Advances in the pathophysiology of primary immune thrombocytopenia

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ABSTRACT

Objectives: Classically, immune thrombocytopenia (ITP) was thought to be caused by the destruction and insufficient production of platelets, as mediated by autoantibodies. More recently other immune mechanisms that contribute to the disease have been discovered. This review attempts to address the main unresolved questions in ITP.

Methods: We review the most current knowledge of the pathophysiology of ITP. Immunological effects of available therapies are also described.

Discussion: The trigger may be a loss of tolerance due to molecular mimicry with cross-reaction of antibodies arising from infectious agents or drugs, genetic factors, and/or platelet Toll receptors. This loss of tolerance activates autoreactive effector B and T lymphocytes, which in turn initiates platelet destruction, mediated by cytotoxic T lymphocytes and the release of pro-inflammatory cytokines (IL-2/IL-17) by T helper (Th) cells (Th1/Th17). Th2 (anti-inflammatory) and regulatory B (Breg) and Treg cells are also inhibited (with decrease in IL-10/TGF-β), which leads to the disease becoming chronic. Some isotypes of autoantibodies may increase the bleeding risk. Corticosteroids, rituximab, and thrombopoietin receptor agonists (A-TPOs) all increase levels of Tregs and TGF-β. The A-TPOs also increase Breg levels, which could explain why complete remission has been seen in some cases.

Conclusion: A better understanding of the immunomodulatory effects of each ITP therapy is needed to best manage the disease.

KEYWORDS

Immune thrombocytopenia; pathophysiology; autoantibodies; Treg lymphocytes; thrombopoietin receptor agonists

Introduction

Primary immune thrombocytopenia (primary ITP, formerly known as idiopathic thrombocytopenic purpura) is an organ-specific autoimmune disease in which the platelets and their precursors, megakaryocytes, are the targets of a disrupted immune system [1]. It is estimated that approximately 4.5% of the Caucasian population suffers from an autoimmune disease [2]. Primary ITP affects both sexes and occurs in both paediatric and adult populations, with an annual incidence rate of between 16 and 27 new cases per million [3,4]. The prevalence ranges from 4.5 to 10.5 per 100 000 in adults [5,6] and 4.6 per 100 000 in children [7].

Traditionally, the pathophysiological model was based on platelet destruction mediated by autoantibodies [8]. In 1951, Harrington and Hollingsworth [9] demonstrated that ITP was caused by a circulating plasma factor, and in 1965 Shulman et al. [10] identified this factor as platelet-specific immunoglobulin type G (IgG).

In 1994, thrombopoietin (TPO) was discovered [11–14]; the cytokine that stimulates the proliferation of megakaryocytes and platelet release via the TPO receptor (TPO-R, also known as c-Mpl) found in haematopoietic progenitor cells and megakaryocytes [15]. The liver continuously synthesizes endogenous TPO (eTPO) [16] which is subsequently cleared by being attached to circulating platelets [17]. In patients with ITP, rather than a compensatory increase in eTPO, as seen in a megakaryocytic thrombocytopenia [18], a reduced or normal level of eTPO is observed in approximately two-thirds of cases [19]. Therefore, there is a functional deficit of eTPO [20–22]. Furthermore, although high levels of megakaryocytes are commonly found, most of them are immature or have been damaged by autoantibodies.

In parallel to these findings, several studies reported the presence of activated platelet-autoreactive T cells in ITP patients, with a cytokine imbalance towards interleukin (IL)-2 and interferon gamma (IFN-γ) [23–26], indicating its role in modulating the anti-platelet antibody response by B cells in ITP. More recently, the existence of other immunological mechanisms mediated by cytotoxic T lymphocytes has been discovered [27,28], providing the first evidence that the cell-mediated toxicity also participates in the pathogenesis of ITP. These lymphocyte subtypes cause both direct destruction of platelets and inhibition of their formation by inducing megakaryocyte maturation defects in the liver, kidney, spleen, and bone marrow [29].

All this evidence supports the rationale for pharmacological therapy based on first-line corticosteroids...
with or without intravenous immunoglobulin (IVIG) [30,31]. Second-line therapies include splenectomy and thrombopoietin receptor agonists (A-TPOs) (romiplostim, eltrombopag) and, in refractory patients, different immunosuppressants such as azathioprine, rituximab, cyclosporin, cyclophosphamide, or danazol are used [30]. Although in many cases the disease is controlled satisfactorily, there is still a significant percentage of patients who fail to achieve a complete and/or long-lasting remission [32,33].

This paper attempts to address the main unresolved questions in ITP: what is the alteration that initiates platelet destruction, which mechanisms perpetuate the disease, why do patients with the same platelet count have different clinical haemorrhagic manifestations, and why do some patients respond to some therapies while others do not?

We review the most current knowledge of the pathophysiology of ITP, including the alteration of B lymphocytes, the loss of immunological tolerance, and alterations in T cells. Furthermore, the immunological effects of available therapies are described in an attempt to contribute to the decision that clinicians make with regard to choosing the appropriate form of treatment.

**Immune system physiology**

**Role of B and T lymphocytes**

The B and T lymphocytes are the main cells of the specific immune response (Fig. 1). B lymphocytes generate a humoral response [34] when differentiating into plasma cells and memory B cells after recognition of the corresponding antigen. Plasma cells produce large amounts of IgG subtype antibodies, which neutralize bacterial toxins and viruses by agglutination, activation of the complemen system, phagocytosis, and the activity of natural killer cells [34]. There are also some regulatory B lymphocytes (Bregs) which inhibit activation of T cells and monocytes by secreting anti-inflammatory interleukins such as IL-10 [35]. These cells regulate the differentiation of the different subtypes of T helper lymphocytes (Th), the pro-inflammatory differentiation of the antigen-presenting cells (APCs) and other autoimmune responses [36].

T lymphocytes, which mature in the thymus, generate a cellular response. Through their receptors (TCR) they recognize antigens bound to molecules of the major histocompatibility complex (MHC) on the surface of APCs (dendritic cells, monocytes, and macrophages) [34]. T-cell precursors split and differentiate into effector T cells and memory T cells. The effector cells are classified into three main types cytotoxic (CTL) [CD8 positive (+)], which induce apoptosis; helper 1 (Th1) (CD4+), which activate CTLs and macrophages; and helper 2 (Th2) (CD4+), which activate B cells [34]. The effector response of Th1 and Th2 consists of the secretion of various growth factors and ILs. The Th1 are mainly linked to inflammation (IL-2, IL-6, IL-12, IFN-γ, tumour necrosis factor beta (TNF-β)), and Th2 to the allergic response (IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-13) [34].

![Figure 1](image.png)  
**Figure 1** Mechanisms of cellular and humoral immune response: the role of T and B lymphocytes.
There are also regulatory T cells (Treg) that monitor autoreactivity in the peripheral blood and inhibit the activity of both CTL and Th lymphocytes, and inhibit the production of antibodies through direct contact with B lymphocytes or by secreting cytokines such as IL-10 or TGF-β. Treg cells are CD4+CD25+ and can inhibit CD4+CD25− and CD8+ lymphocytes. They are therefore responsible for the ‘tolerance’ of the immune system [34].

Cellular and humoral responses are interrelated (Fig. 1). The Th effector cells stimulate both T and B cells and, in turn, the B cells require both antigen recognition and a signal by Th cells. In addition, Treg and Breg lymphocytes exert reciprocal control mechanisms between humoral and cellular responses [34].

**Natural tolerance mechanisms**

The immune system has several mechanisms that inactivate the specific receptors that recognize self-antigens.

The first mechanism is central, and occurs in the thymus and bone marrow. Normally, in these organs, immature lymphocytes only meet with self-antigens presented by APCs as external antigens are presented in peripheral organs (lymph nodes and spleen). The TCR is capable of huge clonotypic variation that allows recognition of over 108 different antigens [37]. After a process of positive and negative selection in the thymus, T cells with a TCR of low-or-intermediate affinity for the self-peptide/MHC complex are positively selected and allowed to mature. However, if the TCR has a high affinity, there is a negative selection that leads to apoptosis of the T cell. After these processes, only low-affinity autoreactive T cells enter the peripheral blood [38].

The second mechanism is peripheral. It consists of the inactivation of circulating autoreactive lymphocytes that have escaped the central process. The Treg cells (previously selected in the thymus), when detecting naive (virgin) T cells that recognize self-antigens, block their differentiation and proliferation by contact-dependent inhibition, whereas when detecting autoreactive effector T cells, Treg cells block their function by secreting IL-10 and TGF-β [37,38].

Finally, there is an additional control mechanism mediated by B cells. Many antibodies are autoreactive, but most of them do not reach circulation thanks to the clonal selection that occurs in the bone marrow [39]. This selection is only effective when self-proteins are present at high concentrations. If the antigens are present at low concentrations, the specific B cells can survive, and therefore an additional peripheral mechanism is required [39]. Since B cell activation depends on Th cells, the removal or anergy of T cells with TCR for the same antigens can, by itself, prevent production of autoantibodies.

**Immune system alterations in ITP**

Approximately 25% of patients with ITP do not have detectable platelet autoantibodies [40], on the one hand due to the lack of standardization of detection methods and on the other hand due to the existence of additional pathophysiological mechanisms. Besides anti-platelet autoantibodies, the alterations include abnormalities in T cells, such as platelet destruction mediated by Th1 and CTL and alterations in the function of Th1 and Treg cells [27], which in turn is associated with clonal activation of autoreactive B cells [41] (Fig. 2 and Table 1).

**Alterations in B lymphocytes**

**Development of anti-platelet autoantibodies**

Although their existence has been known since 1951 [9], in 1971 there was the discovery of IgG autoantibodies directed against the glycoprotein (GP) of platelet membranes (mainly GPIb/IIa, GPIb/IX, and others like GPIa/IIa, GPIV, and GPV) [42]. These opsonic antibodies facilitate binding of phagocytes that destroy platelets. To this end, they bind to the receptor of the constant portion of the IgG (Fc-R) present on the surface of macrophages of the reticuloendothelial system (RES). These GPs are also found in megakaryocytes [43].

The origin of autoreactive B cells may be due to several factors. For example, there are environmental factors, prompted by molecular mimicry between foreign and self-molecules. Some infectious agents with structures (usually of protein nature) similar to those of the host may develop autoreactivity. These include several viruses (human immunodeficiency virus, Epstein–Barr virus, hepatitis C virus) and bacteria (Helicobacter pylori), and even some drugs [1]. Evidence regarding the cross-reactivity of virus-specific antibodies with normal platelet antigens was firstly demonstrated in patients developing ITP after human immunodeficiency virus [44] or varicella zoster virus infection [45]. Molecules that are highly conserved in the phylogeny are the best candidates since they contain common epitopes. Heat shock proteins (HSP) are expressed in all prokaryotic and eukaryotic cells under stress conditions (increased temperature, lack of water or glucose, radiation, etc.) [46]. It is hypothesized that an immune response against a microorganism HSP could cross-react with a self HSP. Anti-HSP antibodies have been found in several autoimmune diseases such as type I diabetes, Crohn’s disease, rheumatoid arthritis, and lupus erythematosus [46]. In ITP, the conversion of HSP60 or HSP70 in autoantigens could lead to the loss of tolerance.

Additionally, genetic susceptibility of the individual is probably involved. For example, ITP has been found to be significantly associated with several polymorphisms: MHC HLAB8DR3 [47], polymorphisms in the genes encoding TNF-β [48], R-Fc [49], DNA methyltransferase 3B (DNMT3B), and IL-1 receptor antagonist [50].
Synergy between autoantibodies and platelet toll-like receptors

Recently, it has been shown that platelets express Toll-like receptors (TLRs), particularly TLR4, TLR-7, and BAFF1, so they can present antigens through MHC-II [51]. TLRs are transmembrane proteins that act as pattern recognition receptors [52]. They have affinity for different groups of pathogen-associated molecular

Table 1 Main alterations of the immune system described in patients with ITP and the possible effects achievable using different available therapies

| Alterations of the immune system in ITP | Therapies that reverse the alteration |
|----------------------------------------|--------------------------------------|
| Related to B Lym. and humoral response  | Anti-platelet autoantibodies (anti GPIIb/IIIa, GPIb/IX, GPIa/IIa, GPIV, GPV) |
| † Circulating B Lym.                    | IVIG, anti-D, corticosteroids         |
| † Splenic B Lym.                       | Rituximab                             |
| † Circulating Breg Lym., ↓ IL-10      | Splenectomy, rituximab               |
| † Fc receptors in macrophages of the RES | Romiplostim                           |
| † Circulating Th1 Lym., ↑ IL-2, IFN-γ and TNF-β | Romiplostim                           |
| † Circulating Th2 Lym., ↓ IL-10 and TGF-β | IVIG                                 |
| † Th1/Th2 ratio                        | Romiplostim                           |
| † Circulating Th17 Lym., ↓ IL-17 and IL-22 | Corticosteroids (high doses)         |
| † Circulating Treg Lym., ↓ IL-10 and TGF-β | Corticosteroids                       |
| † Treg Lym. in bone marrow             | Corticosteroids, rituximab, romiplostim, rapamycin + corticosteroids, corticosteroids + rituximab |
| † Treg Lym. in spleen and thymus       | Corticosteroids, rituximab, romiplostim, rapamycin + corticosteroids, corticosteroids + rituximab |
| † Treg/Th17 ratio                      | Corticosteroids, rituximab, romiplostim, rapamycin + corticosteroids, corticosteroids + rituximab |
| † CD16+CD14hiCD16− monocyte ratio      | Corticosteroids                       |
| † IL-23 by activated macrophages and dendritic cells | Romiplostim                           |
| † Serum-free HSP60                     | Antibiotics anti-H. pylori (in patients with infection) |
| † PDCs                                 |                                      |

*Pre-clinical studies in mice (murine model of ITP); IVIG: intravenous immunoglobulins; Lym: lymphocytes.
patterns (PAMP) [53], such as lipopolysaccharides or nucleic acids of viruses and bacteria. The TLR send intracellular signals that activate different pathways, including mitogen-activated protein (MAP) kinases, signal transducers and activators of transcription, and the pathway of the nuclear factor kappa B [54]. Then, the affected cells produce inflammatory cytokines [ILs, interferons, and tumour necrosis factor alpha (TNF-α)] that send ‘danger signals’ to the immune system [55], triggering a rapid recruitment of effector cells and stimulation of the APC [56].

Recent studies show that TLR can recognize, in addition to PAMP, molecules from injured tissues [53]. The presence of TLRs in platelets is probably because these cells can act as circulating sentinels for infection [51]. This would explain the worsening of the disease observed during infections in some patients with ITP. It is postulated that infections caused by pathogens recognized by TLR increase platelet destruction, multiplied in the presence of anti-platelet antibodies due to the synergy with TLR, which increases R-Fc-mediated phagocytosis [57] (Fig. 3). In turn, the TLR can increase the production of antibodies, since they activate the secretion of factor B lymphocyte stimulator by dendritic cells, which has been correlated with the level of autoantibodies [58].

These mechanisms could also explain why, in some patients, there is spontaneous ITP resolution after treatment with antibacterial agents, since they would inactivate the mechanisms of TLR-mediated destruction.

**Autoantibodies with different isotypes**

It has been hypothesized that not all anti-platelet antibodies are equal, but there are several isotypes associated with different alterations in platelet function. An in vitro study showed that there are differences in SRE-mediated phagocytosis between platelets opsonized by different antibody isotypes [59]. It is further postulated that some isotypes could inhibit platelet activation instead of inducing phagocytosis, which may explain why, at a given platelet count, some patients bleed and others do not. The subtype of antibody could be related also to the severity of the disease, and to different responses to the administration of IVIG.

**Increase in splenic B lymphocytes**

A large number of studies suggest the presence of abnormalities in B lymphocytes. Olsson et al. [60] found an increase in the number of B lymphocytes in the spleens of 29 ITP patients compared to healthy controls. Audia et al. [61] observed a decrease in circulating and splenic B lymphocytes in 18 splenectomized patients with or without rituximab, independently of treatment response. They also observed an increase in the ratio of Th1/Treg lymphocytes, suggesting that rituximab, besides inhibiting B cells, might modulate the T lymphocyte response.

**Decrease in Breg lymphocytes**

In untreated patients with ITP, a decrease in the population of Breg was observed, phenotypically characterized as CD19+CD24highCD38high cells [62,63]. Although this

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**Figure 3** Possible role of platelet TLRs in the pathophysiology of ITP: platelets act as circulating sentinels and, in the event of detecting pathogens or molecules derived from injured tissues, the TLRs that recognize them send ‘danger signals’ to the immune system by stimulating the effector cells and the APCs of the RES; if anti-platelet antibodies are also present, the TLRs stimulate R-Fc-mediated phagocytosis. Adapted from John W. Semple’s Laboratory Homepage, by M. Perera. Retrieved from http://www.angelfire.com/ut/johnsnotes/index.html. Copyright 2009 by John W. Semple. Adapted with permission.
interaction has not yet been demonstrated in humans, a plausible hypothesis in ITP would be that the alteration of Breg cells contributes to the already known disruption in Treg cells, as will be explained below [64].

Two subtypes of B-regulatory lymphocytes were identified in patients with ITP: CD19+CD24+CD38+ and CD19+CD24+FOXP3+. A prior report indicated no difference in splenic B-lymphocytes and between ITP patients and controls, but lower splenic Treg cells in ITP [61]. Altered Breg function has also been described in ITP, with decreased IL-10 secretion and less inhibitory capacity [62]. In mouse models, the IL-10-secreting Breg cells promote the differentiation or recruitment of Treg cells [65,66].

The CD19+CD24+CD38 B-regulatory cells from ITP patients were not significantly different from controls, but CD19+CD24+FOXP3+ Bregs were markedly increased in the spleen. Glucocorticoids and other lympholytic agents in the treatment of ITP before splenic removal are less likely to explain the finding.

This CD19+CD24+FOXP3+ B-regulatory population has been reported in normal human blood [67], and is diminished in the blood of patients with autoimmune disease [68]. Increased splenic CD19+CD24+FOXP3+ Bregs may be due to sequestration, comparable to the sequestration of T-regulatory cells noted in the thymuses of experimental ITP mice [51]. These findings raise the question of the role of B-regulatory subtypes in the alteration of T and B lymphocyte interaction, and immune modulation that results in the antibody and T-lymphocyte destruction of platelets in chronic ITP [69].

**Alterations in T lymphocytes**

To date, there have been three types of T cell alterations in ITP described: an increase in Th1 over Th2 lymphocytes; an increase in Th17 cells; and a decrease in Treg lymphocytes (Fig. 4) [64].

**Increase in Th1 lymphocytes**

Patients with active ITP have an imbalance in the Th1/Th2 response when compared with healthy controls and patients with ITP who have responded to treatment [24]. The pro-inflammatory CD16+ monocytes, which are increased in ITP in detriment of CD14CD16− monocytes [70], release TNF and promote the development of Th1 and the release of IL-17, downregulating Tregs [70]. Th1 release cytokines that activate the cellular response: IL-2, a factor stimulating Th1, Th2, and CTLs; IFN-γ, which induces conversion of macrophages to APC; and TNF-β, which recruits more macrophages [71]. Higher IL-23 levels and increased expression of TLR4 are also observed in monocytes [72]. On the other hand, there is a decrease in Th2 cells and in the inhibitory cytokines IL-10 and TGF-β. In chronic ITP, the levels of TGF-β are inversely related to disease activity [71]. (Fig. 4).

**Increase in Th17 cells**

In ITP there is an increase in pro-inflammatory Th17 cells that secrete IL-17 and IL-22 [72,73], although not all studies have replicated these findings [74,75]. In most autoimmune diseases, there is an imbalance between pro-inflammatory and anti-inflammatory mechanisms. The Th17 cells develop from Th0 through a different pathway than Th1 or Th2. The IL17 plays an important role in many autoimmune diseases [34]. In ITP, several studies have shown increased IL-17 during active disease in both children and adults [73]. IL-22 is also elevated in active phases of disease, and decreases in patients responsive to dexamethasone [76].

**Decrease in Treg lymphocytes**

Phenotypically, Treg cells are CD4+CD25+FoxP3+. They represent 5–10% of all lymphocytes, and suppress humoral and cellular response of autoreactive lymphocytes by secreting IL-10 and TGF-β. Their effect is contrary to that of Th17 cells [78]. ITP patients have decreased Treg levels, which normalize after a response to different therapies [72,77]. The Treg/Th17 ratio correlates with disease activity [79], and lower levels of Treg, IL-10, and TGF-β are associated with lower platelet counts [75,80].

In murine ITP models, it has been observed that Treg reduction occurs only in the spleen, whereas there is an increase in Tregs in the thymus. Treatment of these mice with IVIG increased platelet counts, decreased autoantibody production, and normalized the levels of Tregs in both the thymus and spleen. Therefore,
these models suggest that ITP could be associated with a peripheral Tregs deficiency due to their retention in the thymus, which normalizes after treatment [81].

**Lymphoid organs**

Studies of peripheral blood APCs, lymphocyte subpopulations, and cytokines in the peripheral blood might not mirror the organ-specific environments, such as in the spleen, which is a known site of anti-platelet antibody synthesis. Follicular T-helper cells and Tregs within the proliferative lymphoid nodules (PLNs) and germinal centres (GCs) of spleens from patients with ITP were reduced compared with spleens removed for other reasons [82]. The density of follicular T-helper cells and Tregs was lower in the PLNs, but not GCs, which also contain GP IIb/IIIa within IgM-containing immune complexes tightly bound to follicular dendritic cells, closely approximated to proliferating B-cells. These studies suggest that PLNs are the sites of auto-antigen stimulation related to a lack of T-cell control [82]. The ratio of Th1 cells to Tregs was reported to be increased in the spleens of ITP patients who failed rituximab therapy despite depletion of peripheral blood B-cells [61]. It has also been suggested that rituximab may induce the differentiation of autoimmune plasma cells into long-lived resident cells in the spleen of patients who failed treatment [83]. These studies serve to highlight potential differences between findings in specialized lymphoid organs and those made on components of peripheral blood.

**Relationship between pathophysiology and disease course**

According to its clinical evolution, ITP is classified as ‘newly diagnosed,’ ‘persistent’ (3–12 months) or ‘chronic’ (>12 months) [1]. More aggressive treatment options, such as splenectomy, are usually reserved for patients with chronic ITP [30].

As mentioned previously, autoreactivity against HSP60 may lead to the lack of anti-inflammatory response due to a decreased activation of Tregs, which can lead to the chronic form of the disease. HSP60 serum levels in newly diagnosed ITP are lower than those in patients with chronic ITP or healthy controls [84]. They are also higher in patients with counts >30 x 10^9/l and are positively correlated with platelet counts [84]. Therefore, in the initial stages, it is possible that the autoantibodies generated by molecular mimicry with HSP are the only mechanism causing thrombocytopenia. Since these autoantibodies induce changes in Treg and Breg cells, the disease may become chronic if the inhibited regulatory mechanisms fail to completely eliminate autoreactive cells. In this case, despite the temporary removal of autoantibodies, anti-platelet autoreactivity would manifest again sooner or later.

The natural course of the disease differs between children and adults. In the paediatric population, most cases resolve spontaneously and do not require treatment. Pathophysiologically, this course of ITP would be explained by a transient autoantibody production during a viral infection. Spontaneous remission is associated with the age of onset, being most frequent between 2 and 12 months of age (90%), compared to 1–8 years (71%) or 9–18 years (49%) [85]. Moreover, in children over 1 year of age, there are more remissions if the initial count is very low (<10 x 10^9/l), and if there is a history of other diseases. The remission rate at 6 months is independent of treatment with corticosteroids or IVIG [86]. If the disease becomes chronic, spontaneous remissions in children are less common, about 33%, of which 45% occur in the short term. After splenectomy, 80% achieve remission, but some cases may relapse after several years [87]. Late relapses could be explained by patients continuing to produce low amounts of autoantibodies that cause sustained platelet destruction and inhibited formation; in the long term, destruction is greater than formation [87].

Severe bleeding occurs rarely in children (0.1% developed an intracranial haemorrhage in the IPARC registry[86]), although it is unknown whether this is due to the presence of a certain isotype of autoantibodies.

In adults, spontaneous remission rates are traditionally lower, ranging from 5 to 11% [88], although a recent study in patients treated with romiplostim found an increased rate of up to 32% [89]. At 12 months, approximately 70% develop chronic ITP [90]. The elderly are more likely to suffer chronic disease [87]. At a pathophysiological level, the positivity for autoantibodies at diagnosis is associated with more severe bleeding and a chronic course [90]. It seems plausible that changes in the Th and Treg lymphocytes induced by autoreactive B cells (Fig. 2) alter the inhibitory mechanisms of the immune system, thereby perpetuating the disease.

**Relationship between pathophysiology and treatment**

Treatment of ITP has traditionally been based on corticosteroids, IVIG, and splenectomy [1]. However, the authorization of the A-TPO romiplostim and eltrombopag within Europe in 2009 and 2010, that lead to increased platelet production, resulted in higher response rates of up to 70–80% being achieved even in patients refractory to other treatments [91].

Several recent studies have examined the immunological changes induced by some treatments (Table 1).
Corticosteroids and other immunosuppressive agents

Following response to corticosteroids, associated or not with other immunosuppressive agents such as rituximab or rapamycin, a recovery of the Treg cells population has been observed.

A study of high-dose dexamethasone was found to result in the normalization of the Th1/Th2 ratio, Treg and Th17 cells levels and, by inhibition of RORγt and GATA3, an increased expression of Foxp3 [92]. In patients with rapamycin and low-dose prednisone, there was an increase in Treg and a strong correlation between the levels of TGF-β and Treg after treatment. Furthermore, the upregulation was maintained after discontinuation [93].

A study of rituximab and corticosteroids confirmed the improvement in Treg cells, which was greater and more prolonged than in patients treated with corticosteroids alone [94]. Stasi et al. [95] also observed a rituximab-mediated modulation of Treg cells.

The number of circulating plasmacytoid dendritic cells (PDC) is low in patients with ITP, both with primary or with Helicobacter (H.) pylori-associated disease. However, no reduction is observed in the levels of myeloid dendritic cells (MDC) or CTLs. Interestingly, an increase in PDC was observed in responders but not in non-responders after eradicating H. pylori with an antibiotic, while PDC and MDCs decreased in both responders and in non-responders after administration of prednisolone. In untreated patients, the low number of PDCs persisted [74]. Another study of high-dose dexamethasone found an increase in MDCs, a decrease in PDCs, and a lower expression of CD11c in MDCs after four days of treatment [96].

IVIG

Multiple immunological changes have been described during IVIG therapy. They include inhibition of the production of autoantibodies, their neutralization by anti-idiotypic antibodies, inhibition of complement-mediated damage; modulation of cytokine production, induction of apoptosis of lymphocytes and monocytes, and modulation of the functions of B and T lymphocytes [97]. They also appear to block the activating R-Fc and favour the expression of inhibiting R-Fc [97]. Therefore, they are a well-established therapeutic option, and have been associated with long-term responses [30].

A-TPO

The A-TPOs are indicated in patients who do not respond to splenectomy or in whom splenectomy is not...
contraindicated. Due to their mechanism of action, treatment should be administered on a continuous basis and, once it is suspended, the platelet count is expected to decrease [30]. However, there are an increasing number of published cases of maintenance of response after cessation of romiplostim [98–101], and also some cases published with eltrombopag [100,102]. During the clinical development of romiplostim, 7% of patients had remission, which, at that time, was regarded as spontaneous [91]. A later study designed to assess remission prospectively found a rate of 32% [89].

Two recent studies suggest that A-TPO could induce immunomodulatory changes beyond its stimulatory effect on platelet production. Bao et al. [103] demonstrated that A-TPOs increased Treg activity and decreased levels of IL-2-releasing Th1 cells (CD4+), consistent with an immune response dampening effect by the A-TPO. An increase in circulating TGF-β was also observed, which was correlated with the recovery of platelet counts. This suggests that the platelets could mediate the recovery of Treg cells during treatment with A-TPO, either directly or indirectly by an increased release of TGF-β, as a result of the increased platelet renewal rate. Another study by the same group observed a Breg increase in non-splenectomized patients after a response to A-TPOs [70]. Moreover, in the patients with higher platelet counts, there was an enhancement of the monocyte-suppressor activity of B lymphocytes. They also observed a normalization of pro-inflammatory CD16+ monocytes in responders, while the non-responders continued with increased levels [70].

Both studies suggest that treatment with A-TPO in ITP patients could not only recover platelet counts, but also modulate the immune response or even restore the immune tolerance. Since immune cells do not express TPO-R, the effect should be mediated by other mechanisms. One of the most plausible hypotheses is direct stimulation of Breg cells by the newly generated platelets [64], which would express CD40L due to the activation by the autoantibodies still present [104]. The Breg cells are activated through CD40. Further studies are needed to verify whether the recovery of Breg and Treg is the underlying mechanism that explains complete remission. Such knowledge would allow tailoring of the treatment choice, not only with the aim of recovering platelet levels, but also aiming to completely restore the immune system of the patient.

Conclusions

Patients with ITP have multiple immune system alterations. At the onset of disease, there is a loss of tolerance to platelet GP antigens, caused by molecular mimicry phenomena with infectious agents or drugs, which may be modulated by genetic factors and by the presence of TLR on the platelet surface. As a result, autoreactive effector B and T lymphocytes are activated, and Breg and Treg subpopulations are inhibited, perpetuating the disease in some patients. Activated B lymphocytes release autoantibodies that destroy opsonized platelets, inducing their phagocytosis and inhibiting their maturation from megakaryocytes. In some patients with certain autoantibody isotypes, instead of an increased phagocytosis, there is an inhibition of platelet activation, which increases the probability of bleeding. In T lymphocytes there is an increase in pro-inflammatory Th1 and Th17 subtypes, and a reduction in Th2 and in the cytokines IL-10 and TGF-β that suppress the immune response. Several treatments, such as steroids, rituximab, and A-TPOs could present further benefits to the recovery of platelet counts, thanks to the restoration of subpopulations of T lymphocytes and levels TGF-β. The A-TPOs have also demonstrated a recovery in the levels of Breg lymphocytes, probably by direct stimulation by platelets through CD40. Further studies are needed to verify whether the recovery of Breg and Treg is the underlying mechanism that explains complete remission. Such knowledge would allow tailoring of the treatment choice, not only with the aim of recovering platelet levels, but also aiming to completely restore the immune system of the patient.

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