Pathophysiology and therapeutic options in primary immune thrombocytopenia

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Introduction

The acronym ITP stands for primary immune thrombocytopenia (formerly idiopathic thrombocytopenic purpura), an acquired autoimmune disorder characterised by isolated thrombocytopenia in the absence of conditions known to cause thrombocytopenia, such as infections, other autoimmune disorders, drugs, etc[1]. Manifestations of ITP can be localised haemorrhaging in skin or mucous membranes that are usually of little to no clinical consequence (petechiae, purpura, ecchymoses, epistaxis); more rarely, ITP can be associated with severe bleeding events such as intracranial haemorrhage (ICH). However, most ITP patients are asymptomatic in the presence of platelet counts greater than $50x10^9/L$[2].

ITP can be classified based on patient age (adult or childhood ITP), and duration of thrombocytopenia: newly diagnosed, up to 3 months from diagnosis; persistent, 3-12 months from the time of diagnosis; chronic, >12 months from the time of diagnosis[1]. The clinical features of ITP in adults are usually different from those seen in childhood. ITP in children usually has an abrupt ("acute") onset, often occurring 1 to 2 weeks after a viral infection or 2 to 6 weeks after immunization with the measles, mumps and rubella (MMR) vaccine[3,4], and recovers spontaneously in a few weeks regardless of treatment. In contrast, ITP in adults typically has an insidious onset, with no preceding viral or other illness, and has frequently a chronic course.

Design of prospective, controlled clinical trials has been particularly difficult, since patients with the chronic disease needing treatment are less than 10% of all ITP patients[5]. Nevertheless, randomised trials with several new pharmacologic agents have recently changed this scenario.

In this review we shall summarize the current understanding of the pathophysiology and mechanisms leading to thrombocytopenia and the evolving therapeutic modalities for chronic refractory ITP in adults.

Pathophysiology of ITP

Abnormalities of B and T-cells

In 1951 Harrington and Hollingsworth had observed a child with purpura born to a mother with chronic ITP[6]. Purpura in the child resolved 3 weeks later, although the mother still had ITP. The existence of a humoral anti-platelet factor that had been passed from mother to child was advanced. To test this hypothesis, Harrington received 500 mL of blood from a patient with ITP. Within three hours, his platelet counts dropped below $10x10^9/L$ as he developed chills, fever, headache, confusion and petechiae[7]. His platelet count remained extremely low for four days, finally returning back to normal levels by the fifth day[8]. He performed a similar experiment on volunteers, confirming his original finding.

Harrington's seminal experiment provided the first evidence that platelet destruction in ITP is caused by a plasma-derived factor[9], later identified as anti-platelet antibodies[10,11]. 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[12]. Antibodies against GP IIb/IIIa show clonal restriction in light-chain use[13], and antibodies derived from phage-display libraries show selective usage of a single Ig heavy-chain variable region gene (VH_{3-30})[14]. Sequencing of the antigen-combining regions of these antibodies suggests that they originate from a limited number of B-cell clones by antigen-driven affinity selection and somatic mutation[14]. It should be noted, however, that autoantibodies are not detectable in up to 50% of ITP patients[12,15] and that

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remission in ITP can occur despite the continued presence of platelet autoantibodies[16]. Reasons for these findings may include technical factors (current monoclonal-based assays only detect antibodies with known specificity, typically GPIIb-IIIa and GPIb-IX; variable sensitivity of the assays), removal of autoantibodies by megakaryocytes, and the presence of alternative mechanisms of the thrombocytopenia.

As a matter of fact, several lines of evidence also link T-cells to the pathogenic process in ITP. Platelet-reactive T-cells have been found in the blood of patients with this disorder, with the major target antigen being GP IIb/IIIa[17]. 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[18]. The derivation of these cryptic epitopes *in vivo* and the reason for sustained T-cell activation are unknown. It has been hypothesised that cryptic epitopes, normally not exposed in a self-antigen, may become exposed and recognised by the immune system under certain circumstances, for example, an infection[19]. Other studies have shown that patients with chronic ITP often have increased Th1/Th2 ratio, expansion of oligoclonal T-cells[20,21], and the presence of cytotoxic T-cells against autologous platelets[22].

The emergence of anti-platelet autoantibodies and anti-platelet cytotoxic T-cells is a consequence of a loss of the immunological tolerance for self antigens. Filion *et al.* have shown that autoreactive T-cells directed against GPIIb/IIIa are present in the peripheral blood of all healthy individuals[23], implying that peripheral tolerance mechanisms are crucial to prevent autoreactive T-cells from becoming activated. Several other T cell abnormalities have emerged from the investigation of immune regulation in ITP patients. Among these, CD4+CD25+ regulatory T-cells have an impaired suppressive activity when compared to healthy subjects[24]. 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[22,25].

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[26].

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[27]. 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.

Thrombocytopenia may accompany or follow a variety of infections from which ITP must be differentiated. In adults, the most prevalent infections associated with thrombocytopenia are those from hepatitis C virus (HCV), human immunodeficiency virus (HIV), and *Helicobacter pylori* (*H. pylori*)[28]. In typical cases the thrombocytopenia presents with an insidious onset, has no tendency to remit spontaneously (although its severity may parallel the stage of the infectious disease), and may closely mimic chronic ITP. Response to infection may generate antibodies that cross-react with platelet antigens or immune complexes that bind to platelet Fc receptors[29-32].

Mechanisms leading to the thrombocytopenia

Both *in vitro* and clinical studies have shown that the spleen is the primary site of antibody production[33,34] and is also the dominant organ for the clearance of IgG-coated platelets[35,36]. In a minority of patients, hepatic clearance predominates. Human macrophages express several Fc receptors that bind IgG specifically[37]. 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[38,39]. On the other hand, evidence has accumulated to indicate that the low-

affinity receptors FcγRIIA and FcγRIIIA are primarily responsible for removal of opsonised platelets[40]. 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 opsonised platelets.

The presence of antibodies against GP Ib/IX has been associated with resistance to intravenous immunoglobulin therapy both in a mouse model[41] and in retrospective series of ITP patients[42]. These findings suggest the possibility of direct cytotoxicity or complement fixation as a mechanism of platelet destruction rather than antibody-dependent, Fc receptor-mediated phagocytosis by macrophages.

Interestingly, platelet kinetic studies using indium-111 (^{111}In)-labelled autologous platelets have shown considerable heterogeneity in platelet turnover in patients with chronic ITP[35,36,43,44]. While the platelet lifespan is often markedly decreased in most patients, in some it is only mildly reduced; furthermore, platelet turnover (a measure of platelet production) is frequently subnormal. Overall, approximately 40% of patients with ITP were found to have a reduced platelet turnover[35,36].

If platelet destruction were the only mechanism to cause thrombocytopenia, then platelet production would be expected to increase and offset low platelet counts. It was therefore proposed that thrombocytopenia may result not only from platelet destruction, but also from antibody-mediated damage to megakaryocytes. Evidence to support this hypothesis has accumulated over time.

McMillan *et al.* reported that IgG produced by cells (grown *in vitro*) from the spleens of patients with ITP would bind to megakaryocytes, whereas IgG produced by cells from the spleens of healthy controls did not bind to megakaryocytes[45]. A few years later other investigators demonstrated that antibodies against platelet antigens would bind to megakaryocytes as well[46,47]. More recent *in vitro* experiments have further defined the role of autoantibodies in patients with ITP. Two studies in particular, by Chang *et al.*[48] and McMillan *et al.*[49] support the view that autoantibodies in ITP suppress megakaryocyte production and maturation and platelet release.

Electron microscopy studies have clarified some aspects of the autoantibody-induced damage in bone marrow megakaryocytes from patients with ITP. Extensive megakaryocytic abnormalities were consistently present in a significant percentage of all stages of ITP megakaryocyte[50,51]. In the most recent of these studies, Houwerzijl *et al.* the described features characteristic of nonclassic apoptosis, including mitochondrial swelling with cytoplasmic vacuolisation, distention of demarcation membranes, and condensation of nuclear chromatin[51]. Para-apoptotic changes could be induced in megakaryocytes derived from CD34+ cells grown in ITP plasma, suggesting that autoantibodies may initiate the cascade of programmed cell death. In addition, megakaryocytes may be surrounded by neutrophils and macrophages, suggesting an inflammatory response against these cells.

A role for direct T-cell-mediated cytotoxicity against platelets has been demonstrated *in vitro*[22]. Whether this effect occurs *in vivo* and its relative importance in determining platelet destruction has not been elucidated. There is also evidence that ITP is associated with accumulation and activation of T-cells in the bone marrow that occurs through increased VLA-4 and CX3CR1 expression[52]. It has been advanced that these activated T-cells may mediate the destruction of platelets in the bone marrow[52].

Thrombocytopenia associated with infectious diseases is characterised by antibody-mediated platelet destruction. However, platelet production may be impaired by infection of megakaryocytes (HCV and HIV), decreased production of thrombopoietin (HCV), and splenic sequestration of platelets secondary to portal hypertension (HCV)[28].

Management of patients with ITP

ITP is considered a benign disorder with an approximately 60% higher rate of mortality than gender- and age-matched comparison subjects without ITP[53]. This increased risk of death is largely concentrated in patients 60 years of age or older[54]. However, fatal bleeding occurs only with severe thrombocytopenia and specific therapy may not be necessary unless the platelet count is $<20x10^9$/L or there is extensive bleeding. Another important consideration is that for some patients the morbidity from side effects of therapy may exceed any problems caused by the ITP[5,54]. The management of patients with ITP must therefore take into account the age of

the patient, the severity of the illness, and the anticipated natural history. Current guidelines consider treatment for ITP appropriate for symptomatic patients and for those at significant risk of bleeding[55-59].

Initial treatment

If the clinical presentation is not that of a life-threatening bleeding corticosteroids are considered the standard initial treatment[55]. The current practice in many countries 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 at these "standard" doses, with most responses occurring within the first week of treatment[56]. However, only 10% to 15% of all adult patients with ITP who receive predniso(lo)ne therapy have a durable remission[56]. Two large nonrandomised studies have shown that a short course of treatment with high-dose oral dexamethasone (40 mg/d for 4 consecutive days) was well tolerated and with higher response rates compared to published studies with standard-dose corticosteroids[60,61]. An ongoing randomised trial of the Italian group GIMEMA will define the relative efficacy of the two regimens[62].

Intravenous immunoglobulins (IVIG) are generally recommended for patients with critical bleeding and for those unresponsive to corticosteroids or for whom corticosteroids are contraindicated[55]. Several regimens for IVIG have been used with comparable clinical outcomes. In current practice the standard dose is 1 g/kg per day for one to two days[56]. Intravenous immunoglobulin 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 ($>100\times10^9$/L)[63]. Platelet counts may begin to increase after 1 day and usually reach peak levels within 1 week after treatment[64]. However, responses are generally transient, lasting no longer than 3 to 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[56].

A convenient alternative to IVIG is anti-D immunoglobulin, which is efficacious only in Rh-positive patients and in the presplenectomy setting[65]. Anti-D binds to the Rhesus D erythrocyte antigen. The mechanisms of action of anti-D have not been completely elucidated. A widely accepted model involves immune-mediated clearance of the sensitised erythrocytes in the reticuloendothelial system, competing with antibody-coated platelets for the Fc receptors[66]. The response rate to intravenous anti-D at the dose of 50 μg/kg, defined as a platelet increase $\geq20x10^9$/L, was 78% in the largest series (156 patients) published to date[65]. The increase in platelet count ($95\pm114x10^9$/L) was seen after 72 hours, and lasted more than 21 days in 50% of the responders. At doses of 75 μg/kg, anti-D increases the platelet count more rapidly, and for a longer duration compared with the standard dose of 50 μg/kg[67]. Furthermore, platelet responses occurring within 24 hours are comparable to those reported with IVIG[68]. Subcutaneous anti-D has been tried in a few patients suffering from chronic ITP[69]. 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.

In two different studies from the same center, the repeated use of either maintenance IVIG[69] or maintenance anti-D globulin[70] allowed approximately 40% of adults with ITP to avoid splenectomy. A randomised, controlled trial has subsequently 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[71]. 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[72]. Some sucrose-containing products may also be associated with acute renal failure[73]. Although evidence of haemolysis is present in most patients treated with anti-D, the decline in

haemoglobin concentration rarely exceeds 2 g/dL[65]. Rare cases of massive intravascular haemolysis and disseminated intravascular coagulation have been reported[75]. It has been conjectured that a coexisting autoimmune haemolytic anaemia (Evan's syndrome) could have contributed to these complications[75]. For all these reasons anti-D should not be used, or used with extreme care, in patients with a positive direct anti-globulin test (risk of intravascular haemolysis) and in those with a haemoglobin level that is less than 10 g/dL (risk of developing symptomatic anaemia requiring blood transfusion).

Considering the substantially lower costs and shorter infusion time (minutes compared with hours) of anti-D compared to IVIG, the former is preferred for the long-term use. However, anti-D for use in ITP is currently not licensed in many countries, including the European Union.

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[55,56,76]:

- platelet transfusions (either one therapeutic dose every 4-6 hours or half therapeutic dose/h);
- IVIG (1 g/kg, repeated the following day if the platelet count remains <50x10^9/L);
- intravenous methylprednisolone, 1 g/d for 3 days.

Platelet transfusions may be given without concomitant therapy and can usually stop bleeding irrespective of the increase in platelet counts[77,78]. IVIG and methylprednisolone may be effective both as initial treatment and if bleeding continues after platelet transfusions[79-83]. A limited number of reports suggest intravenous recombinant human factor VIIa can be beneficial in patients with critical bleeding refractory to conventional treatment[84].

Second-line treatment

For decades splenectomy has been considered the second-line treatment in adults with ITP unresponsive to initial corticosteroid therapy. Recently, however, the availability of effective pharmacological agents has challenged the position of splenectomy in the treatment algorithm. Generally accepted criteria for splenectomy include a severe thrombocytopenia (<10-20x10^9/L), a high risk of bleeding for platelet counts less than 30x10^9/L, or the requirement of continuous glucocorticoid therapy to maintain safe platelet counts[55].

There is no doubt about the efficacy of splenectomy in chronic ITP. The initial complete response (platelet count >150×10^9/L) rate is 65-70% and the long term response rate is 60-70%[85,86]. In other words, approximately 50% of patients with chronic ITP can be cured with splenectomy.

No preoperative characteristic has been reported to predict response to splenectomy consistently. Splenic sequestration of ^{111}In-labelled 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%[87]. However, platelet sequestration studies are difficult to standardise and are available in only a few medical centers.

Occasionally, patients may fail to respond to splenectomy because of the failure to remove an accessory spleen. Accessory spleens are found in 12% to 43% of post-splenectomy patients with recurrent or persistent ITP. Recurrence has been reported as late as 21 years after splenectomy, and up to 66% of these patients achieve a complete remission after accessory splenectomy[88].

The presence of residual 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)[85].

Splenectomised patients have a small risk for overwhelming infections, with an estimated mortality of 0.73 per 1,000 patient-years[89]. The risk for serious post-splenectomy infection is greater in children younger than 5 years, who are rarely candidates for splenectomy. Although there are no data on the

efficacy of vaccination, immunisations against encapsulated bacteria (*Streptococcus pneumoniae*, *Haemophilus influenzae B*, and *Neisseria meningitidis*) are generally advised at least 2 weeks before splenectomy[55]. The usefulness of postoperative antibiotic prophylaxis remains a matter of debate. While it is not the standard of care in the United States, lifelong prophylactic antibiotics are recommended in UK guidelines[90].

Treatment of patients with refractory ITP

Patients are considered to have refractory ITP if splenectomy fails (either no initial response or relapse after splenectomy) and they require additional therapy[1]. No curative approach is currently available for refractory ITP, and the objective of treatment is to achieve haemostatically safe platelet counts (>20 to 30x10^9/L) while minimising the adverse side effects of medication[56].

Relapsed patients generally have a poor response to repeat challenge with corticosteroids in the face of substantial toxicity. In recent years, several studies have reported significant response rates both prior to and after splenectomy with the use of rituximab, an anti-CD20 monoclonal antibody. Rituximab specifically targets B-cells, including those producing anti-platelet antibodies[91]. B-cell depletion is transient (lasting 6 to 12 months, in most cases) and has few side effects or toxicities[91]. A systematic analysis of the published literature suggests that rituximab produces an initial response in approximately 60% (range, 25% to 75%) of cases, with no significant difference between splenectomised and non-splenectomised patients[92]. The median response duration is 10.5 months (range, 2-48 months), with a 15-20% rate of long-term complete responses. A French study indicates that the use of rituximab allows to defer splenectomy for at least 1 year in 40% of patients with chronic ITP[93].

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 IFN-α2B, cyclosporin A, and mycophenolate mofetil, alone or in combinations[56]. Responses with these agents are variable and for some of them, such as azathioprine or danazol, they may only be apparent after several weeks or months. More experimental approaches include campath-1H, liposomal doxorubicin, protein A immunoadsorption columns, and peripheral blood stem cell transplantation[56]. The toxicities associated with these agents should be carefully evaluated when further treatment is required.

Thrombopoietin receptor agonists for the treatment of chronic and refractory ITP

As previously discussed, impaired platelet production is a major mechanism of thrombocytopenia in several patients with ITP. Growth factor stimulation of megakaryopoiesis might therefore be a rational approach to increase the platelet count in patients with ITP.

First-generation thrombopoietin receptor agonists included recombinant human TPO (rhTPO), and megakaryocyte growth and differentiation factor (MGDF), a non-glycosylated, truncated form of TPO coupled to polyethylene glycol[94]. Clinical trials with these agents were stopped after the development of IgG4 antibodies to PEG-MGDF was observed in healthy volunteers[95]. Such antibodies cross-reacted with endogenous TPO neutralizing its activity and thereby led to thrombocytopenia. Clinical trial activity was resumed with second-generation thrombopoietic growth factors that have no sequence homology with endogenous TPO, but still bind and activate the TPO receptor. Two of these new agents, romiplostim and eltrombopag, are now licensed for use in patients with chronic ITP.

Romiplostim is a recombinant protein defined as a "peptibody". It is made of 2 disulphide-bonded immunoglobulin Fc fragments each of which is covalently bound at residue 228 with 2 identical peptide sequences linked via polyglycine[96].

Eltrombopag is a small, orally available, hydrazone organic compound. This molecule was identified from high-throughput screening of small-molecule compound collections[97].

Patients enrolled in the trials with the TPO receptor agonists were required to have a platelet count < 30×10^9/L and to have failed a prior ITP therapy, which might have included splenectomy. Response to therapy was variably defined in the various studies.

Romiplostim has been the first agent to receive marketing approval thanks to the results of two pivotal randomised, placebo-controlled, phase III studies[98].

The trials had a 24-week duration; one was in splenectomised and one in nonsplenectomised patients. Patients were randomised (2:1) to receive romiplostim or placebo once weekly by subcutaneous injection over the study period. The initial romiplostim dose was 1 μg/kg and subsequent doses were adjusted based on platelet response to achieve target counts of $50x10^9$ to $200x10^9$/L. The maximum permitted dose was 15 μg/kg.

Responses, defined as a platelet count rise to ≥50×10^9/L during four or more weeks during the 24 weeks study, were observed in 79% (33/42) of splenectomised and 88% (36/41) of non-splenectomised patients, compared with only 14% (3/21) of nonsplenectomised and 0% (0) splenectomised placebo recipients (P<0.001). The majority of the romiplostim-treated patients (87%) were able to discontinue concomitant treatments or substantially reduce dosage (by >25%) compared with only 38% of placebo recipients. Moreover, fewer romiplostim-treated patients required rescue medications compared with placebo subjects (26.2 versus 57.1% of splenectomised and 17.1 versus 61.9% of nonsplenectomised patients).

Subjects who had completed a previous romiplostim study were allowed to enroll in an open-label extension study of romiplostim with doses adjusted weekly depending on the platelet count. Data from 142 patients treated for periods of up to 3 years are available[99]. Altogether, 87% of patients (n=124) achieved a platelet response, defined as a platelet count >$50x10^9$/L and at least double the baseline value in the absence of rescue medication in the previous 8 weeks. On average, this response occurred for 67% of the weeks on study in patients who responded. Bleeding events during treatment were reduced compared to baseline. Long-term romiplostim treatment was generally well tolerated and treatment-related serious events occurred in 13 patients (9%). Thromboembolic events occurred in seven (5%) patients, six of whom had pre-existing risk factors such as cardiovascular disease and/or a history of thrombosis. One patient transiently developed neutralizing antibodies to romiplostim (absent on retesting approximately 4 months after discontinuation of treatment), but these did not cross-react with endogenous TPO or affect the platelet response.

With regard to eltrombopag, the results of two randomised, double-blind, placebo-controlled studies have been published recently. In the first, adults with chronic ITP received standard care plus once-daily eltrombopag 50 mg (n=76) or placebo (n=38) for up to 6 weeks[100]. After 3 weeks, patients with platelet counts less than 50×10^9/L could increase the dose to 75 mg. The primary endpoint was the proportion of patients achieving platelet counts 50×10^9/L or more at day 43. The response rate was 59% in eltrombopag patients and 16% in placebo patients (odds ratio 9.61, 95% CI 3.31-27.86; P<0.0001). Of 34 patients who increased their dose of eltrombopag, 10 (29%) responded. No pre-treatment variable was associated with the achievement of response. Platelet counts generally returned to baseline values within 2 weeks after the end of treatment. The frequency of bleeding events at any time during the study was lower in patients receiving eltrombopag than did those receiving placebo (odds ratio 0.49, 95% CI 0.26-0.89; P=0.021); however, the frequency of grade 3-4 adverse events during treatment and adverse events leading to study discontinuation were similar in both groups[100].

In the second phase III trial, called RAISE, 197 patients with previously-treated chronic ITP and platelets <30×10^9/L were randomised 2:1 to standard of care plus eltrombopag or placebo[101]. Each patient was evaluated for response (platelet count 50-400×10^9/L) over the 6-month period, the primary endpoint being the odds of responding to eltrombopag versus placebo. Irrespective of splenectomy status, more eltrombopag-treated patients responded during the entire 6-month period compared with placebo (odds ratio 8.2, 99% CI 3.59-18.73; P<0.001). A post-hoc analysis using the same response criteria of the romiplostim studies (response defined as platelet count elevations =50 and =400×10^9/L for at least 4 consecutive weeks at any time during the 6-month treatment period) indicated a response rate of 70% in splenectomised patients and 88% of non-splenectomised patients[102]. Eltrombopag also significantly reduced the odds of bleeding by 76% (p<0.001). More patients reduced concomitant ITP medications (predominantly corticosteroids) on eltrombopag than placebo (59% vs 32%; P=0.02), and fewer patients required rescue medication on eltrombopag than placebo (18% vs 40%; P=0.001). Eltrombopag was well tolerated. Nausea and vomiting

were reported more frequently on eltrombopag, whereas serious bleeding events (P=0.03), peripheral edema (P=0.01), and dyspepsia (P=0.01) were reported more frequently on placebo. Three eltrombopag patients experienced thromboembolic events; none were observed on placebo. A mild, transient, elevation of alanine aminotransferase and total bilirubin levels was observed in 9 (7%) and 5 (4%) of eltrombopag-treated patients, respectively.

Results from the EXTEND study (Eltrombopag Extended Dosing Study) have been reported in abstract form[103]. Patients were maintained on their concomitant medication and commenced on 50 mg eltrombopag. Following response, concomitant therapy was reduced and eltrombopag tapered to maintain a platelet count of more than $50x10^9$/L. Evaluable individuals (n=299) had a median treatment duration of 204 days (2-861 days). Overall, 86% of patients (257/299) achieved a platelet count $\geq 50x10^9$/L. Splenectomised and non-splenectomised patients responded equally well (89% and 82%, respectively). Patients on treatment for ≥6 months or ≥12 months achieved platelet counts of $\geq 50x10^9$/L and 2x baseline for 69% (18/26 weeks) and 71% (37/52 weeks) of the time on treatment, respectively. Treatment was generally well tolerated. However, 13 patients (4%) experienced 16 thromboembolic events; 11/13 (85%) experienced the event at a platelet count lower than the maximum platelet count achieved during eltrombopag treatment. The clinical significance of these thromboembolic events is uncertain, as most of the patients had a well defined cardiovascular risk.

Some considerations about the potential risk of chronic therapy with these agents are appropriate. Thrombosis is clearly a major concern, but published data do not suggest a difference in either arterial or venous thrombotic events between treatment and placebo groups. Reversible increase of bone marrow reticulin has been observed in a few patients in the clinical trials with romiplostim[104]. This also requires patient monitoring and a prospective bone marrow study in a larger number of patients to provide a clearer view of the frequency, reversibility, and clinical consequences of bone marrow changes associated with these new agents in patients with ITP. In a mouse model, expression of a constitutively active mutant TPO receptor (MPL), MPL W515A, resulted in the development of a myelofibrosis-like disease[105]. Interestingly, a germline activating mutation of MPL, $MPL^{Ser505Asn}$, has been identified in patients with hereditary thrombocytosis[106]. Affected patients exhibit a significant risk of thrombosis, splenomegaly and bone marrow fibrosis, with reduced life expectancy in comparison with wild-type relatives. Splenomegaly and bone marrow fibrosis were observed predominantly in patients with an age of 20 years old or greater, suggesting that these manifestations develop over several years. It is therefore necessary to monitor closely ITP patients on chronic treatment with TPO receptor agonists for the potential development of bone marrow fibrosis and splenomegaly. It is also uncertain whether treatment with thrombopoietic agents can sustain platelet counts over several years in chronic ITP, or will eventually lead to stem cell depletion and bone marrow failure.

Conclusions

The pathophysiology of ITP is complex and abnormalities of both the B-cell and the T-cell compartments have been identified. The mechanisms of the thrombocytopenia involve both increased platelet destruction and, in a significant proportion of cases, impaired platelet production.

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. Thrombopoietin receptor agonists appear to be very effective in a high percentage of refractory patients and very tolerable. The potential long term side effects of these agents is currently a major limitation in shifting them to second line therapy.

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