Immune thrombocytopenia (ITP) is an acquired bleeding disorder of autoimmune pathophysiology. The causes of ITP could be related to other pathology (viral, bacterial, or systemic), or ITP could develop without any apparent reason. While the immune system dysregulation mechanisms in ITP were described, its etiology remains unclear. Moreover, all existing treatment approaches are not specific for ITP, and its action is highly patient-specific. Here we describe recent findings in the origins and development of ITP and discuss novel experimental and theoretical approaches to diagnosing ITP and predicting therapy effects.

Keywords: autoimmunity; ITP; platelets; acute/chronic ITP; computational modeling; animal models

Introduction

Immune thrombocytopenia (ITP) is an acquired autoimmune disease, characterized by thrombocytopenia with platelet count lower than 100x10^9 plt/l without obvious causes for this [1]. ITP manifests as hemorrhagic syndrome of various severity with such symptoms as ecchymosis, petechiae, spontaneous or post-traumatic bleedings, epistaxis, meno- and metrorrhagias, and rarely with gastrointestinal bleedings and hematuria [1]. ITP is a common disorder with international incidence of 1.6 – 3.2 cases per 100,000 people a year [1–3]. ITP can manifest independently of age, though pediatric patients are more likely to be fully cured [4]. About two-thirds of pediatric patients are fully recovered from ITP through the first year of disease even without any kind of therapy received [4].

A hallmark of ITP is anti-platelet antibodies in patient's plasma [5]. They are specific for platelet glycoproteins [6]. However, the causes of the emergence of antibodies can be either primary or secondary [7]. So, primary and secondary ITP are distinguished [1]. Primary ITP is diagnosed when a separated decrease in platelet count without any predisposing etiology is observed [8]. The secondary ITP is diagnosed when some affecting platelet production or consumption cause was identified [9]. For example, some systemic diseases (systemic lupus erythematosus [10], antiphospholipid syndrome [11]), chronic infections (hepatitis C [12], HIV infection [13], H. pylori [14]), and lymphoproliferative diseases (CLL [15]) are known to affect platelet counts. Also, some therapy agents could affect platelets and thus cause ITP (for example, heparin-induced thrombocytopenia is considered to be one of ITP variants) [16].

Generally, ITP progresses through three phases: acute, persistent and chronic. Newly diagnosed ITP (acute ITP) is the first period of the disease occurring in first three months from clinical manifestation of symptoms; the persistent ITP has a duration of 3-12 months from establishing the diagnosis, and the chronic form of ITP is diagnosed when clinical or/and laboratory markers are present for more than a year [1]. About 75-80% of pediatric patients recover during the first 12 months from the diagnosis, about 15% develop the chronic form, and 0.1% of patients may decease because of severe hemorrhages [3]. The adults are more prone to develop the chronic form of ITP, than children [17].

There is no consistent treatment strategies for ITP, however, there are several recommended lines of therapy, based on the assumption that ITP was developed due to the presence of anti-platelet antibodies or platelet-targeted T-cells [1,18]. The first therapy line consists of injections of glucocorticosteroids (GCS) or intravenous immunoglobulins (IVIGs) [19]. GCS are widely used to reduce the overall immune response [20]. The mechanism of IVIGs action is not yet clear, but it is believed that they competitively inhibit macrophage Fc-receptors and thus reduce antibody-mediated platelet clearance in spleen [21]. Additionally, IVIGs activate T-regulatory cells and inhibit differentiation and functioning of Th17s [22]. The efficacy of the first
line therapy is about 70-80% with high probability of a relapse [23]. When GCS does not show significant efficacy, second line therapy could be used. There are different options for the second line. In some cases, an immune-suppressive therapy is used, i.e. mycophenolate mofetil [24] or cyclosporine [25]. Also, the suppressing B-cellular response anti-CD20 monoclonal antibody, rituximab, is efficient in ITP treatment [26]. Additionally, platelet production in ITP could be increased by agonists of thrombopoietin receptors (TPO-RAs), those stimulate the thrombopoiesis [18,27]. As the last resort, platelet clearance could be significantly reduced by the resection of the spleen (splenectomy) [28,29]. Unfortunately, even the splenectomy might fail to recover a patient’s platelet count [30], and it remains unclear whether the particular treatment approach would be effective.

It is noteworthy that primary ITP being a diagnosis of exclusion, it takes a long period of time to exclude all possible causes and that is why no straightforward diagnostic approach exists. Theoretically, any form of ITP could be diagnosed based on the presence of anti-platelet antibodies in patient’s blood [31]. However, the availability of antiplatelet antibody assay is limited [32]. The transition of acute ITP to the chronic form is of high interest for clinicians as the probability of recovery from the chronic ITP is substantially lower than from the acute form [33]. However, there are still no definite predictors for such course of the disease, although several known risk factors include older age of manifestation, higher platelet count, and pre-manifestation vaccination [34].

Systems biology approach might prove useful to develop diagnostic and predictive criteria for manifestation and progression of ITP and to evaluate the efficacy of the therapy options based on the phenotype of the patient. Here we discuss known mechanisms of ITP development and the in vitro, in vivo and in silico methods to study them.

1. Mechanisms of Immune thrombocytopenia

The molecular mechanisms of ITP development are poorly understood as there is no determined reason for the autoimmunity to unroll. Nevertheless, 60% of pediatric patients had a history of pre-manifestation viral infection [35], or pre-manifestation vaccination against influenza [36], measles virus, rubella virus, and mumps [37]. Though the starting point of acute ITP is uncertain, the mechanisms of autoimmune response were widely studied in chronic patients. Here, the dysregulations in both the humoral (B-cell) and the cellular (T-cell) parts of the immune system have been reported (Figure 1).

![Figure 1. Schematic of immune thrombocytopenia mechanisms. The thrombocytopenia in patients could be caused by both T-cells cytotoxicity (CTLs are more active because T-regulatory cells and T-helpers provide dysregulated stimuli) and B-cells antibody production (plasma cells are producing antiplatelet antibodies of IgG and IgM classes; B-regulatory cells provide more activating signals to B-cells to produce antibodies). CTLs specifically attack platelets and induce their apoptosis. Antibodies from plasma cells opsonize platelets. Consecutively, opsonized platelets are eliminated through the spleen. CTLs – cytotoxic T-lymphocytes; Treg – T-regulatory cell; Th – T-helper; Breg – B-regulatory cell; BAFF – B-cell activating factor.](image-url)
Most ITP patients have high plasma levels of antibodies against platelet and megakaryocyte cell membrane glycoproteins. The most commonly met ones are anti-GPIbα and anti-GPIIb-IIIa [31]. Antibodies of IgG and IgM classes are more frequently found than the IgA class [31,38,39]. It is well established that in ITP, the opsonization of platelets with the antibodies causes their elimination through the spleen in an Fc-dependent manner [40].

It is well-known that B-cells are responsible for the production of antibodies [41]. There are 100-900 naive B cells in 1 µl of human blood and much more in lymphatic vessels and nodes [42]. All of them have specific B-cell receptors (BCRs) specific for some antigens. When a BCR meets its specific ligands and B cell receives additional signals from helper T cells, toll-like receptor ligands and other factors the differentiation of the naive B cells into PCs may start the differentiation of the naive B-cell into a plasma cell [43]. The plasma cells produce specific antibodies against the recognized antigen [44]. Normally, naïve B-cells with BCRs reactive to autologous proteins are driven to be anergic to avoid the autoimmune reaction [45], but in ITP the mechanism of “turning off” the autoreactive B-cells is somehow disrupted. Fang et al. [46] have shown that patients with ITP have higher B-cells (CD19+) counts than healthy donors, as well as less regulatory B-cells, responsible for the suppression of an autoimmune response [41], and more memory B-cells, those are specific for previously met antigens [47]. Therefore, it could be stated, that ITP patients with antiplatelet antibodies have disrupted regulation of B-cellular immunity. Taking into account that the B-cells activating factor (BAFF) and its mRNA are also elevated in ITP patients [48], the autoreactive B-cells survive better.

T-cellular mechanism of ITP

Some ITP patients do not have anti-platelet antibodies [31]. Zhai et al. [49] have shown that the concentrations of the anti-platelet antibodies in the blood of patients with chronic ITP are significantly lower than for the ones with newly diagnosed or persistent ITP. Therefore, it can be expected that not only humoral immunity contributes to the ITP pathogenesis. Indeed, T-cellular immunity dysregulation ITP was earlier described [50,51]. T-cellular mechanism of ITP is assumed to constitute in T-killers’ action against platelet specific antigens, and the T-helpers and T-regulatory cells are also actively involved in the ITP pathogenesis.

T-killers (cytotoxic T-cells, CTLs) are the CD8+ T-cells that are responsible for the cytotoxic immune response [52]. All T-cells recognize specific antigens through their receptors, TCRs (T-cell receptors) [53]. A high-affinity binding of a specific TCR to MHC-peptide complex induces IL-2-dependent proliferation of T-cell with this TCR structure [54]. The mature CTL induces apoptosis in the target cell by means of its secreted perforins and granzymes [52]. Perforins are the proteins that bind to the target cell’s membrane and induce a pore formation to let the serine proteases granzymes (especially granzyme B) enter the cell and thus induce apoptosis [55] through the intrinsic pathway [56].

In order to support the hypothesis of In order to support the hypothesis of CTLs involvement in ITP, Zhang et al. analyzed gene expression in CD8+ cells of patients with chronic ITP [57]. They have shown that c Patients had elevated expression of perforins, granzyme B, TNFα, FasL and TRAIL protein – markers of the CTL activity and ongoing apoptosis. In line with this, Zhu et al. have demonstrated an increased level of IL-21, which is produced upon CTL activation [58]. In another study platelets from patients with chronic ITP demonstrated enhanced markers of apoptosis such as increased mitochondrial collapse, phosphatidylserine exposure, and amounts of pro-apoptotic proteins [59,60]. Altogether, these data points at the active role of CTLs in ITP pathogenesis.

T-helpers (Th) are CD4+ cells that modulate both the B-cellular and the T-cellular responses [61]. Different populations of T-helpers have diverse effects due to their secretory profile: Th1 produces IL-1, Th17 secretes IL-17, etc. [62]. Naive T-helpers, as well as CTLs, are activated upon exposure to the antigen [63]. Activity of the mature T-helpers is diverse: while Th1 promotes the proliferation of cytotoxic lymphocytes, Th2 stimulates the humoral immunity to produce more immunoglobulins [64]. Thus, both Th1 and Th2 can contribute to autoimmune diseases, such as ITP. Th17 [65] and Th22 [66–68] also can be involved in the ITP pathogenesis. Imbalance between Th1 and Th2 is considered to be a marker of immunity dysregulation [69]. In line with this, ITP patients have increased levels of Th1 [58]. Increased levels of Th17 and Th22 also were observed in ITP patients together with increased plasma concentration of IL-1, IL-17, and IL-22 [70,71].

Regulatory T-cells (Tregs) are a subpopulation of T-helpers those are characterized by high expression of Foxp3 gene [72,73]. Tregs regulate proliferation of the B- and T-cells, reducing their activity in case of overreactive immune response [73]. Tregs counts, as well as Foxp3 expression are decreased in ITP patients [74]. And the balance between Th1, Th17, and Treg is disrupted in ITP, probably due to the overproduction of the IL-17A [71] and IL-21 [58], which are negative regulators of Treg differentiation [73].
Basic research approaches to study Immune thrombocytopenia

The approaches to the determination of fundamental ITP mechanisms are diverse with both in vivo, ex vivo, in vitro and in silico assays reported. Here we briefly describe the existing methods of ITP mechanisms evaluation with specific accent on animal models on the one hand and systems biology studies on the other.

In vivo approaches to study ITP pathogenesis

Rodent-based animal studies, especially murine ones, have become the “golden standard” for the studies of the disease pathology in mammals. Murine models of ITP can be roughly divided into acute ITP and chronic ITP models. Acute models are based on the direct injection of the antiplatelet antibodies into animals, while chronic models are mostly based on transgenic mice (Figure 2). Thus, both B- and T-cell dependent mechanisms of ITP development can be mimicked using mice.

Generally, acute ITP is modeled using antiplatelet antibodies-based approaches targeted approaches, which are the most commonly used techniques to study ITP in vivo. The mice are injected with rabbit-produced or rat-produced anti-CD61 or anti-CD41 antibodies once or repeatedly, with MwReg30 anti-CD41 antibody being the most widely used [75]. As no active autoimmune reaction is ongoing in the organism, such models are called “passive” and is among the most trivial model of murine ITP. Antibody injection causes opsonization of platelets and their subsequent elimination by macrophages in spleen or liver [76]. It is noteworthy that anti-CD61 antibodies cause more severe thrombocytopenia than the anti-CD41 antibodies [76], probably due to the fact that anti-CD61 antibodies are responsible for the Fc-dependent elimination of platelets through the spleen [77].

The “passive” ITP models are useful for the studies of therapy impact on the pathology development [78]. This approach allowed to identify the mechanism of action of IVIG in ITP: it appeared that IVIG inhibited the Fc-dependent elimination of platelets through the spleen [77]. However, “passive” models of ITP in mice with single antibody injections are not suitable to study the mechanisms of the transformation of acute ITP to its chronic form as no autoimmune process is ongoing in antibody-injected mice. However, development of the specific regimes of repeated injections of antiplatelet antibodies with escalating dosage regimen can mimic chronic ITP. For this injections of anti-CD41 antibodies (MwReg30) daily with escalating the dose from 7 ug/ml at Day 0 to 21 ug/ml at Day 5 into mice have been done [75]. In these circumstances platelets are constantly consumed what resembles processes ongoing in chronic ITP in human patients. Indeed, such approach is still rather incorrect in contrast to more sophisticated models of chronic ITP in mice [75].

Development of chronic ITP in mice can be reached with repeated injections of antiplatelet antibodies with escalating dosage regimen. The other way to form the chronic ITP is to sensitize the mice to the platelet CD61. In the first model the mice receive the anti-CD41 antibodies (MwReg30) daily with escalating the dose from 7 ug/ml at Day 0 to 21 ug/ml at Day 5 [75]. In this model the platelets are destroyed all of the time, so it seems to be closer to the human disease. Nevertheless, the studies of this model are limited to 1 week for performing the

Figure 2. Existing murine models of primary immune thrombocytopenia. There are several approaches to develop ITP in a mouse. The first one is based on antiplatelet antibodies injections into WT mouse. The second model is based on perfusion of CD25 depleted regulatory T-cells into nude mice, causing a dysregulation in the immune system. On the other hand, an autoimmune reaction can be caused upon transfusion of normal platelets into a CD61-knockout mouse. CD25 depleted Tregs – CD25 depleted regulatory T-cells; anti-CD41 – antibodies against platelet CD41; MwReg30 – rat monoclonal antibody against mice CD41.

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The other approach to the development of the chronic ITP is based on some sensitization of the animal to CD61. For this, a CD61 knockout (CD61 KO) mice are perfused with platelets from WT mice [79,80]. This causes activation of immunity what results in the both clinical and laboratory symptoms of ITP: profound thrombocytopenia, hemorrhagic syndrome up to fatal bleedings [79]. This type of in vivo model is more likely to imitate the "natural" course of ITP from the first manifestation until the clinical outcome. However, even though such model is closely resembling ITP in human patients, sensibilized to CD61 mice do not recover from ITP, while patients can overcome the disease [33].

T-cellular models are less common than B-cellular due to their complexity. The most elegant one is based on the CD25 depleted mice. The CD4+ cells from well characterized CD25 depleted mice [81] are transfused into nude mice. 36% of recipients develop severe isolated thrombocytopenia on the 3rd and consecutive weeks [82,83]. That model is a reflection of a classical ITP manifestation with spontaneous hemorrhagic syndrome and isolated thrombocytopenia. Unlike the CD61-sensibilization based model, Treg-based one does not cause fatal consequences. Despite all of the advances in the in vivo studies, murine immunity significantly differs from human, what severely limits applicability of rodent models [84].

**In silico approaches to studies of ITP**

In silico approach is a convenient tool for studying the biological and physiological systems [85,86]. Computational models of intracellular signaling or intercellular interactions can predict the response of the system to different stimuli. Patient-based computational models could predict treatment efficacy and help the clinician to choose the dosage regimen to improve the quality of life of the patient. Thus, computational modeling could be of use for ITP research. However, there are only two computational models related to ITP.

The first model is designed by Wire et al. [87] for evaluating the dosage regimen for eltrombopag in immune thrombocytopenia patients. This model describes pharmacokinetics (PK) and pharmacodynamics (PD) of the drug and thus allows correction of the therapy dosage in personalized mode. Age and race are among the parameters that define the optimal dosage of eltrombopag in that model. Interestingly, it appeared that the body weight is insignificant to the PK/PD of this TPO-RA. It remains unpredictable if the platelet count would increase effectively, so it is still necessary to monitor the platelet count. Nevertheless, the patient-based regimen of eltrombopag administration is more convenient to be selected and adapted, with this model. This model might be implemented in studying the PK/PD of other types of pharmacology treatment options for immune thrombocytopenia as well.

The second model was published by our group to describe two platelet phenotypes in immune thrombocytopenia [88]. Platelets of ITP patients have been shown to bind to fibrinogen in different ways. Platelets of one group of patients bind fibrinogen less fibrinogen than healthy donors (comparable to Glanzmann’s thrombasthenia patients). The platelets of the other of ITP patients have increased fibrinogen binding. Furthermore, the platelets of ITP patients are pre-activated in the quiescent state which is detected with the increased cytosolic calcium. The cause of the formation of different subtypes of platelets remains unclear. The authors hypothesize that antiplatelet antibodies of different specificity might be the reason. Considering that anti-GPIb antibodies are able to activate platelets, the model can be extended for predicting the platelet phenotype of activation based on the results of the antibody testing in a concrete patient. So that more specific therapeutic approach might be considered.

Finally, models of different phenomena can also be applicable for the studies of ITP. Stepanyan et al. [89] have developed a stochastic computational model of platelets’ life cycle which takes into the account the consumption of platelets due to COVID-19 thrombosis. Briefly, the platelets are produced in the megakaryocytes from the bone marrow with a TPO level dependent manner. The newly produced platelets circulate in the blood for 7-10 days if no event occurs. Then the platelets are either eliminated through the liver what results in TPO level rise, or can be consumed due to ongoing thrombosis in lung microvasculature. This model can be implemented into studying the ITP mechanisms by adding any cells or events that can affect the platelet life cycle. It is of additional interest, how the addition of cytotoxic T-lymphocytes would affect platelet lifespan in such model.

**Conclusion**

ITP is a complex disease with diverse and poorly characterized initiation mechanisms and complex diagnostics. Generally, the treatment of ITP is aimed at suppressing the immune system function, increasing platelet production or decreasing platelet consumption by splenectomy, but there is no treatment aimed at the origin of the decease. Besides, the ITP therapy itself can induce transition from acute to chronic phase of ITP.

Basic science ITP research has flourished in the last couple of decades with a lot of new approached being developed. The murine models are the most common option to study the effects of various novel therapies, while computational modeling might be productive in describing the patient’s phenotype.
based on the clinical and laboratory parameters. So, the computational modeling might be a useful tool to construct a tool for personalized predictions of the disease progression and therapy efficacy.

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**Author Contributions**

O.I.A. planned the study and wrote the paper; A.A.M. analyzed the data and edited the paper; M.A.P. supervised the study, analyzed the data and edited the paper. All authors have read and agreed to the published version of the manuscript. The authors declare no conflict of interests.

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