Immune Thrombocytopenic Purpura of Childhood

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Immune mediated thrombocytopenia (ITP) is a common manifestation of autoimmune disease in children. Although patients often present with bruises, petechiae, and some mucosal bleeding, the incidence of life-threatening hemorrhage is rare (0.2-0.9%) but can be fatal when presenting in vital organs. A wide range of therapeutic regimens are currently in use, including observation alone, as the majority of children recover within 4-6 months regardless of treatment. A growing understanding of the pathophysiology of acute ITP in children has not impacted the controversy surrounding treatment, but has clarified the mechanism of action of the most frequently used agents in chronic ITP. Newer monoclonal antibodies such as Rituxan have proved very useful in chronic or refractory ITP and studies are ongoing to determine the best regimens using this form of immune modulation. Splenectomy and newer agents to boost platelet production are also under study in chronic ITP. Neonates may also have a form of immune thrombocytopenia with extensive bruising and thrombocytopenia called neonatal alloimmune thrombocytopenic purpura (NATP). Rather than autoantibodies, the platelet destruction is secondary to transplacental maternal IgG alloantibodies. During pregnancy mothers may become sensitized to platelet membrane antigens present on fetal platelets. These antibodies may result in serious bleeding, including intracranial hemorrhage in the perinatal period. Once identified, these mothers may require treatment during future pregnancies to minimize serious bleeding in the fetus and neonate. Treatment *in utero* and immediately following delivery is focused on restoring neonatal platelets to a safe level and preventing life-threatening bleeding.

Recent advances in our understanding of the role of individual genetic risk factors and immune dysregulation in immune thrombocytopenic purpura (ITP) have opened the door to new avenues of research in the most common form of hematological autoimmune disease in adults and children. The childhood form of ITP is seen most frequently in patients 1-7 years of age. For this reason, most pediatricians will be confronted at some point by a patient with bruises, petechiae, mucosal bleeding and very anxious parents. Rapid diagnosis, reasonable care plan and education on the etiology and course of this syndrome, will allay the fears of the patient and family members. Much work has been done in the past decade in the pathophysiology and treatment of ITP in adults, but there have been significant advances in the management of childhood ITP as well.

Clarification of the major pathways that lead to childhood ITP have influenced our approach to therapy and may ultimately aid in the early identification of individuals who may need more aggressive intervention versus no treatment at all. By decreasing the risk of hemorrhage and minimizing the long-term side effects of treatment, these insights have greatly improved the care of patients with ITP. This review will briefly discuss current practice in the diagnosis and management of acute and chronic ITP of childhood, as basics of this disease are well known to practicing hematologists and several comprehensive reviews of current practice have recently been published.[1-3] The remainder of this article will examine new insights into pathophysiology of immune thrombocytopenia in children, including neonatal alloimmunization, as well as novel therapeutic approaches currently in use and those emerging on the horizon.

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Diagnosis

In reviewing the current literature, it is clear that the pattern of presentation in acute ITP of childhood has changed very little over the years.[1-3] For decades, physicians and researchers have commented on the rapid onset of bruising and mucosal bleeding in a severely thrombocytopenic child with minimal or no trauma.[4] The remainder of the complete blood count and physical exam should be normal. Although generally healthy at the time of presentation, parents often report a preceding illness or other immune stimulant such as allergic reaction, insect bite, or immunizations, especially the MMR as noted in a large Canadian prospective study.[5] Another common observation supporting an environmental immune trigger in childhood ITP is its seasonal nature: it occurs most in winter and fall, least in summer.

Even in uncomplicated ITP, there is considerable variation in presentation and prognosis. Based on the duration of thrombocytopenia, a distinction is made between "acute" versus "chronic" ITP. Thrombocytopenia lasting less than 6 months is termed acute, and greater than 6 months is termed chronic. Children are more likely to have the acute form of ITP and in 60-75% of the patients, the thrombocytopenia resolves within 2-4 months of diagnosis regardless

of therapy.[1-3] There is an equal incidence of ITP in both males and females in the 1- to 7-year-old age group in acute ITP of childhood. These features are distinctly different from the adult form of the disease, which is more likely to be chronic, with a much greater incidence in females and no seasonal predilection.

Laboratory studies at presentation (**Table 1**) and history are the mainstay of diagnosis in childhood ITP. Although many parents are anxious to rule out leukemia when referred to a hematologist, it is usually not necessary to perform a bone marrow aspirate with isolated thrombocytopenia if a thorough physical examination and review of the blood smear is performed. However, if more than one cell lineage is decreased and the patient has splenomegaly or adenopathy, the bone marrow must be evaluated prior to administration of medications such as steroids, which might confound the diagnosis of leukemia, delay therapy, or trigger an unrecognized tumor lysis syndrome. Other syndromes that might be misdiagnosed as ITP are listed in **Table 2**. Antiplatelet autoantibody testing has flourished over the past 20 years, but it is rarely used to make the diagnosis of acute ITP in children as the other forms of thrombocytopenia listed in **Table 2**, can generally be readily distinguished without this assay. Assays used to identify target antigens on the platelet surface have been used to in clinical research along with measurement of platelet bound autoantibody isotype using flow cytometry to determine whether certain antibodies are more likely to predict a more severe or chronic form of ITP.

Pathophysiology

The majority of children with ITP have a strikingly negative past medical history, with puzzled parents often describing the patient as the "healthiest" one in the family. In a sense, these children are victims of their own exuberant defense system which, in responding to an immune trigger in the previous weeks, has created a permissive state where antiplatelet autoantibodies may emerge. These may be promiscuous antibodies that bind to viral or bacterial antigens and crossreact with platelets, or a previously suppressed autoreactive antibody that has escaped peripheral tolerance as described below.

Recent articles have revealed the dominance of a pro-inflammatory state in ITP following a relatively benign viral or environmental trigger.[6,7] Both the pro-inflammatory cytokines and T cell repertoire seem to persist in some children creating a permissive environment for the emergence of previously suppressed autoantibodies that bind to platelet membrane antigens. A most interesting approach to assess the immune status of ITP at presentation has recently been published by Zehnder et al. Using a whole blood gene expression microarray with 24,473 unique genes per run, they demonstrated the increased expression of gamma interferon dependent genes in the early stage of ITP, supporting a pro-inflammatory or TH1 dominant state.[8] In addition, several articles have documented the persistence of inflam-

Table 1. Common laboratory tests obtained in the thrombocytopenic patient at presentation.

Complete blood count and differential review smear	Rule out: Multilineage involvement leukemia or aplastic/myelodysplasia Evaluate platelet size (giant or "dust-like")
Reticulocyte count	Hemolytic anemia or chronic blood loss
Blood type, Rh, antibody screen	Possible anti-D antibody treatment Autoimmune hemolytic disease
Chemistry panel	Eliminate systemic disease, i.e., hemolytic uremic syndrome, hepatitis, hemolysis, occult malignancy with elevated LDH or uric acid
DIC screen	Sepsis, Kasabach-Merritt syndrome
Quantitative immunoglobulin levels	Rule out: common variable immune deficiency, Wiscott-Aldrich
Viral titers/PCR	Cytomegalovirus, Epstein-Barr virus, human immundeficiency virus
Collagen vascular panel (ANA, anti-DNA)	Older patients, especially those with more chronic onset

Table 2. History and physical findings not consistent with immune thrombocytopenic purpura (ITP) of childhood.

History/Findings	Alternative Diagnosis
Thrombocytopenia present from birth	Amegakaryocytosis Primary thrombocytopenia Giant platelet syndromes
Weight loss and recurrent fevers	Malignancy, immune deficiencies
Bloody diarrhea	Wiskott Aldrich Hemolytic uremic syndrome
Recurrent infections or failure to thrive	Primary immune disorder, HIV
History or presence of jaundice	Autoimmune hemolytic disease Hepatitis, cirrhosis with splenomegaly
Splenomegaly, lymphadenopathy	Autoimmune lymphoproliferative syndrome (ALPS), primary immune disorders, Gauchers malignancies, hypersplenism syndromes
Forearm or hand anomalies	Thrombocytopenia absent radii (TAR) Fanconi's syndrome
Malar rash, dermatomyocytis, polymyocytis, eczema	Collagen vascular disease, Wiskott Aldrich
Cardiac malformation with or without DiGeorge syndrome	Chromosome 22 microdeletions with large platelets, with or without Evans syndrome

matory cytokines[7,9] and disturbed T cell apoptosis in childhood ITP.[10-12]

Given the diversity of immunoglobulin specificity, every child and adult has the potential to form autoantibodies to platelets. For unclear reasons, these self-reactive antiplatelet clones are not deleted during fetal development and persist in the antibody repertoire of the mature individual.[13] *In vivo*, some antiplatelet antibodies are "naturally occurring" antibodies that are kept under tight control by a form of natural immune suppression called peripheral tolerance.

In childhood ITP, the key pathologic event may be failure to suppress these previously sequestered autoantibodies.[14] Specifically, it has been proposed that the T lymphocyte pathways characterized by the $CD25^+$ T regulatory cells may not be fully mature in children 2-5 years of age, thus permitting autoimmune antibody production and antigen presentation by B lymphocytes that had escaped thymic deletion due to their crossreactivity with viral antigens. The T cell profile and cytokines in these patients, at presentation, are most consistent with a protracted T cell helper 1 (TH1) response with elevated interleukin (IL)-1α or IL-1β and decreased IL-4 as has been described in juvenile rheumatoid arthritis (JRA) and early onset diabetes.[6,7,9] In general, it is a polyclonal response with many children producing both IgG and IgM autoantibodies to a variety of platelet epitopes including the more common glycoproteins $\alpha_2\beta_3$ and GPIb complex. Certain Fc gamma receptor IIa and IIIa polymorphisms have an increased association with childhood ITP. This observation may point toward individual differences in the clearance of autoantibody-bound platelets in the onset and duration of ITP. Early studies suggest that these Fc gamma receptor polymorphisms may play a role in predicting response to therapy as well.[7,15]

Recent articles suggest that the generation of a critical $CD4^+CD25^+$ regulatory T cell (T_R) in the thymus during a pro-inflammatory response may be essential in the prevention of auto-reactive effector cells and antibodies.[14] Indeed, children who have thymic hypoplasia as a result of a heterozygous deletion of chromosome 22q11.2 also have a predisposition to autoimmune disease. Absolute numbers of $CD4^+$ $CD25^+$ T cells were markedly higher in healthy infants than in infants with chromosome 22q11.2 deletion syndrome[14] where the incidence of autoimmune disease is 10%.

With or without treatment, the ultimate resolution of thrombocytopenia during the first 6 months or "acute" phase of ITP may rest on the patient's ability to reestablish this network of peripheral tolerance and autoantibody suppression. Current therapy is primarily directed toward decreasing platelet consumption until these regulatory pathways are reestablished.

Megakaryopoiesis in ITP

Increased megakaryocyte number in the bone marrow has been a hallmark of immune-mediated platelet destruction in patients with ITP. Over 20 years ago, researchers demonstrated that more than one third of all adults with ITP had inadequate platelet production despite increased megakaryocyte numbers in the bone marrow.[16-18] It was assumed that autoantibody interfered with platelet formation or egress from the marrow space. A number of studies ultimately confirmed this hypothesis with both *in vitro* and *in vivo* platelet production studies.[19-22]

Like adults, certain pediatric patients also demonstrate decreased megakaryocytopoiesis in the presence of ITP plasma containing anti-GPIb-IX autoantibodies alone or in combination with α_{IIb}-β_{IIIa} (GPIIb-IIIa) autoantibodies, *in vitro* compared to control normal AB plasma (**Figure 1**; see Color Figures, page 516).[21,22] However, those children with ITP mediated by platelet autoantibodies directed against other epitopes (unknown antibody group in **Figure 1**; see Color Figures, page 516), demonstrated little or no suppression. Furthermore, those pediatric patients with α_{IIb}-β_{IIIa} (GPIIb-IIIa) autoantibodies alone demonstrated a mixed response, with certain plasmas being highly suppressive, while others actually appeared to stimulate growth.

McMillan et al recently demonstrated that autoantibody suppression of megakaryocytes by autoantibody may be associated with increased apoptosis.[22] This was not observed in the pediatric ITP megakaryocytopoiesis studies, despite similar autoantibody specificities.[23] It is possible that lymphocyte signaling, receptor occupancy and clustering may also play a more critical role in triggering megakaryocyte apoptosis in the adults. Alternatively, antiplatelet autoantibodies in childhood ITP may not recognize the same epitopes on target molecules, nor have the same affinity and complement binding capacity due to a less mature immune system. Adolescent ITP patients are much more likely to have anti-GPIb autoantibodies and have a clinical course similar to that of the adult with ITP.

The disparities in suppression of megakaryocytopoiesis observed between adult and pediatric ITP patients reinforces the growing evidence that the immunologic trigger, course and outcome for children with immune mediated thrombocytopenia is quite different than that of adults.

Treatment Options in Childhood ITP

Pediatric hematologists must consider a number of factors when considering the management of a child with ITP.[4,24-30] In the absence of hemorrhage, the child with scattered petechiae and superficial bruising may only warrant close observation, as long as the parents are informed of the risks associated with severe thrombocytopenia and can rapidly return to the hospital should bleeding occur. If the family is geographically isolated or unable to closely observe their child, treatment to increase the platelet count above 20,000 and shorten the duration of thrombocytopenia should be considered.[31] Most common treatment options are listed in **Table 3**.

If the decision is made to treat a child with ITP, the most common approach would include either IVIG, anti-D immunoglobulin in the Rh^+ patient, or steroids once one is

Table 3. Comparison of acute immune thrombocytopenic purpura (ITP) treatment regimens in children.

Treatment Response	Prednisone (4 mg/k/day 1-7, max 60 mg)	IV Immunoglobulin (1-2 g/kg)	Anti-D Immunoglobulin (75 μg/kg)
Response > 20,000 at 48 hours	60-70% of patients	70-80% of patients	77% of patients
Common side effects	Weight gain, irritability, hypertension, stomach pain, hyperglycemia	Post-infusion headache, vomiting, allergic reactions, fever, chills	Hemolysis, chills, fever, headache
Rare but severe reactions	Gastic ulcer, reflux, bleeding, hypertension-induced ICH	Anaphylaxis, aseptic meningitis, renal failure	Massive hemolysis with associated back pain myalgia, anemia
Duration of intial response (days)	Wide range of response after 30 days of weaning from initial dose to 0	21-72 days with platelet count greater than 20,000/mm^3	21-48 days based on the 75 μg/kg dose

confident there is no risk of leukemia. Many opt for IVIG in the first few hours, as it may take some time to obtain the red cell antibody studies or Rh status needed prior to giving anti-D immunoglobulin. In addition, if the patient has significant bleeding prior to presentation, hematologists may wish to avoid agents that might lower the hemoglobin even further. Immunoglobulin preparations should be given slowly initially as the large protein load is more commonly associated with headache and vomiting in the 24-48 hours following infusion. Besides the discomfort for the patient, these symptoms are also associated with increased intracranial pressure and may result in an urgent CT scan to rule out central nervous system (CNS) bleeding. Running the IVIG at 1 g/kg over 18-24 hours with hydration can minimize this complication and avert the need for costly studies to rule out intracranial hemorrhage. Mild to moderate neutropenia following IVIG is another common observation, usually resolves after 48 hours and should not prompt the need for a bone marrow aspiration. Either anti-D immunoglobulin or IVIG has roughly the same timeframe for platelet increases to > 20,000 and 50,000/mm^3 and the same durability in the child with acute ITP. Usually children are admitted and observed for 24-48 hours with initial treatment, so there is no benefit to one treatment over another, but with subsequent treatment anti-D immunoglobulin may be given in clinic or infusion centers with observation. The acute massive hemolysis seen with anti-D use has discouraged its use in many centers. Ongoing studies in Europe with subcutaneous anti-D immunoglobulin suggest that these side effects are much decreased if not eliminated all together.

Regardless of which regimen is implemented, it is important to remember that it takes several weeks for the antiplatelet immunoglobulin to go through one half-life even if autoantibody production were to halt at presentation, which would be highly unlikely. Treatment with agents that impact platelet clearance by the spleen and reticuloendothelial (RE) system, lose their effectiveness over 3-4 weeks at which point the platelet count may once again drop below 20,000/mm^3. However, unlike presentation, there will be little to no bleeding and strikingly fewer bruises or petechiae. Many parents are puzzled by this lack of consistency between platelet count and physical findings. The reason for this improved hemostasis lies in the bone marrow, where platelet production has increased 5- to 10-fold over the first 3-4 weeks, resulting in younger more functional thrombocytes. Investigational agents to stimulate platelet production are currently in clinical trials and may prove useful for chronic ITP patients with immune-mediated suppression at the level of the megakaryocyte.[16,32]

Chronic ITP

When the patient remains thrombocytopenic for greater than 6 months, despite therapy, it is both discouraging and frustrating for the patient, family and healthcare team.[33] If the platelet count remains in a hemostatically "safe" range (> 20,000/mm^3), patients are often observed without intervention, as these patients continue to show spontaneous remission rates of ~50%/year.[1,11] Treatment is reserved for clear signs of bleeding: increased bruising, menorrhagia, or prolonged epistaxis.[33,34]

However, older children wishing to participate in sports requiring a higher platelet count may opt for treatment based on quality of life issues and a desire to enter fully into school activities. For many years, the gold standard therapies used in acute ITP[30,33-36] (IVIG, anti-D immunoglobulin, and corticosteroids) were administered as needed to chronic ITP patients, in combination with vincristine or danazol, or on a monthly basis to maintain platelet counts > 50,000/mm^3. Alternatively, splenectomy is successful in resolution of life threatening thrombocytopenia in 75-85% of pediatric patients,[36] but the irreversible nature of this procedure and the increased risk of sepsis, albeit slight, has decreased the frequency of this procedure except in the frankly hemorrhagic patient.

Over the last 5 years, newer agents designed to interrupt the immune dysregulation driving the production of autoantibody have been used in clinical trials involving adults and children with chronic ITP (**Figure 2**; see Color Figures, page 516). These therapies are most commonly humanized monoclonal antibodies that bind to immune receptors on the early B lymphocyte anti-CD20 (Rituxan), anti-CD40, or bind to the T cell such as anti-154 (CD40L/ or its soluble form), anti-tumor necrosis factor (TNF) or its receptor (Enbrel, Remicade).

The success of Rituxan in adult ITP patients has prompted many pediatric hematologists to use this agent in children with chronic ITP in order to improve platelet counts and to avoid splenectomy.[12,37,38] Using a standard dose of 375 mg/m^2 in 4 weekly doses, Wang et al demonstrated a complete response (plt count > 150,000/mm^3), lasting for an average of 13 months, in just over 50% of pediatric patients with chronic ITP.[37] A single annual dose of Rituxan would certainly lessen hospital time and optimize quality of life in these patients.[38-40] Concerns regarding possible infection have proved to be unwarranted, with the exception of those patients who receive Rituxan following or in combination with other immune suppression such as high-dose steroids, azathioprine, or cyclosporin. Other centers are beginning to assess whether the standard dose of 375 mg/m^2 for 4 consecutive weeks is necessary, as this dose was initiated for the treatment of CLL patients with a much larger number of CD20$^+$ cells. Many have suggested that much less Rituxan would be necessary for ITP where there are far fewer CD20$^+$ cells than in CLL. Indeed, a single infusion is usually adequate to clear circulating CD20$^+$ pre-B cells in ITP, but only clinical trials can assess whether a single large dose or serial infusions of 1-4 doses is optimal to treat the pool of CD20$^+$ cells in the spleen and elsewhere.[38,39] It is important to realize that the CD20$^+$ pre-B cells do not produce the antiplatelet antibody, which is the job of the plasma cells; rather, it is highly effective at presenting antigen to T cells, which in turn are driving the autoimmune response. Disruption of this interaction ultimately results in decreased antiplatelet autoantibody with very little to no decrease in normal immunoglobulin production.

Final Thoughts on Childhood ITP

The future of safe and effective management of childhood ITP will be greatly expanded by the optimal application of such therapies that target the selected areas of immune dysfunction, rather than using agents which result in either profound immune suppression or result in serious toxicities. Although we are not yet at a point where molecular markers, cytokine and lymphocyte panels can match ITP patients with personalized treatment regimens, the coordinated efforts of the medical, research, and patient community have brought that goal much closer to reality.

Neonatal Alloimmune Thrombocytopenia (NATP)

Neonatal alloimmune thrombocytopenia is caused by maternal sensitization to paternal alloantigens on fetal platelets (**Table 4**). A correct diagnosis of NATP must first eliminate other cause of thrombocytopenia that may occur during pregnancy. When severe, NATP may result in intracranial hemorrhage leading to hydrocephalus and fetal death. Fetal hydrocephalus, unexplained fetal thrombocytopenia with or without anemia, or recurrent miscarriages should be considered as indicators of possible NATP. Multiparous women with a history of at least one incident of NATP should be monitored carefully for subsequent episodes. Postnatal management involves transfusion of compatible platelets, and washed maternal platelets are often used. Antenatal management is controversial but can include a combination of maternal intravenous gamma globulin (IVIG) administration, intrauterine platelet transfusions and corticosteroid therapy, while monitoring fetal platelet counts closely throughout the pregnancy.

The biological diagnosis is normally made by genotyping of maternal and paternal platelet alloantigens and a serological search for antibodies in maternal plasma or serum that reacts with paternal platelets.

It is intriguing that only a fraction of those mothers negative for the platelet antigen in question deliver infants affected with NATP. For example, in the western world, responsiveness to HPA-1a is most commonly the cause of NATP, yet the frequency of homozygous HPA-1b mothers in the general Caucasian population is 2%, and estimates of the incidence of NATP are no greater than 0.05%. A key to understanding this discrepancy lies in the finding that responsiveness to HPA-1a shows an HLA restriction. Individuals who are homozygous for Pro_{33} (homozygous HPA-1b) and responsive to the predominant HPA-1a antigen are almost exclusively HLA DRB3*0101 or DQB1*02. In the case of DRB3*0101, the calculated risk factor is 141, a risk level equivalent to that of the hallmark of HLA-restriction in autoimmune disease, ankylosing spondylitis and HLA B27. In contrast, responsiveness of homozygous HPA-1a individuals to the HPA-1b allele is not linked to HLA. T cells are the likely candidates for providing HLA-restriction in this case, and Maslanka et al provided elegant evidence that in one case of NATP, T cells that share CDR3 motifs are stimulated by peptides that contain the same

Table 4. Neonatal alloimmune thrombocytopenic purpura: key points.

Incidence: 1 per 3000 in a retrospective study; 1 per 2200 births in one prospective study.
Maternal antibodies produced against paternal antigens on fetal platelets.
Similar to erythroblastosis fetalis except that 50% of cases may occur during first pregnancy.
Most frequently implicated antigens are HPA-1a and HPA-5b (United States/Europe).
Sensitized mothers should expect increasing titers with each subsequent pregnancy when fetal platelets express the paternal antigen, lengthening the duration of neonatal thrombocytopenia.
Sisters of sensitized mothers are at risk due to the HLA-associated rate of alloantibody production: In the case of responsiveness to HPA-1a, there is a high-risk association with HLA DRB3∗0101 or DQB1∗02. In the case of responsiveness to HPA-6b, there is an increased association with HLA DRB1∗1501, DQA1∗0102 or DQB1∗0602.

Leu_{33} polymorphism that is recognized by anti-HPA-1a alloantibodies.[41] Responsiveness to HPA-1a is not the sole cause of NATP. The association of NATP with other alloantigens, such as HPA-3a, HPA-3b, HPA-1b or HPA-2b, has been noted, but is rare. Obviously, differences in allelic gene frequencies between different racial or ethnic populations will have an important impact on the frequency of responsiveness to a particular alloantigen.

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