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STATE OF THE ART

Immune attack on megakaryocytes in immune thrombocytopenia

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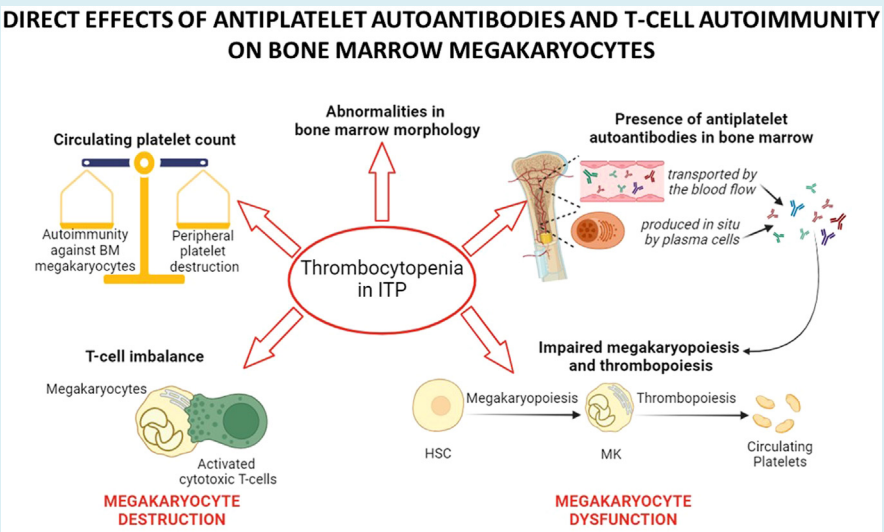
Abstract

A State of the Art lecture titled "Immune Attack on Megakaryocytes in ITP: The Role of Megakaryocyte Impairment" was presented at the International Society on Thrombosis and Haemostasis Congress in 2023. Immune thrombocytopenia (ITP) is an acquired autoimmune disorder caused by autoantibodies against platelet surface glycoproteins that provoke increased clearance of circulating platelets, leading to reduced platelet number. However, there is also evidence of a direct effect of antiplatelet autoantibodies on bone marrow megakaryocytes. Indeed, immunologic cells responsible for autoantibody production reside in the bone marrow; megakaryocytes progressively express during their maturation the same glycoproteins against which ITP autoantibodies are directed, and platelet autoantibodies have been detected in the bone marrow of patients with ITP. *In vitro* studies using ITP sera or monoclonal antibodies against platelet and megakaryocyte surface glycoproteins have shown an impairment of many steps of megakaryopoiesis and thrombopoiesis, such as megakaryocyte differentiation and maturation, migration from the osteoblastic to the vascular niche, adhesion to extracellular matrix proteins, and proplatelet formation, resulting in impaired and ectopic platelet production in the bone marrow and diminished platelet release in the bloodstream. Moreover, cytotoxic T cells may target bone marrow megakaryocytes, resulting in megakaryocyte destruction. Altogether, these findings suggest that antiplatelet autoantibodies and cellular immunity against bone marrow megakaryocytes may significantly contribute to thrombocytopenia in some patients with ITP. Finally, we summarize relevant new data on this topic presented during the 2023 International Society on Thrombosis and Haemostasis Congress. The complete unraveling of the mechanisms of immune attack–induced impairment of megakaryopoiesis and thrombopoiesis may open the way to new therapeutic approaches.



Decreased platelet count in immune thrombocytopenia (ITP) may be caused by both increased peripheral platelet destruction and autoimmunity against bone marrow (BM) megakaryocytes (MKs). Autoimmunity against BM leading to MK dysfunction and/or destruction is mediated by both antiplatelet autoantibodies and cytotoxic T cells. Platelet autoantibodies were detected in the BM of patients with ITP, either transported by the circulation or produced *in situ* by BM plasma cells, both free and bound to MKs. Their binding to MK surface glycoproteins causes an impairment of megakaryopoiesis and thrombopoiesis (Figure 1). The relative importance of these 2 pathogenic mechanisms probably varies among patients with possible distinct subsets of patients with ITP, in agreement with the heterogeneity of ITP in terms of serology, clinical manifestations, and response to treatment. HSC, hematopoietic stem cell.

KEYWORDS
autoantibodies, bone marrow, immune attack, immune thrombocytopenia, megakaryocytes, megakaryopoiesis, thrombopoiesis

Essentials

- Immune thrombocytopenia (ITP) is caused by circulating autoantibodies against platelet and megakaryocyte glycoproteins.
- Antiplatelet autoantibodies have also been detected in the bone marrow of patients with ITP.
- The immunological attack on megakaryocytes impairs many steps of megakaryopoiesis and thrombopoiesis.
- Both increased platelet clearance and impaired megakaryopoiesis cause thrombocytopenia in ITP.

1 | IMMUNE THROMBOCYTOPENIA

Immune thrombocytopenia (ITP) is a complex, multifactorial disorder with heterogeneous clinical manifestations, variable response to therapy and a not completely unraveled pathophysiology. It is characterized by isolated thrombocytopenia (platelet count, <100,000/μL) with normal white blood cells and hemoglobin and by mucocutaneous bleeding manifestations of variable severity. It is an autoimmune disorder caused by the generation of autoantibodies against platelet glycoproteins (GPs) due to the breakdown of self-tolerance. An imbalance in T helper (Th)1-to-Th2 cell ratio and a reduction and dysfunction of regulatory T cells (Tregs), a subset of $CD4^+$ cells that suppress effector T cell activation and help maintain immune tolerance, have been reported in patients with ITP [1–3].

Autoantibodies are found in approximately 60% of patients with ITP and are more frequently directed against GPIIb/IIIa, the fibrinogen receptor (~70%), and/or the GPIb-IX-V complex the von Willebrand factor (VWF) receptor (~25%), and more rarely (~5%) against GPIa/IIa and GPVI, both collagen receptors, and $\alpha_v\beta_3$, the vitronectin receptor [4–6].

ITP has a severe impact on patients' health-related quality of life, with 85% of patients with ITP reporting a reduction in their energy levels, 77% in their exercise capacity, and 75% in their ability to perform daily tasks. Moreover, due to ITP, 49% of patients reduce their working hours, and 29% consider quitting their job [7].

ITP is classified as primary when isolated thrombocytopenia develops in the absence of other disorders or secondary when thrombocytopenia is associated with chronic viral infections, lymphoproliferative disorders, and other autoimmune diseases (eg, systemic lupus erythematosus, rheumatoid arthritis, and antiphospholipid antibody syndrome) [8,9].

Based on its duration, ITP can also be defined as acute (started <6 months earlier), which may remit spontaneously, persistent (lasting >6 months), and chronic (lasting for >12 months), which rarely remits spontaneously [8]. The incidence of ITP in adults is between 1.6 and 3.9 cases per 100,000 persons per year [10–13].

The clinical course is typically fluctuating, with intermittent bleeding episodes that may last for days or weeks [14] and platelet count oscillations, a feature that may help to distinguish ITP from other causes of thrombocytopenia [14,15].

Bleeding manifestations in patients with ITP range from mild skin bruises to life-threatening intracranial hemorrhage, with the severity and frequency of hemorrhages usually correlating with the platelet count [14].

Given that there are no pathognomonic features, ITP is a diagnosis of exclusion, which requires excluding all the other possible causes of isolated thrombocytopenia. The initial evaluation requires a careful personal and familial clinical history and physical examination for hemorrhagic signs, including the inspection of the oral cavity for the prognostic value that oral purpura may have for more severe hemorrhage [16]. Regarding the laboratory approach, whole blood count and peripheral blood smear are the first steps [17], while there is discussion about the utility of antiplatelet autoantibody testing because it has high specificity but rather low sensitivity; thus, it may be of some help to confirm ITP when positive, but it does not allow its exclusion when negative [18].

The lack of specific diagnostic criteria and the clinical heterogeneity of ITP are responsible for the frequent misdiagnosis, with at least 15% of patients being reclassified as having a different diagnosis during follow-up, even by expert centers [13], and for the consequent inappropriate treatment.

2 | PATHOPHYSIOLOGY OF ITP

Concerning ITP pathogenesis, since the early 1950s of the last century, emphasis has been put on increased platelet destruction. The classic experiment carried out by Dr William Harrington, in which he infused himself and his coworkers with blood or plasma from some patients with thrombocytopenic purpura causing rapid and profound thrombocytopenia, showed unequivocally that thrombocytopenia was generated by a soluble factor circulating in patients' blood [19]. The plasma factor could be adsorbed by platelets, it was found in the γ globulin fraction, and the severity of thrombocytopenia was proportional to the amount of plasma infused, all findings strongly supporting the autoimmune nature of this disorder [20]. Other studies showed reduced platelet survival in patients with ITP, with major sites of platelet destruction being the liver and spleen [21–26]. The removal of autoantibody-opsonized platelets from the circulation was shown to occur either through Fc receptor-mediated phagocytosis by splenic macrophages [27–29] or through complement-mediated lysis [30–32].

However, given that no platelet-associated autoantibodies were found in approximately 40% of patients with ITP, alternative mechanisms of peripheral platelet destruction were suggested, including direct T cell-mediated platelet lysis. Indeed, $CD3^+$ T cells from patients with chronic ITP showed increased expression of genes involved in cell-mediated cytotoxicity, such as Fas, granzyme, and perforin, and in Th1 cell response, such as interferon-γ [33,34]. In agreement with increased peripheral destruction, platelet production was reported to be significantly enhanced, and bone marrow megakaryocyte number and volume increased in patients with ITP compared with controls in some studies, even if only in a fraction of patients with ITP (33%-73%) [35–38]. Concordantly, initial studies using radiolabeled autologous platelets reported reduced platelet survival in most patients with active ITP, with the major sites of platelet sequestration being the liver and spleen [21,23,24].

3 | IMMUNE ATTACK ON MEGAKARYOCYTES

After a quarter of a century of predominance of the immune-mediated peripheral platelet destruction theory of ITP pathogenesis, evidence that autoimmunity against bone marrow megakaryocytes could also play a role started to accumulate (Graphical Abstract).

3.1 | Platelet turnover studies

The first observations suggesting a role for suppressed platelet production came from radiolabeled platelet turnover studies. A compilation of 7 studies involving 218 untreated patients with ITP in whom platelet turnover was assessed by autologous instead of allogeneic radiolabeled platelets showed that increased turnover was rather uncommon in patients with ITP (18%), with most subjects exhibiting either normal (50.5%) or depressed (31.5%) platelet production rates [39]. These observations, contradicting previous studies using allogeneic platelets [39], strongly suggested a role of bone marrow impairment in the pathogenesis of thrombocytopenia in ITP.

3.2 | Bone marrow morphologic studies

An increased number of megakaryocytes in bone marrow biopsy specimens, which may indicate a disorder caused primarily by peripheral platelet destruction, has been shown in only a fraction of

patients with ITP (33%-73%), while in others they are normal (27%-65%) or reduced [37,38].

Early light microscopy observations of Wright-stained bone marrow smears from patients with ITP showed immature megakaryocytes with degenerative changes in nuclei, the presence of vacuoles, and a reduction of cytoplasmic granularity, supporting the idea that megakaryocyte damage and abnormal thrombopoiesis could contribute to the reduction of platelet count in ITP [40,41]. Indeed, the infusion of plasma from a patient with ITP into 2 healthy recipients resulted in, besides severe thrombocytopenia, the same degenerative abnormalities in bone marrow megakaryocytes described above, including the reduction of cytoplasmic granularity and the appearance of cytoplasmic vacuoles [41].

In the 1980s electron microscopy studies confirmed abnormal megakaryocytes in the bone marrow of patients with ITP, with distended demarcation membranes, vacuolized cytoplasm, and a disrupted peripheral zone, with some megakaryocytes showing attached monocytes apparently phagocytosing them [42]. More recent ultrastructural observations showed abnormalities in almost 70% of mature megakaryocytes in the bone marrow of patients with ITP compatible with para-apoptosis and apoptosis, including cytoplasmic vacuolization, dilation of the demarcation membrane system (DMS), nuclear chromatin condensation, and positive staining for activated caspase-3. Abnormal ITP megakaryocytes were frequently surrounded by neutrophils and macrophages, suggesting an inflammatory response against them [37]. Cytoplasmic and nuclear morphological abnormalities similar to those found in bone marrow megakaryocytes from patients with ITP were also observed in healthy control megakaryocytes cultured in the presence of ITP plasma, suggesting that antiplatelet autoantibodies may induce megakaryocyte programmed cell death [37].

Indeed, a more recent histological study reported that the assessment of bone marrow megakaryocyte morphology and number has limited utility for the diagnosis of ITP, except for a subset of patients with severe ITP in which abnormal megakaryocyte morphology and increased megakaryocyte number was observed [43]. Altogether these observations highlight that distinct subsets of patients with ITP with different mechanisms causing thrombocytopenia exist, suggesting that ITP is a heterogeneous disorder.

3.3 | Autoantibody binding to bone marrow megakaryocytes

Evidence that ITP autoantibodies can bind to megakaryocytes was first provided in an elegant experiment by Robert McMillan in 1978. Immunoglobulin G (IgG) antibodies purified from serum or cultured human splenic cells of patients with ITP or healthy controls were radiolabeled with iodine-125 and incubated with bone marrow cells from healthy donors. Autoradiography showed spots of radioactivity on megakaryocytes incubated with radiolabeled IgG antibodies from patients with ITP but not with control IgG antibodies [44]. It was later reported that a rabbit antiserum specific for mouse platelets cross-reacted *in vitro* with mouse megakaryocytes, but only with one-half of them, in particular with the mature ones [42]. In fact, megakaryocytes express the major surface GPs against which ITP autoantibodies are directed at different stages of their maturation: early during megakaryocyte differentiation GPIIbIIIa and later GPIb/IX/V [45].

More recently, an immunohistochemical study confirmed that a significant fraction of bone marrow megakaryocytes from patients with ITP (>50%) present IgG antibodies bound to their surface, differently from megakaryocytes from healthy controls. However, high IgG binding was also found on megakaryocytes from thrombocytopenic patients with myelodysplastic syndromes, suggesting that megakaryocyte-associated IgG antibodies may not be specific to ITP [46].

Not only IgG antibodies but also specific antiplatelet autoantibodies (anti-GPIIb/IIIa and anti-GPIb/IX) were found in bone marrow aspirates of 56% of patients with ITP, either bound to cells or free, but not in patients without ITP or healthy controls. Interestingly, in 5 patients with ITP, autoantibodies were found only in the bone marrow and not in peripheral blood, suggesting that autoimmune reactions limited to the bone marrow may occur in certain patients with ITP [47].

Altogether, these studies suggest that in a subset of patients with ITP, antiplatelet antibodies bind to a fraction of bone marrow megakaryocytes, possibly to a greater extent to mature megakaryocytes.

3.4 | The biological plausibility of an immune attack on bone marrow megakaryocytes

Several findings converge in supporting the biological plausibility of an immune attack on bone marrow megakaryocytes in ITP. Plasma cells, the B lineage cells that produce and secrete antibodies, were shown to reside also in the bone marrow where they secrete antibodies [48], and plasma cells producing anti-GPIIb/IIIa autoantibodies were found in the bone marrow of a patient with ITP refractory to rituximab [49]. Moreover, autoimmunity against other bone marrow precursor cells was shown in other hematologic disorders, such as autoimmune neutropenia, immune disease-associated anemia, and myelodysplastic syndromes [50–52].

3.5 | Concise overview of megakaryopoiesis and thrombopoiesis

Megakaryopoiesis is the process by which mature megakaryocytes, polyploid myeloid cells localized primarily in the bone marrow, develop from hematopoietic stem cells, while thrombopoiesis concerns the generation of platelets from mature megakaryocytes (Figure 1A). The bone marrow includes 2 microenvironments, the osteoblastic and the vascular niche, which differ in their cellular and extracellular composition and architecture and influence megakaryopoiesis and thrombopoiesis differently. Megakaryocytes themselves synthesize extracellular matrix components depending on their maturation stage [53]. Hematopoietic stem cells in the bone marrow osteoblastic niche differentiate into megakaryocytes in a thrombopoietin (TPO)-dependent manner. *In vitro* TPO induces the growth of

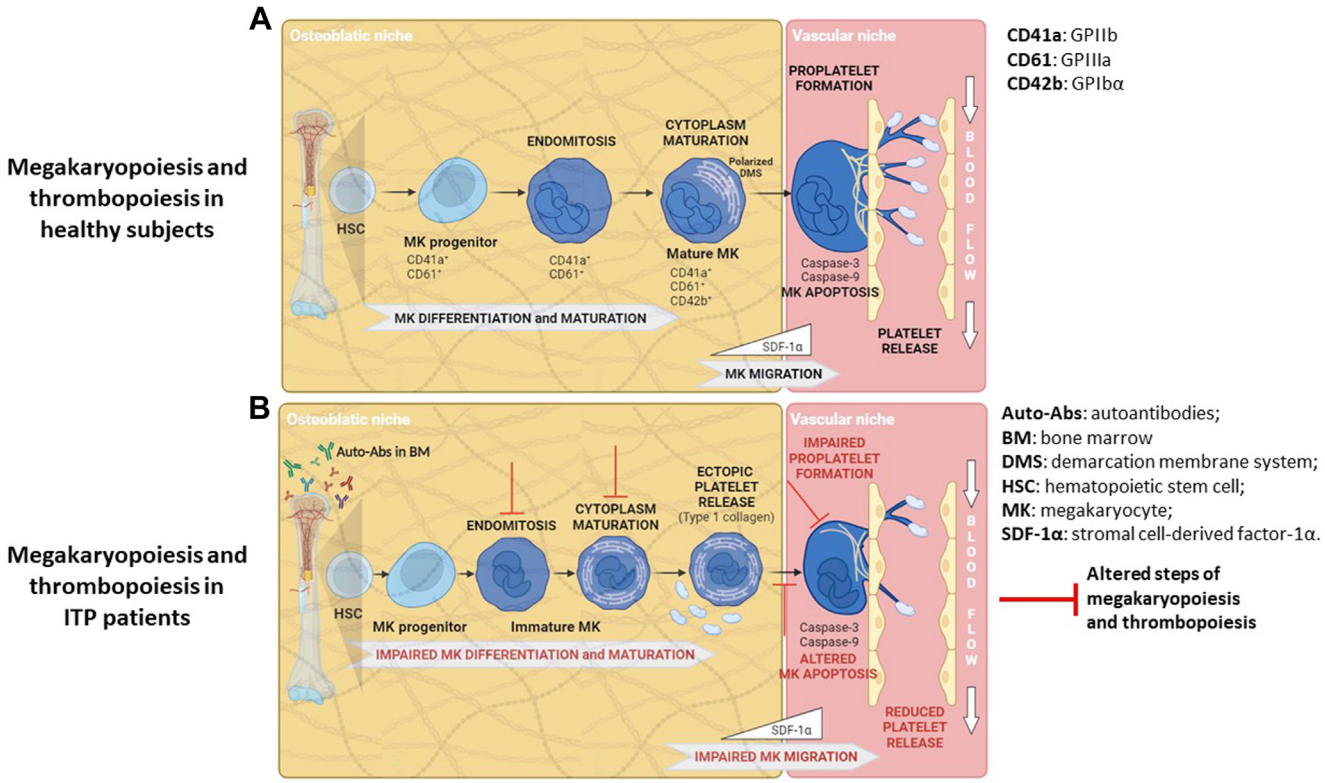


FIGURE 1 Megakaryopoiesis and thrombopoiesis in healthy subjects and patients with immune thrombocytopenia (ITP). (A) Physiological megakaryopoiesis/thrombopoiesis. Hematopoietic stem cells (HSCs) in the bone marrow (BM) differentiate into megakaryocytes (MKs) in a thrombopoietin-dependent manner. Immature MKs undergo maturation, during which they increase in size, undergo endomitosis, ie, become polyploid through cycles of DNA replication without cytokinesis, and develop a highly invaginated membrane system, called demarcation membrane system, which serves as a reservoir for proplatelet formation. The demarcation membrane system has to localize opposite to nuclei in the direction of BM sinusoids (polarization), to avoid ectopic platelet production. MKs express surface glycoproteins (GPs) in different stages of their maturation: in the early stage, they express GPIIb/IIIa, the fibrinogen receptor, followed by the expression of GPIb/IX/V, the von Willebrand factor receptor. In the late stages of maturation, MKs migrate from the osteoblastic to the vascular niche driven by the chemokine SDF-1α, where they extend long branching processes, called proplatelets, into sinusoidal blood vessels that are fragmented by blood flow into platelets. There is controversy about the role of MKs apoptosis in platelet formation: the activation of the proapoptotic proteins caspase-3 and -9 has been reported in mature MKs before proplatelet formation. (B) Altered megakaryopoiesis/thrombopoiesis. Platelet autoantibodies (Auto-Abs), both free and bound to MKs were detected in the BM of patients with ITP, either transported by the circulation or produced *in situ* by BM plasma cells. In ITP, Auto-Abs attack not only circulating platelets but also BM MKs, leading to the impairment of megakaryopoiesis and thrombopoiesis, thus reducing platelet release in the bloodstream and contributing to thrombocytopenia.

megakaryocyte colony-forming units and the generation of mature polyploid megakaryocytes [54]. During maturation, megakaryocytes increase in size and undergo endomitosis, thus becoming polyploid and increasing the content of cytoskeletal proteins. Moreover, organelles and granules are built up, and a highly invaginated membrane system, which serves as a reservoir for proplatelet formation, called DMS, is developed. Subsequently, the displacement of the DMS adjacent to bone marrow sinusoids and opposite to nuclei, called megakaryocyte polarization, which is crucial for allowing the formation of proplatelets only through the endothelium of sinusoids, takes place [55]. Ectopic release of platelets in the bone marrow is also prevented by the binding of megakaryocytes to type I collagen, which is abundant in the osteoblastic niche, through GPIa/IIa, which suppresses proplatelet formation [56]. In the late stages of maturation, megakaryocytes migrate from the osteoblastic to the vascular niche, driven by the chemokine SDF-1α, and there they extend long branching processes, called proplatelets, into sinusoidal blood vessels where they are fragmented by blood flow into platelets [55]. The binding of fibrinogen to GPIIb/IIIa in the vascular niche is crucial for proplatelet formation; in fact, proplatelet formation *in vitro* is impaired by GPIIb/IIIa antagonists [57] and in patients with variant Glanzmann thrombasthenia, a rare hereditary autosomal recessive bleeding disorder due to qualitative abnormalities of GPIIb/IIIa [58]. There is controversy about the role of megakaryocyte apoptosis in platelet formation. The proapoptotic protein caspase-3 is activated in mature megakaryocytes before proplatelet formation, and caspase-3 and -9 inhibitors suppress platelet production, suggesting that the activation of the intrinsic apoptosis pathway in megakaryocytes triggers platelet release [59–62]. Moreover, the inhibition of endoplasmic reticulum stress-induced apoptosis, an apoptotic pathway activated by the accumulation of misfolded proteins, was reported to reduce proplatelet formation, suggesting a role for transient endoplasmic reticulum

stress activation in megakaryocyte maturation and thrombopoiesis [63]. However, opposite findings were also reported, like the observations that the deletion of the prosurvival protein Bcl-x(L) induced megakaryocyte apoptosis and failure to shed platelets, which were restored by the deletion of proapoptotic Bak and Bax [64,65]. The exact role of megakaryocyte apoptosis in platelet formation is thus still controversial, and further studies are required to clarify it.

3.6 | Megakaryopoiesis and thrombopoiesis impairment in ITP

For most of the above-summarized steps of megakaryopoiesis and thrombopoiesis there have been studies showing a potential detrimental effect of ITP autoantibodies (Figure 1B).

3.6.1 | Megakaryocyte proliferation, differentiation, and maturation

Mouse monoclonal antibodies anti-human-GPIbα and anti-$\alpha_v\beta_3$ were shown to inhibit megakaryocyte colony formation from $CD34^+$ cells of healthy subjects *in vitro* [66]. Similar findings were obtained using plasma from patients with ITP containing anti-GPIb or anti-GPIb and anti-GPIIb/IIIa autoantibodies, an effect in part abolished by platelet adsorption of ITP plasmas, and in the presence of 2 human monoclonal autoantibodies specific for GPIIb/IIIa isolated from patients with ITP [67]. These findings were confirmed by another study showing that coincubation of $CD34^+$ cells from healthy donors with plasma from 12 out of 18 patients with chronic ITP produced a significant decrease in megakaryocyte generation and maturation measured as ploidy. This effect was dose-dependent; it was reproduced with IgG antibodies purified from ITP plasma and was largely suppressed by the adsorption of ITP plasma with immobilized GPIIb/IIIa [68]. Contrasting findings were however reported, including a study in which a subgroup of the ITP plasmas increased *in vitro* megakaryocyte production while a reduction of megakaryocyte ploidy was confirmed [69]. Moreover, later studies reported normal megakaryocyte maturation in the presence of recalcified plasma from patients with chronic ITP [70] and a monoclonal anti-$\alpha_v\beta_3$ autoantibody [71]. In summary, the effect of antiplatelet autoantibodies on megakaryocyte proliferation, differentiation, and maturation is quite controversial, perhaps due to the different megakaryocyte culture conditions. These findings are summarized in Table 1.

3.6.2 | Megakaryocyte adhesion to bone marrow extracellular matrix

Megakaryocyte adhesion to collagen, fibrinogen, and VWF is impaired in the presence of anti-GPIa/IIa, anti-GPIIb/IIIa, and anti-GPIb/IX/V autoantibodies, respectively. These results were obtained with both recalcified ITP plasma and purified ITP IgG antibodies [72]. Moreover, a monoclonal anti-$\alpha_v\beta_3$ autoantibody, as well as ITP plasma containing anti-$\alpha_v\beta_3$ autoantibodies, reduced megakaryocyte adhesion to fibrinogen by blocking the phosphorylation of FAK and SRC [71]. In summary, antiplatelet autoantibodies seem to interfere with the physical interaction between megakaryocyte GPs and the corresponding bone marrow extracellular matrix protein, which is crucial for physiological thrombopoiesis.

3.6.3 | Megakaryocyte migration

Integrin $\alpha_v\beta_3$ regulates the adhesion and migration of several cells [73], and a mouse anti-human $\alpha_v\beta_3$ monoclonal antibody and ITP sera containing anti-$\alpha_v\beta_3$ antibodies were shown to inhibit SDF-1α/CXCL12-induced megakaryocyte migration *in vitro*, probably by suppressing AKT signaling [71]. Moreover, in the bone marrow of patients with anti-$\alpha_v\beta_3$ autoantibody–positive ITP fewer megakaryocytes were found in the vicinity of sinusoids compared to controls, even if the total number of megakaryocytes did not differ from that of healthy controls, suggesting that anti-$\alpha_v\beta_3$ antibodies impair the migration of megakaryocytes from the bone marrow to the vascular niche [71] causing an altered megakaryocyte distribution with consequently reduced platelet release in the bloodstream [74].

In summary, anti-$\alpha_v\beta_3$ antibodies impair megakaryocyte migration to the bone marrow vascular niche, a phenomenon possibly contributing to the reduced platelet release in the circulation. Given the key role of integrins in cell migration [75], this phenomenon could also occur for the other antiplatelet autoantibody subtypes.

3.6.4 | Proplatelet formation

Mouse monoclonal antibodies against human-GPIbα and -GPIIb inhibited proplatelet formation by mature human peripheral blood $CD34^+$-derived megakaryocytes in suspension [66]. Also the plasma of patients with ITP dose-dependently reduced proplatelet generation by cord blood–derived megakaryocytes in suspension or adhering to fibrinogen, and the residual proplatelets that formed showed an abnormal morphology with decreased length and branching. Similar effects were obtained with IgG antibodies purified by affinity chromatography from ITP plasma, while platelet adsorption of ITP plasma reversed the inhibitory effect, confirming the role of autoantibodies in the inhibition of thrombopoiesis [70]. Impaired proplatelet formation by megakaryocytes adhering to fibrinogen produced by ITP plasma containing anti-GPIIb/IIIa antibodies was suggested to be due to the interference with GPIIb/IIIa [70,72], while the physiological inhibition of proplatelet formation was lost when megakaryocytes adhering to type I collagen were incubated with ITP plasma bearing anti-GPIa/IIa antibodies [70].

Other studies showed that ITP plasma bearing anti-GPIb/IX/V autoantibodies and a monoclonal anti-$\alpha_v\beta_3$ autoantibody impaired

TABLE 1 Summary of the findings on the effect of immune thrombocytopenia plasma on megakaryocyte proliferation, differentiation, and maturation.

Autoantibody source		Megakaryocyte source	Findings	Reference
Mouse monoclonal antibodies anti-human-GPIbα and anti-$\alpha_v\beta_3$		Peripheral blood $CD34^+$ cells	Inhibition of megakaryocyte colony formation.	[66]
53 ITP plasma	5 $GPIIb/IIIa^+$, 14 $GPIb/IX/V^+$, 19 $GPIIb/IIIa^+$, $GPIb/IX/V^+$, 15 negative	Venous cord blood $CD34^+$ cells	Inhibition of megakaryocyte colony formation.	[67]
18 chronic ITP plasma vs control plasma	9 $GPIIb/IIIa^+$, 5 $GPIb/IX/V^+$, 3 $GPIIb/IIIa^+$, $GPIb/IX/V^+$, 1 ND	Peripheral blood $CD34^+$ cells	Inhibition of megakaryocyte colony formation. Decreased megakaryocyte maturation.	[68]
49 chronic ITP plasma vs control plasma	12 $GPIIb/IIIa^+$, 9 $GPIb/IX/V^+$, 8 $GPIIb/IIIa^+$, $GPIb/IX/V^+$, 20 negative	Human umbilical cord blood $CD34^+$ cells	Normal megakaryocyte colony formation. Decreased megakaryocyte maturation.	[69]
21 recalcified chronic ITP plasma vs recalcified control plasma	4 $GPIIb/IIIa^+$, 1 $GPIb/IX/V^+$, 1 $GPIaIIa^+$, 2 $GPIIb/IIIa^+$, $GPIaIIa^+$, 13 negative	Human umbilical cord blood $CD34^+$ cells	Normal megakaryocyte maturation.	[70]
Monoclonal anti-$\alpha_v\beta_3$ autoantibody		Human umbilical cord blood $CD34^+$ cells	Normal megakaryocyte maturation.	[71]

GP, glycoprotein; ITP, immune thrombocytopenia; ND, not determined.

proplatelet formation on VWF and fibrinogen, respectively [71,72]. Finally, ITP antiplatelet autoantibody-mediated desialylation of GPIIb/IIIa and GPIb/IX/V inhibited megakaryocyte adhesion to fibrinogen and VWF but not to collagen, leading to impaired maturation and proplatelet formation [76].

In summary, these studies show that antiplatelet autoantibodies impair proplatelet formation and suggest that this occurs through the interference with the binding of megakaryocyte GPs to the respective bone marrow extracellular matrix proteins.

3.6.5 | Megakaryocyte apoptosis

Plasma from 26 out of 49 patients with ITP was reported to induce decreased megakaryocyte apoptosis *in vitro*, as shown by reduced caspase-3 and -8 expression and overexpression of the antiapoptotic protein Bcl-xL, with associated impaired megakaryocyte maturation and reduced platelet production [69]. Also, $CD8^+$ T cells from patients with ITP were reported to decrease physiological apoptosis of megakaryocytes, thus impairing platelet production without inducing cell lysis [77]. Subsequent studies, however, reported increased apoptosis of megakaryocytes in the presence of ITP plasma as shown by enhanced chromatin condensation, but no correlation was found with the proplatelet count, suggesting that apoptosis of mature megakaryocytes and proplatelet formation are independent events [70]. In agreement, a monoclonal anti-$\alpha_v\beta_3$ autoantibody did not influence megakaryocyte survival, whereas it decreased proplatelet formation [71]. Ultrastructural abnormalities compatible with para-apoptosis and apoptosis, including cytoplasmic vacuolization, dilation of the DMS, nuclear chromatin condensation, and positive immunohistochemical staining for activated caspase-3, were also observed in almost 70% of mature megakaryocytes in the bone marrow of patients with ITP [37].

In summary, the effects of antibody and cell autoimmunity on megakaryocyte apoptosis and its possible consequences on proplatelet formation are controversial and reflect the contrasting findings on the role of megakaryocyte apoptosis in physiological thrombopoiesis (Table 2).

3.7 | Effect of TPO receptor agonists

The addition of the TPO receptor agonists (TPO-RA) romiplostim and eltrombopag to mature megakaryocytes preincubated with ITP autoantibodies restored the capacity to form proplatelets overcoming the deleterious effects of ITP sera, suggesting that TPO-RA may increase platelet production in ITP by boosting the number of proplatelet-bearing megakaryocytes. Interestingly, restoration of proplatelet formation in the presence of one ITP serum was attained with eltrombopag but not with romiplostim, suggesting that the response to different TPO-RA may be determined by the nature/specificity of the ITP autoantibody [78].

3.8 | Cytotoxic T lymphocytes

While there is clear evidence that cytotoxic T lymphocytes participate in the destruction of circulating platelets in a B cell-independent manner [33,34], only a few studies have explored the effect of cell-mediated autoimmunity on megakaryopoiesis. The analysis of bone

TABLE 2 Summary of the findings on the effect of immune thrombocytopenia plasma on megakaryocyte apoptosis.

Autoantibody source		Megakaryocyte source	Findings	Reference
		ITP megakaryocytes	Decreased megakaryocyte apoptosis by CD8+ T cells from patients with ITP.	[77]
49 chronic ITP plasma vs control plasma	12 GPIIb/IIIa$^+$, 9 GPIb/IX/V$^+$, 8 GPIIb/IIIa$^+$, GPIb/IX/V$^+$, 20 negative	Human umbilical cord blood CD34$^+$ cells	Decreased megakaryocyte apoptosis with consequent impaired megakaryocyte maturation and reduced platelet production.	[69]
21 recalcified chronic ITP plasma vs recalcified control plasma	4 GPIIb/IIIa$^+$, 1 GPIb/IX/V$^+$, 1 GPIaIIa$^+$, 2 GPIIb/IIIa$^+$, GPIaIIa$^+$, 13 negative	Human umbilical cord blood CD34$^+$ cells	Increased megakaryocyte apoptosis but no correlation with proplatelet count.	[70]
11 ITP plasma, ITP bone marrow	1 GPIIb/IIIa$^+$, 1 GPIIb/IIIa$^+$, GPIaIIa$^+$, GPIb/IX/V$^+$, 8 negative, 1 ND	Peripheral blood CD34$^+$ cells	Increased megakaryocyte apoptosis.	[37]
Monoclonal anti-$\alpha_v\beta_3$ autoantibody		Human umbilical cord blood CD34$^+$ cells	No effect on megakaryocyte apoptosis but decreased formations of proplatelets.	[71]

GP, glycoprotein; ITP, immune thrombocytopenia; ND, not determined.

marrow nucleated cells of patients with ITP showed an increased percentage of CD8$^+$ cytotoxic T lymphocytes compared with healthy controls [77,79,80]. Interestingly, these T lymphocytes proliferated much more than control T lymphocytes upon incubation with autologous platelets [77]. Activated T cells expressing the death-signaling protein Fas and surface VLA-4 and CX3CR1, proteins that mediate T cell trafficking, were found in the bone marrow of patients [80] with ITP. The crucial role of VLA-4 in T cell recruitment to target tissues has been reported in other autoimmune diseases, such as multiple sclerosis [81], while CX3CR1 is the specific receptor for fractalkine, a chemokine that regulates T lymphocyte trafficking from peripheral blood to target tissues [82].

In addition, a reduced number of CD4$^+$ Tregs was found in the ITP bone marrow [80,83], similar to what was previously shown in peripheral blood [1,3].

On the contrary, CD4$^+$ Th22, Th17, Th1, and T follicular helper cells, subsets of Th cells with proinflammatory functions that synergistically regulate antiplatelet autoantibody production, were significantly more abundant in the bone marrow of patients with ITP than in the bone marrow of healthy controls [83]. Moreover, the expression of genes involved in Th cell differentiation and T cell chemotaxis, autoantibody response, and complement activation was increased in the bone marrow of patients with ITP [84]. Thus, the increased recruitment of cytotoxic CD8$^+$ T cells to the bone marrow together with the disequilibrium in CD4$^+$ T cells subpopulations, with a reduction of Tregs, could be another mechanism of impaired megakaryopoiesis and thrombopoiesis in ITP. In conclusion, the T cell imbalance in the peripheral blood of patients with ITP is mirrored in the bone marrow, highlighting the importance of the bone marrow as an immune compartment in which cytotoxic T cells, as well as antiplatelet autoantibodies, target bone marrow megakaryocytes resulting in megakaryocyte destruction and dysfunction respectively, both causing impairment in thrombopoiesis.

4 | CONCLUSIONS

There is evidence that both humoral and T cell autoimmunity target bone marrow megakaryocytes in patients with ITP besides the known attack to circulating platelets. While the effect of autoantibodies on megakaryocyte maturation and proliferation is still controversial, their detrimental effect on megakaryocyte interaction with extracellular matrix proteins, their migration from the osteoblastic to the vascular niche, and their ability to produce proplatelets is rather well established. Thus, megakaryocyte dysfunction together with megakaryocyte destruction mediated by cytotoxic T cells represents an additional pathogenic mechanism, besides peripheral platelet destruction, contributing to thrombocytopenia in patients with ITP.

5 | INTERNATIONAL SOCIETY ON THROMBOSIS AND HAEMOSTASIS CONGRESS REPORT

Novel data on the immune attack of megakaryocytes in ITP were presented at the 2023 International Society on Thrombosis and Haemostasis meeting in Montreal. The inhibiting effect of ITP sera bearing anti-GPIa/IIa and anti-GPIIb/IIIa autoantibodies on megakaryocyte DMS polarization, megakaryocyte interaction with bone marrow extracellular matrix proteins, such as type I collagen and fibrinogen, and migration toward SDF-1α was reported [85]. This was the first study analyzing the effect of antiplatelet autoantibodies on DMS polarization and of anti-GPIa/IIa and anti-GPIIb/IIIa autoantibodies on SDF-1α-induced megakaryocyte migration [71]. This study also showed that ITP sera with anti-GPI/aIIa, anti-GPIIb/IIIa, or anti-GPIb/IX/V autoantibodies inhibit *in vitro* proplatelet formation by megakaryocytes adhering to fibrinogen, demonstrating that the inhibitory effect of the autoantibody is independent on its GP target

and suggesting that anti-integrin autoantibodies exert their detrimental effect on thrombopoiesis through a common mechanism [85].

6 | FUTURE DIRECTIONS

Thrombocytopenia in ITP is caused by a dysregulation of both the humoral and cell-mediated immune response with the consequent attack by antiplatelet autoantibodies and cellular immunity to circulating platelets and bone marrow megakaryocytes leading to peripheral platelet destruction and decreased platelet production. The relative importance of these 2 mechanisms probably varies among patients, in agreement with the heterogeneity of ITP in terms of serology, clinical manifestations, and response to treatment [86]. It can be hypothesized that different ITP patient subsets have distinct pathogenetic mechanisms causing the reduction in the number of circulating platelets involving either platelets, megakaryocytes, or both.

The possibility of rapidly characterizing the effect of individual patient ITP autoantibodies on megakaryopoiesis might help in choosing the most effective therapeutic approach, thus allowing personalized therapy. Thus, it could be hypothesized that the effect on megakaryopoiesis of individual ITP patient sera could be tested in 3-dimensional *in vitro* bone marrow models, similar to what was previously done for some forms of inherited thrombocytopenia [87], helping to personalize the therapeutic approach. Indeed, different TPO mimetics are available to treat thrombocytopenia in patients with ITP, and given their difference in structure and binding site, these molecules may have a differential impact on impaired megakaryopoiesis in individual patients with ITP, possibly depending on the nature/specificity of the ITP autoantibody [78].

Finally, some observations suggest that the impairment of megakaryopoiesis by antiplatelet autoantibodies may not be due to the interference with the physical interaction between megakaryocyte GPs and the corresponding specific bone marrow extracellular protein but by a common mechanism impairing megakaryopoiesis; future studies unraveling this hypothesis could lead to the identification of novel therapeutic targets.

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E.P. and P.G. reviewed the literature and wrote the manuscript.

RELATIONSHIP DISCLOSURE

There are no competing interests to disclose.

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