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Mechanisms of Pathogenesis

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ABSTRACT

The mechanism of idiopathic (autoimmune) thrombocytopenic purpura (ITP) has historically been attributed to platelet autoantibody production and the resultant platelet destruction. More recent evidence suggests a multifactorial pathogenesis. A complex picture of the immune processes involved in autoimmunity has emerged over the last decade with the identification and characterization of immunoregulatory elements (receptors, cytokines, and other signaling molecules) and cell trafficking patterns. An understanding of the interplay of cellular and humoral immune responses in the breakdown of self-tolerance has brought to light unrecognized mechanisms of the autoimmune destruction of platelets in ITP and potential targets for future therapeutic advances. The failure of the bone marrow to maximally increase platelet production also appears to play an important role in the thrombocytopenia of ITP. Treatment strategies targeting the thrombopoietin receptor to increase platelet production are a promising new approach to the management of ITP. The Oncologist 2009;14:12–21

INTRODUCTION

Idiopathic (or autoimmune) thrombocytopenic purpura (ITP) is an acquired disorder characterized by mild to severe thrombocytopenia, a relatively normal appearing bone marrow, and mucocutaneous bleeding. Characteristically, there is a rapid increase in the platelet count following treatment with corticosteroids or high-dose i.v. immunoglobulins. Chronic ITP, more common in adults, persists for at least 6 months and occurs in the absence of other abnormalities [1]. The triggering event for ITP is unknown [2, 3], but continued research is providing new insights into the underlying immunopathogenic processes as well as the cellular and molecular mechanisms involved in megakaryocytopenesis and platelet turnover.

Although historically ITP-associated thrombocytopenia was attributed solely to increased rates of destruction of antibody-coated platelets, it has become evident that suboptimal platelet production also plays a role [4–6]. This review...
Autoimmunity in Chronic ITP

In the early 1950s, Dr. William Harrington and several colleagues injected themselves with blood from patients with chronic ITP and within hours developed severe thrombocytopenia that persisted for several days [7, 8]. A factor in the globulin fraction of the blood was later identified as an important mediator of this effect. We now know that both humoral and cellular immunity are involved in the destruction of platelets in chronic ITP, and there is evidence that impaired T-cell expansion underlies the autoimmune response [9]. Indeed, a complex interplay involving components of multiple aspects of the immune system has been identified (Table 1).

Autoreactive B Lymphocytes Secrete Antiplatelet Antibodies

The most commonly occurring autoantibodies (~75%) in patients with ITP are directed against the platelet surface glycoprotein (gp) complexes gpIIb–IIIa and gpIb–IX [10]. Antibodies against other glycoproteins (Ia–IIa, IV, and V) have been identified, and multiple platelet antigen specificities can be found in most patients [11–13].

Although antibodies are primarily of the IgG subtype, IgM and IgA may be found [13]. Platelets are targeted by the attachment of autobodybs to their surface gp antigens, bound to Fcγ receptors expressed on tissue macrophages of the reticuloendothelial system and cleared from the circulation. Gamma camera imaging of ITP patients injected with 111In-labeled autologous platelets revealed that uptake occurs primarily in the spleen and liver [14]. Complement-induced lysis following antibody binding may also play a role [15]. After platelet internalization and degradation, macrophages express platelet epitopes on their surface and secrete cytokines that stimulate initiating CD4+ T-cell clones and clones with additional specificities [1, 16]. Unique to patients with ITP, autoreactive CD4+ T cells recognize several distinct epitopes on gpIIb–IIIa, leading to autoimmune response expansion and accelerated platelet destruction. The trigger for the initiating autoantibody response is unknown, although autoreactive T helper (Th) cells that interact with antibody-producing B cells are required [3].

Platelet-associated autoantibodies are detected in 50%–70% of patients with ITP [17–19], emphasizing the limitations of the currently available assays and/or suggesting that other or additional mechanisms are involved. A quantitative assay for nonspecific platelet-associated IgG had a positive predictive value of only 46% in patients with ITP and it could be detected in disease states other than ITP, including hematologic malignancy and infection [20]. Assays for antibodies targeting gpIIb–IIIa, gpIb–IX, and gpIIa–IIIa may be more specific [17, 18, 21], but have limited sensitivity, and the diagnosis remains dependent on clinical presentation for the most part.

Dysfunctional Cellular Immunity: The Role of Autoreactive T Cells

Accumulating evidence indicates that CD4+ Th cells orchestrate the autoreactive B-cell response. B cells produce antibodies directed at normal platelet antigens in response to signaling from CD4+ Th cells [3]. CD4+ T cells autoreactive to platelet gpIIb–IIIa have been identified in patients with ITP. The trigger for T-cell dysregulation in ITP is unclear.

The native form of gpIIb–IIIa does not normally elicit a T-cell response; however, autoantibodies against gpIIb–IIIa are detected in patients with ITP, as are circulating memory B cells capable of producing autoantibodies [3]. Autoreactive T cells may be found in healthy individuals, but appear to be activated only in patients with autoimmune disease. This phenomenon could be explained by a cryptic epitope model in which self-antigens normally hidden from the immune system are presented at an increased concentration. In ITP patients, epitopes within gpIIb and gpIIIa molecules could in this way elicit a response.

The cytokine profile secreted by CD4+ cells in patients with ITP is consistent with Th-cell activation. A predominantly Th1 (proinflammatory) response is seen in ITP [22], a pattern seen in most organ-specific autoimmune diseases. An increased Th1–Th2 ratio has been observed in patients with active ITP [23], and an increase in interferon-γ and interleukin-2 receptor-β gene expression in patients with ITP has been detected [24]. Andersson et al. [25] found elevated levels of transforming growth factor β1, a potent immuno-
suppressive cytokine, in patients with ITP in remission versus controls and patients with active disease.

**T Cell–Mediated Cytotoxicity**

Cytotoxic lymphocytes appear to be abnormally activated by autoreactive lymphocyte clones. CD3⁺ lymphocytes from patients with ITP show increased expression of cytotoxic genes such as tumor necrosis factor α, perforin, and granzyme A and granzyme B [24, 26]. Platelets from patients with active ITP displayed in vitro lysis when incubated with CD3⁺CD8⁺ T effector cells but not with CD3⁻CD16⁺CD56⁺ natural killer (NK) cells. Killer cell immunoglobulin like receptor (KIR) genes showed increased expression, and CD3⁺ lymphocytes expressing KIRs were greater in number in ITP patients in remission than in patients with active ITP or normal controls [27]. The KIR family of genes downregulate cytotoxic T-lymphocyte and NK cell responses, preventing lysis of target cells. Megakaryocytes may also be damaged in ITP by autologous CD8⁺ T cells [28]. These findings suggest that cytotoxic T cells play a part in at least some patients with ITP, and downregulation of the auto-T-cell response is a potentially effective therapeutic strategy.

**NK Cell Activity**

An increase in the number of NK cells and expansion of the CD56⁺CD3⁻ NK cell and CD56⁺CD3⁺ cytotoxic T-cell subsets has been reported in patients with active ITP [29]. Patients with ITP requiring therapy (including a subgroup refractory to standard therapy) were compared with patients with stable disease and healthy controls. CD56⁺CD3⁻ NK cells were substantially greater in number in patients with therapy-dependent ITP and in those with refractory disease versus patients with stable disease or controls. A high expression of major histocompatibility complex class II molecules was also observed in those patients refractory to standard therapy, suggesting in vivo activation. Although still speculative, these findings imply that the pathogenesis of ITP may include NK cells destroying the IgG-coated targets.

**MEGAKARYOPOIESIS AND THROMBOPOIESIS**

Platelet production, or thrombopoiesis, occurs in the bone marrow where committed stem cells differentiate into megakaryocytes. Megakaryocyte progenitors ultimately differentiate into mature megakaryocytes and release platelets into the bone marrow sinusoids. A stable platelet count occurs only when platelet production equals platelet consumption and destruction. In ITP, the increased demand for platelets, triggered by autoimmune-mediated platelet destruction, appears to result in an increased megakaryocyte mass with more and larger megakaryocytes and an increased mean ploidy [30]. However, there is evidence that platelet production is suboptimal in many patients with ITP.

**Platelet Kinetics in ITP**

Harker and Finch first described methodology for labeling platelets with radioactive ⁵¹Cr to measure platelet disappearance rates and, by implication, production (or “turnover”) [31]. By this method, at a stable platelet count:

\[
\text{turnover} = \frac{\text{platelet count} \times 10^9 \text{ per liter} \times 90\%}{\text{platelet survival (days)} \times \text{platelet recovery (％)}}
\]

whereby platelet turnover (per μl per day) is calculated from the peripheral platelet count corrected for splenic sequestration, or “recovery,” by 90% based on studies in otherwise normal splenectomized individuals. Recovery in individual subjects is calculated from platelet activity (per ml) extrapolated to time zero, multiplied by the estimated blood volume and divided by the labeled platelet activity injected [31]. This calculation gives a fairly accurate measurement of effective platelet production, that is, the number of platelets that are released into the circulation by the bone marrow [6].

By these techniques, a shorter platelet life span (i.e., higher platelet turnover rate) is consistently seen in ITP patients compared with the 8- to 10-day platelet survival duration in healthy controls [4, 31–34]. Although early studies using allogeneic donor platelets suggested an extremely short platelet survival time, later studies using autologous platelets showed a shorter platelet survival time, but on the order of days as opposed to hours. Calculation of platelet turnover using longer platelet survival times results in destruction rates not as marked as previously thought and production rates that are modestly increased, if at all [35].

Ballem and colleagues compared ⁵¹Cr- or ¹¹¹In-labeled autologous and homologous (normal donor) platelet survival in 13 patients with chronic ITP, showing that the survival duration of homologous platelets was significantly shorter than that of autologous platelets [32]. These findings suggest that platelets circulating in patients with ITP are relatively “protected” once leaving the bone marrow, compared with donor platelets from normal volunteers, and suggest that ineffective platelet production may be a significant factor in the pathogenesis of ITP. Some surface antigens are coexpressed on platelets, megakaryocytes, and megakaryocyte precursors (e.g., gpIIb–IIIa and gpIb–IX) and recognized by autoantibodies, and may lead to impaired megakaryopoiesis [10, 19, 36], maturation, and platelet release. Newly formed platelets may be vulnerable to intramedullary reticuloendothelial removal, decreasing the numbers released into the circulation.
Our group measured survival of autologous $^{51}$Cr- and/or $^{111}$In-labeled platelets from 19 patients pre- and post-treatment with prednisone or splenectomy to determine how kinetics changed with increased platelet counts [6]. Whereas prednisone improved platelet counts by a mean factor of three, platelet survival was unchanged; therefore, effective platelet production had to have increased. Although this does not differentiate increased platelet production by the megakaryocytes from increased release of platelets by the bone marrow, this finding is consistent with the observation by Li and coworkers that abnormalities of megakaryopoiesis observed in coculture of autologous bone marrow mononuclear cells and CD8$^+$ T cells from patients with chronic ITP can be corrected by the addition of dexamethasone [28]. Following successful splenectomy, platelet survival improved, sometimes to normal, while platelet production appeared to remain unchanged.

Increased apoptosis or other forms of programmed cell death may play a role in the ineffective thrombopoiesis in the bone marrow of patients with ITP. An ultrastructural analysis of megakaryocytes of patients with ITP showed that 80% of mature megakaryocytes had features of apoptosis and para-apoptosis, suggesting that the low platelet production rate in ITP may be a result of greater apoptosis of platelet-producing megakaryocytes [37].

**Autoantibodies Suppress Megakaryopoiesis**

Chang and colleagues [38] showed that plasma from patients with ITP containing autoantibodies against gpIb and gpIIb–IIIa significantly suppressed megakaryopoiesis in vitro. They proposed that platelet autoantibodies may affect megakaryocyte maturation or survival, leading to decreased platelet production. McMillan et al. [18] studied the effects of plasma from 18 patients with chronic ITP on megakaryocyte production of CD34$^+$ cell cultures from healthy volunteers. Cells were cultured in a medium containing pegylated recombinant human megakaryocyte growth and development factor (PEG-rhMGDF) and 10% plasma from either the ITP patients or healthy volunteers. In vitro megakaryocyte production was significantly lower when plasma from 12 of the 18 ITP patients containing either anti-gpIIb–IIIa antibodies, anti-gpIb–1X antibodies, or both was added to the medium.

**Thrombopoietin and ITP**

Thrombopoietin (TPO) is a lineage-specific cytokine and the major cytokine regulating platelet production in the body (Fig. 1). TPO, also referred to as cellular myeloproliferative leukemia proto-oncogene (c-Mpl) ligand and MGDF, is a 332-amino acid glycoprotein produced primarily in the liver, with smaller amounts produced in the kidney and bone marrow. It exerts biological effects on hematopoietic stem cells as well as megakaryocytes [39–41].

TPO is involved in nearly every step of megakaryocyte development, supporting cell survival and cell cycling and modulating apoptosis and cell cycle regulators [41]. In vitro, only the receptor-binding region of TPO is required for thrombopoietic activity [42, 43].

Binding of TPO to its receptor, c-Mpl, on hematopoietic stem cells and bone marrow megakaryocytes sets in motion a cascade of molecular signaling events that culminate in platelet production [40, 41] (Fig. 2). Intracellular Janus ki-
Figure 2. Biochemical signaling pathways activated by thrombopoietin. Thrombopoietin binds to its receptor, c-Mpl, on bone marrow megakaryocytes causing the activation of the tyrosine kinase JAK2 and the tyrosine phosphorylation of a number of targets, including STAT proteins and the MAPK, ERK1 and ERK2, and Akt pathways.

Abbreviations: AK1, adenylate kinase 1; ERK, extracellular signal-related kinase; GRB2, growth factor receptor-bound protein 2; JAK, Janus kinase; meg, megakaryocyte; MAPK, mitogen-activated protein kinase; P, phosphate; SOS, son of sevenless; STAT, signal transducer and activator of transcription.
thrombocytopenia in patients with ITP suggest an opportunity to stimulate platelet production as a treatment approach [52, 57]. Several candidate molecules have been investigated including recombinant TPO preparations and TPO receptor agonists [58–60].

Two recombinant TPO preparations have been evaluated in the clinic—recombinant human (rh)TPO (Pharmaicia, Peapack, NJ) and PEG-rhMGDF (Amgen, Inc., Thousand Oaks, CA). Although they were active in raising the platelet count in ITP, the development of antibodies crossreactive with native TPO in a few normal individuals resulted in prolonged pancytopenia, limiting the usefulness of this class of drugs [61–63]. Newer TPO receptor agonists have no sequence homology with the native molecule and are not expected to provoke crossreactive antibodies. Results from recent trials with these agents provide in vivo evidence that boosting platelet production is an effective therapeutic strategy [59, 60, 64, 65]. Four TPO receptor agonists are currently being evaluated in patients with ITP—romiplostim (Amgen, Thousand Oaks, CA), eltrombopag or SB-497115 (GlaxoSmithKline, Collegeville, PA), AKR-501 (MGI Pharma, Bloomington, MN), and LGD-4665 (Ligand Pharmaceuticals, San Diego, CA).

Romiplostim consists of a novel compound, a “peptibody,” consisting of two identical c-Mpl receptor-targeting peptides linked with an Fc carrier domain to increase the plasma half-life. Phase II trials in patients with ITP have shown that romiplostim is well tolerated and is associated with increased platelet production, with the target platelet range achieved in 63% (10 of 16) of patients treated with 1/\(H_9262\)g/kg or 3/\(H_9262\)g/kg of romiplostim [66]. Results of two randomized, placebo-controlled, double-blind phase III trials conducted in both splenectomized and nonsplenectomized patients demonstrated significantly higher response rates with romiplostim than with placebo. In both trials, romiplostim-treated patients had a significant decrease in rescue medication use while on study, and most were able to decrease or discontinue concurrent ITP therapy [67, 68]. Although most studies report an acceptable tolerability and safety profile, a treatment-related increase in bone marrow reticulin deposition has been reported [69, 70].

**Table 2.** Idiopathic thrombocytopenic purpura treatment options and their mechanisms of action

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Treatment option</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interfere with clearance of antibody-coated platelets</td>
<td>Splenectomy, Corticosteroids, i.v. immunoglobulin, Anti–D immunoglobulin, Danazol, Vinca alkaloids</td>
</tr>
<tr>
<td>Clear antibody from circulation</td>
<td>Plasmapheresisa</td>
</tr>
<tr>
<td>Nonspecific T-cell immunosuppression</td>
<td>Azathioprine, Cyclophosphamide, Cyclosporine, Corticosteroids, Danazol</td>
</tr>
<tr>
<td>Interfere with B- or T-cell participation in antibody synthesis</td>
<td>Antibody to CD20 (rituximab), Antibody to CD40 ligand, i.v. immunoglobulin, Mycophenolate mofetil</td>
</tr>
<tr>
<td>Increase platelet supply</td>
<td>Platelet transfusiona</td>
</tr>
<tr>
<td>Increase platelet production</td>
<td>Bone marrow transplantation, Recombinant thrombopoietin agonists (rhTPO, PEG-rhMGDF), Small molecule thrombopoietin receptor agonists (romiplostim, eltrombopag, AKR-501), Corticosteroids</td>
</tr>
</tbody>
</table>

aTransient benefit.

Abbreviations: PEG-rhMGDF, pegylated recombinant human megakaryocyte growth and development factor; rhTPO, recombinant human thrombopoietin.
59% of the patients receiving eltrombopag achieved a cebo-controlled phase III trial of 114 patients with chronic ITP, and patients with AMT. Compared with healthy volunteers, serum TPO levels are markedly elevated in AMT patients. TPO levels in ITP patients are only slightly higher despite similar lower platelet counts.

Abbreviations: AMT, amegakaryocytic thrombocytopenia; ITP, idiopathic thrombocytopenic purpura; TPO, thrombopoietin.

From Mukai HY, Kojima H, Todokoro K et al. Serum thrombopoietin (TPO) levels in patients with amegakaryocytic thrombocytopenia are much higher than those with immune thrombocytopenic purpura. Thromb Haemost 1996;76:675–678, with permission.

Eltrombopag is a small (molecular weight, 442), orally bioavailable, nonpeptide TPO receptor agonist [59, 71]. A recently reported, placebo-controlled phase II trial evaluated eltrombopag in 118 adults with chronic ITP. A significantly higher proportion of patients receiving either 50 mg or 75 mg of eltrombopag achieved a platelet count ≥50,000/μl, compared with those receiving placebo (p < .001), and by day 15, >80% of these eltrombopag-treated patients had reached this endpoint [60]. In a subsequent placebo-controlled phase III trial of 114 patients with chronic ITP, 59% of the patients receiving eltrombopag achieved a platelet count of ≥50,000/μl, compared with 16% of the patients receiving placebo, with a significantly lower incidence of bleeding reported in eltrombopag-treated patients (p = .029) [72]. The long-term safety of eltrombopag is being evaluated in an ongoing, open-label extension trial (Eltrombopag extended dosing study, EXTEND) [73]. Across all trials, headache was the most frequently observed adverse event in both the eltrombopag and placebo groups (placebo, 6%–11%; eltrombopag, 50 mg, 3%–20%), but overall tolerability was good, with most reported adverse events being mild in severity [60, 72–74]. Grade 4 elevations of hepatobiliary laboratory values were reported in one patient during eltrombopag therapy in clinical trials [60, 76]. Although potentially a class-related effect, there was no evidence of clinically relevant abnormalities of the bone marrow resulting from eltrombopag treatment reported in a published report of the EXTEND trial [73].

In both the romiplostim and eltrombopag clinical trials, questions have been raised about the hematologic adverse effects related to TPO receptor agonists as a class. As with other drugs that are effective at elevating platelet counts, transient rebound thrombocytopenia may occur with romiplostim or eltrombopag after discontinuation [75, 77, 78]. Thromboembolic events have been observed during treatment with eltrombopag, but there does not appear to be a higher risk than that reported for the general ITP patient population [52, 79]. Similarly, the incidence of thrombotic events with romiplostim in patients with ITP does not appear to be greater than that seen in patients on placebo in clinical trials [64, 67, 68].

AKR-501 (molecular weight, 455) [80] and LGD-4665 are two additional, small molecule TPO agonists currently being evaluated in phase II trials. An increase >50% over the baseline platelet count was demonstrated in five of six healthy volunteers in an early trial of AKR-501 [65]. Similarly, a significant increase in platelet count was observed following single-dose administration of LGD-4665 in healthy male volunteers in a phase I trial [81]. Ongoing clinical trials will help elucidate the adverse event profile of these agents, including TPO receptor agonist-related class effects.

CONCLUSIONS

Immune-mediated platelet destruction in ITP occurs by a complex process involving multiple components of the immune system. The initiating event for the dysregulation remains unclear, although recent evidence helps to explain the processes by which the disorder may perpetuate itself. Platelet kinetic studies suggesting impaired platelet production in ITP have been confirmed by findings of decreased TPO, impaired megakaryocyte growth, and abnormal apoptosis. Positive preliminary findings from ongoing clinical trials of TPO receptor agonists in ITP showing sustained increases in platelet counts in the majority of treated patients provide strong support for the role of TPO, megakaryopoiesis, and thrombopoiesis in ITP pathogenesis.

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