness caused by antibodies to GPIV (CD36) in patients who are GPIV deficient.\textsuperscript{29,31} These patients are difficult to support because virtually all platelet products available for transfusion would be incompatible (GPIV positive). In some cases, GPIV-negative platelets were obtained by large-scale screening of donor
populations with a higher frequency of GPIV deficiency (African), and transfusion of those platelets resulted in good platelet increments for the patients.91

**Immunization to blood group antigens**

Several studies have documented the effect of ABO incompatibility on platelet transfusion therapy. In an early report of 91 alloimmunized thrombocytopenic patients receiving 389 transfusions, 24-hour transfused platelet recovery was reduced by 23% in ABO-incompatible donor–recipient pairs (donor-group A, B, or AB; recipient-group O, B, or A).92 In the aggregate, patients receiving multiple platelet transfusions have been thought to demonstrate a statistically significant but clinically unimportant decrease in platelet recovery after transfusion of ABO-incompatible platelets (especially group A). In a subset, however, as many as 20% of group O patients could develop severe refractoriness to group A platelets.14,20,93 Failure to respond to HLA-matched platelet transfusions in the absence of nonimmune clinical factors should prompt the clinician to examine the ABO group of the recipient and of the donors to determine whether ABO incompatibility might be responsible for the unexpected poor responses.

One report from Japan described a group O patient who failed to respond to two of 12 ABO-incompatible HLA-matched platelet transfusions.14 Further evaluation determined that the platelets in the two unsuccessful transfusions were from donors who expressed unusually high amounts of group B substance on their platelets (high expressers), up to 20 times that found on group B, HLA-matched platelets that were successfully transfused in this patient.

Heal et al.93 have examined the importance of ABO blood groups in platelet transfusion therapy. Forty patients with hematologic diseases receiving platelet transfusions were randomly assigned to receive either ABO-identical or ABO-unmatched platelets. The responses in the group of patients receiving ABO-unmatched transfusions (i.e., when either the recipient would be expected to have isoagglutinins to the ABH antigens on the transfused platelets, or the donor had such antibodies directed at the recipient’s blood type) were significantly worse than those observed in patients receiving ABO-identical platelet transfusions. Analysis of the first 25 transfusions in each group showed a significantly better response in the ABO-identical arm (mean corrected count increment [CCI], 6600 vs. 5200; p < 0.01). This effect was most important in the first 10 transfusion episodes and tended to predict subsequent alloimmunization and refractoriness to platelet transfusions.94 This finding seemed to be in conflict with earlier reports in which a minor, clinically insignificant impact of ABO mismatching was observed. The newer data were then reanalyzed using the earlier definitions of ABO compatibility (the patient lacks isoagglutinins to recipient ABO antigens) and incompatibility (the patient has isoagglutinins that are reactive with donor ABO antigens). In the reanalysis, no benefits of ABO compatibility were detected,95 suggesting that there is a significant negative impact of both major and minor ABO incompatibility in platelet transfusions. An increased frequency of refractoriness in patients receiving ABO-unmatched platelet transfusions was also observed in a study of 26 patients (69% vs. 58%; p = 0.001).96

The mechanism for platelet destruction in platelet ABO-incompatible transfusions (the recipient has isoagglutinins against donor ABH) is not difficult to ascertain. Presumably, IgM and IgG anti-A or anti-B in the recipient interact with A and B substances on the transfused platelets, resulting in their destruction. Explanations offered for the biphasic survival curves of ABO-incompatible platelets include (1) the elution of a portion of group A substance from the platelet surface; (2) the nonhomogeneous distribution of group A substance on donor platelets, with resultant rapid destruction of the subpopulation with highest expression; and (3) secondary injury to a subset of transfused platelets caused by the reaction between anti-A isoagglutinins and A red cells in the platelet concentrate.13,18

The suboptimal response of the plasma-incompatible transfusions (the donor has isoagglutinins against recipient ABH) is more difficult to explain. One report postulates that immune complexes involving soluble recipient ABH substance and donor A or B antibodies form. These immune complexes secondarily interact with the transfused platelets via the FcγRIIα receptor, or the complement receptors C1q-R and C1q-R, and mediate their destruction.97 Some experimental evidence supports this theory, in that anti-A has been detected in an immune complex fraction of group A recipient plasma after transfusion with group O platelets, and these immune complexes bind to IgG FcγRIIα and the cC1q-R and gC1q-R receptors on group O platelets.95 Indeed, in at least one study, plasma-incompatible platelet transfusions were even less effective than platelet-incompatible transfusions.94 Immune complexes involving other plasma proteins such as C2, C4, albumin, and fibrinogen likewise have been implicated in refractory responses to platelet transfusion.97,98

Adverse effects of ABO-incompatible platelet transfusions may extend beyond reducing platelet transfusion increments and stimulating alloimmunization. In one retrospective cohort study of cardiac surgery patients, those patients who received mismatched ABO platelets were compared with those who received ABO-identical platelets. The mismatched group experienced significantly longer hospital stays, more days of fever, increased healthcare costs, and more red cell transfusions. Other negative outcomes including mortality and time in the intensive care unit were also increased in the mismatched group, but these differences failed to reach statistical significance. The authors postulated that the negative effects of ABO-mismatched platelet transfusions were again related to immune complexes that stimulated inflammatory pathways, leading to increased postoperative morbidity in these patients.99 Although intriguing, these findings have yet to be confirmed by other groups. In fact, a later retrospective study involving more cardiac surgery patients failed to identify negative effects of ABO-mismatched platelet transfusions.100

An indisputable risk of ABO plasma-incompatible transfusions, particularly those involving group O donors and non-group O recipients, is acute hemolytic transfusion reaction caused by high-titer isoagglutinins in the donor plasma. With the marked shift from pooled whole blood–derived platelet concentrates to apheresis platelets, the risk of transfusing large volumes of ABO-incompatible plasma with potent ABO isoagglutinins is increased. Many blood providers have taken steps to alleviate this risk by reducing the volume of incompatible plasma or screening group O donors for high-titer isoagglutinins.101

**Transfusion refractoriness**

*Alloimmunization* is an immune response in a recipient stimulated by foreign donor antigens. In the platelet transfusion setting, these responses involve the production of antibodies directed at donor platelets. *Platelet refractoriness* describes a clinical condition in which patients do not achieve the anticipated platelet count increment from a platelet transfusion. It is possible to be alloimmunized...
to platelet antigens without being refractory to platelet transfusions, and also to be refractory to platelet transfusions without being alloimmunized. *Alloimmune refractoriness* occurs when the level of alloimmunization, as measured by the breadth of antibody response to platelet antigens, is sufficient to affect the majority of randomly selected platelet products.

The detection of alloimmunization is straightforward using standard laboratory techniques to detect antibodies in the patient’s serum that are reactive with HLA or platelet-specific antigens. In contrast, the definition of the refractory state is less precise. A standard dose of platelets (six units of pooled whole blood–derived platelet concentrates or one apheresis platelet unit) generally increases the platelet count by about 5000–7000 platelets/μL/unit in a 70-kg adult. This would result in a post-transfusion increment of about 30,000–40,000 platelets/μL 1 hour after platelet transfusion. The TRAP study defined the refractory state as a corrected count increment (CCI) which normalizes transfusion responses for patient blood volume estimated using body surface area and platelet dose, of <5000 after two sequential ABO-compatible platelet transfusions. Another measure of platelet transfusion response is the percent platelet recovery (PPR). Similar to the CCI, the PPR uses platelet dose and patient blood volume; the latter is estimated using the patient’s body weight in kilograms rather than body surface area. Studies in normal autologous platelet donors show an average 1 hour PPR of approximately 66%. Recovery less than 20–30% at 1 hour after the transfusion indicates a refractory response.

The PPR and CCI have been criticized as measures of post-transfusion platelet response because both calculations, which correct for patient size and dose of platelets, fail to provide information about the impact of these two variables on the post-transfusion platelet count increment. For example, a small dose of platelets given to a large patient might result in an acceptable PPR or CCI but a poor absolute platelet count increment. In order to better examine the impact of patient size and platelet dose, as well as other factors that affect the quality of the platelets (storage time, postcollection manipulations, etc.), regression analysis of post-transfusion platelet increments is suggested. It is also recommended by the same authors that because both the CCI and PPR calculations are biased in favor of platelet preparation techniques that provide fewer platelets, neither should be used to define platelet refractoriness. Regardless of which method is used, the actual platelet increment in patients who are highly refractory because of alloimmunization is extremely small to negligible.

It is important to recognize that refractoriness does not necessarily imply alloimmunization. Indeed, only about 30% of refractory responses to platelet transfusion is attributable to alloimmunization, the remainder being due to nonimmune factors that result in shortened platelet survival and/or markedly decreased platelet recoveries in patients who receive transfusions. Multiple linear regression analysis has been used to demonstrate a number of factors related to clinical refractoriness, including HLA alloimmunization as well as splenomegaly, amphotericin therapy, disseminated intravascular coagulation, or recent allogeneic marrow transplantation. Other studies have also demonstrated factors with negative effects on transfused platelets such as sepsis, fever, and drugs. Individual patients may have significantly different responses to the same nonimmune causes of refractoriness, with some patients experiencing minimal impact and others having markedly impaired response to platelet transfusions. Some patients, particularly those with multiple clinical complications (e.g., sepsis and fever), appear to respond poorly to platelets that are approaching the end of the recommended storage interval (five days). Such patients may experience an improvement in their platelet response after receiving “fresh platelets”—platelets that were collected less than 48 hours earlier.

A cause of non-alloimmune refractoriness that can be overlooked in the infected, neutropenic, hematopoietic–oncology patient is development of drug-dependent platelet-reactive antibodies. Vancomycin, a drug often used to treat serious Gram-positive bacterial infections in such patients, has been implicated. Drug-dependent platelet-reactive antibodies should be suspected in refractory patients when there is no evidence of alloimmunization or they fail to respond to HLA-selected platelets, and the refractory responses are temporally related to therapy with a drug.

**Treatment of the refractory alloimmunized patient**

In the modern era wherein universal leukocyte reduction of blood products for patients receiving multiple platelet transfusions has become standard practice, the rate of alloimmunization (primarily to HLA class I antigens) remains about 20%, and the rate of refractoriness due to alloimmunity is about half that rate. The refractoriness, if related to HLA immunization, can be transient or persistent. Several approaches can be considered to provide such patients with adequate platelet support—including provision of HLA-matched platelets or platelets selected by crossmatch tests.

**Platelet selection**

**HLA-selected platelet transfusions**

A standard approach to supporting a patient who is refractory to whole blood–derived platelet transfusions is to supply HLA-matched apheresis platelet concentrates. Because the primary cause of immune refractoriness to platelet transfusion is alloimmunization to class I HLA antigens, it follows that avoidance of incompatible HLA specifics should result in a more successful platelet transfusion response. In practice, up to 90% of alloimmune refractory patients benefit from an HLA-matched product. This was first demonstrated in a study that showed that patients who were refractory to platelets from unselected donors could be successfully supported by HLA-matched family member platelet transfusions.

Certain “private” HLA antigens can be segregated into so-called cross-reactive groups (CREGs), defined by antibodies directed against shared “public” determinants. Indeed, these shared determinants are the basis for the cross-reactivity and are different from the private determinants, which account for the highly polymorphic HLA system. Selection of platelet donors with antigens in the same CREGs as the antigens in the patient, so-called cross-reactive antigens, was demonstrated to be nearly as successful as supporting alloimmune platelet refractory patients as HLA-identical transfusions. This appeared to be due to the relative inability of the patient’s immune system to recognize these cross-reactive antigens as different, thereby greatly increasing the number of potentially successful platelet donors in a given pool.

A disadvantage of relying on HLA-matched platelets, even when using selective mismatching with CREG associations, is that a pool of 1000–3000 or more HLA-typed potential apheresis donors is generally necessary to find sufficient HLA-compatible matches to support a typical patient. Moreover, donor selection on the basis
of HLA type can lead to the exclusion of donors whose HLA types, although different from that of the recipient, may still be effective.

In alloimmune refractory patients, the best increases in CCI occur with the subset of grade A and B1U or B2U HLA-matched platelets, but platelets mismatched for some antigens (e.g., B44 and B45) that are poorly expressed on platelets can also be successful.\(^\text{117}\) Although alloimmunized patients with high panel-reactive (HLA) antibody (PRA) values benefit only from platelet products that lack any incompatible class I antigens (match grades A, B1U, B1X, B2U, and B2UX; see Table 18.3), patients with lesser degrees of sensitization can sometimes benefit from less well-matched platelets. In one study, 73% of HLA single-antigen mismatched platelet transfusions (grade C match) were successful when provided to patients with PRA values less than 60%.\(^\text{118}\) On the basis of these data, some experts suggest extending donor searches for alloimmunized patients to include single-antigen mismatches (grade C matches), particularly if the PRA is less than 60%.

Even with the additional donors that the above-cited studies may make available, HLA-matched platelets are frequently unavailable for many refractory patients. Use of the patient’s HLA antibody specificity as an additional basis for selection of platelet products has been demonstrated to increase the numbers of potentially compatible donors. Antibody specificity prediction (ASP) allows procurement of platelet products from donors who lack HLA antigens to which the patient has raised an antibody. Such platelet donors often have frank mismatches for some or all of the class I antigens in the refractory patient. One study compared ASP platelets to those selected by standard HLA matching criteria and by platelet crossmatching. HLA-matched, crossmatched, and ASP-selected platelet transfusions were found to have similar platelet recoveries, whereas randomly selected control platelets had significantly lower PPR. For 29 alloimmunized patients, the mean number of potential donors found in a file of 7247 HLA-typed donors was only six when grade A HLA matches were required and 39 when BU matches were added. However, 1426 potential donors (20% of total) were identified by the ASP method. The authors suggest that careful HLA antibody specificity identification could greatly enhance the number of potential donors by identifying nonmatched products that lack these HLA antigens.\(^\text{119}\) Other investigators using a computerized analysis of the lymphocytotoxicity (LCT) assay for private and public HLA class I epitopes in platelet recipients confirmed that there is value in carefully identifying HLA antibody specificities, allowing selection of many more donors by simply avoiding the HLA antigens against which the antibodies are directed.\(^\text{120}\) Current sensitive techniques for detecting and identifying class I HLA antibodies using flow cytometry or Luminex provide a precise way of determining the relative strength of multiple HLA antibodies that may be present in patient sera.\(^\text{121-123}\)

Regardless of the method planned to select HLA-compatible platelets for alloimmune refractory patients, the HLA type of the patient should be determined before myeloablative therapy, and HLA antibody screening should be obtained periodically (preferably weekly) so that HLA-selected platelets can be provided when and if alloimmune refractoriness is diagnosed.

In a small number of patients who fail to respond to HLA-matched platelets and for whom no other nonimmune explanation can be found for refractoriness, platelet-specific alloantibodies may be the cause of the poor platelet increments. Approximately 8% of platelet multitransfused patients develop platelet-specific antibodies.\(^\text{55}\) Many of these patients are also alloimmunized against HLA. One report described six patients who were highly alloimmunized to HLA but also had human platelet alloantibodies to HPA-1b or HPA-5b.\(^\text{124}\) These patients were successfully supported with a pool of HLA-matched platelets that were typed for the HPA antigens.

### Platelet crossmatching

Although the use of HLA-matched, selectively HLA-mismatched, and ASP-selected donors provides support for the majority of patients who are alloimmune refractory, there are limitations to these strategies. As many as 20–25% of refractory patients fail to respond adequately to HLA-matched platelets. In the absence of nonimmunologic clinical factors that can decrease platelet transfusion recovery and survival, these failures might be explained by ABO incompatibility, platelet-specific antibodies, and undetected HLA incompatibility.\(^\text{125}\) These immune causes of transfusion failure cannot be addressed with HLA matching alone. In addition, many facilities do not have access to adequately sized HLA-typed donor files, so alternative methods of selecting compatible platelets for alloimmune refractory patients must be considered.

Many platelet and leukocyte antibody detection methods have been assessed as platelet compatibility tests. One method that has gained wide acceptance is the commercially available solid-phase red cell adherence (SPRCA) assay. The test is rapid and sensitive, particularly for the detection of HLA antibodies, and is therefore suitable for routine platelet crossmatching.\(^\text{126,127}\) Good correlation between test results and posttransfusion platelet counts has been achieved with the SPRCA assay.\(^\text{127,128}\)

To date, few studies have compared the efficacy of HLA matching to that of platelet crossmatching for supporting alloimmune refractory patients. One multicenter study\(^\text{129}\) compared HLA selection and platelet crossmatching using three different techniques—the indirect immunofluorescence test for platelets, an enzyme-linked immunosorbent assay (ELISA), and a radioimmunoassay were used to test apheresis platelet concentrates. At least one pair of apheresis platelet components was transfused to each patient. One unit was selected by HLA matching, and the other by crossmatching. The results of the two donor selection methods were compared. Although the difference between 1-hour posttransfusion recoveries was not statistically significant, after 24 hours the HLA-selected transfusions were significantly more successful. This was even more apparent when only A and BU matches were considered. The authors concluded that when HLA-typed donors are available, A or BU matches were preferable to randomly chosen products selected by any of the crossmatch methods used in their study.

### Table 18.3 Classification of Donor/Recipient Pairs on the Basis of HLA Match

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>All four antigens in donor are identical to those in recipient.</td>
</tr>
<tr>
<td>B1U</td>
<td>Only three antigens are detected in donor; all are present and identical in recipient.</td>
</tr>
<tr>
<td>B1X</td>
<td>Three donor antigens are identical to recipient; the fourth antigen is cross-reactive with recipient.</td>
</tr>
<tr>
<td>B2U</td>
<td>Only two antigens are detected in donor; both are present and identical in recipient.</td>
</tr>
<tr>
<td>B2UX</td>
<td>Only three antigens are detected in donor; two are identical with recipient, and the third is cross-reactive.</td>
</tr>
<tr>
<td>B2X</td>
<td>Two donor antigens are identical to recipient; the third and fourth antigens are cross-reactive with recipient.</td>
</tr>
<tr>
<td>C</td>
<td>One antigen of donor is not present in recipient and non-cross-reactive with recipient.</td>
</tr>
<tr>
<td>D</td>
<td>Two antigens of donor are not present in recipient and non-cross-reactive with recipient.</td>
</tr>
</tbody>
</table>
The quality of the HLA match is paramount to the transfusion success rate as shown in a study in which HLA matching apparently did not improve on the success rate of crossmatch-selected platelets.\textsuperscript{128} Here, however, the HLA-selected platelets were seldom of A or BU match grade with the recipients.

The degree to which a patient is alloimmunized has an impact on the success of platelet transfusions selected by crossmatching assays. Using platelet concentrates from whole blood units and a radio-labeled antiglobulin technique, one study found that 70% of transfusions selected by the test were successful in supporting moderately alloimmunized patients.\textsuperscript{130} In contrast, others found that only 41% of transfusions selected by either an ELISA or the SPRCA test were successful in a highly alloimmunized group of leukemia patients.\textsuperscript{131} Moreover, only 15–24% of platelet concentrate segments tested were negative in these tests, indicating that many whole blood–derived platelet concentrates had to be screened in order to identify sufficient compatible platelets to pool for the transfusions. In the latter study, the most compatible platelet products selected by crossmatching were less successful in supporting the refractory patients than were HLA-matched platelets.

A report of an automated version of the SPRCA assay demonstrated how large numbers of platelet concentrates could be routinely tested to identify compatible pools of whole blood–derived platelets for refractory patients. In this study, a daily inventory of 100–120 whole blood–derived buffy coats were assessed to provide platelet support for 40 consecutive alloimmune refractory patients.\textsuperscript{132} Platelets collected from routinely obtained donor samples, rather than from the concentrates themselves, were tested with patient serum. Using this system, up to 94 different platelet samples could be tested for each patient. Buffy coat platelets from five or six donors whose platelets yielded negative results were then pooled and transfused to the patient. The authors were able to demonstrate significant improvement in both 1-hour and 18- to 24-hour increments with the crossmatch-negative pools compared with random pools given in the month before the patients met the criteria for refractoriness in the study (two consecutive 1-hour posttransfusion CCIs <5000).

The cost of the kits for achieving each “successful” platelet increment (posttransfusion platelet count >10,000/μL) was about 450 euros. This did not include any costs for labor or administration of the crossmatching program. The authors noted that although the automated crossmatching strategy allowed rapid identification of compatible platelet pools from available inventories, a larger study, preferably a randomized controlled trial of this method versus HLA matching, would be necessary to confirm the value of this approach and more accurately capture the costs involved.

Experience using both the LCT assay and the SPRCA for analyzing antibodies to HLA and platelet antigens has been reported to aid in identifying patients who will benefit from either HLA-selected or crossmatch-selected platelets.\textsuperscript{133} With the LCT assay, investigators found that if the PRA was <70%, adequate transfusion responses were seen in approximately 80% of transfusions that were selected simply by HLA criteria (ASP and CREGs). However, when the PRA was >70%, only about 25% of patients did well. When analyzing the results using crossmatching, patients with PRAs <80% did well with crossmatch-selected platelets; but when the PRA was 80–100%, there were many failures with the crossmatch-compatible products. It was suggested that at high levels of alloimmunization, crossmatching misses significant antibodies. One report compares the MAIPA assay to the LCT test for the detection of HLA antibodies. This study showed the MAIPA assay to be more sensitive, detecting apparent HLA antibodies missed by the LCT assay. Moreover, these MAIPA-positive, LCT-negative HLA antibodies may be clinically relevant and affect the posttransfusion platelet count increment.\textsuperscript{134} The advent of more sensitive methods for screening patients for HLA antibodies, when used to identify patients who are alloimmune refractory to platelet transfusions, may prove to be superior to the LCT assay for this purpose.\textsuperscript{122,123,135}

With the considerable effort and expense entailed in their procurement, HLA- or crossmatch-selected platelet products should be reserved for those refractory patients who have definite evidence of alloimmunization by either HLA or platelet antibody testing. In the latter case, alloimmunity can be established in the context of performing a platelet crossmatch test if at least some of the platelet products tested with patient serum are reactive in the assay. In practice, when refractoriness is first recognized during use of random donor platelets, provision of a few HLA-selected products may be warranted before the results of definitive testing to document alloimmunization are available. In the event that such testing fails to document alloimmunization (i.e., no HLA- or platelet-reactive antibodies are identified), then support with randomly selected platelet products should resume.

Although the incidence of platelet-specific antibodies causing patients to be refractory to most or all attempted platelet transfusions is very small, this possibility should be investigated when most of the attempted crossmatches are positive or when HLA-matched transfusions fail. If platelet-specific antibodies are present, donors of known platelet antigen phenotype or family members, who may be more likely to share the patient’s phenotype, should be tested.

Despite the effort and expense involved in providing platelets selected by crossmatching to refractory patients, as is the case with platelets selected by HLA matching, a positive impact on patient outcomes has not been demonstrated. A recent review of platelet crossmatching studies reported from the past four decades concluded that none provided adequate evidence that either mortality or morbidity related to bleeding could be reduced by using crossmatch-compatible platelets in refractory patients, regardless of the method chosen.\textsuperscript{136}

Overcoming established alloimmunization

Strategies attempting to reverse established alloimmunization to HLA antigens have met with only limited success to date. Previously attempted methods include (1) methods to temporarily block the immune-mediated destruction of platelets, (2) suppression of the immune response to decrease the production of relevant antibodies, and (3) provision of modified platelets or alternative (nonplatelet) hemostatic compounds.

Blocking immune destruction of platelets

Several studies investigated the utility of high-dose intravenous immunoglobulin (IVIG) to increase the platelet count in association with transfusion in refractory patients. The results of these studies have been highly variable.\textsuperscript{137–139} Initial uncontrolled trials were conducted with very small numbers of patients using whole blood–derived platelet transfusions. Studies using somewhat larger numbers of patients have been inconclusive. One study treated 11 refractory patients with 0.4 g/kg/day for 5 days with no benefit in the 1-hour posttransfusion recoveries.\textsuperscript{137} Another found a modest beneficial effect of IVIG in a randomized placebo-controlled trial with 12 alloimmunized thrombocytopenic patients.\textsuperscript{138} The same donor platelets were used before and after treatment with IVIG.
The posttreatment 1-hour CCLs in the IVIG group (seven patients) were significantly greater than in the five patients receiving placebo treatment—8413 and 1050, respectively. However, by 24 hours after the transfusion, there was no residual benefit of IVIG. The authors concluded that the use of IVIG could not replace HLA-matched platelet transfusions in supporting alloimmunized refractory patients.

Using very high-dose (6 g/kg) IVIG, other investigators could reverse the refractory response in some patients who failed to respond to HLA-selected LCT-compatible platelet transfusions. Before treatment with IVIG, the patients had been refractory to HLA-matched platelet products. Following therapy, 13 of 19 patients responded with improvement in 1-hour posttransfusion platelet count increment using HLA-matched platelets. Those patients with PRAs <85% responded better than those who were more highly alloimmunized.

After initial reports of success with IVIG to improve platelet responses in immune refractory patients, other investigators examined the role of Rh immune globulin (RhIG) infusions in preventing refractory responses from developing. In this randomized control trial, patients treated with weekly intravenous RhIG infusions had rates of refractoriness similar to those treated with placebo, indicating that this form of reticuloendothelial system blockade was not helpful in preventing refractory responses.

**Suppression of the immune response to decrease antibody production**

Other strategies evaluated to overcome the platelet refractory state include use of vinblastine-loaded platelet transfusions, treatment with cyclosporin A, immunoabsorption using staphylococcal protein A columns, and plasmapheresis. A more recent report describes the coincident improvement in platelet response in a patient treated with bortezomib for multiple myeloma, and a second one describes a patient whose alloimmune refractoriness resolved after conditioning for allogeneic stem cell transplantation. Each strategy has had some limited success, and none can be recommended for routine use in this situation.

**Provision of modified platelets or alternative (nonplatelet) hemostatic compounds**

A novel approach has been developed involving transfusion with acid-treated whole blood–derived platelets. Treatment of platelets with citric acid modifies HLA class I antigens on the platelet surface, making them less recognizable to the immune system. A small number of patients have been treated with such products but with very limited success. Several potential platelet substitutes are currently being investigated. These include lyophilized platelets, infusable platelet membranes, thromboerythrocytes, and thrombospheres. Whether these will have clinical relevance remains to be determined by future investigation.

The role of platelet growth factors in the treatment of patients who have undergone highly myeloablative therapy is not totally defined. Studies support the use of both interleukin-11 (IL11) and recombinant human thrombopoietin in shortening the duration and blunting the severity of thrombocytopenia after chemotherapy. However, toxicity and the development of thrombopoietin-neutralizing antibodies have halted trials using the latter agent. Less success has been demonstrated with these agents in the setting of myeloablation with stem cell transplantation. These drugs may play more of a role in preventing rather than managing platelet refractoriness because responsive patients would require fewer platelet transfusions. Currently, only one product, recombinant human IL11 (Neumega, Genetics Institute, Cambridge, MA), is licensed in the United States for the prevention of severe thrombocytopenia and the reduction of the need for platelet transfusion following myelosuppressive but not myeloablative chemotherapy in patients with nonmyeloid malignancies.

There have been only limited case reports and no formal trials of the newer thrombopoietin mimetic agents romiplostim or eltrombopag for refractory patients.

**Prevention of alloimmunity**

Alloimmunization to HLA antigens appears to be transient in some patients; however, for most patients, once the alloimmune refractory state is established, it is very difficult to reverse. Therapy for the refractory state is expensive and frequently requires more transfusions than therapy for patients who are not refractory. Therefore, attention has turned to prevention of alloimmunization as a more practical way to ensure continued successful platelet support. Figure 18.2 represents an approach to managing platelet transfusions that encompasses both prevention of and platelet selection for refractoriness to platelet transfusions.

Several strategies have been used to prevent alloimmunization to platelet products. Antigenic exposure can be limited by the use of apheresis platelet transfusions. Both animal studies and trials involving humans have shown that HLA alloimmunization can at least be delayed (if not reduced) by the provision of apheresis platelets. Apheresis platelet products provide adequate platelet doses for adults from single donors rather than pools of whole blood–derived platelet concentrates, which typically expose patients to 5–8 different donors. Indeed, the majority of platelet transfusions in the United States are now provided from apheresis collections, partly in order to reduce the rates of alloimmunization and refractoriness.

A second promising method is treatment of blood components with ultraviolet B (UVB) irradiation. UVB irradiation prevents the interaction of donor dendritic cells with recipient T lymphocytes, which is the major route of primary HLA alloimmunization to platelet transfusions. Additional studies have provided evidence that the inability of donor and recipient immune cells to interact following UVB irradiation may indicate a state of immunologic tolerance to a blood transfusion. Preliminary studies with UVB-treated platelets in thrombocytopenic patients with cancer demonstrated a reduced rate of HLA antibody formation. The TRAP study found that UVB irradiation could reduce the incidence of alloimmunization and platelet refractoriness. Despite its effectiveness in reducing alloimmunization, UVB irradiation of platelet products is currently not approved for use in the United States.

Because the major pathway of primary alloimmunization against HLA class I involves antigen presentation in the context of HLA class II molecules on donor APCs, significant work has centered on removing leukocytes from the donor blood components as a means of preventing alloimmunization. Clinical trials have demonstrated that reduction of leukocytes to a level <5 x 10⁶ in blood components is sufficient to reduce the risk of HLA alloimmunization. Removal of leukocytes is most often accomplished by passing the components through third-generation leukocyte reduction filters. These filters remove the leukocytes by a combination of adhesion and pore size and are capable of producing a three- to four-log reduction in leukocytes. This process results in products that easily meet the US Food and Drug Administration’s requirement for leukocyte-reduced blood components (<5 x 10⁶ total...
leukocytes). Although such filters can be used at the bedside, the preferred method is prestorage leukocyte reduction in the laboratory. The latter allows leukocyte-reduced blood components to be manufactured according to good manufacturing practice regulations with well-defined quality control. It also prevents leukocyte fragments, shown to be capable of passing through filters and sensitizing recipients in animal studies, from accumulating in stored blood components.\textsuperscript{158} Leukocyte reduction of platelet products can be accomplished by using either of two techniques. With apheresis technology (by which the equivalent of six whole blood–derived platelet concentrates can be obtained from a single donor), the product is leukocyte reduced as it is collected. Alternatively, pools of whole blood–derived platelet concentrates can be filtered before a five-day storage period.

The majority of trials to date support the use of leukocyte reduction to reduce alloimmunization to blood components. In 1998, 18 clinical trials (some randomized controlled and some nonrandomized) using leukocyte-reduced red cells and platelets

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**Algorithm for Managing Platelet Support**

Whole-blood-derived or apheresis platelet transfusions, preferably leukocyte-reduced

- Measure platelet count
  - 10 minutes to 1 hour posttransfusion or at 18-24 hours posttransfusion

**Poor response $\times 2$**

- (CCI $< 5000$ or inadequate platelet count increment)
- Screen patient for HLA antibodies

**Adequate response at 10 minutes to 1 hour and 18-24 hours posttransfusion**

- (CCI $> 5000$ and adequate platelet count increment)
- Continue support with whole-blood-derived or apheresis platelet transfusions

- **PRA $> 30\%$**
  - Use HLA-selected apheresis platelets (HLA matched-A, BU or CREG selected products).
  - Expand pool using ASP method selecting products lacking antigens reactive with patient's HLA antibodies.
  - Products selected by a crossmatch method can be substituted.

- **PRA $< 30\%$**
  - Assess for nonimmune clinical factors and correct, if present.
  - Use fresh ($< 48$ hours old) ABO-compatible platelets.
  - Screen patient for platelet-specific antibodies and avoid relevant specificities, if possible.

**Poor response**

- Assess for nonimmune clinical factors and correct, if present.
- Use fresh ($< 48$ hours old) ABO-compatible platelets.
- Screen patient for platelet-specific antibodies and avoid relevant specificities, if possible.
- Continue to provide HLA- (or crossmatch) selected products.

**Adequate response**

- Continue support with HLA- (or crossmatch) selected products.

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*Figure 18.2* Algorithm for managing platelet support.
to prevent alloimmunization were reviewed.159 Fourteen of the trials showed positive results using leukocyte-reduced blood to prevent HLA alloimmunization and platelet refractoriness, whereas three of the trials showed no benefit. In addition, a recent meta-analysis of eight randomized controlled trials demonstrated a clear, protective effect of white cell reduction in the prevention of HLA alloimmunization and platelet refractoriness.160

The most definitive clinical trial demonstrating the importance of leukocyte reduction in the prevention of alloimmunization and refractoriness to platelet transfusion was the TRAP study.55 This trial compared four groups of patients: (1) patients receiving pooled platelet concentrates (control); (2) patients receiving leukocyte-reduced (filtered), pooled platelet concentrates; (3) patients receiving UVB-irradiated pooled platelet concentrates; and (4) patients receiving filtered platelets obtained by apheresis. All patients received leukocyte-reduced red cell components. Evidence of alloimmunization (by LCT assay) developed in 45% of the control group compared with 17–21% in the treated groups (p ≤ 0.0001 for each treated group as compared with controls). There were no differences among the treated groups in rates of alloimmunization. In the control group, 13% of patients became refractory compared to 3–5% in the treated groups (p ≤ 0.03 for each treated group as compared with controls). Individual treatments did not differ.

Several other important findings were noted in the TRAP study: (1) Clinical outcomes were similar in all four groups of the study, with no significant difference in the incidence of deaths caused by hemorrhage; (2) the incidence of refractoriness to platelet transfusions was low in the control group relative to previous reported studies; (3) patients who had never been pregnant had a lower risk of developing refractoriness than those who had previous pregnancies; (4) only about 8% of the patients in the study developed platelet-specific antibodies, and the treatment groups had no impact on this type of immunization; (5) the presence of platelet-specific antibodies did not correlate with refractoriness; and (6) a small percentage of patients in all groups were refractory to platelet transfusions yet did not have lymphocyteotoxic antibodies—most likely, these patients had either clinical factors or drug-related causes of refractoriness.

Another large, single-institution, retrospective study was reported from Canada, where universal leukocyte reduction (ULR) of blood components was instituted between 1997 and 1999.61 The study examined rates of alloimmunization and refractoriness in cohorts of patients undergoing chemotherapy for acute leukemia or stem cell transplantation before and after the introduction of ULR. Investigators found that alloimmunization to HLA, alloimmune refractoriness, and the need for HLA-matched platelets were all significantly reduced in the ULR cohort. Even taking into account the lower numbers of platelet transfusions given to the ULR patients, which likely resulted, in part, from changing the platelet transfusion trigger from 20,000/µL to 10,000/µL, leukocyte-reduced transfusions remained a significant independent variable driving the lower rates of alloimmunization and refractoriness in this patient population.

From the studies cited above, it is clear that both leukocyte reduction and UVB irradiation are capable of reducing the incidence of alloimmunization and platelet refractoriness. At the present time, leukocyte reduction of blood components is the most practical means of reducing the incidence of alloimmune platelet refractoriness and should be recommended for all patients at risk.

The identification and characterization of platelet antigens together with increased understanding of immunologic responses to these markers have been crucial in developing optimal platelet transfusion practices. Our understanding of the causes and mechanisms of both the immunologic and nonimmunologic aspects of the refractory response allows for improved strategies in the care of the potentially refractory patient.

Summary
Platelets express a variety of immunogenic markers on the cell surface. Some of these antigens are shared with other cell types as in the case of HLA antigens, which are shared with virtually all nucleated cells in the body. Other antigens are observed to be essentially platelet specific. This chapter has reviewed the antigens expressed on the platelet, the various patterns of alloimmunization to these antigens, and the impact on platelet transfusion responses. Strategies to treat and prevent alloimmunization were also summarized.

Disclaimer
The author has disclosed no conflicts of interest.

Key references
A full reference list for this chapter is available at: http://www.wiley.com/go/simon/transfusion
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