CHAPTER 21
Management of immune-mediated thrombocytopenia

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Introduction
Thrombocytopenia, defined as a platelet count below the normal range, is one of the most common reasons for hematological consultation. Low circulating platelet numbers can be caused by platelet underproduction, sequestration, hemodilution, consumption, or destruction. This chapter explores the problem of immune-mediated thrombocytopenia, including autoimmune conditions (e.g., primary immune thrombocytopenic purpura [ITP]), secondary immune conditions (e.g., drug-induced immune thrombocytopenia [DITP] and heparin-induced thrombocytopenia [HIT]), and alloimmune conditions (e.g., neonatal alloimmune thrombocytopenia [NAIT]). Immune thrombocytopenia is characterized by a shortened platelet lifespan caused by platelet–antibody interactions and, in primary ITP, reduced platelet production likely because of immune-mediated alterations to bone marrow megakaryocytes. We begin with a review of routine and specialized laboratory testing used for the investigation of thrombocytopenic conditions, followed by a description of the most common immune-mediated thrombocytopenic syndromes and a review of current management.

Laboratory tests for the investigation of thrombocytopenia

Complete blood cell count and blood film
Platelets usually are quantitated during a complete blood cell count with a particle counter. A normal platelet count usually is 150,000–400,000/μL, although the reference range may be lower in Mediterranean populations (125,000–300,000/μL) that have larger sized platelets. The platelet count usually remains fairly stable throughout a normal human lifespan.1 An exception occurs during pregnancy, when the platelet count decreases somewhat, perhaps the result of increased plasma volume (hemodilution). An elevated platelet count also is normal 10–14 days after a major surgical procedure (postoperative thrombocytosis, 250,000–1,000,000/μL) before return to preoperative baseline by three weeks after the operation.2,3 Thus, a platelet count of only 170,000/μL 10 days after an operation in a patient with dyspnea who had received postoperative heparin prophylaxis could represent pulmonary embolism complicating HIT. A useful general rule is that isolated thrombocytopenia usually is caused by increased platelet consumption or destruction, whereas bicytopenia or pancytopenia usually is attributable to marrow dysfunction, hypersplenism, or hemodilution. Isolated, severe thrombocytopenia (platelet count less than 20,000/μL) often indicates platelet destruction by autoantibodies, alloantibodies, or drug-dependent immunoglobulin G (IgG) antibodies. Such severe thrombocytopenia occasionally occurs in patients with HIT4 or septicemia, although platelet count nadirs are typically more than 20,000/μL in these two disorders characterized by in vivo platelet activation caused by heparin-dependent IgG antibodies or thrombin, respectively.

Examining the blood film is important to exclude pseudothrombocytopenia (spurious thrombocytopenia resulting from antibodies that cause ex vivo platelet agglutination) and to suggest various nonimmune causes of thrombocytopenia, such as toxic leukocytes indicating infection or fragmented red cells suggesting microangiopathic hemolysis. In contrast, primary immune thrombocytopenia is usually characterized by a reduction in platelet number with otherwise unremarkable morphologic features of all cell lines.

Platelet size and platelet RNA
A particle counter also is used to determine average platelet size, or mean platelet volume (MPV), which usually ranges from 7.0 to 10.5 fl. Disorders of increased platelet destruction usually are characterized by large platelets, and MPV ranges from 10 to 15 fl. Normal-sized or small platelets are common in disorders of underproduction or sequestration of platelets.

Young platelets contain residual amounts of RNA, which can be detected by means of flow cytometric analysis of platelets labeled with either thiazole orange or auramine-O. However, such quantitation of reticulated platelets (immature platelet count [IPC]) has not gained the acceptance that red cell reticulocyte assays have. Recent studies5 have reported that elevated IPC is associated with greater risk of major adverse cardiovascular events (although a causal vs. confounded relationship remains uncertain6), and that reduced IPC may be used to identify subgroups of patients with ITP who have a marked defect in platelet production.7

Bone marrow examination
Disorders of increased platelet destruction are characterized by normal or increased numbers of megakaryocytes in the marrow.8 Sometimes examination of the marrow yields enough information to determine the cause of the thrombocytopenia, such as myelodysplasia or megaloblastic anemia.

Measurement of platelet life span

A platelet survival study is the definitive test for classifying the cause of thrombocytopenia. Indium-111 is the radiolabel of choice because of its higher labeling efficiency and efficient range of γ emissions. Indium-111 is not released from platelets by platelet autoantibodies. Three patterns of platelet survival can be observed: (1) normal platelet recovery (60% to 75%) and a normal survival time (7 to 10 days) characterize thrombocytopenia caused by underproduction; (2) markedly reduced platelet lifespan (hours) is found in patients with thrombocytopenia caused by increased platelet destruction; and (3) reduced platelet recovery (10% to 30%) with a normal or near-normal platelet survival time is consistent with platelet sequestration (hypersplenism). Platelet survival studies are rarely performed because these tests are complex and physicians usually infer the mechanism of the thrombocytopenia from the clinical situation.

Platelet–antibody assays

There are two broad categories of platelet–antibody assays: (1) platelet-associated IgG assays, and (2) assays that identify the protein target of the antibody (direct binding assays). Measurement of platelet-associated IgG (PAIgG) has been widely available for many years. It can be used to detect surface-associated immunoglobulin or complement using a labeled anti-immunoglobulin (and/or anticomplement) probe, or total PAIgG measured after platelet lysis. These assays have limited diagnostic usefulness, because a positive test result does not differentiate immune from nonimmune thrombocytopenia.9

Direct binding assays can quantify binding of a labeled anti-immunoglobulin probe to the platelet surface. The labeled immunoglobulin probe is labeled with a radioisotope, fluorescent marker, or enzyme. The labeled probe (e.g., anti-IgG, anti-IgM, or anticomplement) is incubated with washed test platelets, unbound probe is washed away, and the amount bound to the platelets is measured.9 Although these assays are simple, a disadvantage is that platelet membranes nonspecifically adsorb proteins, including the labeled probe. Furthermore, even monoclonal anti-immunoglobulin probes may not differentiate immune from non-immune thrombocytopenic disorders.9 Nevertheless, this type of assay can be diagnostically useful in special situations, such as detecting a drug-dependent increase in platelet surface-bound IgG in the presence of patient serum.10

Protein-specific platelet–antibody assays

The diagnostic usefulness of platelet–antibody assays has increased dramatically with the introduction of various protein-specific assays that help identify the platelet protein target of antibodies with either monoclonal antibodies or electrophoretic techniques. Current protein-specific antibody assays use detergents to extract the glycoprotein (GP) target from the membrane. This process increases the specificity of the test, but may affect antigenicity because the use of detergents and certain inhibitors (e.g., EDTA) may affect the structure of certain GP targets, such as the GPⅡbⅢa complex, reducing the reactivity of some antibodies to HPA-1a.11

Various monoclonal antibody-based assays can be used to detect platelet antibodies. Perhaps most widely used is the monoclonal antibody immobilization of platelet antigen (MAIPA) assay. An improved modified MAIPA assay12 is shown in Figure 21.1A. A technically simpler assay is the antigen capture enzyme immunoassay, in which platelet GP monoclonal antibodies interact with detergent-solubilized platelet samples (Figure 21.1B), rather than intact platelets.10,13 Simplicity and improved specificity are advantages of these assays, especially the antigen capture assay. In addition to investigation of autoimmune thrombocytopenia, these assays can be adapted to study alloimmune disorders with a panel of platelet glycoproteins of known alloantigen phenotype, or drug-induced thrombocytopenia through demonstration of drug-dependent binding of antibody to specific platelet glycoproteins.10 A disadvantage is that the identity of the target protein (and thus the monoclonal antibody to be used in the test) must be known in advance, and a number of different monoclonal antibodies are often needed. These assays also can give false-negative results if the patient antibody competes for the same epitope recognized by the monoclonal antibody. Some human sera contain antibodies that recognize murine IgG, which is why the modified MAIPA and antigen capture assays are preferred to the original MAIPA.12

Immunoprecipitation

Immunoprecipitation has the advantage of being able to identify novel protein targets of platelet antibodies and does not require the use of a known monoclonal antibody in advance. It is performed by incubating patient serum or plasma with platelets labeled with iodine-125 or tagged with nonradioactive biotin.14 The proteins are then solubilized by the addition of detergent, and the antibody–protein complex is precipitated by addition of an anti-immunoglobulin bound to a solid phase (e.g., immobilized staphylococcal protein A). The labeled protein–antibody complexes are washed, and the platelet proteins are separated by denaturing gel electrophoresis and detected by autoradiography or use of enzyme-conjugated streptavidin. The target antigen is identified according to its electrophoretic mobility. Either the patient’s platelets are used (direct immunoprecipitation), or patient serum or plasma is mixed with target platelets (indirect immunoprecipitation).

Immunoprecipitation offers advantages over immunoblotting because the antibody reacts with native, rather than denatured, platelet proteins. In particular, this technique has allowed detection of clinically significant antibodies against previously unrecognized platelet proteins, such as anti-HPA-15 (anti-Gov) on CD109, a 175-kD glycosylphosphatidylinositol (GPI)-anchored protein.15 The main disadvantage of immunoprecipitation is technical difficulty. In addition, not all platelet proteins are optimally labeled (e.g., GPⅤI).

Immunoblotting (Western blotting)

In immunoblotting, patient serum is allowed to interact with platelet proteins that have been electrophoretically separated and then immobilized onto a solid phase (nitrocellulose). The test serum is added, and binding of patient antibody to specific protein bands is detected with labeled anti-immunoglobulin. Although immunoblotting offers the advantage of simplicity and the opportunity to store the immobilized proteins for long periods, a major disadvantage is that protein antigens are denatured in this method. This can destroy some platelet antigens (e.g., HPA-5 and -15) and may modify or expose epitopes to which normal sera can react (e.g., vinculin and talin), making interpretation difficult.15,16

Surface plasmon resonance (SPR)

A problem with many immunoblot assays is the requirement for wash steps. Even relatively gentle washes and dilutions can disrupt the binding of lower affinity antibodies to their glycoprotein targets. SPR was developed to investigate interactions between biomolecules in real time. The change in refractive index at the interface between
a gold surface and media can be measured as molecules in the fluid phase are passed over their targets bound to the surface. SPR has been used to investigate serum platelet antibodies, which may be low avidity and thus may escape detection by conventional protein binding assays. For example, SPR was used to confirm the diagnosis of NAIT in women who were suspected of having the disease but tested negative in other conventional immunoassays.17,18 A dis-

Figure 21.1 Modified monoclonal antibody immobilization of platelet antigen (MAIPA) assay and antigen capture assay. “Direct” versions of both assays are shown, that is, the patient’s platelets are tested for the presence of platelet glycoprotein (GP)-bound autoantibodies. (In indirect assays, the patient’s serum or plasma is added to target platelets to detect the presence of serum or plasma platelet glycoprotein antibodies.) (A) In the MAIPA assay, the antihuman GP monoclonal antibodies (MoAbs) are added to washed intact patient platelets before detergent lysis.12 (B) In the antigen capture assay, the serum or plasma is added to target platelets to detect the presence of serum or plasma platelet glycoprotein antibodies. (A) In the MAIPA assay, the glycoproteins that can be isolated effi-
ci

ciently. SPR has not yet been ad-

Tests for heparin-induced thrombocytopenia

Special tests are required to detect the antibodies that cause HIT. These assays are classified as platelet activation or platelet factor 4 (PF4)-dependent antigen assays.19,20

Platelet activation assays

Heparin-induced thrombocytopenia antibodies have potent hepa-

rin-dependent, platelet-activating properties. Aggregation of plate-

lets (prepared as citrate-anticoagulated platelet-rich plasma) by

patient plasma detected with conventional platelet aggre-
grometry once was widely used for HIT; however, the sensitivity and speci-

city of this assay are relatively low for detecting HIT antibodies. In

contrast, assays performed with washed platelets that have been resuspended in calcium-containing buffer, such as the platelet serotonin release assay (SRA) or heparin-induced platelet activation assay (HIPA), are both sensitive and specific for detecting clinically significant HIT antibodies.19,20 These assays are not widely available because they are technically demanding, require careful platelet donor selection, and require a panel of strong and weak positive serum controls. HIT antibodies have a characteristic activation profile: platelet activation at therapeutic, but not high, heparin concentrations and inhibition of activation by Fc receptor-blocking monoclonal antibodies. Strong serum-induced platelet activation that occurs in the absence of heparin is a feature of severe HIT, including “delayed-onset” and “spontaneous” HIT (discussed in this chapter).

PF4–heparin enzyme immunoassay

In 1992, Amiral et al.21 identified PF4–heparin complexes as the antigen of HIT. Both commercial and in-house antigen assays based on enzyme immunoassay (EIA) methods have since become available. An intriguing feature of the HIT antigen is that certain polyanions other than heparin render PF4 antigenic. This is the basis of the PF4-polyvinylsulfonate EIA for PF4–heparin antibodies.22

Figure 21.2 compares the operating characteristics (sensitivity–specificity trade-offs) for three types of platelet–antibody assays.
Classical PAIgG assays provide limited diagnostic information, whereas the newer protein-specific platelet–antibody assays have moderate sensitivity and relatively high specificity for immune thrombocytopenia. Both the antigen and washed-platelet activation assays are useful for diagnosis of HIT. Conventional assays for platelet-associated immunoglobulin G (PAIgG) have minimal diagnostic value; that is, sensitivity is similar to 1/50 dilution. In contrast, the modified monoclonal antibody immobilization of platelet antigen (MAIPA) assay and antigen capture (AC) tests have moderate usefulness for diagnosis of autoimmune thrombocytopenia (high specificity but low to moderate sensitivity). Conventional assays for platelet-associated immunoglobulin G (PAIgG) have minimal diagnostic value; that is, sensitivity is similar to 1 – specificity. Open symbols are the diagnostic cutoff actually used for each assay. The data used for the MAIPA, AC, and PAIgG assays are from published sources. Source: Warkentin (2001).

**Platelet genotyping**

Serologic assays are generally reliable for detecting platelet alloantibodies. They also have been used to identify platelet antigens (platelet phenotyping). However, a disadvantage of antibody-based analysis is that there may not be sufficient platelets available from a patient with severe thrombocytopenia (e.g., posttransfusion purpura [PTP]) to allow determination of the reciprocal platelet alloantigen phenotype. Secondly, specific phenotyping sera are available for only a few of the platelet antigens. Molecular techniques provide a reliable alternative to serologic phenotyping. Genomic DNA is used to determine the corresponding platelet alloantigen genotype and is readily available from a number of sources. Molecular techniques are useful in the evaluation of suspected NAIT. Analysis of fetal cells (obtained by amniocentesis, chorionic villus sampling, or sampling of fetal blood) can determine whether a fetus is at risk for this complication. Small amounts of tissue can be studied (e.g., 5–10 mL of amniotic fluid) because the technique of polymerase chain reaction (PCR) greatly amplifies the DNA. The possibility of significant maternal DNA contamination of a fetal sample can be assessed with the forensic technique of variable-number tandem repeat analysis of the sample.

**Polymerase chain reaction and restriction fragment length polymorphism**

For all platelet alloantigen polymorphisms but one (HPA-4), the single-base substitution responsible for the change in the expressed amino acid is associated with a restriction endonuclease recognition site. Accordingly, restriction fragment length polymorphism (RFLP) analysis was the first genotyping assay developed to identify platelet alloantigens. In the PCR–RFLP method, a section of DNA that encompasses the polymorphism is amplified using a pair of sense–antisense primers. The amplified product is subjected to restriction enzyme digestion, which cuts the amplified DNA into fragments depending on the nucleotide sequence. These are separated using agarose gel electrophoresis to identify the size and number of fragments, which indicate the platelet antigen genotype. One limitation is the possibility that another polymorphism within the amplified fragment could confound the genotyping by interfering with the restriction enzyme. Phenotyping methods do not appear to be affected, suggesting that multiple methods for antigen typing may be warranted.

**Allele-specific polymerase chain reaction**

Because PCR–RFLP is a comparatively labor-intensive technique, allele- or sequence-specific PCR (SSP-PCR) is commonly used. For this technique, one of the primers is specific for the particular allele to be amplified. When the specific nucleotide corresponding to the allelic polymorphism is positioned at the 3’ end of the oligonucleotide primer, efficient amplification occurs only when the
primer is 100% complementary to the genomic sequence. The advantage of this method is that the PCR product is visualized directly in agarose gels to determine the genotype. Appropriate controls must be included in the assay, including additional primers to amplify a ubiquitous gene (e.g., human growth hormone) to ensure that all assay constituents are working properly.

Real-time polymerase chain reaction
A modification of SSP-PCR is real-time PCR. Real-time PCR has improved both the speed and accuracy of genotyping. The use of specifically designed hybridization probes tagged with fluorescent dyes for the PCR allows direct determination of the platelet genotype without gel electrophoresis or restriction enzymes in a single reaction. One hybridization probe, the donor, is tagged with a dye (fluorescein) and emits light at a specific wavelength when excited by a light source. The other probe, the acceptor, is tagged with a different dye. It straddles the single nucleotide polymorphism of interest and is 100% homologous to one of the platelet alleles. The acceptor probe usually is one nucleotide away from the donor probe in a head-to-tail arrangement, that is, the dyes are juxtaposed. The energy emitted by the dye of the donor probe is transferred to the adjacent dye of the acceptor probe, which emits light at a different wavelength. The intensity of the second light emitted is proportional to the amount of double-stranded DNA present. Unbound donor probe in the mixture can be excited but cannot transfer energy to the acceptor probe. On completion of the PCR, the platelet genotype is determined by means of melting curve analysis. Because the acceptor probe straddles the polymorphism and is 100% homologous to one of the alleles, the melting curve has a different temperature midpoint (Tm) depending on whether a mismatch is present. The acceptor probe can have as much as 5°C to 8°C difference in Tm between the two platelet alleles. In all, three melting curves are seen, one for each polymorphism and a composite melting curve when the heterozygous situation is present. The advantage of fluorescence-based real-time PCR with melting curve analysis is that the PCR product is measured directly and no further manipulation is required. Any additional polymorphism in the region of the acceptor probe is detected in the melting curve analysis. Rapid thermal cycling and DNA extraction permit determination of a platelet genotype within two hours of specimen collection.

Immune-mediated thrombocytopenic syndromes
Immune thrombocytopenia results from antibody-mediated platelet destruction. The IgG-sensitized platelets are phagocytosed by monocytes and macrophages of the reticuloendothelial system. Reticuloendothelial cells are located throughout the body but are concentrated in the spleen, liver, lungs, and marrow. Pathogenic antibodies bind to platelets via their Fab termini, usually against specific autoantigen or alloantigen epitopes. Sometimes the target antigen is induced by a drug or drug metabolite. The result is a ternary complex that involves IgG, a drug, and a specific region on a platelet glycoprotein. Heparin-induced thrombocytopenia is an exception to these generalizations: Although HIT antibodies bind to PF4–heparin complexes via the Fab terminus, the Fc portion of IgG interacts with platelet FcγRIIa receptors, resulting in platelet activation.

The rate of platelet destruction is determined by the quantity and subclass distribution of IgG on the platelet, the amount of complement, and the efficiency of reticuloendothelial clearance. The severity of thrombocytopenia reflects the balance between the rate of platelet destruction and the compensatory marrow thrombopoiesis.

Immune thrombocytopenia
Immune thrombocytopenia (ITP), previously known as primary idiopathic thrombocytopenic purpura, is a common disorder characterized by increased platelet destruction and impaired platelet production. It is a diagnosis of exclusion, defined by isolated thrombocytopenia with no other clinically apparent cause. Secondary ITP can occur in the setting of human immunodeficiency virus (HIV), hepatitis C, and Helicobacter pylori infections; systemic lupus erythematosus (SLE); drugs; and lymphoproliferative disorders. Causes of non-immune thrombocytopenia that are often confused with ITP include myelodysplasia, splenomegaly, and familial thrombocytopenia. Although platelet–antibody assays allow for the detection of platelet GP-reactive autoantibodies in up to 75% of patients, the false-negative rate (at least 25%) gives this test a low sensitivity (e.g., a negative test does not exclude the diagnosis of ITP).

Pathogenesis
Over 50 years ago, Harrington et al. showed that ITP plasma infused into healthy volunteers caused acute severe thrombocytopenia. The platelet-destroying plasma factor was later shown to be IgG, although in some patients, IgM and IgA antibodies may be pathogenic. The immune target of the autoantibodies usually is one of the two major platelet glycoprotein complexes, with GPIb/IIIa implicated more often than GPIb/IX. Other less common autoantigen targets include GPla/IIa and, possibly, nonprotein targets such as glycosphingolipids. The autoantibodies bind to platelets by way of their Fab terminus and cause premature platelet destruction via Fc receptor (FcγR)-mediated phagocytosis by macrophages in the spleen and other reticuloendothelial tissues. Platelet autoantibodies can also activate complement in vitro. Another proposed mechanism of platelet destruction is direct platelet lysis by cytotoxic T cells.

Platelet production is also impaired in ITP. Platelet turnover is lower than expected, as shown by radiolabeled autologous platelet studies, and some patients who respond to thrombopoietin (TPO)-based treatments have demonstrated an increase in the number of reticulated platelets, suggesting that this compensation was not maximal. An increased megakaryocyte mass that has been observed in some patients may account for the relative TPO deficiency, because these cells bind free TPO; conversely, platelet underproduction may result from megakaryocyte injury caused by autoantibodies.

The cause of autoantibody formation in ITP is not known. Light-chain and immunoglobulin subclass restriction of platelet-reactive antibodies suggests an oligoclonal origin of autoantibodies in chronic ITP. Furthermore, the autoepitopes involved may be fairly restricted in scope. Autoantibodies are produced by B lymphocytes, and the loss of T-cell tolerance to platelet proteins is a key feature.

Clinical and laboratory features
Stages of ITP have recently been defined by the international ITP Working Group: newly diagnosed ITP (within three months), persistent ITP (3–12 months), and chronic ITP (more than 12 months). The prevalence of ITP has been estimated at 12.1 (95% confidence interval [CI], 11.1–13.0) per 100,000 adults. If clinical
Many patients do not bleed, even with low platelet counts (<30 × 10^9/L), and may not need treatment.

The urgency of treatment will dictate the type of therapy: Platelet transfusions may be useful when immediate (but transient) hemorrhage is needed, intravenous immunoglobulin (IVIG) will start to increase the platelet count in most patients by 12–24 hours, and corticosteroids result in a platelet count response within 3–7 days.

Most adults have chronic ITP with low (but not infrequent) rates of spontaneous remission (up to 20%), whereas most children have acute ITP that improves spontaneously or with minimal treatment.

Many adults will require treatment beyond first-line therapies, but the minimal amount of treatment should be used to maintain a hemostatic platelet count in patients with chronic ITP.

Drugs that interfere with platelet function—particularly aspirin, nonsteroidal anti-inflammatory agents, and alcohol—should be avoided.

Corticosteroids

Corticosteroids, either prednisone (1 mg/kg/day tapered over 6–8 weeks) or high-dose dexamethasone (40 mg/day for four consecutive days, repeated monthly if necessary), are considered first-line treatments. Two prospective studies compared conventional and lower dose prednisone therapy (1 mg/kg/day vs. 0.25 mg/kg/day in one trial; 1.5 mg/kg/day vs. 0.5 mg/kg/day in the other), and in neither study was there a significant difference in remission at 6-month follow-up evaluation. In the larger study, however, the higher dose regimen showed a trend to a higher rate of complete remission (46% vs. 35%) as well as higher platelet counts at 14-day follow-up evaluation (77% vs. 51% having a platelet count greater than 50,000/μL).

High-dose dexamethasone has been used with success in patients with newly diagnosed ITP. In one study (n = 125), 84% of patients with acute ITP treated with a single course of high-dose dexamethasone (40 mg/day for four days) achieved a platelet count response (>50,000/μL), and of those, 50% had a response that lasted from 2 to 5 years. In another study (n = 37), six courses of monthly high-dose dexamethasone resulted in an overall response rate of 83.8%, and a sustained response of 64.9% after two years. However, repeated cycles of high-dose dexamethasone were often poorly tolerated, and these high rates of durable response have not been consistent in practice. In patients with chronic ITP, responses with high-dose dexamethasone are variable. In one study, all 10 treated patients achieved a response that lasted six months following six cycles of high-dose dexamethasone (40 mg/day for four days). However, other subsequent studies were less encouraging. Using the same regimen, Stasi et al. reported that 13 of 32 patients (40.6%) had transient responses only, and in the study by Warner et al., none of nine patients responded, and five could not tolerate the treatment. Overall, only approximately 20% of adult patients with ITP have a durable remission following corticosteroid therapy.

Adverse effects of corticosteroids include facial swelling, weight gain, and behavioral changes in up to 20% of patients. Less common (1–5%) complications include infection, myopathy, hyperglycemia, psychosis, hypertension, hypokalemia, and osteoporosis. Osteonecrosis, most commonly involving the femoral head, occurs as a late side effect in approximately 5% of patients who undergo prolonged therapy, but it may occur even after intensive short-term exposure.

Intravenous immunoglobulin

High-dose IVIG has been used to treat patients with ITP since 1981. Reticuloendothelial blockade is the principal mechanism of action of IVIG, but many other mechanisms have been proposed, including the reduction of antibody synthesis, IgG molecules against the Fab regions of pathogenic autoantibodies ("anti-idiotypic antibodies"), cytokine-induced pro- or anti-inflammatory effects, up- or downregulation of various FcγRs, or the formation of soluble immune complexes. In a mouse model of ITP, the inhibitory IgG receptor, FcγRIIB, was required for IVIG to cause an elevation in platelet count, and the transfer of IVIG-prime dendritic cells has been shown to recapitulate the effect of IVIG.

The use of IVIG is indicated for patients with ITP with bleeding or for whom an invasive procedure is planned to raise the platelet count quickly. A French study of 122 adults with ITP showed that IVIG (0.7 g/kg/day for three days) raised the platelet count to over 50,000/μL within five days more frequently than did corticosteroids (methylprednisolone, 15 mg/kg/day for three days). The usual dose of IVIG is 1–2 g/kg, given either as 0.4 g/kg for five consecutive days or as 1 g/kg over two days. One trial showed no difference between one and two doses of 1 g/kg. Thus, our approach is to give 1 g/kg as a single dose and to repeat the dose 1 or 2 days later if no significant platelet count increase has occurred.

IVIG increases the platelet count to greater than 50,000/μL in approximately 80% of adult patients. Responses are usually transient, lasting 2–6 weeks. Tachyphylaxis after repeated courses may be observed in patients with severe ITP.

Common but mild side effects include headache in 10% of patients, backache, nausea, flushing, and fever. Aseptic meningitis may occur rarely. Chest pain, hypertension, hypotension, bronchospasm, and laryngeal edema have been reported. A boxed warning concerning the risk of thrombosis with IVIG was issued by the US Food and Drug Administration (FDA) in 2013 (http://www.
infection transmission is theoretically possible. Transfusion-related IVIG is prepared from pooled plasma from thousands of donors, suitable alternative. However, most patients do not have excessive IVIG or corticosteroids, and TPO receptor agonists may be a hemostasis usually can be achieved with preoperative high-dose and (3) prevention of thromboembolism. Adequate perioperative mobilization, prophylactic low-molecular-weight (LMW) heparin should be considered when the platelet count is recovering.

Rituximab
Rituximab is a chimeric monoclonal anti-CD20 currently indicated for the treatment of lymphoma and rheumatoid arthritis. The Fab portion binds to CD20 on B lymphocytes, which results in FcyR-mediated B-cell lysis by complement-dependent and antibody-dependent pathways. In ITP, rituximab depletes CD20-positive B cells, which are responsible for platelet autoantibody production. It can also correct T-cell dysfunction which has been associated with platelet count improvements after rituximab. Rituximab may be a suitable alternative to splenectomy for some patients because of its association with lasting remissions in up to 20% of patients.

Table 21.1 Comparison of high-dose IVIG and intravenous RhIg for management of immune thrombocytopenic purpura

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>High-Dose IVIG</th>
<th>Intravenous RhIg</th>
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<tbody>
<tr>
<td>Side effects</td>
<td>Common: headache, hypertension, fever and chills</td>
<td>Common: mild hemolysis</td>
</tr>
<tr>
<td>Response rate</td>
<td>Rare: hemolysis, renal failure, myocardial infarction, stroke</td>
<td>Rare: severe hemolysis necessitating transfusion or hemodialysis, DIC ~80%</td>
</tr>
<tr>
<td>Response duration</td>
<td>~80%</td>
<td>~80%</td>
</tr>
<tr>
<td>Pattern of platelet increase</td>
<td>Usually transient</td>
<td>Usually transient</td>
</tr>
<tr>
<td>Influence of ABO, Rh type</td>
<td>Faster increase, higher peak, shorter duration of response</td>
<td>Slower increase, lower peak, longer duration of response</td>
</tr>
<tr>
<td>Influence of splenectomy</td>
<td>No influence</td>
<td>Only D-positive patients respond</td>
</tr>
<tr>
<td>Suitable for emergency</td>
<td>Unknown</td>
<td>Minimal response in patients without a spleen</td>
</tr>
<tr>
<td>management of ITP</td>
<td>Recommended</td>
<td>Not recommended</td>
</tr>
</tbody>
</table>

IVIG = intravenous immune globulin; RhIg = Rh immune globulin; DIC = disseminated intravascular coagulation; ITP = immune thrombocytopenic purpura.

(fda.gov), because arterial and venous thrombosis have rarely been reported, particularly in elderly patients (Table 21.1). Because IVIG is prepared from pooled plasma from thousands of donors, infection transmission is theoretically possible. Transfusion-related acute lung injury following IVIG also has been described.

**Intravenous Rh immune globulin**
Like IVIG, the mechanism of action of intravenous Rh immune globulin (RhIg) is believed to be reticuloendothelial blockade, which occurs through occupancy of the reticuloendothelial cell FcγRs by IgG-sensitized red cells. The therapy is only effective in D-positive individuals.

Doses of 50–75 μg/kg of RhIg have been shown to produce a rapid increase in platelet count. Hemolysis is an expected side effect, and rarely severe hemolysis with disseminated intravascular coagulation and renal failure has been reported with fatal outcomes. Because of this, RhIg was voluntarily withdrawn from some European markets in 2009 (Table 21.1).

**Second-line therapies**

**Splenectomy**
Splenectomy is frequently successful, because the spleen is the major site of both autoantibody production and platelet destruction. Complete remission occurs in 70% of patients within 4–6 weeks. In complete responders, normal platelet counts are reached within seven days in 90% and within six weeks in 98% of patients; spontaneous remission is encountered rarely thereafter. In a further 5–10% of patients, partial remission is achieved. In a systematic review of the efficacy of splenectomy for patients with ITP, younger age was an independent predictor of response to splenectomy, however, other investigators have shown that response to IVIG and splenic clearance of platelets (determined by radionuclide platelet imaging techniques) are good predictors of response to splenectomy. The risk of relapse after a complete remission following splenectomy is low (approximately 10–15% at 10 years). Splenectomy is most often done using a laparoscopic approach.

Perioperative management of splenectomy involves (1) optimization of the platelet count before surgery, (2) vaccination to prevent the risk of overwhelming post-splenectomy infection, and (3) prevention of thromboembolism. Adequate perioperative hemoablation usually can be achieved with preoperative high-dose IVIG or corticosteroids, and TPO receptor agonists may be a suitable alternative. However, most patients do not have excessive bleeding; therefore, platelet transfusions should be reserved for the treatment of perioperative bleeding.

The presence of residual accessory spleen should be considered when splenectomy fails, or when patients relapse months or years after surgery. Postsplenectomy blood film changes (e.g., Howell–Jolly bodies) do not necessarily eliminate the possibility of a residual accessory spleen. A durable platelet count response following accessory splenectomy performed because of postsplenectomy relapse of ITP is uncommon and can be expected in less than 50% of patients.

Perioperative morbidity after splenectomy is less than 10%. The most frequent complications are pleuropulmonary (pneumonia, subphrenic abscess, and pleural effusion) in 4% of patients, major bleeding in 1.5%, and thromboembolism in 1%. Morbidity is lower with a laparoscopic approach by an experienced surgeon. Overall mortality is approximately 1% following laparotomy and 0.2% following laparoscopic splenectomy.

Bacterial infection is the most feared complication of splenectomy. The risk of overwhelming post-splenectomy infection is approximately 1–3%. In a population-based Danish cohort study of 3812 patients who underwent splenectomy, the overall incidence rate of infection was 7.7 per 100 person-years (odds ratio compared to unsplenectomized patients with an indication for splenectomy, 1.7 [95% CI, 1.5–2.1] in the first 90 days). In a review of the literature up to 1996, the frequency of infection was 2.1% and mortality was 1.2% among 484 patients who had splenectomy for ITP. Life-threatening infections may occur many years after splenectomy, suggesting that the risk of severe infection may persist. To reduce the risk of bacterial infection, all patients who undergo splenectomy should receive vaccinations against the encapsulated bacteria Streptococcus pneumoniae, Haemophilus influenzae type B (HIB), and Neisseria meningitidis.

Postsplenectomy thromboembolism is the most common cause of postoperative mortality; thus, for patients with delayed postoperative mobilization, prophylactic low-molecular-weight (LMW) heparin should be considered when the platelet count is recovering.
A systematic review of observational studies described outcomes in adults who had ITP for 1–360 months (half had splenectomy). A complete response to rituximab (platelet count greater than 150,000/µL) was seen in 43.6% of patients (95% CI, 29.5–57.7%) and an overall response (platelet count >50,000/µL) in 62.5% (95% CI, 52.6–72.5%). Responses lasted a median of 10 months. Data from randomized trials were less optimistic. In a recent meta-analysis, rituximab plus standard of care was more often associated with a complete platelet count response (platelets >100 x 10^3/µL without rescue treatment) compared with standard of care alone (46.8% vs. 32.5%; p = 0.002); however, responses were generally not sustained past 12 months.

Results with rituximab in children are less encouraging. In a prospective single-arm study of 36 children with chronic ITP treated with rituximab, 11 (30.6%) achieved a platelet count of 50,000/µL or greater for four consecutive weeks.

Toxicities of rituximab include infusional reactions, hepatitis B reactivation, and a possible association with progressive multifocal leukoencephalopathy. Rituximab has been shown to impair vaccine responses for up to six months.

**Thrombopoietin receptor agonists**

TPO receptor agonists are a relatively new class of medications indicated for the treatment of patients with chronic ITP. Romiplostim is administered by weekly subcutaneous injection, and eltrombopag is a daily pill. The clinical success of these medications has been one of the most important advances in ITP treatments since the discovery of IVIG. In phase III trials, each has been shown to be effective in increasing platelet counts compared with placebo or standard of care in 60–80% of patients. Responses are sustained even after long-term follow-up. The platelet count response is usually maintained as long as the medication is continued; however, once it is stopped, platelet counts typically drop to pretreatment levels, at which point patients may be at increased risk of bleeding. However, some patients can successfully stop TPO receptor agonists and maintain durable platelet count responses.

Rare side effects include thrombosis and bone marrow reticulin formation; however, the true risk of these complications remains uncertain. Potential long-term toxicities and high cost limit the prolonged use of these medications.

TPO receptor agonists bind to and activate the c-Mpl receptor on hematopoietic stem cells and megakaryocytes, ultimately leading to increased platelet production. They do not resemble endogenous TPO, and thus cannot induce the formation of TPO-reactive autoantibodies, which was a problem with early formulations of pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF).

Where TPO receptor agonists should be positioned for the treatment of patients with ITP remains uncertain. They are good treatment options for patients who have failed second-line therapies, including splenectomy and rituximab. The use of these medications at an earlier stage of disease may improve short-term outcomes.

**Other treatments**

**Danazol**

Danazol is an attenuated androgen with mild virilizing effects that can be used to treat men and nonpregnant women with ITP. Its mechanism of action is to decrease FcγR numbers and the rate of FcγR-mediated clearance of IgG-sensitized cells. Danazol decreases the number of monocyte IgG Fc receptors. Usually, 400 to 800 mg is administered daily in divided doses, although some physicians use low doses (50 mg/day). A response usually occurs within two months, although when low doses are used, as long as six months may be needed.

Danazol appears to produce a sustained increase in platelet count in approximately 30–40% of treated patients. The response rate may be higher among patients with associated rheumatologic disorders. Danazol must be continued to maintain the platelet count response, although attempts at dose reduction should be made. Danazol is generally well tolerated but may be associated with virilizing side effects in women, liver dysfunction, and rash.

**Vinca alkaloids**

Vinca alkaloids (vincristine and vinblastine) can produce generally short-lived increases in platelet count in approximately 65% of patients. These drugs bind to platelet microtubules and so might work by being delivered to, and thereby inhibiting, reticuloendothelial macrophages. Repeated doses of vinca alkaloids are rarely used to treat patients with chronic ITP, mostly because of dose-dependent neuropathy.

**Immunosuppressive agents**

Cyclophosphamide, azathioprine, mycophenolate, and cyclosporine are immunosuppressive agents that have been used to treat patients with refractory ITP. Cyclophosphamide is an alkylating agent given in doses of 1–2 mg/kg/day, although high-dose cyclophosphamide has been used in combination with autologous stem cell transplantation. Azathioprine and mycophenolate are purine antimetabolites that inhibit lymphocyte proliferation. Cyclosporine is a calcineurin inhibitor that selectively blocks T-cell-dependent biosynthesis of lymphokines, particularly interleukin-2, at the level of messenger RNA transcription.

These drugs, alone or in combination, have shown moderate success, with up to two-thirds of patients exhibiting a platelet count response. However, only 20–30% have responses that persist after stopping the drug(s). Responses may occur as early as two weeks and as late as three months after initiation of treatment.

Azathioprine can have myelosuppressive effects on hematopoietic cells and may cause severe bone marrow toxicity in patients who lack activity of the enzyme thiopurine methyltransferase (TPMT) (1 in 300 individuals). Mycophenolate may also be associated with leukopenia. The concern over leukemic transformation has limited use of cyclophosphamide. Animal studies suggest that intermittent cyclophosphamide may be less leukemogenic than daily administration. Cyclophosphamide also can cause hemorrhagic cystitis and hepatic toxicity. Cyclosporine is associated with hypertension and renal failure in some patients.

**Special treatment situations**

**Emergency treatment of a bleeding patient**

Any patient with ITP who has life-threatening bleeding needs immediate platelet transfusion. The patient then should receive IVIG (1 g/kg) over 4–6 hours to block the reticuloendothelial system, and corticosteroids should be considered for long-term disease control.

**Preparation for invasive procedures**

High-dose IVIG usually is the treatment of choice for severely thrombocytopenic patients with ITP who need urgent surgery or an
invasive procedure. Prophylactic platelet transfusion should be administered only when maximal hemostasis is required, because transfusion-induced platelet count increments are usually transient. For situations in which at least 2 or 3 days are available before the planned procedure, less expensive and equally effective options include corticosteroids. TPO receptor agonists may be a reasonable alternative but must be started 2–3 weeks in advance.

**Immune thrombocytopenia in pregnancy**

Thrombocytopenia during pregnancy usually is not caused by ITP. Rather, a benign condition known as gestational thrombocytopenia is more common, occurring in approximately 5% of pregnancies at term. In this condition, maternal platelet counts do not fall below 70,000/μL. Newborns of mothers with incidental thrombocytopenia are not at increased risk of neonatal thrombocytopenia. The second most likely cause of thrombocytopenia is pregnancy-related disorders (e.g., preeclampsia and syndrome of hemorrhysis, elevated liver enzymes, and low platelet count [HELLP syndrome]). Preeclampsia occurs in approximately 10% of pregnancies and causes thrombocytopenia in one-fourth of affected mothers. Preeclampsia is associated with increased maternal and fetal morbidity and mortality. Secondary causes of immune thrombocytopenia that can occur in young women, such as SLE or HIV infection, should also be considered in the appropriate clinical context.

ITP in pregnancy is an important disorder because of the treatment implications for the mother and the possibility of fetal or neonatal thrombocytopenia caused by transplacental passage of IgG platelet autoantibodies. In the past, infants of mothers with ITP were delivered by cesarean section; however, today, this procedure is not routinely recommended for two reasons. Firstly, the frequency of severe fetal thrombocytopenia (platelet count less than 20,000/μL) is low (approximately 4%). Secondly, there is no evidence that cesarean section leads to less intracranial bleeding compared with vaginal delivery.

Many pregnant women with ITP do not need specific treatment, unless the platelet count decreases below 20,000/μL or there is evidence of impaired hemostasis. The two preferred treatment options are intermittent high-dose IVIG and low-dose prednisone; however, IVIG is generally the treatment of choice (1 g/kg every 2–4 weeks) because side effects are more common with prednisone and include teratogenicity (first trimester) and preeclampsia (second and third trimesters). At delivery, thrombocytopenic patients should not be subjected to epidural analgesia. Platelet transfusions are almost never needed at delivery. In the largest cohort of infants born to mothers with ITP (119 pregnancies in 97 women), 10% of infants had a platelet count below 50,000/μL at birth, 15% required hemostatic treatments, and 1% died.

There are no good predictors of fetal thrombocytopenia, not even the severity of maternal thrombocytopenia (indeed, women cured of ITP by splenectomy can bear infants with passive autoimmune thrombocytopenia).

If therapy for maternal thrombocytopenia is needed, IVIG is preferred. Splenectomy is rarely indicated for severe, refractory thrombocytopenia in pregnancy. Cesarean section delivery should be reserved for obstetrical indications.

**Immune thrombocytopenia in children**

Acute ITP of childhood is a relatively common, generally self-limited, immune thrombocytopenic disorder with a peak incidence between 2 and 6 years of age. Boys and girls are equally affected, except for infants, in which males predominate. Most children (80–90%) with acute ITP recover completely within six months; the others have persistent thrombocytopenia. Acute ITP of childhood likely is a transient autoimmune disorder.

The typical clinical manifestations are bleeding and bruising following a viral infection. The mortality is approximately 0.4%, and most deaths are caused by intracranial hemorrhage. Laboratory abnormalities include isolated thrombocytopenia and normal or increased MPV. A marrow examination usually is not performed on a child with typical clinical features of ITP, but is indicated for unexpected clinical or laboratory findings. Normal or increased numbers of megakaryocytes are observed. Sometimes, morphologically distinct lymphoid cells, called hematogones, constitute up to one-half of the marrow cells and may cause confusion with acute leukemia. These nonneoplastic cells have the surface immunophenotypic profile of immature lymphocytes.

Acute ITP in children usually is a benign disease. Although rare, intracranial hemorrhage is the most feared complication. Clinical trials have focused on the time to increase the platelet count to safe levels as a surrogate endpoint for avoiding intracranial hemorrhage.

In a meta-analysis of six randomized trials (n = 410 children), IVIG was associated with a more rapid platelet count increase compared with corticosteroids.

High-dose corticosteroid therapy (e.g., intravenous methylprednisolone at 30 mg/kg/day for 3 days; or oral methylprednisolone at 30–50 mg/kg/day for 7 days) also rapidly increases the platelet count in children with acute severe ITP. Combined treatment with IVIG and pulse methylprednisolone may be indicated for those children felt to be at very high risk of intracranial hemorrhage.

Approximately 10–20% of children with ITP eventually have chronic ITP, defined as a platelet count less than 100,000/μL for more than six months. As many as one-third of children who meet this definition can still enter late spontaneous remission, sometimes as long as 5–10 years after diagnosis. Chronic ITP in children resembles adult chronic ITP.

Some patients have no symptoms despite marked thrombocytopenia. These children generally do not need treatment. For children with symptoms, options include long-term or intermittent administration of corticosteroids, maintenance IVIG or RhIG, splenectomy, vinca alkaloids, and immunosuppressive agents.

Intravenous immune globulin (2 g/kg over 2–5 days) increases the platelet count of most children with chronic ITP and repeated courses of IVIG have been used to defer or avoid splenectomy. This practice also appears to be cost-effective. Unfortunately, approximately 25% of patients become refractory. Low-dose, alternate-day corticosteroid therapy may improve the results with maintenance IVIG.

Intravenous RhIG is effective in increasing the platelet count of most Rh-positive children with chronic ITP and, at a dose of 75 μg/kg, appears to be as effective as IVIG. The benefit is transient, and the median duration of benefit is approximately three weeks. RhIG can also be considered splenectomy-sparing in some patients. However, RhIG is generally ineffective following splenectomy.

Splenectomy is sometimes performed in children with chronic ITP who cannot be maintained on low-dose maintenance
glucocorticoids, IVIG, or RhIG therapy. Because of the high probability of a cure (approximately 70%), some physicians perform splenectomy before instituting potentially toxic immunosuppressive therapy. However, in general, splenectomy is avoided in children, especially young children, because of the high risk of postsplenectomy sepsis, the possibility of late spontaneous reimplantation, and the efficacy of intermittent maintenance therapy with IVIG or RhIG. As with adults, children must receive vaccines for *S. pneumoniae*, *N. meningitides*, and *H. influenzae* type b at least two weeks before splenectomy.122

Results of use of immunosuppressive agents (azathioprine or cyclophosphamide) and vinca alkaloids to treat children with chronic ITP are variable and based on uncontrolled studies.117 Pulse corticosteroids have been used in children, although few durable remissions occur. In one study, three of seven children achieved a platelet count above 50,000/μL six months after receiving high-dose dexamethasone. One child had a response that was maintained to one year.123 Children with chronic ITP refractory to splenectomy occasionally obtain benefit from cyclosporine. Danazol can be tried in the adolescent patient. About one-third of pediatric patients with chronic ITP responded to rituximab in one study.84

**Table 21.2** Drug-induced immune thrombocytopenic purpura (DITP)

<table>
<thead>
<tr>
<th>Syndrome and Drug(s)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heparin-induced thrombocytopenia</strong></td>
<td>Prothrombotic reaction caused by heparin-dependent platelet-activating IgG antibodies that recognize platelet factor 4/heparin complexes; caused less often by low-molecular-weight heparin and fondaparinux than by unfractionated heparin</td>
</tr>
<tr>
<td><strong>Drug-induced immune thrombocytopenic purpura (DITP)</strong></td>
<td>Prothrombotic reaction caused by IgG antibodies that recognize drug (or drug metabolite) bound to platelet glycoprotein (GP); patients have severe thrombocytopenia and mucocutaneous bleeding.</td>
</tr>
<tr>
<td>Quinine</td>
<td>Quinine-dependent anti-GPIIb/IIIa and GPIb/IX IgG implicated; drug is widely available (e.g., tonic water)</td>
</tr>
<tr>
<td>Quinidine</td>
<td>Antibodies usually distinct from quinidine-dependent antibodies</td>
</tr>
<tr>
<td>Rifampin</td>
<td>Rifampin-dependent anti-GPIIb/IIIa and GPIb/IX IgG implicated</td>
</tr>
<tr>
<td>Sulfa antibiotics</td>
<td>Occurs in ~1 of 25,000 patients receiving trimethoprim-sulfamethoxazole</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>Vancomycin-dependent anti-GPIIb/IIIa IgG</td>
</tr>
<tr>
<td>Iodinated contrast</td>
<td>Severe thrombocytopenia begins after radiologic procedure</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>IgG recognizes metabolite of acetaminophen</td>
</tr>
<tr>
<td>Many others</td>
<td>See published lists155,154</td>
</tr>
<tr>
<td><strong>Atypical DITP</strong></td>
<td>Abrupt onset of severe thrombocytopenia (platelet count nadir, 15,000 to 35,000/μL) perhaps by naturally occurring ligand-induced binding site antibodies</td>
</tr>
<tr>
<td>Gold</td>
<td>Thrombocytopenia can persist for months after gold therapy is stopped</td>
</tr>
<tr>
<td>MMR vaccine</td>
<td>Transient autoimmune thrombocytopenia (anti-GPIIb/IIIa) that occurs a few weeks after vaccination (resembles childhood acute ITP)</td>
</tr>
<tr>
<td><strong>DITP: hapten mechanism</strong></td>
<td>Indicates that IgG recognizes drug that remains bound to platelet surface even following platelet washing</td>
</tr>
<tr>
<td>Penicillin</td>
<td>Not well-established</td>
</tr>
<tr>
<td><strong>Drug-induced TTP/HUS</strong></td>
<td>Estimated frequency, 1/2,000 to 1/5000</td>
</tr>
<tr>
<td>Ticlopidine</td>
<td>Estimated frequency, 120,000</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>Quinine-dependent IgG against platelets and other cells found</td>
</tr>
<tr>
<td>Quinine</td>
<td>May be pathogenic factor in transplantation-associated TTP/HUS</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>Others</td>
</tr>
</tbody>
</table>

MMR = measles-mumps-rubella; ITP = immune thrombocytopenic purpura; TTP = thrombotic thrombocytopenic purpura; HUS = hemolytic uremic syndrome.

**Table 21.2** Drug-induced immune thrombocytopenic syndromes

**Typical drug-induced immune thrombocytopenia**

The most common drugs that have been confirmed by laboratory testing to cause DITP are quinine, quinidine, trimethoprim/sulfamethoxazole, vancomycin, penicillin, rifampin, carbamazepine, and cephradine.126 The risk of DITP is approximately 1 case in 1000 for quinine and 1 in 25,000 for sulfamethoxazole-trimethoprim.129 Quinine is present in certain beverages (e.g., tonic water), and consequently patients may not be aware of exposure.

Clinical criteria supporting a diagnosis of DITP are (1) thrombocytopenia occurs during drug treatment and is corrected completely after discontinuation of the drug; (2) the implicated drug was the only one used when thrombocytopenia occurred, or platelet count recovery occurred or persisted despite continuation or reintroduction of the other drugs used; (3) other causes of thrombocytopenia are excluded; and (4) reexposure to the implicated agent resulted in recurrent thrombocytopenia.125 For reasons of patient safety, drug reexposure is rarely performed deliberately, but sometimes the outcome of unintentional reexposure can provide important diagnostic information (e.g., recurrent thrombocytopenia following ingestion of tonic water suggests quinine-induced thrombocytopenia). Meeting all four criteria provides definite evidence of causation, whereas meeting the first three criteria suggests a probable cause.125

Although many dozens of drugs have been suspected as causing DITP, laboratory evidence confirming the presence of drug-dependent platelet antibodies exists for relatively few drugs.128 Supporting evidence for the drugs claimed to cause DITP is available (http://www.ouhsc.edu/platelets/).

Drug-induced immune-mediated thrombocytopenia typically begins abruptly and is severe; most patients have a platelet count less than 20,000/μL.124,125,130 Although the interval between starting the drug and development of thrombocytopenia usually is 1 or 2 weeks, occasionally it can be several months or even longer after
administration of the drug is started. Sometimes, thrombocytopenia persists for several weeks even after the drug is stopped, possibly because some of the IgG antibodies formed have drug-independent platelet reactivity.

The pathogenesis of DITP involves formation of a ternary complex involving a platelet glycoprotein (usually, the GPIb/IIIa complex, less often GPIb/IX), drug (or drug metabolite), and the Fab terminus of IgG. Such a mechanism has been invoked for quinine, quindine, sulfonamide, rifampin, vancomycin, and pentamidine, among others. Unlike the mechanism of HIT, platelet FcεRs are not involved. Furthermore, the drug does not function as a hapten; that is, drug-dependent IgG does not bind to platelets that have been washed after pretreatment with the implicated drug. Limited evidence of a hapten mechanism of DITP has been suggested only for penicillin; that is, penicillin-dependent IgG binds to platelets that have been washed after pretreatment with penicillin. A proposed model suggests that drug-dependent antibodies are derived from a pool of naturally occurring antibodies with weak affinity for self antigens residing on platelet membrane glycoproteins; certain drugs are able to affect both antibody and antigen in such a way that their interaction is greatly enhanced, provided that B cells expressing such antibodies are induced to produce these.

In a case of suspected DITP, as many drugs as possible should be discontinued. If further drug treatment is necessary, an immunologically non-cross-reactive substitute should be prescribed. Platelet transfusions should be given to patients with life-threatening bleeding or who are judged to be at high risk of bleeding (e.g., severe thrombocytopenia plus "wet purpura"). High-dose IVIG, 1 g/kg given over 6–8 hours for two consecutive days, may be of value, but can be ineffective if the relevant drug is not discontinued. Corticosteroids are relatively ineffective in the management of this condition.

Atypical drug-induced immune thrombocytopenia

Glycoprotein IIb/IIIa antagonist-induced thrombocytopenia

Thrombocytopenia is a relatively common side effect of the three approved GPIIb/IIIa antagonists—abciximab, tirofiban, and eptifibatide. Often, the thrombocytopenia begins within hours of a first exposure; this is caused by naturally occurring antibodies. In other patients, the thrombocytopenia begins about one week after initial—or abruptly upon repeat—drug administration; this clinical presentation reflects drug-induced antibody formation.

Abciximab (ReoPro, Eli Lilly) is a humanized chimeric Fab fragment of a murine monoclonal antibody specific for an epitope on GPIIIa. It is used to prevent restenosis after coronary angioplasty. Approximately 0.5% of patients have moderate or severe thrombocytopenia within several hours of treatment with this drug. Although approximately 20% of the normal population have antibodies that react against the papain cleavage site in abciximab, the remainder (1.6% overall) react against murine sequences incorporated into abciximab that confer specificity for GPIIb/IIIa. It is this latter group of antibodies that evinces pathogenicity. Perhaps surprisingly, given the degree of thrombocytopenia and use of a major platelet glycoprotein inhibitor, most patients do not have petechiae or bleeding (although fatal bleeding episodes have been reported). Treatment with platelet transfusions and, perhaps, IVIG may benefit bleeding patients. For approximately one-third of patients with apparent abciximab-associated thrombocytopenia, examination of the blood film shows platelet clumping. This finding suggests pseudothrombocytopenia (ex vivo platelet clumping) caused by abciximab. Such patients are not at risk for bleeding and do not need treatment.

Tirofiban and eptifibatide are synthetic compounds that mimic or contain the RGD (arg-gly-asp) peptide and bind tightly to the RGD recognition site in GPIIb/IIIa. As with abciximab, the frequency of naturally occurring antibodies (approximately 1–2%) correlates roughly with their risk of inducing abrupt-onset thrombocytopenia upon first exposure. Delayed-onset thrombocytopenia beginning about one week after exposure has also been reported. For tirofiban, some drug-dependent antibodies produce platelet activation and increased risk of ischemic events.

Drug-induced autoimmune thrombocytopenia

Approximately 1–3% of patients treated with gold have thrombocytopenia that sometimes persists for weeks or months despite stopping the drug; thus, the disorder resembles chronic ITP. It remains uncertain whether this is true drug-induced autoimmune thrombocytopenia or is caused by gold-dependent IgG antibodies that are slowly released from tissues. Procainamide, α-methyldopa, sulphonamide antibiotics, and interferons alfa and beta also may cause autoimmune thrombocytopenia. In extremely rare instances, measles–mumps–rubella vaccination causes an acute ITP-like illness in which anti-GPIIb/IIIa IgG is formed.

Drug-induced thrombotic microangiopathy

Drugs may cause a thrombotic microangiopathy (TMA) that closely resembles TTP or HUS. Two general mechanisms for drug-induced TMA include immune (idiiosyncratic, non-dose-dependent) and toxic (dose-dependent). Immune-mediated drug-induced TMA is caused by quinine, with endothelium and other target cells affected by quinine-dependent antibodies. Paradoxically, immune-mediated TMA can even be caused by the antplatelet agents ticlopidine and clopidogrel. Ticlopidine-induced TTP is estimated to occur in 1 in 2000–5000 patients who receive this drug after coronary stenting. The characteristic onset is between one and eight weeks after administration of the drug is started. Clopidogrel-induced TTP occurs in approximately 1 in 20,000 recipients, generally within the first two weeks of treatment. Autoantibodies to von Willebrand factor–cleaving metalloproteinase have been identified in these patients and may contribute to the pathogenesis.

Other drugs that cause an illness that resembles HUS due to toxic effects include antineoplastic chemotherapy (e.g., gemcitabine, mitomycin C, cisplatin, and bleomycin) and immunosuppressive agents (e.g., cyclosporine and tacrolimus). Because some of the medical conditions leading to use of these drugs can be associated with microangiopathic blood film changes (neoplasia, organ rejection, graft-vs.-host disease, and vasculitis), causal relationships to the various drugs listed can be problematic.

The mortality of drug-induced TMA is high. Early recognition and discontinuation of the drug are essential. Response to plasma exchange has been observed. Many physicians would also give corticosteroids, although the efficacy remains unknown. Specific therapy for drug-induced HUS (dialysis, plasmapheresis, and corticosteroids) is individualized depending on the clinical situation. The prognosis is poor for drug-induced TTP in the setting of hematopoietic stem cell transplantation (HSCT).

Heparin-induced thrombocytopenia

Heparin-induced thrombocytopenia is a relatively common, IgG-mediated, adverse reaction to heparin that has a strong association with venous and arterial thrombosis. Heparin-induced
thrombocytopenia is a clinicopathologic syndrome; that is, the diagnosis is made most reliably on both clinical and serologic grounds.147–149 Thus, HIT antibody formation without thrombocytopenia or other abnormalities is not HIT, whereas HIT antibody formation accompanied by an otherwise unexplained 50% or greater postoperative decrease in platelet count (even if the platelet count remains greater than 150,000/µL) or complicated by necrotizing skin lesions at heparin injection sites146 are examples of HIT syndrome. Indeed, the thrombocytopenia usually is much less severe in HIT4 than in DITP130 or GPIIb/IIIa antagonist-induced thrombocytopenia134 (Figure 21.3). Another contrast from DITP is that even when severe thrombocytopenia occurs in HIT, petechiae and other types of bleeding typically are not observed.146,150 Indeed, even the one characteristic hemorrhagic complication of HIT—bilateral adrenal hemorrhage—is caused by thrombosis (adrenal vein thrombosis leading to hemorrhagic necrosis).146 HIT is an antibody-mediated disorder, and a minimum of five days is required for an immunizing exposure to heparin to generate sufficient levels of antibodies to cause thrombocytopenia.146 Sometimes, onset of thrombocytopenia and thrombosis begins—or worsens—after all heparin has been stopped (“delayed-onset” or “autoimmune-like” HIT).151–153 Some patients who have not previously been exposed to heparin will develop thrombocytopenia, thrombosis, and have detectable platelet-activating anti-PF4/heparin antibodies; such patients with “spontaneous HIT” have often had recent infection or surgery.154

The target antigen recognized by HIT antibodies consists of a multimolecular complex between PF4 (a platelet α-granule protein of the CXC family of chemokines) and heparin.2,3 The HIT antibodies bind to one or more PF4 regions that have undergone conformational modification through binding to heparin. The formation of the antigen is somewhat nonspecific, because PF4 can be rendered antigenic by binding to certain other polyanions, such as pentosan polysulfate or polyvinylsulfonate.22 At least 12–14 saccharide units are needed for heparin to form the antigen complex with PF4. This may explain why LMW heparin preparations are less immunogenic than unfractionated heparin and are less likely to cause HIT.2,3 Although LMW heparin sometimes causes HIT, it is likely that very small heparin preparations (e.g., fondaparinux, a synthetic anti-Factor Xa-binding pentasaccharide) or specially engineered heparins (e.g., highly sulfated heparin moieties bridged with nonsulfonlated spacer regions) only rarely cause HIT.155,156

Figure 21.4 illustrates several possible mechanisms to explain the intense thrombin generation that occurs in HIT.157,158 These include the formation of procoagulant, platelet-derived microparticles159 that result from cell signaling triggered by clustering of platelet FcγRIIa. Recent data suggest that in situ formation of IgG-PF4/heparin complexes on the platelet surface leads to platelet activation.160 In vivo platelet activation is suggested by expression of P-selectin by circulating platelets in HIT as well as by increased levels of circulating microparticles. Tissue factor expression by endothelium161 or monocytes162 activated by HIT antibodies that recognize PF4 bound to surface glycosaminoglycans comprises other possible procoagulant events.

Marked in vivo thrombin generation helps explain several clinical features of HIT, including its association with venous and arterial thrombosis (hypercoagulable state), the occurrence of decompen-sated DIC with low fibrinogen levels and/or elevated prothrombin time in approximately 10–20% of patients, and the potential for deep vein thrombosis (DVT) to progress to venous limb gangrene, particularly in patients treated with warfarin.158 This last syndrome results from impaired procoagulant–antiocoagulant balance: Warfarin-induced protein C depletion leads to microvascular thrombosis caused by ongoing intense thrombin generation. Patients with warfarin-induced venous gangrene typically have a supratherapeutic international normalized ratio (INR), usually more than 3.5. The explanation is a concomitant severe decrease in factor VII that parallels the decrease in protein C. The importance of in vivo thrombin generation in HIT provides a rationale for consensus recommendations that an agent that reduces thrombin generation or directly inactivates thrombin be used for the management of this syndrome.147,148,163

The frequency of HIT varies among different patient populations. Medical patients appear to develop HIT less often than do surgical

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**Figure 21.3** Platelet count nadirs (log10 scale) and clinical profile of classic drug-induced immune thrombocytopenic purpura (DITP) and heparin-induced thrombocytopenia (HIT). Classic DITP (e.g., caused by quinine, vancomycin, or glycoprotein IIb/IIIa antagonists, among many others) typically produces severe thrombocytopenia (platelet count nadir, ∼10,000/µL) and associated mucocutaneous bleeding. In contrast, HIT typically results in mild to moderate thrombocytopenia (median platelet count nadir, ∼60,000/µL) and associated venous or arterial thrombosis. Note that the relative heights of the two peaks are not drawn to scale; HIT is much more common than all other causes of DITP combined. Source: Warkentin (2007). Reproduced with permission of Massachusetts Medical Society.
patients, and female gender is a minor risk factor for HIT.\footnote{\textsuperscript{164}} The frequency of HIT can be as high as 5\% when multiple risk factors are present (thromboprophylaxis with unfractionated heparin administered for two weeks to a female patient following major surgery), whereas the risk is very low or negligible in other settings (e.g., administration of LMW heparin during pregnancy).\textsuperscript{2,3,165}

Iceberg model of HIT

Figure 21.5 illustrates the relation between clinical HIT and detectability of anti-PF4/heparin antibodies by either washed platelet activation assay or PF4-dependent EIA.\textsuperscript{166} The key concept is that whereas both types of assay have high sensitivity for diagnosis of clinical HIT, the former assay has greater diagnostic specificity. It is estimated that substantial overdiagnosis (~50\%) of HIT will result\textsuperscript{167} if a positive EIA of any magnitude is considered diagnostic of HIT irrespective of the patient’s clinical likelihood of HIT, as judged using a clinical scoring system (the 4Ts)\textsuperscript{168} and the gold standard assay (platelet SRA).

Management of heparin-induced thrombocytopenia-associated thrombosis

Results of prospective and retrospective studies\textsuperscript{3,4,150} indicate that approximately 50–75\% of patients with HIT have new, progressive, or recurrent thrombosis. Thus, the need for an alternative anticoagulant is a common issue in the care of these patients.

\textit{Lepirudin} is a direct thrombin inhibitor (DTI) derived from hirudin (leech anticoagulant protein) manufactured by means of recombinant technology. Although it is approved for treatment of HIT complicated by thrombosis, it was discontinued by the manufacturer in 2012.

\textit{Argatroban} (Novastan, GlaxoSmithKline) is a small-molecule DTI approved in the United States for the management and prevention of HIT-associated thrombosis.\textsuperscript{148,169} Features of argatroban include its short half-life (40–50 minutes) as well as its hepatic route of metabolism. No dose adjustments are needed for patients with moderate renal failure, although dose reduction is needed in the presence of hepatic insufficiency. Anticoagulant monitoring is usually performed using the aPTT; although simple, aPTT monitoring can result in systematic underdosing of argatroban in patients with baseline (pretreatment) elevation in aPTT, for example because of severe HIT-associated disseminated intravascular coagulation (“aPTT confounding”).\textsuperscript{170,171} Argatroban also prolongs the INR, which complicates the transition from argatroban to warfarin therapy. It is important to postpone introduction of warfarin until the thrombocytopenia has substantially recovered (platelet count at least 150,000/\textmu L).\textsuperscript{148}

\textit{Danaparoid sodium} (Orgaran, Aspen Pharmacare) is a mixture of anticoagulant glycosaminoglycans with predominant anti–Factor Xa activity that decreases thrombin generation in patients with HIT.\textsuperscript{172} A randomized trial showed a higher thrombosis resolution rate among patients treated with danaparoid and warfarin than among those treated with dextran and warfarin, especially for patients with severe thrombosis (92\% vs. 33\%; \(p < 0.001\)).\textsuperscript{173} Although some HIT sera show in vitro cross-reactivity with

Figure 21.4 Pathogenesis of heparin-induced thrombocytopenia. Two explanations for thrombosis in HIT are presented. Activation of platelets by platelet factor 4 (PF4)–heparin 1G antibodies leads to formation of procoagulant, platelet-derived microparticles. Together with neutralization of heparin by PF4 released from activated platelets, there results a marked increase in thrombin generation. Such a “hypercoagulability state” is characterized by an increased risk of venous and arterial thrombosis, as well as predisposition to warfarin-associated microvascular thrombosis (e.g., venous limb gangrene). However, it is also possible that other unique pathogenetic mechanisms operative in HIT explain unusual thromboses, such as arterial “white clots.” For example, HIT antibodies have been shown to activate endothelium and monocytes (leading to cell surface tissue factor expression), although this stimulation may be largely indirect through poorly defined mechanisms involving platelet activation and, possibly, formation of platelet-derived microparticles. Furthermore, aggregates of platelets and polymorphonuclear (PMN) leukocytes have been described in HIT. To what extent these cooperative interactions between platelets, microparticles, PMN leukocytes, monocytes, and endothelium lead to arterial or venous thrombotic events in HIT, either in large or small vessels, remains unclear. Source: Warkentin and Kelton (1996).\textsuperscript{153} Reproduced with permission of American College of Physicians.
There are several important contraindications to therapy for acute HIT, including warfarin monotherapy and LMW heparin.\textsuperscript{148,163} Warfarin therapy can lead to acute depletion of protein C, which can cause microvascular thrombosis in HIT and lead to venous limb gangrene.\textsuperscript{159} However, once thrombocytopenia has resolved, it is reasonable to overlap warfarin cautiously with one of the agents that can reduce thrombin generation in HIT (danaparoid, fondaparinux, and argatroban). Use of LMW heparin is contraindicated because of a high chance of treatment failure (approximately 50% of patients have further thrombocytopenia or thrombosis).\textsuperscript{148} Prophylactic platelet transfusion is relatively contraindicated because even patients with severe thrombocytopenia do not usually have evidence of hemostatic dysfunction, such as petechiae, and platelet transfusions theoretically may contribute to increase risk of thrombosis.\textsuperscript{148,149} However, recent observational studies have questioned whether platelet transfusion in HIT is associated with thrombotic events.\textsuperscript{179,180}

Management of isolated heparin-induced thrombocytopenia
Isolated HIT is defined as HIT that is recognized because of thrombocytopenia without evidence of HIT-associated thrombosis.\textsuperscript{150} Unfortunately, simply stopping administration of heparin or substituting warfarin for heparin is inadequate treatment for these patients. In a large retrospective cohort study,\textsuperscript{150} the risk of thrombosis among these patients was approximately 10% at 2 days, 40% at 7 days, and 53% at 30-day follow-up evaluations. Other investigators\textsuperscript{181} with a similar approach subsequently found a 38% rate of thrombotic events despite stopping administration of heparin. The frequency of thrombosis surprisingly was not any lower in the subgroup of patients for whom heparin was stopped fairly promptly (<48 hours) after the onset of thrombocytopenia than it was among patients with later cessation of heparin (45 vs. 34%; \(p = 0.26\)).

For patients strongly suspected (or confirmed) to have isolated HIT, the authors discontinue heparin, start administration of an alternative rapidly acting anticoagulant, and screen for subclinical DVT by means of compression ultrasonography (approximately
50% of patients are shown to have DVT with this approach).148 Whether or not thrombosis is found, a therapeutic dose of anti-coagulant is given to these patients. This is because prophylactic-dose anticoagulation appears to have a higher failure rate than does therapeutic-dose anticoagulation.174 After platelet count recovery, the absence of venous thrombosis is confirmed before discharge. For patients found at initial or follow-up imaging to have venous thrombosis, overlapping warfarin anticoagulation is usually begun for longer term antithrombotic control, although recent reports have indicated subsequent outpatient treatment with one of the new oral anticoagulants (e.g., rivaroxaban or dabigatran).182,183

Reexposure to heparin in a patient with a history of heparin-induced thrombocytopenia
Patients who have circulating HIT antibodies can have an abrupt decrease in platelet count if heparin is administered. However, the risk of such abrupt-onset HIT on reexposure to heparin is restricted to the first few months after use of heparin. This is because HIT antibodies begin to decline after heparin is discontinued, and usually they are no longer detectable by the 3-month follow-up evaluation.184 Under exceptional circumstances (e.g., need to perform heart or vascular surgery), it is recommended to readminister heparin to a patient with previous HIT, provided that HIT antibodies are no longer detectable with a sensitive and reliable assay (e.g., SRA or PF4-dependent ELA).148,184,185 Such patients often do not form HIT antibodies after the brief reexposure to heparin, and if they do, these antibodies are not formed before Day 5. Nevertheless, it seems prudent to limit the heparin reexposure to the operation itself and to use an alternative anticoagulant for perioperative anticoagulation.

Alloimmune thrombocytopenia
Alloantigens are genetically determined molecular variations of proteins or carbohydrates that can be recognized immunologically by some healthy persons. Exposure to alloantigens occurs during pregnancy, transfusion, or transplantation. If alloantibodies form against platelet alloantigens, alloimmune thrombocytopenia can result from platelet clearance mediated by the reticuloendothelial system. Five alloimmune thrombocytopenic disorders have been described (Table 21.3),186 the most common being NAIT.

Alloantigens
More than 20 platelet alloantigens have been identified.186–191 Table 21.4 classifies the platelet alloantigens by glycoprotein localization and gene frequency, the latter divided into public and private (or low frequency, arbitrarily less than 0.02). A database of genetically confirmed alloantigens is maintained by the European Bioinformatics Institute with 28 antigens in the current list (http://www.ebi.ac.uk). More than one-half of the alloantigens that have been identified are located on one of the two glycoproteins that constitute the GPIIb/IIIa complex (platelet fibrinogen receptor). One of these alloantigens, HPA-1a (previously, PlA1), is located on GPIIa. It is responsible for most alloimmune thrombocytopenia in populations of European ancestry, including almost all patients with severe alloimmune thrombocytopenia. The other major platelet glycoprotein complex (GPIIb/IX, von Willebrand factor–binding complex) is rarely implicated in alloimmune thrombocytopenia. However, the GPIa/IIa complex (platelet collagen receptor), which bears the HPA-5a/5b (Bra/b; Zav/b) alloantigen system, is a relatively common cause of moderately severe alloimmune thrombocytopenia.192 The HPA-15a/15b (Gov/b) alloantigen system is expressed on CD109. It has been shown to be a relatively common cause of alloimmune thrombocytopenia that, like HPA-5a/5b, tends not to be severe.193

Immunogenetics and frequency of alloimmune thrombocytopenia
The HPA-1a alloantigen is far more immunogenic than its corresponding allele, HPA-1b. For example, consider the frequency of NAIT caused by either anti-HPA-1a or anti-HPA-1b alloantibodies in relation to the genotype frequency of HPA-1a (0.85) and HPA-1b (0.15). Thus, a homozygous HPA-1bb (PlA1-negative) female, representing approximately 2% (0.15 × 0.15) of the population, would have an 85% probability of being exposed to the HPA-1a alloantigen during pregnancy. In contrast, a homozygous HPA-1aa female, representing approximately 72% (0.85 × 0.85) of the population, would have a 15% probability of being exposed to the HPA-1b alloantigen during pregnancy. Thus, if both alloantigens were equally immunogenic, one would expect anti-HPA-1b to occur approximately six times more often than anti-HPA-1a: (0.72 × 0.15)/(0.02 × 0.85) = 6.4. However, the opposite is actually observed: anti-HPA-1a is far more common than anti-HPA-1b (Table 21.5).192,194 In a study of 348 cases of suspected NAIT,192 only one case caused by anti-HPA-1b was found, compared with 144 cases of proven or suspected NAIT secondary to anti-HPA-1a. Thus, the observed ratio of NAIT caused by anti-HPA-1a/anti-HPA-1b (1:144, or 0.007) is almost 1000 times less than predicted by the theoretical ratio (6.4).

Immunogenetics is a major factor determining alloimmunization against HPA-1a. There is a strong association between formation of anti-HPA-1a and HLA-DRB3*0101 and HLA-DQB1*0201 (odds ratio, 25 and 40, respectively).195 In contrast, no HLA association exists for immunization against HPA-1b.196 Thus, it appears that persons with certain HLA genotypes are much more likely to generate an alloimmune response when GPIIIa bears the leucine substitution that determines the HPA-1a phenotype.

Overall, on the basis of the observed allelic frequencies, the expected theoretical ratio of NAIT for anti-HPA-5a, compared with anti-HPA-5a, should be approximately 8 (Table 21.5). A similar ratio (47:3, or 15.7) has been observed. However, although the expected and observed ratios are similar (contrasting the HPA-1a/1b system), a role for immunogenetics and alloimmunization exists also for the HPA-5a/5b system.197

Severity of alloimmune thrombocytopenia
In general, the severity of thrombocytopenia is greater for alloimmune thrombocytopenia that involves the GPIIIa/IIa complex, compared with the GPIIa/IIa complex (Table 21.6).186,192,194,198 Because there are approximately 20 times more GPIIIa/IIa molecules compared with GPIIa/IIa complexes (40,000 vs. 2000), this

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**Table 21.3** Five alloimmune thrombocytopenic syndromes

<table>
<thead>
<tr>
<th>Classical Alloimmune Thrombocytopenic Syndromes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal alloimmune thrombocytopenia</td>
</tr>
<tr>
<td>Posttransfusion purpura</td>
</tr>
<tr>
<td>Other Alloimmune Thrombocytopenic Syndromes</td>
</tr>
<tr>
<td>Passive alloimmune thrombocytopenia</td>
</tr>
<tr>
<td>Transplantation-associated alloimmune thrombocytopenia</td>
</tr>
</tbody>
</table>

Table 21.4 Platelet antigens classified according to glycoprotein location and gene frequency

<table>
<thead>
<tr>
<th>Platelet-Specific Alloantigen (Alternative Nomenclature)</th>
<th>GP</th>
<th>Gene Frequency in Whites</th>
<th>NAIT</th>
<th>PTP</th>
<th>PAT</th>
<th>TAT</th>
<th>PTR</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPIb/IIa: Public (Gene Frequency &gt; 0.02)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPA-1a (PlA1, Zwa)</td>
<td>Ilα</td>
<td>0.85</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>HPA-1b (PlA1, Zwb)</td>
<td>Ilα</td>
<td>0.15</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>(+)</td>
</tr>
<tr>
<td>HPA-3a (Bakε, Lekε)</td>
<td>Ilb</td>
<td>0.61</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>(+)</td>
</tr>
<tr>
<td>HPA-3b (Bakδ)</td>
<td>Ilβ</td>
<td>0.39</td>
<td>?</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>(+)</td>
</tr>
<tr>
<td>HPA-4a (Penε, Yukδ)</td>
<td>Ilα</td>
<td>&gt;0.99</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>(+)</td>
</tr>
<tr>
<td>GPIb/IIa: Private/Low-Frequency (Gene Frequency &lt; 0.02)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPA-4b (Penα, Yukα)</td>
<td>Ilα</td>
<td>&lt;0.01</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HPA-6b (Tuα, Catf)</td>
<td>Ilα</td>
<td>0.003</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HPA-7b (Bakδ)</td>
<td>Ilβ</td>
<td>&lt;0.01</td>
<td>++</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HPA-7c (Hτc)</td>
<td>Ilα</td>
<td>&lt;0.01</td>
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<td>—</td>
<td>—</td>
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<td>—</td>
</tr>
<tr>
<td>HPA-8b (Srβ)</td>
<td>Ilα</td>
<td>&lt;0.003</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HPA-9b (Maxα)</td>
<td>Ilb</td>
<td>0.002</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HPA-10b (Saα)</td>
<td>Ilα</td>
<td>&lt;0.01</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HPA-11b (Gnb)</td>
<td>Ilα</td>
<td>&lt;0.001</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HPA-14b (Oεb)</td>
<td>Ilα</td>
<td>&lt;0.005</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HPA-16b (Dvu)</td>
<td>Ilα</td>
<td>&lt;0.01</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Vα⁺</td>
<td>Ilβ Iliα</td>
<td>&lt;0.002</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>GP Ia/IIa: Public</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPA-5a (Br⁺, Zav⁺)</td>
<td>Ia</td>
<td>0.89</td>
<td>+</td>
<td>?</td>
<td>—</td>
<td>—</td>
<td>(+)</td>
</tr>
<tr>
<td>HPA-5b (Br⁺, Zav⁺, Hc⁺)</td>
<td>Ia</td>
<td>0.11</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>GP Ia/IIa: Private</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPA-13b (Sil)</td>
<td>Ia</td>
<td>0.0025</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Swa⁺</td>
<td>Ia</td>
<td>&lt;0.002</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>GP Ib/IX: Public</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPA-2a (Kο⁺)</td>
<td>Ibx</td>
<td>0.89</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>?</td>
</tr>
<tr>
<td>HPA 2b (Kο⁺, Sib⁺)</td>
<td>Ibx</td>
<td>0.11</td>
<td>+</td>
<td>?</td>
<td>—</td>
<td>—</td>
<td>(+)</td>
</tr>
<tr>
<td>GP Ib/IX: Private</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPA-12b (Iy)</td>
<td>Ibx</td>
<td>&lt;0.01</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CD109: public</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPA-15a (Govα)</td>
<td>CD109</td>
<td>0.53</td>
<td>+</td>
<td>?</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HPA-15b (Govβ)</td>
<td>CD109</td>
<td>0.47</td>
<td>+</td>
<td>?</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>GP 38</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dy⁺</td>
<td>38 kD</td>
<td>&lt;0.01</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Platelet Nonspecific Alloantigens</td>
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<td></td>
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<tr>
<td>ABO</td>
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<td>—</td>
<td>—</td>
<td>—</td>
<td>++</td>
<td>—</td>
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<tr>
<td>HLA</td>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>++</td>
<td>—</td>
</tr>
</tbody>
</table>

GP, glycoprotein; NAIT, neonatal alloimmune thrombocytopenia; PTP, posttransfusion purpura; PAT, passive alloimmune thrombocytopenia; TAT, transplantation-associated alloimmune thrombocytopenia; PTR, platelet transfusion refractoriness.

+, relatively common; +, established but rare; —, not reported; (+), probable association, but definitive link inconclusive; ?, possible association but not established. Also see the current list of polymorphisms on the European Bioinformatics Institute website (http://www.ebi.ac.uk/ipd/hpa/table1.html)


Table 21.5 Observed frequencies of neonatal alloimmune thrombocytopenia and posttransfusion purpura in relation to expected (theoretic) frequency of the HPA-1ab (PlA1); HPA-3ab (Br⁺, Zav⁺); and HPA-3ab (Bakε, Lekε) alloantigen systems

<table>
<thead>
<tr>
<th>Target Alloantigen</th>
<th>Percentage of Pregnancies at Theoretic Risk of NAIT (Descending Order)</th>
<th>Observed Cases of NAIT¹</th>
<th>Observed Cases of PTP⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPA-3b</td>
<td>14.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HPA-1b</td>
<td>10.8</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>HPA-3a</td>
<td>9.3</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>HPA-5b</td>
<td>8.7</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>HPA-1α</td>
<td>1.9</td>
<td>44</td>
<td>105</td>
</tr>
<tr>
<td>HPA-5a</td>
<td>1.1</td>
<td>0</td>
<td>7</td>
</tr>
</tbody>
</table>

¹ Percentage of pregnancies at theoretic risk of NAIT for a given target alloantigen is determined as follows: x(1 – x)² x 100, where x is the gene frequency of the target alloantigen. Note the lack of correlation between the theoretical and observed risk for NAIT.

² Data are from Mueller-Eckhardt et al. and represent serologic investigations using a defined protocol over an 18-month period ending June 30, 1988.

³ For comparison, the serologic findings for cases of PTP are shown for which only one platelet alloantigen specificity was identified (from January 1990 to August 2006 at the Blood Center of Wisconsin).

⁴ NAIT, neonatal alloimmune thrombocytopenia; PTP, posttransfusion purpura.


suggests that greater numbers of alloantibodies binding to the more numerous GPIb/IIa receptors result in greater platelet destruction. Similarly, thrombocytopenia is less severe when alloimmunization is to HPA-15 on CD109 (2000 receptors). Neonatal alloimmune thrombocytopenia

NAIT is a transient but potentially life-threatening thrombocytopenic disorder limited to fetal and neonatal life. It is caused by maternal IgG alloantibodies that cross the placenta and cause premature destruction of platelets bearing paternally derived platelet alloantigens (analogous to hemolytic disease of the fetus and newborn). NAIT occurs in approximately 1 to 1.5 per 1000 live births.

Approximately 75% of cases in a population of European ancestry are caused by fetomaternal incompatibility for the platelet-specific alloantigen HPA-1a, and 20% by HPA-5b. Other alloantigens implicated in NAIT, including private alloantigens identified in only one or a few families (e.g., HPA6b [Tu⁺/Ca⁺] and HPA-7b [Mo⁺]), are shown in Table 21.4. In East Asian populations, anti-HPA-4b (Penα) is more common than anti-HPA-1a. Although HLA or ABO alloantibodies have been claimed to cause NAIT, in most...
The common HPA antigens.204 A minority of NAIT cases unresolved following investigations for large studies indicates that low-frequency antigens account for only mothers with infants believed to have had NAIT, anti-HPA-1a bocytopenia. Indeed, for approximately one-fourth of HPA-1bb (PlA1-negative) status confers risk of NAIT caused by anti-HPA-1a. The third step is to determine whether the mother has certain platelet alloantigens that often are associated with alloimmunization. Commonly, the mother lacks and paternal platelets to determine whether they are incompatible for a major platelet alloantigen. Commonly, the mother lacks platelet alloantigens in her serum. Sometimes, no alloantibodies can be detected in maternal serum despite severe neonatal thrombocytopenia. Indeed, for approximately one-fourth of HPA-1bb mothers with infants believed to have had NAIT, anti-HPA-1a cannot be detected.192 The potential for low-incidence platelet-specific alloantigens to explain fetomaternal incompatibility means that maternal serum should be tested against paternal platelets whenever possible. Although maternal immunization to low-frequency antigens can explain some cases of NAIT,203 evidence from large studies indicates that low-frequency antigens account for only a minority of NAIT cases unresolved following investigations for the common HPA antigens.204

Table 21.6 Severity of thrombocytopenia by platelet count nadirs (x1000/μl) in relation to target glycoprotein for various alloimmune thrombocytopenic syndromes

<table>
<thead>
<tr>
<th>Glycoprotein</th>
<th>NAIT</th>
<th>PTP</th>
<th>PAT</th>
<th>TAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPIb/IIa</td>
<td>17 (n = 81)</td>
<td>6 (n = 43)</td>
<td>8 (n = 9)</td>
<td>8 (n = 4)</td>
</tr>
<tr>
<td>HPA-1a (IIa)</td>
<td>9 (n = 2)</td>
<td>5 (n = 4)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HPA-3ab (IIb)</td>
<td>10 (n = 5)</td>
<td>3 (n = 4)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HPA-4ab (IIia)</td>
<td>13 (n = 7)</td>
<td>6 (n = 1)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mean</td>
<td>16</td>
<td>6</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>GPIa/IIa</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HPA-5b</td>
<td>44 (n = 48)</td>
<td>26 (n = 1)</td>
<td>35 (n = 1)</td>
<td>43 (n = 1)</td>
</tr>
<tr>
<td>HPA-5a</td>
<td>35 (n = 5)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mean</td>
<td>43</td>
<td>26</td>
<td>35</td>
<td>43</td>
</tr>
</tbody>
</table>
*Data from Warkentin and Smith177 and Brunner-Bolliger et al.178 The data show that alloimmune thrombocytopenic syndromes that involve GPIb/IIa are more likely to cause severe thrombocytopenia than are those involving GPIa/IIa. The data are combined for alloimmune thrombocytopenic syndromes involving either allele of the HPA-3ab and HPA-4ab alloantigen systems, whereas the data are shown separately for the alleles of the HPA-1ab and HPA-5ab systems. NAIT, neonatal alloimmune thrombocytopenia; PTP, posttransfusion purpura; PAT, passive alloimmune thrombocytopenia; TAT, transplantation-associated alloimmune thrombocytopenia.

The typical clinical presentation of NAIT is isolated severe thrombocytopenia in an otherwise healthy neonate, especially if fetomaternal incompatibility involves an alloantigen on the GPIb/IIa complex (Table 21.6). Petechiae are found in 90%; gastrointestinal tract hemorrhage in 30%; and hemoptysis, hematuria, and retinal bleeding in fewer than 10% of patients. Isolated intraocular hemorrhage is rare.202 Approximately 15% have intracranial hemorrhage.192 The thrombocytopenia usually resolves within 1–3 weeks. Serious sequelae of fetal and neonatal intracranial bleeding include hydrocephalus, porencephalic cysts, and epilepsy. First-born offspring constitute approximately one-half of patients. This suggests that, unlike the situation for hemolytic disease of the fetus and newborn, sensitization can occur early during the first pregnancy.192 Subsequently affected siblings usually have thrombocytopenia to a similar or greater extent, an observation used to emphasize preventive treatment in subsequent pregnancies. In the case of heterozygous fathers of affected children, an antigen risk assessment should be performed using fetal cells in future pregnancies.

Laboratory investigation of suspected NAIT involves three steps. Firstly, there must be a high index of suspicion: Isolated thrombocytopenia in an otherwise well infant should be assumed to indicate NAIT until proved otherwise. The second step is to type maternal and paternal platelets to determine whether they are incompatible for a major platelet alloantigen. Commonly, the mother lacks certain platelet alloantigens that often are associated with alloimmune thrombocytopenia; for example, maternal homozygous HPA-1bb (PIA1-negative) status confers risk of NAIT caused by anti-HPA-1a. The third step is to determine whether the mother has platelet alloantibodies in her serum. Sometimes, no alloantibodies can be detected in maternal serum despite severe neonatal thrombocytopenia. Indeed, for approximately one-fourth of HPA-1bb mothers with infants believed to have had NAIT, anti-HPA-1a cannot be detected.192 Debate continues as to whether the titer of alloantibodies predicts severity of fetal thrombocytopenia. The maternal alloantibody is sometimes detectable for years following the birth of the child with NAIT. Patients with a history of NAIT must not donate blood because their plasma can trigger passive alloimmune thrombocytopenia. It is possible that these patients may also be at risk for transfusion reactions and PTP should they receive incompatible blood products in future.

Neonatal treatment

The optimal treatment of a neonate in whom NAIT is suspected because of severe thrombocytopenia is to increase the platelet count urgently to safe levels, even before serologic confirmation of the diagnosis. In some centers (e.g., National Blood Service in the United Kingdom), HPA-1bb and HPA-5bb platelets can be obtained upon request. These should be effective for more than 95% of patients.205 When matched platelets are not available, washed and irradiated maternal platelets should be given to the neonate. These platelets are obtained by apheresis, and are washed to remove the maternal alloantibodies. Irradiation is performed to prevent graft-versus-host disease caused by maternal lymphocytes. In an emergency, immediate administration of whole blood–derived platelets obtained from random donors may be of benefit to a bleeding infant.206,207

Giving high-dose IVIG to the neonate increases the platelet count of approximately 65% of patients.208 This treatment should be combined with maternally derived platelets. Corticosteroids are not recommended.

Prenatal management

About one-half of the time, NAIT is suspected during the prenatal period, usually because the mother previously bore an affected infant, although the diagnosis sometimes is suggested in utero when fetal ultrasonography shows cerebral hemorrhage, hydrocephalus, or hydrops fetalis. One tenet of management is that thrombocytopenia in a subsequently affected offspring is generally as severe as, or more severe than, a previously affected sibling. Neonatal alloimmune thrombocytopenia caused by anti-HPA-1a is more likely to cause fetal morbidity and mortality than that caused by anti-HPA-5b and usually requires more aggressive treatment. When the father is known to be heterozygous for the implicated alloantigen (a situation that occurs approximately 25% and 20% of the time for...
NAIT involving the HPA-1a/1b and HPA-5a/5b systems, respectively), prenatal fetal typing is important, because it identifies the infant who is homozygous for the maternal antigen and is not at risk, obviating further treatment. For pregnancies at risk, general advice to the mother includes avoiding aspirin and nonsteroidal anti-inflammatory medications.

The general approach that has been taken to manage pregnancies at high risk of severe NAIT205,209 are regular administration of high-dose IVIG, repeated in-utero platelet transfusions, or both. The initial step is to obtain a fetal platelet count by means of percutaneous umbilical blood sampling, generally starting at 20–24 weeks of gestation. Because of the risk of fetal exsanguination, maternal platelets should be on hand for transfusion if the fetal platelet count is shown to be less than 50,000/µL.209 IVIG is given at a dosage of 1 g/kg/week starting within one week of documentation of fetal thrombocytopenia. Fetal blood sampling is repeated 4–6 weeks later; if no response is seen, glucocorticoid salvage treatment (prednisone, 60 mg/day) is started.209 However, not all fetuses respond to this approach.

Another approach, which has been used in certain European centers, involves regular intrauterine platelet transfusions by means of percutaneous umbilical blood sampling, including a short time before delivery. This approach has led to good outcomes in situations in which previous siblings were severely affected.210 However, each fetal platelet transfusion carries risk of hemorrhage and death211 that likely depends on the experience of the fetomaternal unit. There is no consensus on which approach is preferred.

Regardless of the antenatal management, there is consensus that delivery should be by means of elective cesarean section, performed as soon as fetal maturity is documented. The major reason for this mode of delivery is that it allows an organized, multidisciplinary approach to the peripartum care of the newborn. This approach includes urgent determination of the cord platelet count; provision of washed, irradiated maternal platelets (or antigen-negative platelets); and, usually, the use of high-dose IVIG (1 g/kg/day for two consecutive days) to treat severe neonatal thrombocytopenia.

**Posttransfusion purpura**

Posttransfusion purpura is a very rare disorder that typically manifests as severe thrombocytopenia and bleeding that begin 5–10 days after blood transfusion—usually red blood cells (RBCs)—in a patient previously sensitized by pregnancy or transfusion.194 In 85–95% of cases, women are affected; the median age is 52 years. The observation that previous blood transfusions can be sensitizing explains why, on occasion, males develop PTP. Sometimes the presumably sensitizing transfusion occurs only a few weeks earlier; consequently, PTP can present after just a few weeks of intermittent transfusions.194

Although thrombocytopenia usually lasts 1–4 weeks, the duration can be as short as three days212 to as long as four months or more. The platelet count usually is less than 10,000/µL (Table 21.6). Mucocutaneous bleeding (wet purpura, petechiae, epistaxis, gastrointestinal, and urinary tract) is common, and approximately 5–10% of patients die, usually because of intracranial hemorrhage. Because effective treatments are available (discussed further in this chapter), it is important to diagnose PTP promptly to minimize morbidity and mortality. Diagnostic confusion with HIT can result because both syndromes can present 5–10 days following surgery, and sometimes PF4/heparin antibodies are present because of concomitant exposure to heparin.213–215

**Pathogenesis**

Almost invariably, high-titer, platelet-specific alloantibodies are found in the patient’s serum or plasma. Anti-HPA-1a is detected in 60% of cases, although several other platelet alloantigens have been implicated (HPA-1b, -2b, -3a, -3b, -4b, -5a, -5b, and -15b, and the isoantigen CD-36 [Nak]).194 More than one specificity is observed in approximately 15% of cases. As in NAIT, the HLA-DRB3*0101 antigen is found in most HPA-1a-negative patients with PTP. As reported for NAIT, antibodies to HPA-3a may be difficult to detect in some patients with PTP.216

Although platelet-specific alloantibodies are causative, the pathogenesis of PTP remains obscure, and the quandary is that autologous platelets are destroyed. The currently favored hypothesis is that PTP represents a situation in which alloantibodies resulting from reexposure to an incompatible platelet alloantigen have autospecificity (“pseudospecificity”). Although the platelet-specific alloantibodies are detectable for years following an episode of PTP, the autoreactive (or panreactive) antibodies are detectable only during the acute (thrombocytopenic) phase of PTP. In keeping with this view, Taaning and Tønnesen217 reported that panreactive GPIIb/IIIa antibodies are readily detected during, but not after, an episode of PTP. Kiefel et al.218 reported that antibody with allospecificity for HPA-1a, but not for HPA-1b, could be eluted from both autologous and donor HPA-1bb platelets that had been sensitized with acute-phase serum from a PTP patient, suggesting that use of adsorption and elution methods may help distinguish a reactivity profile of PTP sera from that seen with NAIT. One report219 suggests that such alloantibodies with autoreactivity could arise spontaneously, because a woman with HPA-1bb platelets and no history of blood transfusion developed “ITP” with antibodies showing specificity for HPA-1a. Cure of the thrombocytopenia by splenectomy was accompanied by disappearance of the HPA-1a-like antibodies. Studies by another group of investigators220 indicate that two distinct types of antibodies—some with alloreactivity and others with autoreactivity—develop during the acute phase of PTP.

**Treatment**

High-dose IVIG is the treatment of choice for PTP. More than 90% of patients respond, attaining a platelet count greater than 100,000/µL in an average of four days.221 Plasmapheresis may also be used. Although some physicians also give corticosteroids, this agent probably does not influence the course of disease and should be considered adjunctive rather than primary therapy. In rare instances, splenectomy may be considered for a patient refractory to IVIG, corticosteroids, and compatible platelet transfusion.222

Whole blood–derived (unselected) platelets—which are likely to bear the HPA-1a antigen—are usually destroyed quickly, and can cause febrile or even anaphylactoid reactions. Antigen-negative platelets are the preferred component; however, the efficacy of HPA-1a-negative platelet transfusions (for patients with PTP caused by anti-HPA-1a) is also uncertain. Some reports indicate lack of benefit.223 RBC units should be washed224 or filtered225 before administration to remove platelet antigens. Only four patients are known to have developed recurrent PTP with subsequent transfusions.194 Accordingly, for a patient who has recovered from PTP, future precautions usually include avoidance of incompatible blood components (only autologous, washed, or platelet alloantigen-compatible RBCs are given, or platelet alloantigen-compatible plasma or platelet products are given). However, PTP recurrence is
uncommon even if incompatible blood is given, possibly because residual high-titer platelet alloantibodies immediately clear the alloantigens. Patients with a history of PTP must not donate blood because their plasma can trigger passive alloimmune thrombocytopenia (PAT).

**Passive alloimmune thrombocytopenia**

PAT is characterized by an abrupt onset of thrombocytopenia within a few hours after transfusion of a blood component, most often RBCs or plasma.\(^{186}\) PAT is caused by the passive transfer of platelet-reactive alloantibodies in the component that rapidly become the incompatible recipient platelets. In one study, glycoprotein-specific platelet-antibody studies confirmed that the alloantibodies were bound to the recipient’s platelets in vivo.\(^{225}\) Furthermore, although the alloantibody can be detected in the blood donor’s plasma, it may not be detectable in the recipient’s plasma. This finding suggests that almost 100% of the transfused alloantibody binds to target platelets soon after transfusion.\(^{198,225}\) Although anti-HPA-1a is most commonly implicated, antibodies to HPA-3a and -5b have also been reported in this syndrome.\(^{186,198,225}\) In general, the severity of bleeding parallels the degree of thrombocytopenia; thus, spontaneous mucocutaneous bleeding usually occurs only in patients with severe thrombocytopenia caused by anti-HPA-1a. The duration of thrombocytopenia is generally less than one week. It is important to investigate suspected passive alloimmune thrombocytopenia, because the risk that numerous recipients can develop this syndrome means that the implicated blood donor must not donate blood in the future.

**Transplantation-associated alloimmune thrombocytopenia**

In rare instances, alloimmune mechanisms explain thrombocytopenia that occurs in the setting of HSCT or transplantation of solid organs.

**Hematopoietic transplantation**

Panzer et al.\(^ {226}\) reported a 32-year-old man with chronic myeloid leukemia who had severe thrombocytopenia (platelet count, 17,000/μL) beginning 18 months after allogeneic marrow transplantation from his HLA-matched sister. High-dose IVIG produced transient increases in platelet count, and persisting remission followed splenectomy. Antibodies with HPA-1a specificity were eluted from the patient’s platelets. This led to further investigations, which showed that a small number of residual, non-neoplastic lymphoid cells of host origin produced anti-HPA-1a against the HPA-1a-positive platelets formed by donor-derived megakaryocytes. Thus, host-versus-donor alloimmune thrombocytopenia resulted from mixed chimerism, in which residual host lymphoid cells derived from the HPA-1a-negative individual developed an alloimmune response against platelets derived from the engrafted HPA-1a-positive marrow.

A similar situation attributable to anti-HPA-5b after allogeneic marrow transplantation for chronic myeloid leukemia has been reported.\(^ {227}\) However, in this patient, HPA-5b alloantibodies were detectable both before and after transplantation, and the early posttransplantation thrombocytopenia gradually improved as elutable anti-HPA-5b became more difficult to detect.

Alloimmune thrombocytopenia may have played a role in two cases with transfusion-refractory thrombocytopenia associated with a rise in titer of anti-HPA-1a (compared with the pretransplantation state) that developed following autologous HSCT performed for metastatic carcinoma of the breast. However, these reports are not conclusive, because it is difficult to distinguish a PTP-like illness (which implies destruction of engrafted autologous donor marrow–derived platelets) from typical posttransfusion platelet transfusion refractoriness.\(^ {228}\)

**Solid-organ transplantation**

In rare instances, immunocompetent lymphoid cells within a transplanted solid organ cause alloimmune thrombocytopenia in the recipient of the organ. A dramatic scenario was reported by West et al.\(^ {229}\) All three-organ recipients (two of a kidney and one of a liver) had severe thrombocytopenia and bleeding within 5–8 days after transplantation from a multiparous female organ donor with normal platelet counts. The two recipients of renal transplants had thrombocytopenia refractory to high-dose IVIG and platelet transfusions. One of these patients died, but the other recovered after splenectomy performed 50 days after transplantation. The liver transplant recipient had organ rejection, which was accompanied by correction of the platelet count when he received a new liver allograft. HPA-1a alloantibodies were detected in the organ donor and posttransplant (but not pretransplant) recipient serum. These cases illustrate that passenger immunocompetent lymphoid cells occasionally induce severe alloimmune thrombocytopenia when introduced into an alloincompatible recipient.

**Platelet transfusion refractoriness**

Platelet transfusion refractoriness, which is failure to achieve the expected platelet increment after two consecutive platelet transfusion episodes, has several explanations (Table 21.7). Refractoriness is primarily due to anti-MHC class I antibodies produced by the recipient following multiple transfusions. However, nonimmune, patient-dependent factors are probably the most important, which means that poor platelet count recoveries can persist even when HLA alloimmunization is prevented with leukocyte-reduced blood components\(^ {230}\) and when HLA- or ABO-compatible platelets are given. High-titer anti-HLA antibodies can cause transfusion refractoriness in a surgical setting, and their effect is not removed by hemodilution or absorption with multiple platelet transfusions.\(^ {231}\)

There is anecdotal evidence that platelet-specific alloantibodies sometimes cause refractoriness. However, prospective studies have shown that this is a relatively infrequent occurrence. For example, Novotny et al.\(^ {232,233}\) found that even when HLA alloantibody formation was largely prevented with blood components filtered before storage, platelet-specific alloantibodies at most explained 4 of 79 (5%) cases of refractoriness. There are occasions, however, on which

<table>
<thead>
<tr>
<th>Table 21.7 General causes of platelet transfusion refractoriness, listed in probable descending order of frequency</th>
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<tr>
<td><strong>Nonimmune mechanisms</strong></td>
</tr>
<tr>
<td>Septicemia, fever, disseminated intravascular coagulation, amphotericin B therapy, hypersplenism, and fixed platelet count requirements in severe thrombocytopenia</td>
</tr>
<tr>
<td><strong>Platelet-nonspecific alloantibodies</strong></td>
</tr>
<tr>
<td>HLA alloantibodies</td>
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<tr>
<td>ABO alloantibodies</td>
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<tr>
<td>Platelet-specific alloantibodies</td>
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<tr>
<td>Drug-dependent antibodies (e.g., vancomycin)</td>
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<tr>
<td>Platelet-reactive antibodies</td>
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</tbody>
</table>

the transfusion service needs to provide HLA- and platelet-specific antigen-compatible platelet products to manage some of these patients. In one study, antibodies to HPA-15 were found in 2.3% of multiply transfused patients, and were implicated in failure to obtain an adequate platelet count in one patient.

Summary
A variety of platelet–antibody assays have improved the ability of the clinician to make an accurate diagnosis of immune thrombocytopenia in many diverse clinical settings that can involve pathogenic autoantibodies, alloantibodies, and drug-dependent antibodies. The treatment decisions that arise depend upon several relevant factors, including the nature of the specific diagnosis, the expected prognosis, and the presence of clinically evident bleeding or thrombosis.

Acknowledgment
We thank Natalie Ramsay for her help organizing the reference list for this chapter.

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

184 Warkentin TE, Kelton JG. Temporal aspects of heparin-induced thrombocyto-