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## Pathogenesis of Immune Thrombocytopenia

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**Keywords:** immune thrombocytopenia, platelets, pathogenesis, T cells, B cells, macrophages, dendritic cells, regulatory cells

**Abbreviations:** ADP: adenosine diphosphate, AID: autoimmune diseases, AML: acute myeloid leukemia, APRIL: a proliferation inducing ligand, BAFF: B cell activating factor, Breg: regulatory B cells, CMV: cytomegalovirus, DC: dendritic cells, EBV: Epstein Barr virus, FcγR: Fcγ receptor, GP: glycoproteins, HCV: hepatitis C virus, HIV: human immunodeficiency virus,IDO: indoleamine 2,3-dioxygenase, ITP: immune thrombocytopenia, MDS: myelodysplastic syndrome, MHC: major histocompatibility complex, moDC: monocyte-derived dendritic cells, MSC: mesenchymal stromal cells, RTX: rituximab, TCR: T cell receptor, TIMP-3: tissue inhibitor of metalloproteinase 3, TFH: T follicular helper cells, TLR: toll-like receptor, TPO: thrombopoietin, TPO-RA: thrombopoietin receptor agonists, TRAP: thrombin receptor activating peptide, Treg: regulatory T cells.

## **Abstract**

Immune thrombocytopenia (ITP) is a rare autoimmune disease due to an abnormal T cell response, notably supported by splenic T follicular helper cells, that stimulates the proliferation and differentiation of autoreactive B cells. The antiplatelet autoantibodies they produce facilitate platelet phagocytosis by macrophages, essentially in the spleen. Macrophages contribute to the perpetuation of the auto-immune response as the main antigen-presenting cell during ITP. CD8<sup>+</sup> T cells also participate to thrombocytopenia by increasing platelet apoptosis. Besides this peripheral platelet destruction, inappropriate bone marrow production also exacerbates thrombocytopenia, due to an immune response against megakaryocytes. Moreover, the level of circulating thrombopoietin, the main growth factor of megakaryocytes, is low during ITP. In this review, the major mechanisms leading to thrombocytopenia, the role of the different immune cells and the different targets of treatments are described.

## 1. Introduction

Immune thrombocytopenia (ITP) is an autoimmune disease (AID) characterized by a low platelet count ( $<100$  G/L). It causes bleeding, especially in the skin and the mucosa, in 2/3 of patients. The prevalence of ITP in adults is about  $10/10^5$  [1] with an incidence rate ranging from 1.6 to  $3.9/10^5$ /year [2-4]. Adults generally present a chronic course ( $>1$  year in 80%) whereas in children the disease is most often acute. ITP is a diagnosis of exclusion which needs to rule out thrombocytopenia secondary to medications (including heparin-induced thrombocytopenia), disseminated intravascular coagulation, vitamin B9 and B12 deficiency, congenital thrombocytopenia, spleen sequestration, portal hypertension and bone marrow disorders such as myelodysplastic syndromes. Secondary ITP refers to autoimmune thrombocytopenia occurring in the course of other diseases, mainly infections (Human Immunodeficiency Virus (HIV), Hepatitis C Virus (HCV), Epstein Barr virus (EBV), Cytomegalovirus (CMV), *Helicobacter pylori*, other AID (lupus, Evans syndrome, Sjögren's syndrome, antiphospholipid syndrome), hematologic malignancies (mainly non-Hodgkin lymphoma, most particularly chronic lymphocytic leukemia) or primary immune deficiency (common variable immune deficiency (CVID), autoimmune lymphoproliferative syndrome (ALPS)). Guidelines have standardized ITP nomenclature: newly-diagnosed ITP refers to a disease lasting less than 3 months, persistent ITP between 3 to 12 months from diagnosis, chronic ITP when the disease lasts for more than 1 year and refractory ITP if patient is at risk of or displays bleeding despite splenectomy. Severe ITP refers to the presence of bleeding that requires treatment or treatment escalation [5].

Steroids are the first-line therapy and represent a useful diagnostic test, as a transient response is observed in more than 80% of cases. Intravenous immunoglobulin (IVIg) and anti-D immunoglobulin are generally used as emergency rescue therapies with a transient response [6]. Splenectomy remains a cornerstone treatment that should be proposed when ITP

lasts more than one year, as the chances of spontaneous remission are nearly null at this time [7]. Importantly, splenectomy gives the highest long-term response rate of about 66% [8]. However, drugs such as rituximab (RTX, off-label use in ITP) or thrombopoietin receptor agonists (TPO-RA) have changed the management of ITP, particularly for persistent ITP, when drugs with a high benefit/risk ratio must be favored [9, 10]. These drugs are thus more and more used to postpone splenectomy. Other therapies such as dapsone, danazol, hydroxychloroquine and vinca alkaloids can be useful in specific situations [11]. The aim of this review is to describe the general mechanisms involved in ITP pathogenesis with a focus on the role of the different immune cells; these are summarized in **Figure 1**.

## **2. Triggering the immune response: genetic background and environmental factors**

### **2.1. Genetic predisposing factors**

As in other AID, specific genetic backgrounds have been reported to be associated with ITP (**Table 1**). The major histocompatibility complex (MHC) [12-15], Fc $\gamma$  receptors (Fc $\gamma$ R) [16-21], transcription factors [22], chemokines [23], pro-inflammatory cytokines [24-29] or anti-inflammatory cytokines [27, 30, 31] together with their receptors [27, 28, 32] display polymorphisms that are often observed in ITP cohorts, or specifically during the chronic course of the disease. Polymorphisms of regulator proteins such as the phosphatase PTPN22 are also overrepresented in ITP patients [33-35]. Specific epitopes on human platelet antigens (HPA) have also been linked to acute or chronic ITP [36, 37]. More recently, variations in the level of some microRNAs, non-coding RNAs that regulate genes at the post-transcriptional level, have been reported in ITP. These variations of microRNAs lead to the dysregulation of cytokines involved in the immune response, such as IFN- $\gamma$ , IL-21, IL18 or TGF- $\beta$  [38-40].

However, most of these data were obtained from small cohorts or from patients of specific ethnic origins, and should thus be interpreted carefully. Analyses of exome sequencing are in process, to identify new genes that might be implicated in the development of ITP.

Polymorphisms have also been associated to the response to medications. Indeed, *FCGRA-V/V* polymorphism is overrepresented in responders to RTX [41], while *FCGR2B-I/I* is associated with a better response to IVIg during pediatric ITP [42]. However, treatment choice at an individual level is not yet decided on gene polymorphisms.

## **2.2. Environmental factors**

Different mechanisms participate in the initiation of the autoimmune process triggered by infections (**Table 2**). Molecular mimicry between infectious components and platelet glycoproteins (GP) has been clearly demonstrated *in vitro*: between the GP120 of HIV or the core envelope 1 protein of HCV and the platelet glycoprotein GPIIb/IIIa [43, 44]. By computer analyses, homologies have been observed between the sequences of different viral proteins from *Herpes simplex virus*, *Varicella zoster virus*, EBV, CMV and the GPIIb/IIIa. Interestingly, some peptides derived from these proteins are recognized by antiplatelet antibodies *in vitro* [45]. Molecular mimicry is mainly involved in secondary acute ITP, accounting for 70, 50 and 30% of neonatal CMV, EBV and HIV infections, respectively [45-47]. However, in some cases, the viral infection triggers an autoimmune response that subsequently perpetuates itself despite virus clearance. As a result, radical treatments such as splenectomy could be needed to treat CMV-secondary ITP despite the eradication of the virus [48]. In mice, it has been shown that the worsening of thrombocytopenia following viral infection can be non-specific. Indeed, the platelet count drops as a result of the increase in macrophage phagocytosis secondary to stimulation by IFN- $\gamma$  elicited by viruses [49, 50].

It has been shown that toll-like receptor 7 (TLR7), which recognizes single-stranded viral RNA, is involved in ITP pathogenesis. Stimulation of TLR7 expressed on antigen-presenting cells is responsible for an increase in BAFF (B cell activating factor) secretion which stimulates autoreactive B cells, thus boosting antiplatelet antibody production [51]. Moreover, TLR7 ligation induces IL-12 secretion by antigen-presenting cells, which drives the T cell response towards Th1 polarization [52]. Thus, during viral infections, signals needed to trigger an autoimmune response are present: danger signals consistent with viral ssARN recognized by TLR7, immunogenic viral proteins that share molecular homology with platelet glycoproteins and an appropriate cytokine environment that stimulates both B and T cells. On the contrary, a prospective study reported that influenza vaccination tended to decrease the occurrence of ITP, with an adjusted odds ratio of 0.7 (95% CI 0.5-1.1) in the year following vaccination [53].

Bacteria, like viruses, can also play a role in ITP. For example, the virulence protein CagA of *Helicobacter pylori* cross-reacts with antiplatelet antibodies [54]. Mechanisms other than molecular mimicry are also triggered by infections: *H. pylori* decreases the expression of the inhibitory receptor FcγRIIb on monocytes [55], CMV and HIV inhibit megakaryopoiesis [46, 48] and chronic HCV infection induces circulating immune complexes that non-specifically bind to platelets, thus accelerating their clearance [56]. In a more theoretical perspective, all these observations suggest that cross-reactive antibodies against platelets can develop in response to exogenous antigens caused by infection, resulting in somatic hypermutation of immunoglobulins. It also raised the question of why some individuals are prone to develop autoantibodies or autoreactive T-cells.

### **3. The innate immune response in ITP: the role of macrophages, dendritic cells and NK cells**

### 3.1. Macrophages

Macrophages play a dual role in the pathogenesis of ITP (**Table 3**): as effector cells, macrophages contribute to platelet destruction, and as antigen presenting cells, they stimulate the adaptive immune response. First reports localized macrophages in the red pulp and the marginal zone, and described the presence of platelets in their cytoplasm during ITP [57]. At the same time, the ability of splenocytes to phagocyte platelets was observed *in vitro* [58] and was subsequently demonstrated with blood leukocytes from ITP patients [59].

Splenic macrophages play a key role in the stimulation of T cells in ITP. *Ex vivo* analyses of splenic macrophages reveal that they spontaneously stimulate anti-GPIIb/IIIa specific T cells, whereas splenic DC or B cells need to be loaded with GPIIb/IIIa-derived peptides to induce T cell proliferation [60]. The authors used monocyte-derived macrophages to demonstrate that platelets need to be recognized by antibodies to trigger an immune response and that their uptake by macrophages relies on Fc $\gamma$ RI ligation [60].

Fc $\gamma$ R are not only involved in phagocytosis, but also regulate the immune response. Indeed, the Fc $\gamma$ R family is composed of several receptors, whose ligation with IgG or IgG immune complexes leads to opposite signals [61]. Activating receptors are represented by Fc $\gamma$ RI (CD64), Fc $\gamma$ RIIa/c (CD32a/c) and Fc $\gamma$ RIII (CD16) whereas Fc $\gamma$ RIIb (CD32b) gives an inhibitory signal [61]. Most immune cells express both activating and inhibitory receptors that tune immune cell activation depending on their relative expression [62]. During ITP, monocytes display both an increase in Fc $\gamma$ RI and in the Fc $\gamma$ RIIa/Fc $\gamma$ RIIb ratio, associated with an increase in their phagocytic capability. This pro-inflammatory profile is reversed upon treatment with dexamethasone [63]. In the spleen, we observed similar expression of the different Fc $\gamma$ R between ITP and controls, with a trend for a higher expression of Fc $\gamma$ RI during ITP. However, CD86 and HLA-DR expressions were higher during ITP, which is consistent with a higher activation [64].



### 3.2. Dendritic cells

During ITP, dendritic cells (DC) are supposed to participate to the auto-immune response, as monocyte-derived dendritic cells (moDC) are able to phagocyte apoptotic platelets and to stimulate specific T cells. Although their phagocytic capacity is similar to that of DC from healthy controls, their expression of the costimulatory molecules CD86 [65] and CD80 is higher and they produce larger amounts of IL-12 than do controls [66]. CD80 and CD86 overexpression together with the increase in IL-12 production are reduced upon treatment with dexamethasone [66]. However, as previously mentioned, *ex vivo* isolated splenic DC failed to spontaneously induce the proliferation of T cells, whereas splenic macrophages were able to do so [60]. To obtain T cell proliferation, DC should first be loaded with GPIIb/IIIa-trypsin-digested peptides [60], indicating that macrophages are more potent antigen-presenting cells in ITP, probably due to their high phagocytic activity against platelets within the spleen (**table 3**).

DC can also dampen the immune response as they are endowed with tolerogenic capacities. This function is partly supported by indoleamine 2,3-dioxygenase (IDO), an enzyme that converts tryptophan, an essential amino-acid for T cell survival, into pro-apoptotic metabolites such as kynurenine. Tolerogenic DC also favor the conversion of naive T cells into regulatory T cells (Treg), which maintain this tolerogenic state by increasing IDO expression through the ligation of CTLA-4, which they constitutively express, to CD80 and CD86. During ITP, mature moDC display lower IDO expression than do controls [67, 68]. This reduced expression is associated with a decrease in the conversion of naïve CD4<sup>+</sup> T cells into Treg [67]. Furthermore, the functionality of Treg is impaired with a decreased ability to inhibit T cell proliferation and to secrete IL-10 [67]. Interestingly, these dysfunctions are reversed by the addition of CTLA-4 Ig in the co-culture of DC and T cells, with an increase in

IDO expression, kynurenine production and Treg conversion, thus dampening the proliferation of effector T cells and increasing their apoptosis [68].

The regulation of DC activation *in vivo* is a crucial step to abrogate the autoimmune process. The tissue inhibitor of metalloproteinase 3 (TIMP-3), a factor secreted by DC, has been shown to modulate T cell polarization by favoring Th2 commitment [69]. TIMP-3 is upregulated in moDC cultured with IL-4 and leads to a decrease in CD86 expression and in IL-12 and TNF- $\alpha$  secretion [69]. Interestingly, serum TIMP-3 levels are lower in ITP patients than in controls, which could account for the overexpression of CD86 on DC [65] and the Th1 polarization observed in ITP.

Concerning the quantification of DC subsets in the blood, both plasmacytoid DC (Lineage<sup>-</sup>HLA-DR<sup>+</sup>CD11c<sup>-</sup>CD123<sup>+</sup>) and myeloid DC (Lineage<sup>-</sup>HLA-DR<sup>+</sup>CD11c<sup>+</sup>CD123<sup>-</sup>) levels are similar in ITP and controls [70]. Dexamethasone induces a decrease in plasmacytoid DC, a DC subset that plays a key role in AID such as systemic lupus erythematosus [71].

### **3.3. NK cells**

The literature concerning NK cells in ITP is scarce and gives contradictory results. One study reported a normal range of circulating NK cells but a functional deficiency consistent with a decreased ability to lyse K562 target cells [72]. Another study showed that the number of circulating NK cells was increased in ITP patients and correlated with the severity of the disease, but their functional activity was not assessed [73]. A recent study observed similar levels of circulating NK cells with preserved cytotoxic functions in ITP and controls but lower IFN- $\gamma$  production in ITP [74]. Importantly, it has been demonstrated that contrary to CD8<sup>+</sup> T cells, NK cells were not involved in platelet lysis [75]. Further studies are thus needed to better understand the role of NK cells in ITP pathogenesis, notably whether they can modulate platelet production.

## **4. The adaptive immune response: the roles of B and T cells**

### **4.1. B cell involvement**

In the 1950s, Harrington demonstrated that the infusion of serum from ITP patients to healthy volunteers triggered profound thrombocytopenia [76]. This was the first evidence of the involvement of a humoral factor in ITP pathogenesis, that was subsequently identified as an IgG [77]. Antiplatelet antibodies of other isotypes (IgA and IgM) can also be detected but usually in association with IgG [78]. Of note, platelet phagocytosis is greater with IgG than with IgM, particularly in the presence of complement [79]. Antiplatelet antibodies mainly bind to glycoproteins (GPs) or GP complexes (especially GPIIb/IIIa and GPIb/IX/V, and less commonly GPIa/IIa, IV or VI) [78, 80]. Antiplatelet antibodies participate in platelet destruction, as the opsonization of platelets facilitates their phagocytosis by macrophages. Moreover, antiplatelet antibodies also mediate complement-dependent cytotoxicity, as the deposition and activation of complement on the membrane of platelets leads to their lysis [81, 82].

Antiplatelet antibodies are produced by B cells clones with a restriction on the specific heavy- and light-chain usage, which harbor somatic hypermutations [83]. These features are the foot print of a germinal center derived process, in which T and B cells interact for inducing affinity maturation. Germinal centers are expanded and probably dysregulated in the spleen of ITP [84, 85]. Recently, we showed that splenic T follicular helper cells (TFH) are expanded during ITP and support B cell differentiation and the production of anti-platelet antibody through CD40L/CD40 interaction and the production of IL-21 [85].

Antibody producing B cells are found in different places. After their priming in the spleen, a proportion of anti-GPIIb/IIIa secreting cells can be detected in the blood [86] and probably reached specific niches such as the bone marrow [87]. Long-lived plasmocytes also reside in

the spleen, which is the major production site for antiplatelet antibodies [84, 88]. The cytokines BAFF and APRIL are the main factors involved in B cell survival, maturation and stimulation. Serum BAFF levels are increased during ITP and correlate with disease activity [26, 89]. BAFF is a potent stimulator of splenocytes, whose BAFF receptor (BAFF-R) RNA expression is 15 times higher than that in peripheral blood mononuclear cells [89]. Importantly BAFF increases the survival of splenic plasma-cells *in vivo* and *in vitro* [84, 90] and could play a role in the resistance of B-cell depletion therapy.

B cell involvement in ITP pathogenesis has led to the therapeutic use of B cell depleting therapy such as RTX, a chimeric monoclonal antibody targeting CD20, which is expressed by the B cell lineage except by pro-B cells and plasma cells. The binding of RTX to CD20 triggers B cell depletion by different mechanisms including apoptosis, complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity [91, 92]. In AID, the complete depletion of B cells in the blood, the spleen and in the bone marrow is achieved within the first weeks following RTX infusion [84, 93, 94]. In ITP, the response rates to RTX are 40, 30 and 20% at 1, 2 and 5 years of follow-up respectively [95, 96]. The causes of RTX failure are not fully understood. Despite splenic B cell depletion after RTX, long-lived plasma cells that do not express CD20 represent the major residual cells of the B cell pool in the spleen and produce antiplatelet antibodies [84, 94]. Thus, the maintenance of these long-lived plasma cells in the spleen and in the bone marrow could account for the persistence of the thrombocytopenia in some RTX-refractory patients [87]. However, it is also known that in ITP, a clinical response can be achieved despite antiplatelet antibody maintenance [97].

## **4.2. T cell involvement**

### **4.2.1. T cell polarization**

The involvement of T cells in the pathogenesis of ITP has been known for many years (**Table 3**). Th1 polarization was first demonstrated by the increase in serum IFN- $\gamma$  and IL-2 in ITP patients [98]. Similar results were obtained by measuring RNA levels [99] or intracellular IFN- $\gamma$  expression in T cells [100]. A decrease in Th2 polarization is also implicated, so that the Th1/Th2 ratio of T cells in the circulation [100-102] and in the spleen [101] are increased. We also observed increased Th1 polarization in splenic CD4<sup>+</sup> T cells, which was even more pronounced in RTX-refractory patients and associated with an increase in CD8<sup>+</sup>IFN- $\gamma$ <sup>+</sup> cells (Tc1) in these patients [103]. Other T-cell subsets, listed below, have been sought to play a role in the pathogenesis, but their implications is less clear. The literature concerning Th17 cells in ITP contains contradictory results. The first reports on the topic reported similar levels of serum IL-17 [104], IL-17 mRNA and intracellular cytokine expression between ITP and controls [105]. However, in these two studies, the widely accepted Th1 polarization was not found [104, 105]. Another study that focused on Th17 precursors, CD4<sup>+</sup>CD161<sup>+</sup> T cells, also showed circulating levels comparable to those of controls [106]. Then, the following studies showed increased Th17 commitment [107-111] together with Th1 polarization [110, 111]. In the spleen, no such increase in Th17 cells was observed [103].

Th22 represent a subset of CD4<sup>+</sup> T cells that produce IL-22, IL-26 and IL-13, the former being the main cytokine, which belongs to the IL-10 family. IL-22 is secreted not only by Th22, but also by Th17, Th1, NK and NKT cells. IL-22 preferentially acts on tissue cells, such as keratinocytes, hepatocytes, respiratory and gastrointestinal cells, exerting both protective and pathogenic effects in chronic inflammatory diseases, depending on the cytokine environment [112]. In ITP, publications reported an increase in IL-22 levels in serum together with an increase in CD4<sup>+</sup>IL-22<sup>+</sup> T cells [113, 114]. Moreover, the serum IL-22 level correlated with numbers of Th1 [113] and Th17 cells [114]. Therefore, besides Th1 polarization, which is crucial for macrophage stimulation and probably promotes platelet

phagocytosis, other CD4<sup>+</sup> T cell subsets seem to be associated with ITP. However, their roles in ITP pathogenesis need to be specified.

#### **4.2.2. T follicular helper cells**

As previously mentioned, TFH play a crucial role in ITP pathogenesis by supporting B cell differentiation and antiplatelet antibody production [85]. Indeed, we observed an expansion of TFH, defined as CD3<sup>+</sup>CD4<sup>+</sup>CXCR5<sup>+</sup>ICOS<sup>+</sup>PD-1<sup>+</sup> cells, within the germinal center of splenic follicles. Their frequency correlated positively with the percentage of splenic germinal center B cells and plasma cells. TFH express CD40L and are the main producers of IL-21 within the CD4<sup>+</sup> T cell compartment. *In vitro*, we showed that in the presence of IL-21 and CD40 stimulation, splenic B cells of ITP patients differentiate into germinal center B cells and plasmablasts, and produce anti-GPIIb/IIIa antibodies [85], demonstrating the major role of TFH in the pathogenesis of ITP.

#### **4.2.3. Cytotoxic T cells**

The involvement of CD8<sup>+</sup>T cells in ITP pathogenesis is now well established. Cytotoxic T lymphocytes act on the peripheral destruction of platelets and impair their production in the bone marrow. CD8<sup>+</sup> T cells display an increase in the expression of proteins involved in their cytotoxic capacity such as perforin, granzyme A and granzyme B, leading to platelet apoptosis in co-culture [75, 115, 116]. On the other hand, when CD8<sup>+</sup>T cells are co-cultured *in vitro* with megakaryocytes, platelet production is impaired [117]. *In vivo*, the recruitment of T cells into the bone marrow is increased in ITP patients [118]. Associated with the activation of CD8<sup>+</sup> T cells, plasma levels of granzyme A and granzyme B are increased in some ITP patients [119].

Concerning CD8<sup>+</sup> T cell commitment, similarly to what is observed with CD4<sup>+</sup> T cells, the Tc1/Tc2 ratio is increased in the blood [102] and in the spleen [101]. In our experience, splenic Tc1 polarization is mainly observed in RTX-refractory patients and associated with an increase in effector memory CD8<sup>+</sup> T cells and a restriction of their TCR repertoire [103]. These results argue for the specific involvement of CD8<sup>+</sup> T cells in platelet destruction in these patients, which might account for the failure of B cell depleting therapy [103].

#### **4.2.4. T cell antigens**

The antigens that are recognized by T cells during ITP are derived from platelet GP. Indeed, when antigen presenting cells are loaded with platelets, a strong proliferation of CD4<sup>+</sup> T cells is observed in ITP patients but not in healthy controls [120]. The stimulation of CD4<sup>+</sup> T cells with trypsin-digested GPIIb/IIIa also triggers their proliferation, whereas the use of the native protein does not [121]. The immunodominant epitopes responsible for CD4<sup>+</sup> T cell proliferation have been characterized and derived from the amino-terminal portion of the GPIIb and GPIIIa proteins [122]. The analysis of the CD4<sup>+</sup> T cell repertoire reveals an oligoclonal pattern, which is related to these antiplatelet clones [102, 123]. These alterations of the TCR repertoire are associated with a non-response to splenectomy [124] and are reversed in patients who respond to RTX [102].

Splenic CD8<sup>+</sup> T cells also display an oligoclonal profile, but especially in patients that are refractory to RTX [103]. One study identified a platelet peptide derived from GPIb. However, this peptide is probably not specific to ITP but depends on the MHC allele, being restricted to HLA-B7 in this case [125].

### **4.3. Disturbance of immune regulation**

#### **4.3.1. Regulatory T cells**

Regulatory T cells (Treg) consist of a T cell subset with a CD4<sup>+</sup>CD25<sup>high</sup>Foxp3<sup>+</sup> phenotype that displays immunoregulatory properties with the ability to inhibit effector cells such as CD4<sup>+</sup>, CD8<sup>+</sup> T cells and B cells [126] and to induce tolerogenic DC [127]. A decrease in their number and/or function has been shown in ITP (**Table 3**). A low frequency of circulating Treg has been reported in most of the publications [70, 128-130]. However, depending on the phenotype that was retained to define Treg (CD4<sup>+</sup>CD25<sup>high</sup>, CD4<sup>+</sup>Foxp3<sup>+</sup> or CD4<sup>+</sup>CD25<sup>high</sup>FoxP3<sup>+</sup>), levels similar to those in controls have also been observed [94, 131, 132]. Treatment with dexamethasone [70] or RTX [130, 133] leads to an increase in circulating Treg levels in responder patients. Treg functionality, assessed by their ability to repress the proliferation of T cells upon polyclonal stimulation, is decreased in circulating Treg during ITP [130-132]. The secretion of IL-10 and IL-35, two anti-inflammatory cytokines produced by Treg, are decreased [134, 135]. Interestingly, the functional Treg deficiency can be reversed in patients who respond to RTX [130] or TPO-RA [131]. Not only circulating Treg are defective in ITP : the number of splenic Treg [94] and their functions [128] are reduced, and Treg frequency is also decreased in the bone marrow of ITP patients [118].

To determine whether the correction of Treg deficiency upon treatment was a cause or a consequence of ITP remission, serum TGF- $\beta$ 1 was measured in ITP patients treated with TPO-RA, a treatment that is not supposed to directly act on immune cells [131]. In fact, as platelets are the main producers of TGF- $\beta$ , the increase in serum TGF- $\beta$ 1 correlated with the rise in the platelet count. Thus, rather than being a direct effect of treatment, the increase in platelet turnover could promote the development of Treg, particularly Th3, a TGF- $\beta$  induced subset. However, in other AID, the correction of Treg deficiency obtained upon immunomodulatory therapy is generally achieved without modification of the platelet number and is related either to the treatment itself or to disease remission [136]. Thus, the link



between platelet count and Treg is probably specific to ITP. The ability of TPO to increase Treg was also demonstrated in a murine model of ITP. The transfer of splenocytes from *GPIIIA* KO mice immunized against GPIIIa into severe combined immunodeficient mice led to thrombocytopenia mediated by a humoral and cellular response. These mice presented a peripheral deficiency in Treg linked to their retention in the thymus [137]. Upon treatment with TPO, the correction of the thrombocytopenia was associated with a decrease in antiplatelet antibody production and an increase in splenic Treg [138]. The underlying mechanisms involved in this improvement in Treg frequency remain to be determined.

During AID, such as type 1 diabetes, Treg have been used as immunomodulatory therapy. Their infusion after *ex vivo* expansion attenuated AID symptoms [139]. In ITP, it has been shown that GPIIb/IIIa-specific Treg able to modulate DC functions can be generated and expanded from naïve T cells [140]. This result opens up new fields in the treatment of ITP, particularly for refractory patients.

#### **4.3.2. Regulatory B cells**

B cells can regulate immunity negatively or positively, however the evaluation of the secretion of the anti-inflammatory cytokine IL-10 alone is not sufficient to identify Breg, as it can be produced by most of the B cell subsets upon stimulation. The identification of regulatory B cells (Breg) in humans remains challenging as no unambiguous phenotype has been described. Recent studies, in mice, have shown that plasma cells were the major producer of IL-10 and IL-35 [141]. However, in humans, transitional B cells with a CD19<sup>+</sup>CD24<sup>high</sup>CD38<sup>high</sup> phenotype have been described as the major source of IL-10, able to inhibit T cell proliferation and Th1 commitment, and to induce Treg [142-144]. In ITP, a decrease in transitional CD19<sup>+</sup>CD24<sup>high</sup>CD38<sup>high</sup>IL-10 producing B cells has been observed in active non-splenectomized ITP patients. In this study, IL10 producing B-cells were able to

decrease TNF- $\alpha$  expression on monocytes [145] (**Table 3**). Of note, both quantitative and functional deficiencies were corrected with the normalization of the platelet count on treatment with TPO-RA [145]. In a murine model of arthritis, it has been shown that the deficiency in IL-10-producing B cells affects the T cell compartment, leading to a Treg impairment associated with an increase in Th1/Th17 polarization [146]. Whether the decrease in IL-10-producing B is implicated in Treg deficiency and in the skewed polarization of T cells observed in ITP patients remains to be demonstrated. Some authors also pointed out that AID flare-up sometimes observed after RTX could be due to the depletion of IL-10-producing B cells [147-149], as observed in a murine model of multiple sclerosis [150]. However, such a role has not yet been investigated in humans.

#### **4.3.3. Mesenchymal stem cells**

Mesenchymal stem cells (MSC) are pluripotent cells endowed with self-renewal properties. They are able to differentiate into adipocytes, osteoblasts and chondrocytes. These cells can be obtained from bone marrow or cord blood, and display many regulatory properties leading to the inhibition of T cells, the generation of Treg from naïve T cells and the induction of tolerogenic DC. These immunoregulatory capabilities are triggered in an inflammatory environment (notably by IFN- $\gamma$ , TNF- $\alpha$  and LPS) and are mediated by mechanisms dependent on close contact with the target cells *via* nitric oxide and prostaglandin E2 secretions, IDO induction and the Fas/FasL pathway [151]. Bone marrow derived MSC from ITP patients display an abnormal morphology with increased apoptosis and a decreased proliferation rate [152, 153]. Moreover, their ability to induce Treg and to stimulate IL-10 production is diminished, leading to lower inhibition of the proliferation of activated-T cell [152, 153].

## **5. ITP: not only the peripheral destruction of platelets but also insufficient production**

Besides the peripheral destruction of platelets, mechanisms leading to insufficient platelet production are also involved in ITP pathogenesis. This relative deficiency in megakaryopoiesis and thrombopoiesis is due to an immune response against megakaryocytes together with insufficient stimulation by thrombopoietin.

### **5.1. Thrombopoietin regulation**

Contrary to what was expected because of their peripheral destruction, the evaluation of platelet survival in ITP revealed that platelet turnover was normal or reduced [154]. This can be explained by the original regulation of thrombopoietin (TPO), the main growth factor of megakaryocytes. TPO is produced by the liver [155] with normal serum value of about 0.7 fmol/ml in adults [156, 157]. Recently, it has been shown that TPO production is regulated *via* the Ashwell-Morell receptor: during the senescence of platelets, there is a progressive desialylation of their membrane proteins, leading to their binding to the Ashwell-Morell receptor and subsequently to their phagocytosis and the induction of TPO production through a Jak2/STAT3 signaling pathway [158]. TPO is released into the circulation and binds to its receptor, c-Mpl, expressed on megakaryocytes and on platelets. The ligation of TPO to c-Mpl on platelets leads to the endocytosis and catabolism of both the receptor and the growth factor. Thus, the higher the platelet pool reaching the circulation is, the lower the TPO level that can stimulate megakaryocytes is [159]. Therefore, with similar platelet counts, patients suffering from aplastic anemia, a bone marrow disorder, show a rise in TPO level to around 12 fmol/ml whereas ITP patients show only a slight increase (1.25 fmol/ml) when compared with controls (0.75 fmol/ml) [156]. This is related to the fact that in ITP, the megakaryocyte mass is close to normal, with a large number of platelets reaching the circulation, but with a short lifespan, leading to thrombocytopenia. Thus, most of the TPO binds to c-Mpl expressed

on platelets, resulting in insufficient levels to increase platelet production by megakaryocytes. In contrast, a decrease in both megakaryocyte and platelet mass is present in aplastic anemia, resulting in an increase in circulating TPO levels, which are unable to overcome the thrombocytopenia because of the primary bone marrow insufficiency.

During ITP, it is worth noticing that the desialylation of GPIb/IX by autoantibodies could contribute to platelet destruction through the recognition by the Ashwell-Morell receptor in the liver, a mechanism that differs from the Fc $\gamma$ R-mediated phagocytosis within the spleen [160] and could contribute to different responses to treatment, notably IVIg.

## **5.2. Intrinsic megakaryocyte dysfunction**

In some patients who do not have antiplatelet antibodies, an intrinsic impairment of platelet production has been demonstrated [161]. In these patients, despite a normal proliferation of megakaryocyte precursors, normal expression of glycoproteins and similar level of endomitosis compared to controls, their ability to generate proplatelets is decreased. These results suggest a defect in late-differentiation megakaryopoiesis in some patients, which could explain their resistance to immunomodulatory treatments.

## **5.3. Immune response against megakaryocytes**

### **5.3.1. Antibody-mediated immune response**

As megakaryocytes express GPIIb/IIIa and GPIb/IX, they are also targeted by anti-platelet antibodies [162]. *In vivo*, megakaryocytes display abnormal features consistent with an increase in immature forms, impaired platelet production, degenerative changes and signs of para-apoptosis [163]. All these features are reproduced *in vitro* by adding plasma from ITP patients to cultured megakaryocytes [163]. In fact, antiplatelet IgG contained in the plasma of

adult ITP patients decreases not only megakaryocyte formation and their polyploidy [164, 165] but also proplatelet formation [166], a phenomenon that is reversed by TPO-RA [167].

Whether a disturbance of apoptotic pathways contributes to bone marrow impairment during ITP is matter of debate, as another study demonstrated that the effects of plasma from ITP patients on megakaryopoiesis were not reversed by the inhibition of caspases [165]. Moreover, apoptosis is a physiological mechanism during the late phases of megakaryopoiesis that leads to platelet production, which makes it difficult to interpret the results of these studies [168].

The exploration of bone marrow from patients with ITP also reveals macrophages surrounding megakaryocytes with images of phagocytosis. This finding suggests that antibody-dependent cell-mediated cytotoxicity is involved in the central impairment of platelet production [163].

### **5.3.2. CD8<sup>+</sup> T cell-mediated immune response**

The decrease in megakaryocyte maturation does not depend on the humoral response alone; it is also mediated by a cellular response. Indeed, T cell recruitment into the bone marrow during ITP is increased, subsequent to VLA-4 and CX3CR1 expression by T cells, CX3CR1 being the receptor of fractalkine, a cytokine produced in the bone marrow [118]. However, the effects of CD8<sup>+</sup> T cells on megakaryocytes in humans are different from those in murine models. In humans, cytotoxic T cells are responsible for the impairment of platelet production by decreasing the physiological apoptosis of megakaryocytes, which usually leads to platelet formation [117]. In contrast, in a murine model of ITP, there was a decrease in the number of megakaryocytes in the bone marrow, and those present displayed features of apoptosis [169]. However, the mechanisms causing the decrease in megakaryocyte apoptosis in humans are unknown.

## **6. Platelet functions in ITP**

### **6.1. Platelet destruction**

As previously described, the decrease in platelet count in ITP is related to different processes: the recognition of platelets by autoantibodies that facilitate their phagocytosis by macrophages, mainly in the spleen [58] and their lysis secondary to complement activation [81, 82]. Platelets also display increased levels of apoptosis [65], probably due to CD8<sup>+</sup> T cell cytotoxicity [75, 115, 116, 170]

### **6.2. Platelets as B cell co-stimulators**

Platelets are not only involved in primary hemostasis, but also play a role in the immune response [171], especially by the constitutive expression of CD154 (CD40L), which is furthermore upregulated in ITP [172]. The interaction of CD154 with CD40 expressed on B cells triggers their proliferation and anti-platelet antibody secretion, demonstrating that platelets are potent stimulators of autoreactive B cells in ITP [172].

### **6.3. Platelet activation**

The difference in platelet activation between bone marrow disorders such as myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) and ITP have been investigated [173]. Despite the absence of differences in bleeding symptoms between MDS/AML and ITP patients with similar platelet counts, the fraction of immature platelets is greater in ITP, with a higher expression of GPIb. Moreover, when compared with platelets from MDS/AML patients, those from ITP patients are more activated *in vivo* and are more responsive to stimulation by adenosine diphosphate (ADP) or thrombin receptor activating

peptide (TRAP) *ex vivo*, and platelets from ITP patients with bleeding symptoms display lower reactivity to stimulation than do platelets from patients without bleeding.

Such differences between patients' phenotypes could be linked to antiplatelet antibodies, which can interfere with platelet function. Indeed, it has been shown that 20% of ITP patients have antiplatelet antibodies that inhibit either ADP- or ristocetin-induced aggregation [174]. These inhibitory antibodies are mainly directed against GPIIb/IIIa.

The impact of ITP treatments on platelet activation has been investigated in three papers that focused on the effect of the TPO-RA eltrombopag [175-177]. In accordance with previous reports, a higher immature platelet fraction and higher platelet activation were found in ITP patients at baseline. While eltrombopag corrected thrombocytopenia, it did not trigger platelet hyper-reactivity despite a transient increase in the activation marker, P-selectin (CD62). Thus, the correction of the platelet count should not be associated with an increase in the thrombotic risk in ITP patients.

## **7. Current and future treatments of ITP**

### **7.1. First line therapies**

Treatments are usually started depending on the presence of bleeding manifestations and/or when the platelet count is below 20-30 G/L.

**Steroids**, known for years to repress multiple inflammatory genes in immune cells [178] are used as first line therapy and represent a good diagnosis test as a transient response is achieved in almost 80% cases [179]. Most particularly in ITP, they have been shown to correct the Th1/Treg imbalance [180] and to shift the balance between activator and inhibitor Fc $\gamma$ R on circulating monocytes [63]. Steroids also increase the proportion of suppressor cells such as circulating Treg [70], and more recently, they have been shown to increase myeloid-

derived suppressor cells in a murine model of ITP [181]. Interestingly, steroids also act on hemostasis by reducing the activation threshold of platelets during ITP [182].

**IVIg** are highly effective drugs (response rate around 80%) that are recommended as emergency therapies depending on the bleeding score [183]. They are usually used concomitantly with steroids to increase their efficacy. Their mechanisms of actions are complex and supported by functional blockade of activating Fc $\gamma$ R, by the upregulation of the inhibitory Fc $\gamma$ RIIb, by the enhancement of autoantibody clearance due to the blockade of the neonatal FcR, by modulating cytokine production, DC maturation and by neutralizing autoantibodies [184]. However, most of the mechanisms of action in ITP have been extrapolated from animal models. In human ITP, it has been shown that IVIg, *in vivo*, increase platelet lifespan by decreasing splenic platelet clearance [185]. However, IVIg do not increase the expression of the inhibitory Fc $\gamma$ RIIb on monocytes [186] and on splenocytes but decrease the phagocytic function of splenic macrophages [64].

The precise mechanisms of action of **anti-D** are not known but they are thought to block Fc $\gamma$ R, thus decreasing opsonized platelet clearance in rhesus D<sup>+</sup> ITP patients. Similarly to IVIg, a transient response is observed in around 80% cases. Of note, rozrolimupab, a mixture of 25 recombinant human monoclonal rhesus D antibodies was shown to be efficient in almost two-thirds of ITP patients [187], thus appearing as an interesting alternative to human plasma-derived therapies.

## **7.2. Second line therapies**

**Splenectomy** remains a cornerstone therapy of ITP, with a prolonged response in 60-70% cases [8]. The main mechanism of action is the removal of the site of destruction of platelets. Moreover, patients who respond to splenectomy also have a reduction in their T cell clonal



expansion [124]. As the spleen is also a niche of specific antiplatelet secreting long-lived plasma cells [188], splenectomy allows their removal.

Because of the major role of B cells in the pathogenesis of ITP, B cell depleting therapies such as **rituximab (RTX)** have been used for years [97]. Response rates of 40% and 30% at 1 and 2 year of follow-up are usually obtained [95], and 20% of patients are still responders after 5 years [96]. RTX acts by depleting B cells, but it is worth noticing that CD20 is not expressed by plasma cells. One explanation of the clinical efficiency of RTX is that B cell depletion abrogate the generation of short-lived plasma cells that produce antiplatelet antibodies. On the contrary, when antiplatelet antibodies are produced by long-lived plasma cells, B cell depletion is not efficient, as confirmed by their persistence in the spleen [84] or in the bone marrow [87] of RTX-refractory ITP patients. Interestingly, RTX does not only deplete B cells but is also able to reverse T cell polarization abnormalities [102], to increase Treg functionality [130] in responder patients, and to decrease splenic and circulating TFH [189]. Another B cell depleting therapy, veltuzumab, has shown its efficiency in ITP [190]. To our knowledge, no data are available concerning the potential effect of obinutuzumab, another CD20 targeting therapy, in ITP.

**Thrombopoietin receptor agonists (TPO-RAs)** have a response rate as high as 70-80% and are thus increasingly used in ITP. Their main mechanism of action is the increase of platelet production by megakaryocytes [191]. However, prolonged response after their discontinuation has been observed in up to 15% of ITP patients [192, 193], raising the question of their potential immunomodulating properties. Such a phenomenon could be supported by the improvement of Treg function [131] and/or by the modulation of the expression of the activator and inhibitor Fc $\gamma$ Rs on monocytes [194]. Romiplostim and eltrombopag are currently available, but new TPO-RA are under development such as avatrombopag [195], lusutrombopag [196] or hetrombopag [197].

Different **immunosuppressant drugs** such as azathioprine, ciclosporin A, cyclophosphamide or mycophenolate mofetil have been used in refractory ITP patients [179]. Their mechanisms of action have not been specifically investigated in ITP, but mostly rely on T and B cell inhibition.

### **7.3. Future treatments**

Because of high level of BAFF [26] and an increased expression of its receptors (BAFF-R and TACI) on autoreactive B cells and CD4 T cells during ITP [198], BAFF targeting therapies are promising in ITP. Belimumab, an anti-BAFF monoclonal antibody licensed in lupus, and blisibimob, a selective peptibody antagonist of BAFF, are under investigation in ITP (clinicaltrials.gov; NCT01440361 and NCT01609452).

With regards to the major role of plasma cells in ITP, most particularly their persistence in RTX-refractory patients, plasma cell targeting therapies such as bortezomib, a proteasome inhibitor, or daratumumab, an anti-CD38 monoclonal antibody licensed in multiple myeloma, could be of particular interest in refractory ITP. However, bortezomib-induced thrombocytopenia is a limiting factor to its use during ITP.

New strategies to target Fc $\gamma$ R<sub>s</sub> or their transduction signals have been developed. Previous reports have shown efficacy of CD16 (Fc $\gamma$ R<sub>III</sub>) targeting therapy [199]. However, due to the murine source of the antibody, a loss of response was observed with time, promoting the development of a humanized monoclonal antibody [200], that is now under investigation in humans (clinicaltrials.gov; NCT00244257). The spleen tyrosine kinase (Syk) is a protein tyrosine kinase involved in the transduction signal of Fc $\gamma$ R. Its inhibition by fostamatinib (R935788) is well tolerated and leads to an increase in platelet count in more than half of the patients [201].

TFH, which play a key role in ITP pathogenesis [85], particularly *via* the CD40L/CD40 axis and IL-21 production, could be promising targets: an antagonistic anti-CD40 monoclonal antibody (BI655064) is currently under investigation in ITP (clinicaltrials.gov; NCT02009761), while an anti-IL-21 monoclonal antibody is being tested in various autoimmune diseases (rheumatoid arthritis, lupus, Crohn's disease and diabetes notably).

The effects of MSC obtained from cord blood on immune response disturbances in ITP have recently been reported [202]. *In vitro*, MSC decreased B and T cell proliferation, shifted the T cell response from a Th1 to a Th2 pattern and decreased CD40L expression on T cells. The expression of CD80 on B cells and anti-platelet antibody production were decreased and abnormalities of megakaryocytes were also reversed. Moreover, the infusion of MSC into two ITP patients led to a transient amelioration in one and a sustained response in the other. If such results are confirmed, MSC could be a good alternative in the future for refractory ITP patients.

## **8. Concluding remarks**

Rather than a homogenous disease, ITP should be considered a syndrome in which the multiple pathways leading to thrombocytopenia are differently involved from one patient to another, thus accounting for, in part, the different responses to treatments. Even if the primary triggering factor is usually unknown, infections can act as a starter for an antiplatelet immune response, initially caused by molecular mimicry or bystander stimulation that will subsequently proceed despite infection resolution. This autoimmune response occurs mainly on specific genetic backgrounds and involves both the innate and the adaptive immune system, including humoral and cellular responses. The autoimmune response is not counteracted because of a deficiency in immune regulation mechanisms involving Treg, IL-10-producing B cells and tolerogenic DC. Furthermore, the peripheral destruction of platelets

is not overcome by an increase in platelet production because of an immune response against megakaryocytes, mediated by both autoantibodies and CD8<sup>+</sup> T cells, and because of inappropriate TPO levels.

As a result of the better understanding of ITP pathogenesis, new drugs such as TPO-RA have been developed, and new supportive strategies such as Treg or MSC infusions will probably extend the therapeutic arsenal in the near future.

Moreover, the identification of the main mechanisms involved in thrombocytopenia at the patient level, *i. e.* auto-antibody *vs.* CD8<sup>+</sup> T cell-mediated platelet destruction or peripheral *vs.* central mechanisms, will modify the therapeutic strategy and lead to tailored therapies.

« **Take-home messages** »

- ITP should be considered a syndrome rather than a disease: thrombocytopenia is the common feature resulting from different mechanisms leading to peripheral platelet destruction by the immune system and/or inappropriate platelet production in the bone marrow
- Platelet destruction is due to their recognition by auto-antibodies that facilitate phagocytosis by macrophages and implies complement-dependent cytotoxicity, and to an increase in their apoptosis mediated by CD8<sup>+</sup> T cells.
- Inappropriate platelet production results from an immune response mediated by autoantibodies and CD8<sup>+</sup> T cells that target megakaryocytes, but also because of relatively low levels of thrombopoietin, the main growth factor of megakaryocytes.
- Deciphering the immune response at the individual level will lead to better use of treatments with major clinical implications.

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## Tables

**Table 1.** Polymorphisms associated with ITP

Molecules	Particularity / population	References
HLA	DRB1*0410: overrepresented in ITP, associated with a lower response to steroids, Japanese, adults	[12]
	B8, DR3: related to chronic ITP, American, teenagers and adults	[13]
	A2: overrepresented in ITP, particularly chronic ITP, particularly in females, European, adults	[15]
MICA	MICA*183: overrepresented in ITP, Brazilian, adults (MICA= NKG2D ligand expressed on NK and CD8 <sup>+</sup> T cells)	[14]
Fc $\gamma$ R	FCGR3A -158VV: overrepresented in ITP, increase in IgG1 and IgG3 affinity, Brazilian, Canadian and Greek, children	[16, 17, 21]
	FCGR2C-ORF: overrepresented in ITP, Dutch, children and adults	[18]
	FCGR2B -232I/T: related to chronic ITP, Dutch, children	[19]
	FCGR2A H131R: overrepresented in childhood ITP, Caucasian, children	[20]
T-bet (TBX21)	TBX21 T-1993C: related to chronic ITP, Chinese, children and adults	[22]
SDF-1	rs2839693 A/A and rs266085 C/T: underrepresented in ITP rs2297630 A/G: underrepresented in chronic ITP, Taiwan, children	[23]
IL-10	GCC haplotype: related to acute ITP, increase in serum IL-10, Italian, children	[30]
	IL-10 -627AC: related to chronic ITP susceptibility, Chinese, children	[31]
TGF- $\beta$	TGFB1 -10TT: overrepresented in ITP, Turkish, teenagers and adults	[27]
IL-4	IL4 intron 3:related to chronic ITP, Chinese,children	[31]
IFN- $\gamma$	IFNG -874AG: overrepresented in ITP, Turkish, teenagers and adults	[27]
TNF- $\alpha$	TNFA -308AG: overrepresented in ITP, Caucasian, adults	[24]
	TNFA -308AG: overrepresented in ITP, Turkish, teenagers and adults	[27]
	TNFA -308AG: associated with persistent ITP, Indian, adults	[29]
IL-17	IL17 -7488TC: related to chronic ITP, Japanese, adults	[25]
IL-2	IL2 -330G: overrepresented in ITP, increase in serum IL-2 and IFN- $\gamma$ , Brazilian, adults	[28]
IL-1RA	IL1RN VNTR: overrepresented in ITP, increase in serum IL-1, Brazilian, adults	[28]
	IL1RA A1/A2: overrepresented in ITP, Turkish, teenagers and adults	[27]
IL-23R	IL23R rs1884444 GT/TT: overrepresented in ITP, Chinese, teenagers and adults	[32]
BAFF	BAFF -871TT: overrepresented in ITP, increase in serum BAFF, German, adults	[26]
HPA	HPA-5b (located on GPIa): related to acute ITP, Brazilian, children and adults	[36]
	HPA-2a (located on GPIb): related to refractory chronic ITP, German, adults	[37]
PTPN22	PTNP22 -1858T: overrepresented in ITP, Egyptian, children	[33]
	PTNP22 -1858T: overrepresented in ITP, Caucasian and Hispanic American, adults	[34]
	PTPN22 -1123G: overrepresented in ITP, Chinese, children and adults	[35]
miRNA	Disturbed expression of 22 miRNA, CXCL13 and IL-21 are among the targets, Swedish, adults	[38]
	MIR409-3p: decreased expression in ITP, regulates IFN- $\gamma$ gene, Chinese, children and adults	[39]
	MIR130A: decreased expression in active chronic ITP, regulates TGF- $\beta$ and IL-18 genes, Chinese, adults	[40]

**Table 2.** The role of infections in ITP pathogenesis

<b>Infectious agent</b>	<b>Mechanism</b>	<b>References</b>
HIV	- GP120 / GPIIIa molecular mimicry - Circulating immune complexes - Megakaryocyte infection	[43, 46]
HCV	- <i>Core envelope 1 protein</i> / GPIIIa molecular mimicry - Circulating immune complexes	[44, 56]
CMV	- Molecular mimicry? - Megakaryopoiesis inhibition - Platelet dysfunction	[45, 48]
EBV	- Molecular mimicry	[45]
<i>H. pylori</i>	- Molecular mimicry (CagA)	[54]
	- Decrease in Fc $\gamma$ RIIb expression on monocytes	[55]



**Table 3.** Actors in ITP pathogenesis and their respective roles

Cells	Role/specific feature	Targets/effects	References
B cells	- Autoantibody production	- GPIIb/IIIa, GPIb/IX, GPIa/IIa expressed on platelets (CDC, phagocytosis) and megakaryocytes (ADCC and apoptosis)	[76-82, 84, 88, 166, 167]
	- BAFF/BAFFR dysregulation	- Auto-reactive B cell stimulation	[26, 89]
CD4 <sup>+</sup> T cells	- Th1 polarization, $\nearrow$ Th1/Th2 ratio - Oligoclonal TCR repertoire - Anti-apoptotic pathways	- Autoreactive T cell survival	[98-103, 123, 124]
	- Th17 polarization	- Autoreactive T cell survival?	[108-111]
	- Class-switch <i>via</i> CD40L-CD40	- Autoreactive B cell stimulation	[83]
	- splenic T follicular helper cell expansion	- B cell proliferation and differentiation - antiplatelet production stimulation	[85]
CD8 <sup>+</sup> T cells	- Platelet apoptosis - Increase in plasma granzymes - Tc1 polarization, $\nearrow$ Tc1/Tc2 ratio	- Platelets - Megakaryocytes	[75, 102, 103, 115, 116, 117-119]
Macrophages	- Phagocytosis	- Opsonized platelets - Opsonized megakaryocytes	[57-60]
	- Antigen presenting cells - Increase in activation markers (CD86, HLA-DR)	- Autoreactive T cell stimulation - Epitope spreading	[60, 78]
Dendritic cells	- Increase in activation markers (CD86, CD80) - Increase in IL-12 secretion - Decrease in IDO expression - Decrease in TIMP-3	- Autoreactive T cell stimulation - Th1 commitment - Treg deficiency	[65-70]
Treg	- Quantitative and/or functional deficiency in the blood, in the spleen and in the bone marrow	- Failure to inhibit the autoimmune response	[70, 94, 118, 128-135]
Breg	- IL-10 production deficiency	- Treg production deficiency?	[145]
Platelets	- CD40L expression	- Autoreactive B cell stimulation	[172]

## Figure legend

### Figure 1: Immune thrombocytopenia pathogenesis.

**Platelet peripheral destruction:** splenic macrophages phagocyte opsonized platelets and subsequently present a platelet-derived antigen that activates autoreactive CD4<sup>+</sup> T cells. These CD4<sup>+</sup> T cells participate in the stimulation of B cells, which recognize native platelet antigens expressed on the surface of follicular dendritic cells. The interaction between CD40L/CD40 expressed by CD4<sup>+</sup> T cells, namely TFH, and B cells, respectively, leads to class-switch recombination and somatic hypermutations. The stimulation of auto-reactive B cells can also be mediated by platelets that overexpress CD40L. Auto-reactive B cells differentiate into plasma cells that will stay in the spleen or reach the bone marrow or other niches to produce anti-platelet antibodies. Circulating CD8<sup>+</sup> T cells, most probably within the spleen, participate in platelet destruction. Circulating auto-reactive T cells harbor a restricted repertoire and are engaged in anti-apoptotic pathways. Circulating and splenic T cell responses are skewed towards Th1 and Tc1, with an increase in Th1/Th2 and Tc1/Tc2 ratios. Because of the decrease in Treg number and/or function observed in the blood, the spleen and the bone marrow, the autoimmune response is not counteracted.

**Bone marrow impairment:** a decrease in megakaryocyte maturation and in platelet production relies on a specific immune response, supported by different mechanisms: autoantibodies that bind to GPIIb/IIIa and GPIb/IX expressed by megakaryocytes are responsible for antibody-dependent cellular cytotoxicity by macrophages that surround megakaryocytes; T cells driven to the bone marrow by CX3CR1, the receptor for fractalkine, and VLA4, notably CD8<sup>+</sup> T cells, decrease platelet production although the mechanisms involved are not known.

Besides the autoimmune response against megakaryocytes, inappropriate levels of thrombopoietin (TPO), the major growth factor of megakaryocytes, contribute to insufficient

platelet production. TPO, which is produced at a constant level by the liver, binds to its receptor, c-Mpl, expressed on platelets. As the platelet pool that reaches the circulation is close to normal in ITP, the level of TPO that remains free to bind to c-Mpl on megakaryocyte is too low to increase platelet production.

Figure 1

