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Review Article

Idiopathic thrombocytopenic purpura: Current concepts in pathophysiology and management

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Summary

Idiopathic thrombocytopenic purpura (ITP) is characterized by a low platelet count, which is the result of both increased platelet destruction and insufficient platelet production. Although the development of autoantibodies against platelet glycoproteins remains central in the pathophysiology of ITP, several abnormalities involving the cellular mechanisms of immune modulation have been identified. Conventional treatments for ITP aim at reducing platelet destruction, either by immunosuppression or splenectomy. Two new thrombopoietic agents, AMG 531 and eltrombopag, have been used in clinical trials to stimulate platelet production in ITP patients not responsive to standard treatments. These new molecules bear no structural resemblance to thrombopoietin, but still bind and activate the thrombopoietin receptor. This review will focus on the pathophysiology and treatment of ITP in adults, highlighting recent advances in both fields.

Keywords

Thrombocytopenia, platelet immunology, immunity

Thromb Haemost 2008; 99: 4–13

Introduction

Primary immune thrombocytopenia, commonly referred to as idiopathic thrombocytopenic purpura (ITP), is an acquired autoimmune disorder defined by isolated thrombocytopenia and the exclusion of other causes of thrombocytopenia (1).

ITP can be classified based on patient age (adult or childhood ITP), and duration of thrombocytopenia (acute or chronic). The clinical features of ITP in adults are different from the clinical features seen in childhood. In children, ITP is usually an acute, self-limiting disease, often occurring 2–3 weeks after a viral infection or immunization. In contrast, ITP in adults typically has an insidious onset, with no preceding viral or other illness, and has a chronic course.

Using a lower-threshold platelet count of 50×10^9/l, the annual incidence of ITP among adults has been reported to be 1.6–3.2 cases per 100,000 per year (2, 3). The median age at diagnosis is 56 years, with a female preponderance in those younger than 60 years and equal proportions between sexes among older patients (2, 3). Many cases of ITP in adults are diagnosed incidentally after a routine complete blood cell count (4). Individuals with a platelet count stably between 100 and 150×10^9/l for at least six months are not likely to develop a more severe thrombocytopenia; it is not clear whether they have a higher risk than the general population of developing another autoimmune disorder (4). In adults, the symptoms and signs are highly variable and range from the completely asymptomatic patient to frank haemorrhage from any site, the most serious of which is intracranial (5).

Design of prospective, controlled clinical trials has been particularly difficult, since patients with the chronic disease are less than 10% of all ITP patients (6) and present considerable clinical variability. Accordingly, several issues regarding the optimal treatment of these patients have remained unresolved, and principles of management have been mainly based on expert opinions (1, 7–10). Nevertheless, ongoing randomized trials with several new pharmacologic agents promise to rapidly change this scenario.

The aim of this article is to provide an updated review of ITP in adults, focusing on our current understanding of the mechanisms of the thrombocytopenia and on the evolving therapeutic modalities for chronic refractory ITP.

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Received August 22, 2007
Accepted after minor revision October 10, 2007

Prepublished online December 5, 2007
doi:10.1160/TH07–08–0513

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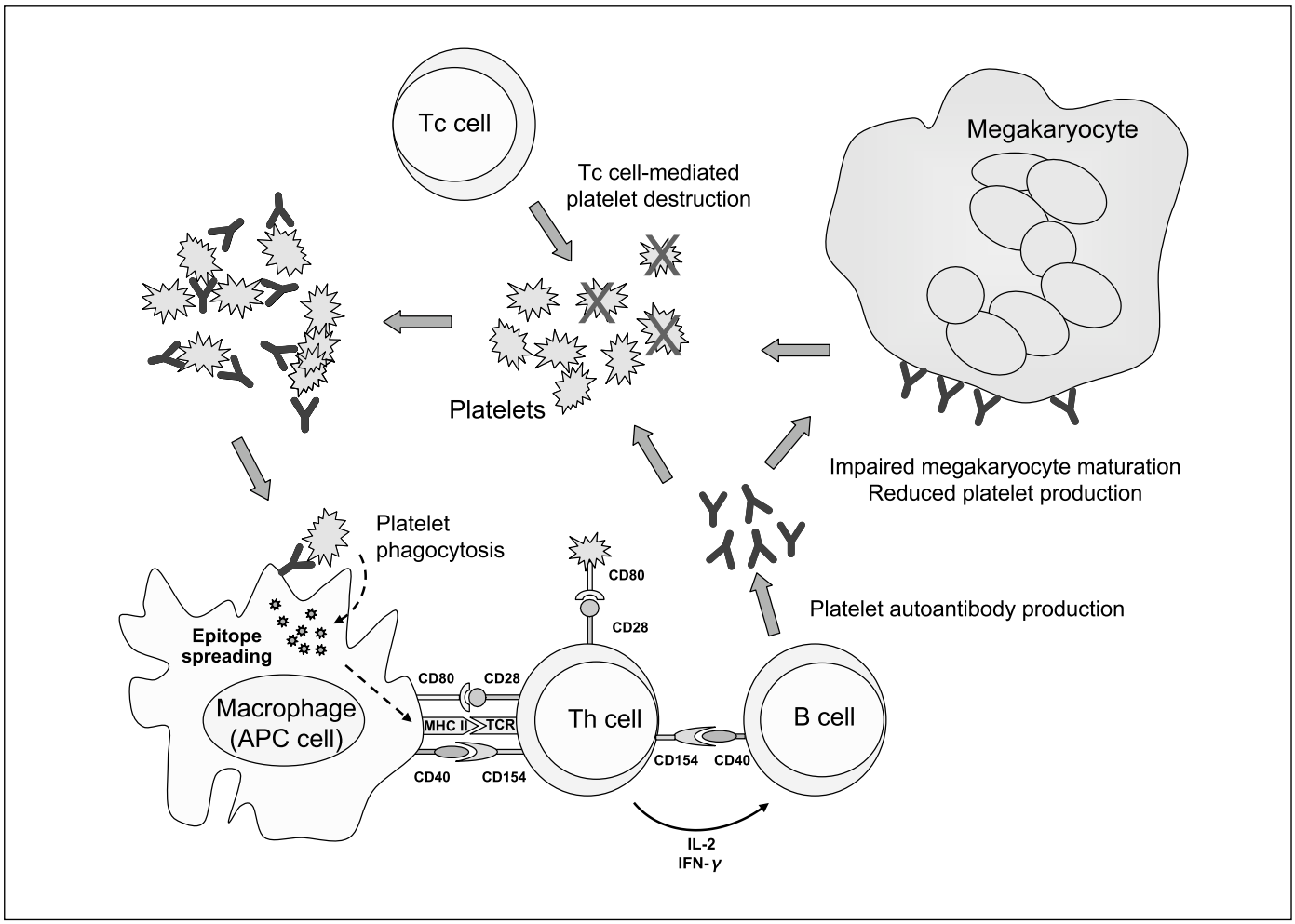

Figure 1: Simplified representation of the pathophysiology of ITP. The primary mechanism for the loss of tolerance in ITP remains unknown. The emergence of antiplatelet autoantibodies remains the central pathogenetic mechanism. Platelet glycoproteins are cleaved to peptides by macrophages or another antigen-presenting cell (APC) and expressed on the APC cell surface via MHC class II molecules. APCs are crucial in generating a number of new or cryptic epitopes ("epitope spreading"). The T-cell receptor (TCR) of the Th cell can then bind the peptide-MHC complex and signal activation that upregulates CD154 (CD40 ligand) to interact with CD40 on the APC and cause additional costimulatory interactions to occur. An additional co-stimulatory signal can originate from the binding of the CD80 molecule, overexpressed on the cell membrane of ITP platelets, with CD28 expressed on Th cells. The activated Th cell produces cytokines (interleukin-2 and interferon-γ) that promote B-cell differentiation and autoantibody production. Autoantibodies opsonize platelets, which are taken up and destroyed by macrophages predominantly in the spleen. They also bind bone marrow megakaryocytes, thereby impairing megakaryocyte maturation and platelet production. An alternative pathway of platelet destruction is by autoreactive cytotoxic T cells, although the relevance of this mechanism *in vivo* is not known.

Pathophysiology of ITP

Although the precipitating etiology for the loss of tolerance in ITP remains elusive, major advances have been achieved regarding our understanding of the pathogenetic mechanisms of the disease. The simplistic view of ITP as a disorder determined solely by platelet autoantibodies and characterized by increased platelet destruction has been challenged by both clinical and experimental data. A much more complex situation has been envisaged (Fig. 1), in which impaired platelet production and T cell-mediated platelet destruction play a significant role, and dysfunction in more than one step of the immune regulation occurs (11).

Role of B cells

Harrington's seminal experiment published in 1951 provided the first evidence that platelet destruction in ITP is caused by a plasma-derived factor (12), later identified as antiplatelet antibodies (13, 14). The most commonly identified antigenic targets of these autoantibodies are platelet glycoproteins (GP) IIb/IIIa and Ib/IX, with a number of ITP patients having antibodies directed to multiple platelet antigens (15). It should be noted, however, that autoantibodies are not detectable in up to 50% of ITP patients (15, 16) and that remission in ITP can occur despite the presence of platelet autoantibodies (17). Antibodies against GP IIb/IIIa show clonal restriction in light-chain use (18), and antibodies derived from phage-display libraries show highly constrained VH gene use (19, 20). Sequencing of the antigen-com-

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bining regions of these antibodies suggests that they originate from a limited number of B-cell clones by antigen-driven affinity selection and somatic mutation (20).

Role of T cells

T cells also appear to be related to the pathogenic process in ITP, as a series of studies have demonstrated that B-cell production of antiplatelet antibodies requires antigen-specific CD4+ T-cell help (21). Platelet-reactive T cells have been found in the blood of patients with this disorder, with the major target antigen being GP IIb/IIIa (22). In these patients, T cells stimulate the synthesis of antibody after exposure to fragments of GP IIb/IIIa but not after exposure to native proteins (23). The derivation of these cryptic epitopes *in vivo* and the reason for sustained T-cell activation are unknown. It has been hypothesized that cryptic epitopes, normally not exposed in a self-antigen, may become exposed and recognized by the immune system under certain circumstances, for example, an infection (24). Ancillary evidence for the involvement of T cells comes from studies showing that patients with chronic ITP often have increased numbers of HLA-DR+ T cells, increased numbers of soluble interleukin-2 receptors (25), an increased Th1/Th2 ratio, and expansion of oligoclonal T-cells (26, 27). Finally, CD3+ T cells from ITP patients were found to have an increased expression of genes involved in cell-mediated cytotoxicity, and the presence of cytotoxic T cells against autologous platelets was shown in patients with active ITP (28).

Immunological tolerance failure

The emergence of antiplatelet autoantibodies and antiplatelet cytotoxic T cells is a consequence of a loss of the immunological tolerance for self antigens. T cells recognizing peptides generated from native GPIIb/IIIa by normal processing pathways are hypothesized to be deleted in the thymus (negative selection), since GPIIb/IIIa has been shown to be expressed on the cell surface of the epithelial cells of the thymic stroma early in intrauterine life (29). However, Filion et al. have shown that autoreactive T cells directed against GPIIb/IIIa are present in the peripheral blood of all healthy individuals (30), implying that peripheral tolerance mechanisms are crucial to prevent autoreactive T cells from becoming activated. As a matter of fact, several abnormal findings have emerged from the investigation of immune regulation in ITP patients. Among these, CD4+CD25+ regulatory T cells are decreased in number and with impaired suppressive activity when compared to healthy subjects (31). Also, CD3+ T lymphocytes from patients with active ITP present an altered expression of genes associated with apoptosis and are significantly more resistant to dexamethasone-induced suppression compared to normal lymphocytes (28, 32).

As far as B cells are concerned, the expansion of autoreactive clones is suppressed in the bone marrow. If some B cells escape this suppression or deletion, peripheral mechanisms, most importantly the functional balance between activating and inhibitory Fc receptors (FcR), may also be launched to maintain tolerance (33).

The role of antigen-presenting cells (APCs) for the loss of tolerance in ITP remains unclear, but a model has been advanced in which APCs are crucial in generating a number of new or cryptic epitopes from platelet glycoproteins (34). In this model, APCs expressing these novel peptides, along with co-stimulatory molecules, induce the activation of T cells that recognize these additional platelet antigens. Thus, this acquired recognition of new self-determinants, or epitope spreading, may play an important role in the initiation and perpetuation of ITP. T-cell clones that react with cryptic epitopes may escape the negative selection in the thymus when self-determinants are present at a sub-threshold concentration.

Role of cell-cell interactions

Intercellular communication is essential for both the activation and effector phases of T cells, B cells and macrophages. Optimal T-cell activation requires two signals. The first signal is based on T-cell receptor (TCR) recognition of complexes of peptide/major histocompatibility complex (MHC). The second signal is provided by interaction of CD28, CD2 and LFA-1 on T cells with their costimulatory ligands B7, LFA-3 and ICAM-1, respectively, on antigen-presenting cells. The activated Th cell produces cytokines that promote B-cell differentiation and antibody production. Ongoing interaction between T cells and B cells through CD40-CD40L (CD154) is necessary to maintain active platelet autoimmunity (11). Platelets themselves express CD154. Normally, surface levels of CD154 are low, but these increase after platelet activation (35, 36). Increased amounts of CD154 and its messenger RNA were found in the platelets and megakaryocytes of patients with ITP, and were able to drive the activation of autoreactive B lymphocytes, suggesting a possible active role of platelets in the autoimmune process (37). Finally, an enhanced expression of CD80 (B7–1) on platelets from patients with ITP was observed, implicating a role of B7/CD28 costimulation in the pathogenesis of this disorder (38).

Mechanisms leading to the thrombocytopenia

Autoantibody-dependent phagocytosis is thought to be the primary mechanism of platelet destruction in ITP (34). Both in-vitro and clinical studies have shown that the spleen is the primary site of antibody production (39, 40) and is also the dominant organ for the clearance of IgG-coated platelets (41, 42). In a minority of patients, hepatic clearance predominates. Human macrophages express several Fc receptors that bind IgG specifically (43). Functionally, there are two different classes of Fc receptors: the activation and the inhibitory receptors, which transmit their signals via immunoreceptor tyrosine-based activation (ITAM) or inhibitory motifs (ITIM), respectively. Clinical data, along with information gained from animal models, suggest that the FcγRI, the high affinity receptor, does not play a relevant role in ITP (44, 45). On the other hand, evidence has accumulated to indicate that the low-affinity receptors FcγRIIA and FcγRIIIA are primarily responsible for removal of opsonized platelets (46). Engagement of FcγRIIA on the surface of human macrophages by anti-GPIIb/IIIa-coated platelets triggers intracellular signaling through the tyrosine kinase Syk, that leads to engulfment of the opsonized platelets.

The presence of antibodies against GP Ib/IX has been associated with resistance to intravenous immunoglobulin (IVIG) therapy both in a mouse model (47) and in retrospective series of ITP patients (48). These findings suggest the possibility of direct cy-

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totoxicity or complement fixation as a mechanism of platelet destruction rather than antibody-dependent, Fc receptor-mediated phagocytosis by macrophages.

Recently, in-vitro studies have produced evidence for direct T cell-mediated cytotoxicity against platelets (28). Whether this effect occurs *in vivo*, its relative importance in determining platelet destruction, and whether the same cytotoxic T cells exert a role against bone marrow megacaryocytes has not been elucidated.

Studies by several groups of investigators (41, 42, 49, 50) using indium-111 (^{111}In)-labeled autologous platelets showed considerable heterogeneity in platelet turnover in chronic ITP. Although the platelet lifespan is often markedly decreased, in some patients the lifespan is only mildly reduced; furthermore, platelet turnover (a measure of platelet production) is frequently subnormal. Overall, approximately 40% of patients with ITP had a reduced platelet turnover (41, 42). In keeping with this finding, autoantibodies against platelet glycoproteins have been shown to interfere with the maturation of megakaryocytes, resulting in reduced platelet production (51, 52). In-vitro studies have shown that antibodies that target the GpIb-IX-V complex may induce thrombocytopenia both by inhibiting megakaryopoiesis (52), and by inhibiting proplatelet formation (53).

Current therapeutic options for patients with ITP

There is no evidence based on randomized trials to guide management decisions, and for some patients the morbidity from side effects of therapy may exceed any problems caused by the ITP (5, 6). Treatment of patients with ITP must take into account the age of the patient, the severity of the illness, and the anticipated natural history. Adult patients, particularly those older than 60 years of age, have a higher incidence of major or fatal bleeding than children (5). However, specific therapy may not be necessary unless the platelet count is <20 x 10^9/l or there is extensive bleeding. In fact, at the present time treatment for ITP is considered appropriate for symptomatic patients and for those at risk of bleeding (1, 7–10).

Initial treatment

Once the decision to treat a patient with ITP has been made, and provided the patient's situation is not life-threatening, corticosteroids are the standard initial treatment (1). The mechanism of action of corticosteroids in ITP is still obscure, although they have been shown to impair the clearance of antibody-coated platelets by mononuclear macrophages (54), decrease autoantibody production (55), and improve the integrity of leaking capillaries (56). The current practice is to initiate treatment with oral prednisolone or prednisone, 1 to 2 mg/kg per day, given as single or divided doses. Approximately two thirds of patients achieve a complete or partial response with corticosteroids, and most responses occur within the first week of treatment (7). However, only 10–15% of all adult patients with ITP who receive prednisone therapy have a durable remission (7). Two large non-randomized studies have shown that a short course of treatment with high-dose oral dexamethasone (40 mg/day for 4 consecutive days) was well tolerated and effective compared to standard-dose therapy (57, 58). Despite this success, the use of this regimen as first-line therapy has not been validated.

IVIGs are generally recommended for patients with critical bleeding and for those unresponsive to corticosteroids (1). The preponderance of evidence continues to support the original postulate that the beneficial effect of IVIG in ITP is a transient impairment of reticuloendothelial clearance function, also referred to as macrophage "blockade" mechanism (59). Samuelsson et al. have demonstrated that IVIG requires the presence of the inhibitory low affinity IgG receptor FcγRIIB for prevention of thrombocytopenia in a murine model of passive ITP (60). They have also demonstrated that some monocytes from IVIG-treated mice begin to express the FcγRIIB at elevated levels within four hours of IVIG administration. This increase in FcγRIIB results in a change in the ratio of expression of the inhibitory FcγRIIB as compared to stimulatory FcγRIII. The way IVIG functions via FcγRIIB is still unclear. Genetic and biochemical studies have identified the SH2 domain-containing inositol 5-phosphatase (SHIP1) as a critical effector in FcγRIIB inhibitory signaling (61). Whether or not this pathway is involved in IVIG action, however, has not been established, and in a murine model of ITP the individual expression of SHIP1 (and two other potential signaling mediators, SHP-1 and Btk) was not required for IVIG action in amelioration of immune thrombocytopenia (62). Other immunomodulatory effects of IVIG include inhibition of complement binding to platelets, interference of immune complexes binding to platelets, immune activity of anti-idiotype antibodies, and impaired release of pro-inflammatory cytokines from monocytes (59). Several regimens for IVIG have been used. In current practice the standard dose is 1 g/kg per day for one to two days (7). IVIG is effective in elevating the platelet count to more than 50 × 10^9/l in approximately 80% of patients. In more than half of responders, the platelet count becomes normal (>100 × 10^9/l) (63). Platelet counts may begin to increase after one day and usually reach peak levels within one week after treatment (64). However, response is generally transient, lasting no longer than 3–4 weeks, after which the platelet counts decrease to pretreatment levels. Thus, IVIG therapy is ideal when a rapid increase in platelet count is desired in patients with life-threatening bleeding and can also be combined with steroids and platelet transfusions in these situations (7).

The platelet count also can be supported by anti-D immunoglobulin, which is active only in Rh-positive patients and in the presplenectomy setting (65). Anti-D binds to the erythrocyte D-antigen. Immune-mediated clearance of the sensitized erythrocytes occupies the Fc receptors in the reticuloendothelial system, minimizing removal of antibody-coated platelets (59). The response rate to intravenous anti-D at the dose of 50 μg/kg was 70% in the largest series published to date (65). The increase in platelet count was seen after 72 hours, and lasted more than 21 days in 50% of the responders. At doses of 75 μg/kg, anti-D not only increases the platelet count more rapidly, and for a longer duration compared with the standard dose of 50 μg/kg, but platelet responses within 24 hours occur in the majority of patients, faster than those that have been reported with corticosteroids and as fast as those reported with IVIG (66). Subcutaneous anti-D has been tried in a few patients suffering from chronic ITP (67).

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None of the patients treated with this alternate route of administration developed haemolysis or any other significant reaction. In addition, subcutaneous delivery of anti-D seemed to produce largely the same beneficial effect observed with intravenous delivery.

The repeated use of either maintenance IVIG (68) or maintenance anti-D globulin (69) allows approximately 40% of adults with ITP to avoid splenectomy altogether. Considering the substantially lower costs and shorter infusion time (minutes compared with hours) of anti-D compared to IVIG, the former should probably be preferred for the long-term use. A randomized, controlled trial has tested the potential of anti-D to avoid or defer the need for splenectomy in newly diagnosed adults with ITP and a platelet count <30,000/µl. There were no differences in the rates of spontaneous remission or the need for splenectomy between the anti-D group and the routine care group (70). However, splenectomy was performed prematurely, not according to protocol, in 11 of 14 patients. This may have affected the inability to show a difference between the two groups with regard to rates of splenectomy.

IVIG and particularly anti-D can cause mild alloimmune haemolysis, and IVIG may also cause headache, nausea, and vomiting, symptoms that may cause concern for the possible occurrence of intracranial haemorrhage (71). Some sucrose-containing products may also be associated with acute renal failure (72). Anti-D immunoglobulin can rarely cause intravascular haemolysis and disseminated intravascular coagulation (73). Anti-D should not be used, or used with extreme care, in patients with a positive direct antiglobulin test and a haemoglobin level that is less than 10 g/dl, due to the risk of increasing the severity of the anemia.

Emergency treatment is indicated for internal or profound mucocutaneous bleeding. Hospitalization is required, and general measures should be instituted to reduce the risk of bleeding, including avoidance of drugs that inhibit platelet function, control of blood pressure, and other factors. Although no systematic studies have evaluated the efficacy of different regimens, there is general agreement that appropriate interventions should include the following (1, 7, 74):

- IVIG, (1 g/kg, repeated the following day if the platelet count remains < 50 x 10^9/l)
- Intravenous methylprednisolone, 1 g/d for 3 days
- Platelet transfusions (either 10 U every 4–6 hours or 3 U/h)

Although patients with ITP are assumed to have a rapid platelet destruction, transfused platelets may provide temporary critical haemostatic support (75). Platelet transfusions usually are given after IVIG and are often effective in controlling bleeding, irrespective of the increase in platelet counts. If critical bleeding continues after initial management with platelet transfusions, IVIG, and methylprednisolone, intravenous recombinant human factor VIIa may be effective (76–80).

Second-line treatment

Splenectomy is traditionally considered to be the second-line treatment in adults with ITP in whom achieving a safe platelet count with initial prednisone therapy has failed. Generally accepted criteria for splenectomy include a severe thrombocytopenia (<10 x 10^9/l), a high risk of bleeding for platelet counts less than 30 x 10^9/l, or the requirement of continuous glucocorticoid therapy to maintain safe platelet counts (1).

A systematic review of 47 case series with 2,623 patients indicates a complete response (platelet count >150 × 10^9/l) rate of 66% (range, 37% to 100%) (81). Of 707 evaluable patients with at least five years of follow-up, 456 (64%) retained their response status. Most relapses occur during the first two years after splenectomy, but even after that a small percentage of patients continued to relapse. No preoperative characteristic that has been reported predicted response to splenectomy consistently. Younger age was linked to a good response, but no specific age could be defined (81).

Splenic sequestration of ^{111}In-labeled platelets was a good prognostic factor in many studies in which this scanning method was applied. In the large study by Najean et al., a splenic pattern of platelet destruction had a positive predictive value of 93%, whereas a hepatic or diffuse pattern of platelet destruction had a negative predictive value of 77% (82). However, platelet sequestration studies are difficult to perform and are available in only a few medical centers. Furthermore, the specificity of the test is not high enough to recommend it routinely for patients in whom splenectomy has been considered.

Occasionally, patients may fail to respond to splenectomy because of the failure to remove an accessory spleen. In other patients, a small, inactive accessory spleen may grow or new splenic foci may develop from splenic cells shed at the time of surgery and cause the late onset of thrombocytopenia. In either case, the presence of splenic tissue can be diagnosed by examination of the blood smear for Howell-Jolly bodies that appear in the red cells of asplenic individuals. Persistent splenic tissue can be confirmed by a radionuclide scan.

An alternative to conventional open splenectomy is laparoscopic splenectomy. Several studies have shown reduced blood loss with this procedure and more rapid recovery time, and suggested a lower mortality rate compared with open splenectomy (0.2% and 1.0%, respectively) (81).

Splenectomized patients have a small risk for overwhelming infections, with an estimated mortality of 0.73 per 1,000 patient-years (83). The risk for serious postsplenectomy infection is greater in children younger than five years, who are therefore treated with prophylactic penicillin after splenectomy. Although there are no data on the efficacy of vaccination, immunizations against encapsulated bacteria (*Streptococcus pneumoniae*, *Haemophilus influenzae B*, and *Neisseria meningitides*) are generally advised at least two weeks before splenectomy (1). The usefulness of postoperative antibiotic prophylaxis is a matter of controversy, and although it is not the standard of care in the United States, life-long prophylactic antibiotics are recommended in UK guidelines (84).

Treatment of patients in whom splenectomy fails

Patients can be defined as having chronic refractory ITP if splenectomy fails and the patients require additional therapy. About 30% of adult patients with ITP may belong to this category (85). The goals of therapy for refractory ITP are clearly different from

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those for patients at initial presentation because the chance of inducing a durable, complete, and unmaintained remission is much lower. The objective of treatment for patients with refractory ITP is to achieve stable adequate platelet counts (>20 to 30 x 10^9/l) while minimizing the adverse side effects of medication (7).

Patients who have a relapse are generally treated again with corticosteroids, which are only transiently effective, have low response rates and substantial toxicity with daily use. In recent years several studies have reported the use of anti-CD20 monoclonal antibody rituximab in patients with chronic ITP, both prior to and after splenectomy. Rituximab eliminates normal B cells, including those producing antiplatelet antibodies (86). This B cell depletion is transient (lasting 6 to 12 months, in most cases) and has few side effects or toxicities (86). A recent report indicates that rituximab exerts significant effects on cellular immunity as well (27). Pretreatment abnormalities of T cells in ITP patients were reverted in responders at three and six months after anti-CD20 therapy, whereas they remained unchanged in non-responders. Furthermore, a strong association was found between failure to rituximab therapy and oligoclonal expansion of T cells (27). An attractive hypothesis to explain this finding is that rituximab may be sufficiently immunomodulatory to produce a clinical remission at an early stage of ITP, when expansion of pathogenic T cells has not reached a "critical" level and is still dependent upon B-cell costimulation. In contrast, when T-lymphocyte clones are more expanded they continue to drive antibody production irrespective of the cytokine microenvironment produced by B cells. The results of a large clinical trial with rituximab are consistent with such a speculation (87). In that study patients with a short disease history, in whom presumably T-cell expansion was not yet critical, were much more likely to respond to treatment than those with an ITP duration of many years. Therefore, non-responsiveness to anti-CD20 therapy in ITP may be related to an inability to modify the oligoclonal nature of the T-cell subsets.

A systematic analysis of the published literature suggests that rituximab produces an initial response in approximately 60% (range, 25–75%) of cases, with no significant difference between splenectomized and non-splenectomized patients (88). The median response duration is 10.5 months (range, 2–48 months), with a 15–20% rate of long-term complete responses. A recent French study indicates that the use of rituximab allows to defer splenectomy for at least one year in 40% of patients with chronic ITP (89).

Several other drugs have been reported to have some activity (although not consistently) in refractory ITP. These include the attenuated androgen danazol, dapsone, azathioprine, cyclophosphamide, vinca alkaloids, recombinant interferon-γ2B, cyclosporin A, and mycophenolate mofetil, alone or in combinations (7). Responses with these agents do not usually exceed 30–35% and, if seen, they may only be apparent after several weeks. More experimental approaches include campath-1H, liposomal doxorubicin, protein A immunoadsorption columns, and peripheral blood stem cell transplantation (7). The toxicities associated with these agents should be carefully evaluated when further treatment is required.

Management of the thrombocytopenia complicating other immune disorders

Thrombocytopenia can complicate other disorders of immune dysregulation, including autoimmune haemolysis (the combination is called Evans' syndrome), systemic lupus erythematosus (SLE), the antiphospholipid syndrome, and common variable immunodeficiency. The approach to treatment should parallel considerations given for the treatment of primary ITP. However, for some of these conditions (e.g. Evans' syndrome), the thrombocytopenia may be less responsive to first-line treatment (corticosteroids and splenectomy). In the case of thrombocytopenia complicating SLE, danazol and hydroxychloroquine, when used with glucocorticoids, resulted in sustained remission rates of 50% and 64% of cases, respectively (90). The role of splenectomy in SLE remains controversial, and it should be used only when the thrombocytopenia is severe and persistent and is the predominant reason for treatment and when the SLE is otherwise well controlled (90). Thrombocytopenia is a common finding in patients with the antiphospholipid syndrome, and requires treatment strategies similar to those used for patients with ITP (91). An issue of clinical importance in evaluating thrombocytopenia associated with the antiphospholipid syndrome is the risk for future development of thrombosis, since moderate thrombocytopenia does not prevent thrombosis in these patients (92).

Immune thrombocytopenia also occurs with increased frequency in patients with common variable immunodeficiency (93). Common variable immunodeficiency should be suspected in patients with even mild recurrent infections and allergies. Splenectomy and immunosuppressive agents should be avoided to the extent possible (94).

A novel therapeutic approach: thrombopoietic agents

Current treatments for ITP aim at suppressing the production of autoantibodies and/or inhibiting macrophage-mediated destruction of opsonized platelets. As discussed above, however, several patients have impaired platelet production rather that increased platelet destruction. Additionally, ITP patients have normal or slightly elevated thrombopoietin (TPO) levels whether measured in plasma or serum, but the levels are always lower than the concentrations found in thrombocytopenias resulting from megakaryocytic hypoplasia (95–97). This finding probably results from active TPO uptake and destruction by the expanded megakaryocyte mass in ITP. On these grounds, growth factor stimulation of megakaryopoiesis might be expected to increase the platelet count in patients with ITP.

First-generation thrombopoietins used in clinical trials included recombinant human TPO (rhTPO), and a non-glycosylated, truncated form of TPO coupled to polyethylene glycol. The recombinant protein, called "megakaryocyte growth and differentiation factor" (MGDF), had important differences compared to native TPO that probably explain its immunogenic potential (98). Clinical trials were discontinued after the development of TPO autoantibodies was observed in healthy volunteers (99), and did not yield a clinically approved therapeutic thrombopoietin. Recently, however, intense clinical trial activity has resumed with second-generation thrombopoietic growth factors. These

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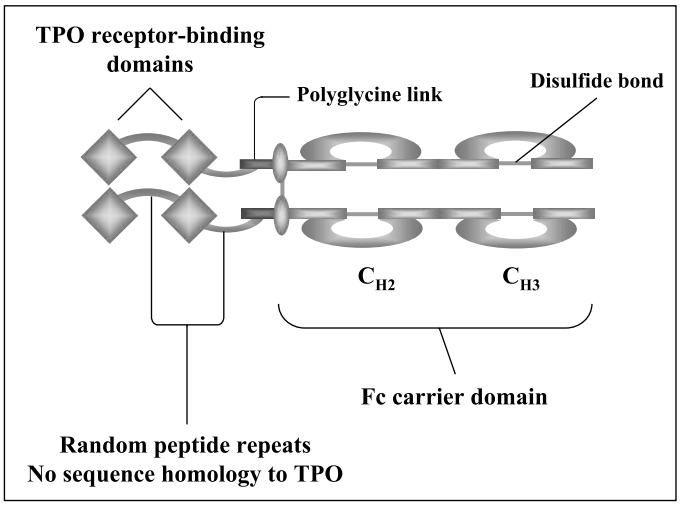

Figure 2: Structure of AMG 531. AMG 531 is made of two disulphide-bonded immunoglobulin Fc fragments each of which is covalently bound at residue 228 with two identical peptide sequences linked via polyglycine.

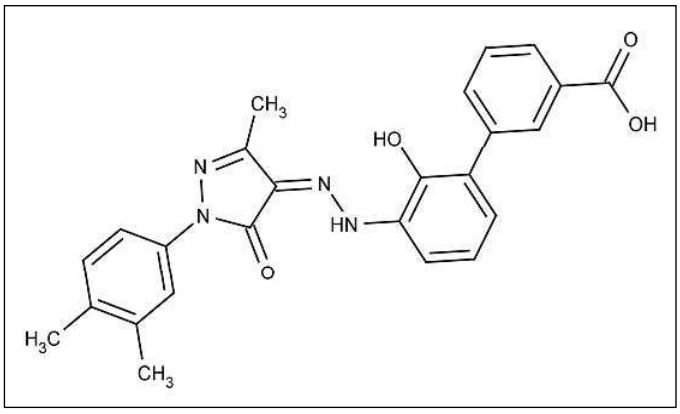

Figure 3: Chemical structure of eltrombopag. The IUPAC name of the molecule is 3-[(5E)-5-([2-(3,4-dimethylphenyl)-5-methyl-3-oxo-2,3-dihydro-1H-pyrazol-4-yl]hydrazono)-6-oxocyclohexa-1,3-dien-1-yl]benzoic acid. The molecular weight is 442 Da, and the empirical formula is $C_{25}H_{22}N_4O_4$. The compound has an acidic (COOH) group at one end, lipophilic (CH3) groups at the other end, and a metal chelate group in the center. The above information and the figure are public domain available at http://chem.sis.nlm.nih.gov/chemidplus/chemidheavy.jsp.

new molecules bear no structural resemblance to TPO, but still bind and activate the TPO receptor. Studies have been completed for two TPO mimetics, AMG 531 and eltrombopag.

AMG 531 is a recombinant protein defined as a "peptibody" (Fig. 2). It is made of two disulphide-bonded immunoglobulin Fc fragments each of which is covalently bound at residue 228 with two identical peptide sequences linked via polyglycine (100). The carrier Fc component of the molecule binds to the FcRn salvage receptor and undergoes endothelial recirculation, resulting in a substantially longer half-life than the peptide alone. Results of phase 1/2 trials were published recently, demonstrating that AMG531 given as a weekly subcutaneous injection for 1–6 weeks leads to doubling of platelet counts and an increase to more than 50 x10^9/l in approximately 75% of patients with minimal side effects (101, 102).

Patients from the AMG 531 phase 1–3 studies have been enrolled in an open-label study of long-term administration of the drug. The planned interim analysis included 36 patients (safety subset), 29 of whom treated for longer than 48 weeks, whose previous study was a phase II trial (103). The analysis indicates that they had a stable mean platelet count of greater than 100 x 10^9/l, were on a stable weekly dose, and half were able to stop other concomitant immunosuppressive drugs. The most frequently reported complication of therapy was mild headache; in two patients there was a mild-to-moderate increase in bone marrow reticulin but without collagen fibrosis and with normal cytogenetic findings (101). Results of a randomized, placebo-controlled phase III study of six months of treatment with AMG 531 in splenectomized and non-splenectomized ITP patients have not yet been reported. The potential of AMG 531 as a splenectomy-sparing agent is being investigated in an ongoing phase IIIb trial, in which the thrombopoietic agent is compared to medical standard of care for non-splenectomized subjects with ITP (see http://clinicaltrials.gov/ct/show/NCT00415532 for details). The treatment duration is 52 weeks, and is followed by a six-month safety follow-up study.

Eltrombopag (formerly SB497115) is a small, orally available, hydrazone organic compound (Fig. 3). This molecule was identified from high-throughput screening of small-molecule compound collections, utilizing haematopoetic cell lines co-transfected with the human TPO receptor, a luciferase gene, and several requisite signal-transduction responsive elements (104). In a randomized, double-blind, placebo-controlled phase 2 trial, subjects were given daily oral treatment with placebo or escalating doses of eltrombopag (105). Platelet responses were observed in 70% of patients at the 50 mg dose, and in 81% at the 75 mg dose. Furthermore, a preliminary analysis in 36 patients suggested a trend toward fewer bleeding events on therapy in those receiving the 50 mg and 75 mg doses. No significant adverse events were seen. A subsequent randomized, double-blind, placebo-controlled phase III trial enrolled 114 adults with chronic ITP and baseline platelet counts of <30 x 10^9/l (106). These patients were randomized to either placebo (38 patients) or eltrombopag 50 mg (76 patients) once daily for six weeks. The eltrombopag dose could be increased to 75 mg in patients not responding after an initial three weeks of treatment. All patients had received prior ITP treatment and 52% had received ≥3 prior therapies. At the end of the trial, 16% of placebo patients and 59% of eltrombopag patients achieved the primary endpoint (platelet count ≥50 x 10^9/l). Importantly, there was a significantly lower incidence of bleeding events during treatment with eltrombopag compared to placebo (p=0.029) with clinically significant bleeding (WHO grades 2–4) observed in fewer eltrombopag patients (16%) than placebo patients (36%). The most common adverse event observed in this study was headache, but this could not be attributed to the study medicine. Several eltrombopag trials investigating the short- and long-term treatment of chronic ITP are currently open and enrolling (see http://clinicaltrials.gov/).

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There are other thrombopoietic agents in development, such as AKR-501 (107), which appear promising in normal volunteers. Ongoing phase III clinical trials will reveal the potential of these agents in the management of ITP prior to splenectomy and for long-term maintenance therapy, as well as their relative benefit compared to placebo or standard of care treatment.

Conclusions

Despite some fundamental tenets of the pathophysiology of ITP have been known for half a century, important discoveries at the molecular level have been relatively recent.

Concepts about the mechanisms of the thrombocytopenia in ITP have shifted from the traditional view of increased platelet destruction mediated by antibodies, to a much more complex situation where impaired platelet production and T cell-mediated platelet destruction have emerged as playing a significant role. At the same time, new therapeutic approaches, such as rituximab, have been evaluated in adult patients with ITP. These findings have been particularly important for those patients who would prefer to postpone or avoid splenectomy. Thrombopoietic agents appear to be very effective in a high percentage of even refractory patients with ITP, very tolerable, and safe. They appear to be a major breakthrough in the treatment of patients with ITP.

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