Egypt J Pediatr Allergy Immunol 2014;12(2):49-61.

Review article

Pathophysiology of immune thrombocytopenic purpura: a bird's-eye view.

Amira Abdel Moneam Adly
Pediatrics Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt

ABSTRACT

Immune thrombocytopenic purpura (ITP) is a common autoimmune disorder resulting in isolated thrombocytopenia. It is a bleeding disorder characterized by low platelet counts due to decreased platelet production as well as increased platelet destruction by autoimmune mechanisms. ITP can present either alone (primary) or in the setting of other conditions (secondary) such as infections or altered immune states. ITP is associated with a loss of tolerance to platelet antigens and a phenotype of accelerated platelet destruction and impaired platelet production. Although the etiology of ITP remains unknown, complex dysregulation of the immune system is observed in ITP patients. Antiplatelet antibodies mediate accelerated clearance from the circulation in large part via the reticuloendothelial (monocytic phagocytic) system. In addition, cellular immunity is perturbed and T-cell and cytokine profiles are significantly shifted toward a type 1 and Th17 proinflammatory immune response with impaired regulatory compartment, including Tregs and Bregs, have been reported, suggesting a generalized immune dysregulation in ITP. Understanding how Th1/Th17/Treg differentiation and expansion are controlled is central to uncovering how autoimmunity may be sustained in ITP.

INTRODUCTION

Immune thrombocytopenia (ITP) is recognized as an immune mediated disorder in which platelets are opsonized by autoantibodies and prematurely destroyed by reticuloendothelial system[1]. It is a hematologic disorder affecting children with an incidence of four to five cases per 100,000 children per year[2].It is characterized by immune-mediated accelerated platelet destruction and suppressed platelet production. Although the etiology of ITP is not yet known, and the diagnosis continues to be one of exclusion.[3] A number of studies have provided evidence of disturbed innate and adaptive immune responses in patients with ITP.[4] The pathophysiology of ITP increasingly is understood better[5]. Not surprisingly, it is complex with involvement of many players in the human immune orchestra, including antibodies, cytokines, antigen-presenting cells, costimulatory molecules, and T and B lymphocytes (including T-helper, T-cytotoxic, and T-regulatory lymphocytes) [6].

The triggering event for ITP is unknown[7], but continued research is providing new insights into the underlying immunopathogenic processes as well as the cellular and molecular mechanisms involved in megakaryocytopoiesis and platelet turnover. Although historically ITP-associated thrombocytopenia was attributed solely to increased rates of destruction of antibody- coated platelets, it has become evident that suboptimal platelet production also plays a role [8].

Bleeding is due to decreased platelet production as well as accelerated platelet destruction mediated in part by autoantibody-based destruction mechanisms[9]. Most autoantibodies in ITP are isotype switched and harbor somatic mutations[10], and as such a role for CD4+ helper T cells in disease pathogenesis has been invoked. Consistent with this, ITP patients have activated platelet-autoreactive T cells with increasing cytokine imbalance toward IL-2 and IFN-γ, indicating a shift toward Th1 cells[11]. More recently, increased Th17 cells or IL-17 cytokine were reported in ITP patients[12], implicating a possible role for Th17 cells in ITP immunopathology.

Moreover, a role for cytotoxic T cells in direct lysis of platelets and megakaryocytes in the bone marrow has been proposed [13]. In addition to an increase in the effector T cell arm of the immune response (Th1, Th17 and CD8 cells); a decrease in the regulatory immune compartment of patients with ITP has been described. Specifically, a deficiency in generation and/or defective functions of ITP regulatory T cell (Treg)[14] and regulatory B cells (Bregs)[15].

This brief review will highlights the mechanisms, and their elements, underlying the pathogenesis and cellular kinetics of ITP and discusses the aspects of current understanding of immune dysregulation. Also it addresses the recent findings on the state of the Breg and Treg compartments by which this information may guide therapy in ITP patients in the future.

Implicating agents in ITP

ITP is a heterogeneous group of disorders with potentially differing etiologies, natural histories and response to therapy[3]. Some ITP patients appear to have fundamental disturbances in their innate and adaptive immunity, while in others a specific often times self-liming inciting agent may be involved. Several potential inciting agents have been identified or proposed.

In the case of childhood ITP, many patients give a history of a recent infection. In adults, infection with human immune deficiency virus, Helicobacter pylori and hepatitis C have been implicated in a high percentage of cases of ITP in endemic areas; the causal relationship is strongest in settings where microbial elimination leads to resolution of the associated ITP[16]. Acute ITP can also occur after vaccination. The evidence for vaccine associated ITP is most compelling in those immunized with measles-mumps-rubella vaccine (~1:40,000). There have also been case reports of ITP in children following other vaccinations against hepatitis B, diphtheria-tetanus-pertussis, and hepatitis A, but the relationship is less compelling[17].

It has been hypothesized that ITP results from molecular mimicry engrafted on a normal immune repertoire, such that the immune thrombocytopenia resolves once the inciting antigen has been eliminated. Although microbial antigens may play a role in the induction of vaccine associated ITP, many vaccines also contain adjuvants (e.g., alum) that might potentiate the immune response.

Recent work from Shoenfeld and coworkers suggests that some autoimmune syndromes and inflammatory states might be induced by these immune adjuvants themselves[18]. The name they have given to this activity is Autoimmune/Inflammatory Syndrome Induced by Adjuvants (ASIA). The role that ASIA might play in the initiation of ITP requires further study, but the observation that ITP has been seen after the administration of several different vaccines provides an interest in this possibility.

Identification of factors that precipitate ITP is extremely difficult due to the likely transient nature of the provoking event, the inherent difficulty in diagnosing ITP early in its course, and the prolonged time period over which monitoring would need to occur to capture the onset of ITP. Even in subjects known to be at high risk for ITP due to a personal history of ITP, a strong family history of ITP (rare), or comorbidity with a condition predisposing to secondary ITP, the onset of ITP often occurs seemingly rapidly and between monitoring time points[19].

Despite these limitations, data from numerous studies in recent years are beginning to come together to form a picture of an unbalanced immune response. Not surprisingly, the immune changes observed during ITP are complex. The long-held dogma of platelet-bound Abs leading to Fc_receptor (Fc_R)–mediated clearance of platelets by phagocytes residing in the spleen (and liver) continues to be a central theme in our current understanding of ITP. In addition to this, the evidence supports a wide array of immune shifts involving all components of the immune system, resulting in both shortened platelet survival and inhibition of the production of platelets[20].

The role of the spleen

In 1916, Kaznelson, while a medical student in Vienna, prevailed upon the attending surgeon to perform a splenectomy in a patient with ITP. The splenectomy was successful in normalizing the platelet count and, with other cases, first established the critical role of the spleen in ITP[21]. However, the cause of thrombocytopenia remained unclear. Was the spleen destroying the platelets or did it secrete a suppressive substance that inhibited platelet production and/ or release into the circulation? Doan et al.[22] examined a number of spleens from patients with ITP. They demonstrated sea blue (lipid laden) histiocytes in the spleen, suggesting it was the platelet ‘destroyer’. What directed the spleen to prematurely destroy platelets, however, remained unclear[23]. The spleen, with its unique microvascular architecture, is well suited for immunologic surveillance of the body platelet mass as it has the capacity to store a large number of platelets in an exchangeable pool in close proximity to cells of the immune system[23]. The intrasplenic platelet transit time has been estimated to be around 10 min and is independent of spleen size. The demonstration that splenic tissue from patients with ITP produces antiplatelet IgG suggests that the spleen has capacity of forming these autoantibodies and that this mechanism is active in the disease[24]. In addition, the splenic macrophages have been shown to avidly phagocytose platelets sensitized by serum from ITP patients[25]. The spleen therefore, both produces antiplatelet antibodies and sequesters opsonized platelets. The central role of the spleen in ITP is further illustrated by the fact that 70% of chronic ITP patients enter a durable remission after splenectomy[26].

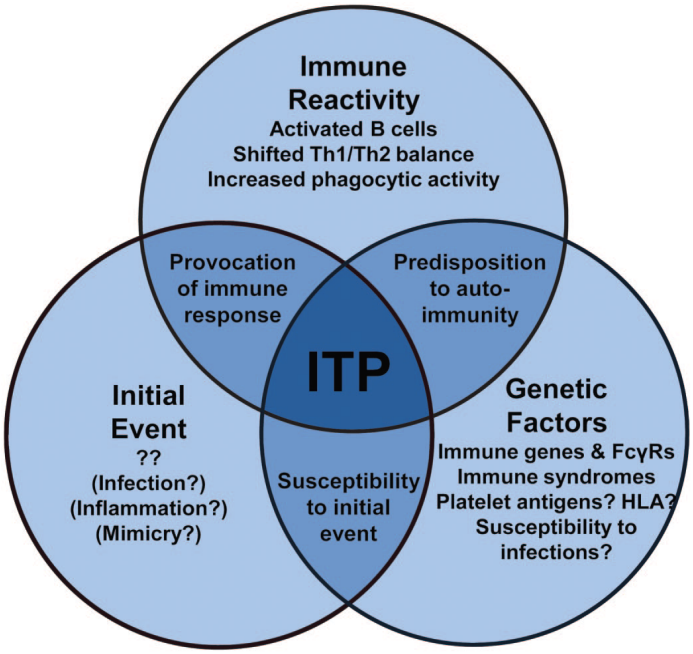

Figure 1. Model of relationship of contributing factors in ITP.
(Johnsen J. Pathogenesis in immune thrombocytopenia: new insights. Hematology Am Soc Hematol Educ Program. 2012;2012:306-12).

Antigen presenting cells

The role of antigen-presenting cells (APCs) for the loss of tolerance in ITP remains unclear, but a model has been advanced in which APCs are crucial in generating a number of new or cryptic epitopes from platelet glycoproteins. In this model, APCs expressing these novel peptides, along with costimulatory molecules, induce the activation of T-cells that recognize these additional platelet antigens. Thus, this acquired recognition of new self-determinants, or epitope spreading, may play an important role in the initiation and perpetuation of ITP. T-cell clones that react with cryptic epitopes may escape the negative selection in the thymus when self-determinants are present at a sub-threshold concentration[5].

Complement system

In general, Abs specifically bound to cell-surface antigens not only mediate clearance from circulation by Fc_Rs, but also can serve to fix complement on cells. Recently, plasma from ITP patients was shown by 2 groups to be capable of fixing complement to platelets in vitro[27]. Furthermore, platelets from ITP patients also exhibit detectable complement, and the ability to fix complement was correlated with the presence of detectable antiplatelet Abs[28]. Therefore, complement-mediated immunity, either by enhanced clearance or direct cell destruction, represents another mechanism by which platelet autoantibodies may lead to immune-mediated platelet destruction. Several studies have demonstrated increased platelet-associated C3 and C4 on ITP platelets but these are thought to be secondary in importance to platelet IgG and/or the result of antiplatelet IgM[29]. Furthermore, there is an association of (especially) C4 deficiency and ITP that has not been well studied and is of additional interest because the C4 genes are in the midst of the HLA region on chromosome 6[30].

Role of T cells

T-cell abnormalities in patients with ITP

A number of T-cell abnormalities have been demonstrated in patients with ITP (summarized in Fig 2) and it is likely that there are three main mechanisms by which T cells could be involved in the thrombocytopenia in patients with ITP. First, a number of studies suggest a Th1 bias, compared with Th2, in adults with chronic ITP. For example, increased numbers of HLA-DR+ T cells, increased soluble IL-2 receptors, and a cytokine profile suggesting the activation of precursor helper T and type 1 helper T cells have been described[31]. Reduced levels of IL-10 have also been described in patients with active disease when compared with those in remission or healthy controls[32] but conversely, raised IL-10 levels have been described in children with chronic ITP[33]. Further evidence of

Th1 involvement in the pathology of ITP is illustrated by an increase in the Th1 cytokines, IL-2 and IFN-c, in patients with ITP when compared with controls[34]. Interestingly, this increase was more marked in patients in remission than in those with active disease.

Overall, they describe significantly increased Th1/Th2 ratios in patients with both active and quiescent disease when compared with controls. These findings may be related to ongoing immune activation as part of autoimmunity. The activity of regulatory T cells and the potential for in vivo T-cell exhaustion because of prolonged in vivo activation has not been well studied in ITP[30].

A second method of potential T-cell involvement is the release of cytokines that interfere with megakaryocyte maturation and/or platelet release. Transforming growth factor (TGF)-b1 level has been inversely correlated with disease activity[32]. The role of TGF-b1 in ITP is thought to be as a potent inhibitor of megakaryocyte maturation. Two studies have shown increased granulocyte-macrophage colony stimulating factor (GM-CSF) levels, and one increased macrophage (M) CSF levels, suggesting that monocyte/macrophage activation is associated with ITP[35]. Circulating cytokines may also alter the response of HLA class II presentation, and/or influence the interaction between B and T lymphocytes causing pre-existing B cells to proliferate and produce high-affinity autoantibodies[36]. Finally, there is evidence to suggests a direct cytotoxic effect of T cells, by DNA microarray screening, Olsson et al.[37] found increased expression of several cytotoxic genes, such as granzyme A, granzyme B and perforin, as well as increased expression of genes involved in the Th1 cell response, such as INFc and IL-2 receptor-b in a small number of patients with ITP when compared with controls. As apparent compensation for this increased cytotoxicity, they also found increased expression of the killer cell immunoglobulin-like receptor (KIR) family on CD3+ T cells in patients with ITP in remission when compared with controls and to those with active ITP. KIRs downregulate cytotoxic T lymphocytes (CTL) and natural killer cell (NK) responses by binding to MHC class 1 molecules, preventing lysis of target cells. These findings suggest that CTLs may be involved in ITP. In a direct assay similar to that measuring NK cell activity by using radiolabelled K562 targets, these investigators assessed platelet destruction in vitro by T cells. They found that six of eight patients with active ITP showed platelet lysis by T cells whereas none of the patients in remission did. The effector cells were found to be CD3+CD8+ T cells[37].

This expanded role of cytotoxic T cells may explain why not all of the patients originally described by Harrington et al.[38] had a fall in their platelet count following the infusion of plasma from patients with ITP, and may also explain a percentage of patients without measurable antiplatelet antibodies, and again points to the heterogeneity of this disease.

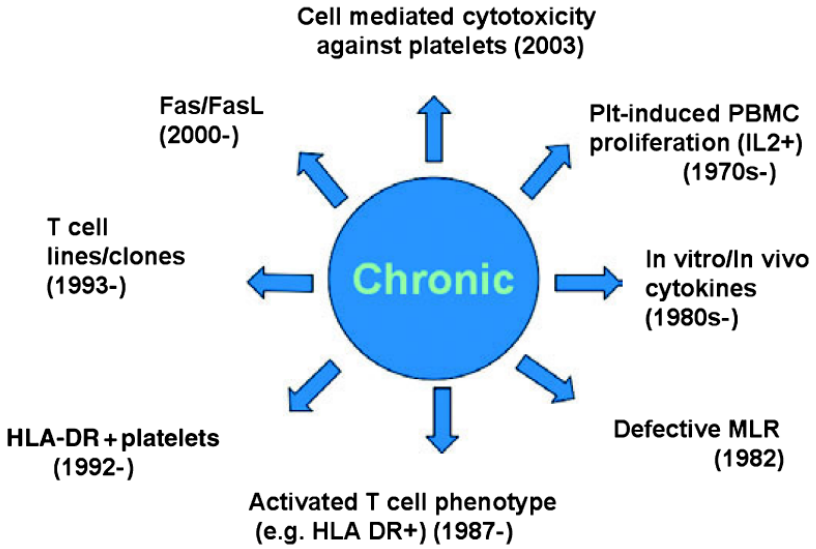

Figure 2. Summary of the variety of T-cell abnormalities found in adults with ITP.
PBMC, peripheral blood mononuclear cells; MLR, mixed lymphocyte reaction; ITP, immune thrombocytopaenic purpura (Cooper N, Bussel J. The pathogenesis of immune thrombocytopaenic purpura. Br J Haematol. 2006 May;133(4):364-74.)

T-cell phenotypes in ITP

From studies of the immune system in animals, immune homeostasis is thought to be maintained via a balance of type 1 (IFNγ, IL-2, TNFα, and TNFβ1 related) and type 2 (IL-4, IL-5, IL-6, IL-10, and IL-13 related) responses. Although the distinctions between type 1 and type 2 T cells and cytokine profiles in humans are less clear, these categories are helpful in broadly placing common immune patterns into context. Type 1 reactions are classically thought to be involved in response to intracellular pathogens, and a type 1 response is one which generally promotes pro-inflammatory, cell-mediated, complement fixing phenotypes. On the other hand, type 2 immunity is thought to function in the fight against extracellular pathogens, and a type 2 response typically elicits an immediate-type hypersensitivity response. During an inflammatory event, dominance of either a type 1 or 2 profile is characteristic, because the prevailing dominant type both expands and engages in negative feedback loops to suppress the "other" T-cell types. Resolution of an immune inflammatory event is then characterized by suppression of the dominant type phenotype and restoration of the type 1/type 2 balance[19].

Therefore, the shifting of the balance of type 1 and type 2 permits tailoring of the immune response to the perceived threat. In ITP, type 1/type 2 ratios are unbalanced (Figure 3). In primary ITP, adult chronic primary ITP patients have high Th1/Th2 ("helper" $CD4^+$ cells) ratios and high Tc1/Tc2 ("cytotoxic" $CD8^+$cells) ratios[39]. Furthermore, the Th1/Th2 ratio imbalance is inversely correlated with disease severity[40], meaning the higher the Th1/Th2 ratio, the lower the platelet count. ITP patients also exhibit decreased numbers of $CD4^+CD25^+$ T-regulatory cells (Tregs), which function to down-regulate T-cell responses[20]. Not surprisingly, the degree of decrease in numbers of Tregs is associated with more severe disease in ITP [41]. In addition to these type 1/2–specific changes, the total CD4:CD8 ratio is also observed to be diminished in ITP[42] and improves with disease remission[39].

More recently, other subsets of T cells distinct from type 1 and type 2 have also been in implicated in autoimmune diseases, including ITP. Similar to type 1 and type 2 T cells, these T-cell subsets are defined by their cytokine secretion profiles. T cells, which secrete IL-17, are pro-inflammatory and of interest in ITP in part due to a large body of evidence implicating IL-17 in autoimmunity. Within IL-17–secreting T-cell subsets, Th17 ($CD4^+$) cells are increased in ITP, as are Tc17 ($CD8^+$) cells[42]. Moreover, the increase in Tc17 cells is correlated with skewing of the CD4:CD8 ratio in ITP[42].

An additional discrete T-cell subset, Th22 ($CD4^+$IFNγ_IL-17_IL-22^+), has recently been identified and found to be up-regulated in several autoimmune diseases. Th22 cells are also significantly increased in ITP patients, and this increase is correlated with the observed increased numbers of Th1 and Th17 cells[43]. Another cytokine, IL-21, is produced by some $CD4^+$ T cells and natural killer T cells and is capable of up-regulating both Th17 cells and B cells. In ITP, IL-21 expression on T cells is elevated in untreated newly diagnosed ITP patients, although circulating IL-21 is unchanged[44].

Simplistically, the evidence supports a type 1 T-cell response with an up-regulated Th17 response in ITP. Interestingly, similar patterns of immune dysregulation can also be seen in other autoimmune disorders[43]. These types of responses, stereotypically proinflammatory, cell-mediated, complement-fixing phenotypes, would be expected to propagate and enhance the ongoing autoantibody mediated platelet immune process. Moreover, the increase in Tc17 cells is correlated with skewing of the CD4:CD8 ratio in ITP[44].

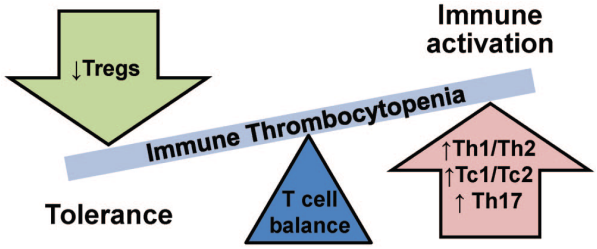

Figure 3. Schematic of shifts in the T-cell balance in ITP.
(Cines DB, Bussel JB, Liebman HA, Luning Prak ET. The ITP syndrome: pathogenic and clinical diversity. Blood. 2009; 113(26):6511-6521).

T cell tolerance failure

An autoantigenic stimulus may occur in the extrathymic periphery that could derive from the host's tissues in the form of an altered self-antigen or from a cross-reactive environmental stimulus that could mimic self-antigens. Alternatively high-affinity atuoreactive CD4+ Th cells may have escaped thymic selection during T-cell ontogeny and may stimulate the anti-platelet autoantibody response[45]. T cells recognizing peptides generated from native GPIIb/IIIa by normal processing pathways are hypothesized to be deleted in the

thymus (negative selection) since GPIIb/IIIa has been shown to be expressed abundantly as early as the 16th week of intrauterine life on epithelial cells of thymic stoma[46]. However, some autoreactive T cells directed against membrane antigens present on bone marrow-derived cells and also expressed in the thymus may not be eliminated by intrathymic deletion[47]. They can be detected in the peripheral, even though they may not cause autoimmune response. Some of these T cells to native GPIIb/IIIa that escape thymic deletion and exist in periphery, are inactivated by a post-thymic mechanism of peripheral tolerance[48] Thus, even though the process of autoreactive T cells deletion or rendered anergic is not totally efficient, there are several peripheral tolerance mechanisms that could potentially suppress autoreactive T and B cells from becoming activated (Fig. 4). These include activation-induced cell death through expression of Fas and FasL, inhibitory action of membrane molecules like CTLA-4, presence of a group of suppressor T cells and some cytokines that help inhibit the activation and proliferation of autoreactive T cells, such as CD4+CD25+ T cells[49].

All these tolerance mechanisms contribute to the inhibition of autoreactive T cells that exist in peripheral tissue and it is their failure that in various ways leads the autoreactive T cells to mount an attack against self-antigen. In clinics, immuno-suppressants such as azathioprine, cyclo-phosphamide and cyclosporine have been used to suppress T cells. Therefore, investigations of these aspects in the T cells tolerance not only help understand the disorder or imbalance of immune system in ITP, but may also help development of novel clinical treatment of ITP by targeting these mechanisms[50].

Defective Treg compartment in ITP

Tregs, as characterized by high level expression of the CD25 surface marker and of the transcription factor forkhead box protein 3 (Foxp3) on CD4+ cells, suppress proliferation of many immune cell types including T and B cells, either directly through cell contact or indirectly through secretion of cytokines, thereby dampening inappropriate immune activation and autoreactivity[51]. Possible reason for decreased Treg numbers can be due to impaired development, proliferation, survival and/or stability of Tregs whereas defective Treg function may be explained by failed cell contact dependent suppression or reduced secretion of cytokines that mediate suppression including IL-10, TGF-β or IL-35[52].

Reduced Treg activity may also be due to increased resistance of effector T cells to suppression, although we specifically demonstrated that effector T cells from ITP patient and healthy controls were equally inhibited by Tregs from healthy controls, arguing against the refractoriness of ITP effector cells to suppression[53]. Indeed, Tregs from ITP patients were less effective that Tregs from healthy controls in inhibiting effector T cell proliferation from either patients or healthy controls, suggesting that reduced Treg activity is due to an intrinsic defect in ITP Tregs[53]. Failure to maintain immune suppression by Tregs may be responsible for the reported platelet autoantigen-specific T cell proliferative responses and the proinflammatory phenotype in ITP patients[53]. Nevertheless, the observed polyclonal Treg dysregulation fails to explain why immune autoreactivity in ITP is directed toward platelets rather than other cell types. Detailed identification and characterization of platelet antigen-specific Tregs in ITP may help clarify why loss of tolerance is toward platelets rather than other tissues[54].

Role of B cells

Autoreactive B lymphocytes secrete antiplatelet antibodies

The most commonly occurring autoantibodies (~75%) in patients with ITP are directed against the platelet surface glycoprotein (gp) complexes gpIIb–IIIa and gpIb–IX[55].

Antibodies against other glycoproteins (Ia–IIa, IV, and V) have been identified, and multiple platelet antigen specificities can be found in most patients Although antibodies are primarily of the IgG subtype, IgM and IgA may be found[56].

Platelets are targeted by the attachment of autoantibodies to their surface gp antigens, bound to Fcγ receptors expressed on tissue macrophages of the reticuloendothelial system and cleared from the circulation. Complement- induced lysis following antibody binding may also play a role[57]. After platelet internalization and degradation, macrophages express platelet epitopes on their surface and secrete cytokines that stimulate initiating $CD4^+$ T-cell clones and clones with additional specificities[5].

Unique to patients with ITP, autoreactive $CD4^+$ T cells recognize several distinct epitopes on gpIIb–IIIa, leading to autoimmune response expansion and accelerated platelet destruction. The trigger for the initiating autoantibody response is unknown, although autoreactive T helper (Th) cells that interact with antibody-producing B cells are required[7]. Platelet-associated autoantibodies are

detected in 50%– 70% of patients with ITP[58], emphasizing the limitations of the currently available assays and/or suggesting that other or additional mechanisms are involved.

Assays for antibodies targeting gpIIb–IIIa, gpIb–IX, and gpIIa–IIIa may be more specific[59], but have limited sensitivity, and the diagnosis remains dependent on clinical presentation for the most part.[9]

Altered Bregs in ITP

Similar to the T regulatory compartment, Bregs inhibit T cell and monocyte activation and they do so in part through secretion of anti-inflammatory IL-10[60], which in turn regulates Th polarization, pro-inflammatory differentiation of other antigen presenting cells (APCs) and autoimmune responses[61]. The alteration that we have detected in ITP patients is both at the phenotypic and functionality of B cells[14]. Specifically, frequency of previously described as Breg population[62], characterized as $CD19^{+}CD24^{hi}CD38^{hi}$ cells, was decreased in nonsplenectomized ITP patients off treatment.

ITP B cells have impaired IL-10 response after stimulation and a reduced ability to dampen of monocyte activation[15]. In mouse models, IL-10 secreting regulatory B cells promote differentiation of Tregs[63] or their recruitment[64], indicating that these two immunoregulatory cell types interact with each other.

Although the ability of human Bregs to control Treg differentiation has not yet been demonstrated, the possibility remains that altered Bregs in ITP patients contributes to compromised Treg compartment[14].

This also brings up the question as to how these two immunoregulatory cell types interact with each other and that there may be a hierarchy amongst these regulatory compartments with Bregs controlling Tregs. Moreover, as with the data in Tregs, altered Breg activity identified in ITP and for that matter in other disease states in humans[62] does not explain the antigen-specific autoreactivity, but rather is consistent with a perturbed immune reactive state.

Autoantibodies Suppress Megakaryopoiesis

Chang and colleagues[65] showed that plasma from patients with ITP containing autoantibodies against gp1b and gpIIb–IIIa significantly suppressed megakaryopoiesis in vitro. They proposed that platelet autoantibodies may affect megakaryocyte maturation or survival, leading to decreased platelet production. It is increasingly clear that cellular immune mechanisms play a pivotal role in ITP[5].

The production of antiplatelet antibodies by B cells requires antigen-specific, CD4-positive, T-cell help (Fig 5). It also is possible that in some ITP cases, cytotoxic T cells play a role in the destruction of platelets. A possible sequence of events in ITP is as follows. A trigger, possibly an infection or toxin, leads to the formation of antibodies/ immune complexes that attach to platelets. Antibody-coated platelets then bind to antigen-presenting cells (macrophages or dendritic cells) through low-affinity Fcg receptors (Fcg RIIA/Fcg RIIIA) and are internalized and degraded. Activated antigen-presenting cells then expose novel peptides on the cell surface and with costimulatory help facilitate the proliferation of platelet antigen-specific, CD4- positive, T-cell clones. These T-cell clones drive autoantibody production by platelet antigen-specific B-cell clones[5]. As part of the platelet destructive process in ITP, cryptic epitopes from platelet antigens are exposed, leading to the formation of secondary platelet antigen-specific T-cell clones, with stimulation of new platelet antigen-specific B-cell clones and broadening of the immune response. The autoantibody profile of individual patients who have ITP reflects activity of polyclonal autoreactive B-cell clones derived by antigen-driven affinity selection and somatic mutation[6].

B cell tolerance failure

Besides T cells, B cell tolerance are also important in the pathogenesis of ITP, because they are the ultimate producer of autoantibodies. Genetic analysis of autoantibodies in idiopathic thrombocytopenic purpura reveals evidence of clonal expansion and somatic mutation[66]. In the germinal-center B cells, the degree of down regulation is negatively correlated to the up regulation of autoantibody IgG response. Central tolerance suppresses the unwanted expansion of autoreactive B cell population. If some B cells escape this suppression or deletion, peripheral mechanism may also be launched to maintain tolerance and one of the pivotal elements for maintaining peripheral tolerance is Fc_RIIB. ITAM-containing FcRs, on the other hand can prime autoimmune diseases and the impairment of the functional balance between activating and inhibitory FcRs leads directly to immune complex-mediated autoimmune diseases[67]. Possible mechanisms that result in the activation of GPIIb/IIIa-reactive T cells are expression of cryptic determinants of GPIIb/IIIa and generation of cross-

reactive B cells. The mechanism is thought to be the generation of cross-reactive B cells, initially primed by foreign protein serving as a molecular mimic that then bind, process, and resent self-protein. Then B cells would have the ability to efficiently concentrate and present to T cells small quantities of determinants that are typically sequestered. Cross-reactive B cells can subsequently prime naive autoreactive T cells if they express the appropriate costimulatory molecules[68]. Autoantibodies can then be abundantly produced by B cells after this T–B interactivation. In principle, autoimmune disorders arise because of the failure to eliminate or deactivate self-reactive lymphocytes, which is reflected in a deficiency of central and/or peripheral tolerance induction mechanism[69]. Central tolerance selection in the thymus may be faulty and allows the release of high affinity autoreactive T cells. Alternatively, an environmental agent can mimic a self-antigen that leads to the breakdown of peripheral tolerance mechanism. In ITP, these antibodies produced by B cells recognize self-antigens on platelet and cause in platelet phagocytosis via the reticuloendothelial system. In clinical therapy, patients may be responsive to splenectomy[70], but treatment with an immunosuppressant that inhibits T- and B-cell function and cooperation, including azathioprine, cyclophosphamide, cyclosporine, mycophenolate mofetil or anti-CD20, may be required. Monoclonal anti- CD20 antibody causes B-cell depletion, which may inhibit T-cell–B-cell interactions[50].

Impaired Platelet Production

Although increased platelet destruction clearly plays a key role in the pathogenesis of ITP, it is now recognized that impaired platelet production also is important in many cases. In adults, as many as 40% of ITP cases may have reduced platelet turnover, reflecting the inhibitory effect of platelet autoantibodies on megakaryopoiesis[71].

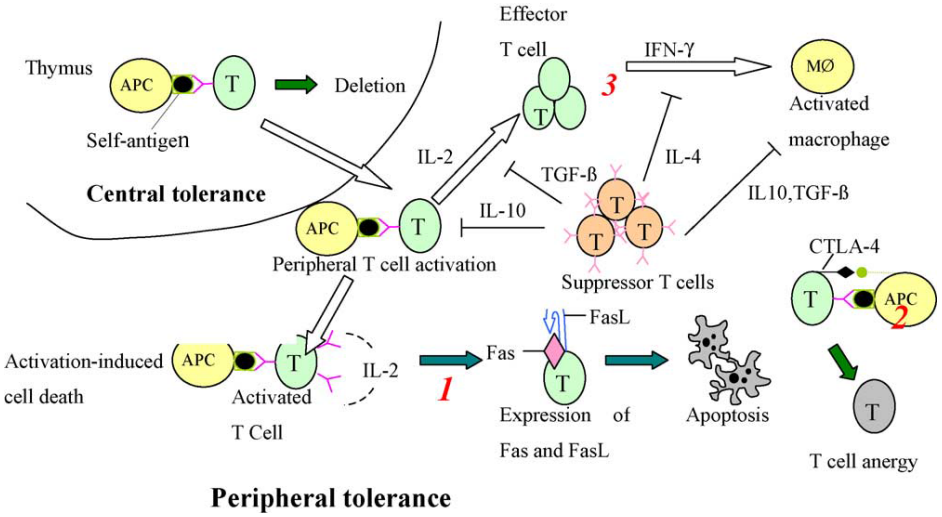

Figure 4. Mechanisms of central and peripheral T cell tolerance.
(1) Fas/FasL pathway induces the activated T cells to apoptosis, (2) inhibitory cell surface molecules help T cell anergy and (3) regulatory T cells secrete a profile of suppressive cytokines (Zhou B, Zhao H, Yang RC, Han ZC. Multi-dysfunctional pathophysiology in ITP. Crit Rev Oncol Hematol. 2005; 54(2):107-16)

Studies of platelet kinetics in children who have ITP are limited but it is possible that a similar situation exists. There also is evidence that platelet autoantibodies may induce thrombocytopenia by inhibiting proplatelet formation[72]. Circulating thrombopoietin (TPO) levels in patients who have ITP typically are normal or increased only slightly, reflecting the normal or only slightly reduced TPO receptor mass in this acquired platelet disorder. In contrast, TPO levels are high in inherited platelet production Disorders[73].

Summary of multi-dysfunctional pathophysiology in ITP

The increase in serum cytokine levels and activated T cells, the alteration of cell communication and the impaired megakaryocytopoiesis in patients with chronic ITP are related to a continual self-antigen-stimulated autoimmune response that is caused by tolerance failure. It is still unclear what causes the breakdown of central and/or peripheral tolerance to trigger autoreactive lymphocytes' response, but both environmental and genetic factors are thought

to be crucial. It is apparent that dysfunction in multi-steps, particularly in T of cellular immunity, play a central role in the final outcome of megakaryocytopoietic suppression and platelet destruction. These steps are tightly connected and should not be viewed in isolation or mutually exclusive from each other. Dysfunction in one step may be a result of concurrent dysfunction of another. For example, Th1 cells increase in the circulation promotes an increase in cytokines IL-2, IL-10 and INF-γ, which result in feedback activation and proliferation of Th1 cells and APCs. Fig. 6 also shows that dysfunction of one item in a step (e.g., induction of mimic antigen by foreign antigen) is not necessarily accompanied by the development of ITP because of the absence of dysfunction of the latter steps. The immune system in human body is extremely delicate and complicated. An error in one step can sometimes be compensated or be restored by other remedy mechanism. ITP occurs only if multi-step dysfunctions exist. Circulating antibodies to the GPIIb/IIIa complex may represent only part of the relevant antibody pool therefore reducing platelet antigen specific antibody is clearly downstream of treatment and too insensitive to predict relapse[50].

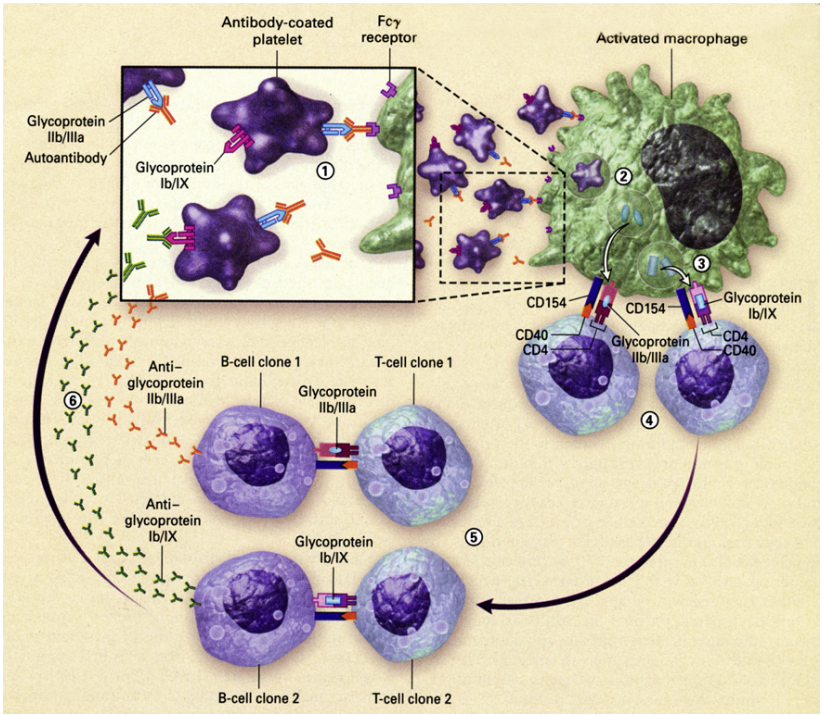

Figure 5. Pathogenesis of epitope spread in ITP.
The factors that initiate autoantibody production are unknown. Most patients have antibodies against several platelet-surface glycoproteins at the time the disease becomes clinically evident. Here, glycoprotein IIb/IIIa is recognized by autoantibody (orange, inset), whereas antibodies that recognize the glycoprotein Ib/IX complex have not been generated at this stage (1). Antibody-coated platelets bind to antigen-presenting cells (macrophages or dendritic cells) through Fcg receptors and then are internalized and degraded (2). Antigen-presenting cells not only degrade glycoprotein IIb/IIIa (light blue oval), thereby amplifying the initial immune response, but also may generate cryptic epitopes from other platelet glycoproteins (light blue cylinder) (3). Activated antigen-presenting cells (4) express these novel peptides on the cell surface along with costimulatory help (represented in part by the interaction between CD154 and CD40) and the relevant cytokines that facilitate the proliferation of the initiating CD4-positive T-cell clones (T-cell clone 1) and those with additional specificities (T-cell clone 2) (5). B-cell immunoglobulin receptors that recognize additional platelet antigens (B-cell clone 2) thereby also are induced to proliferate and synthesize antiglycoprotein Ib/IX antibodies (green) in addition to amplifying the production of anti-glycoprotein IIb/IIIa antibodies (orange) by B-cell clone 1 (6) (Cines DB, Blanchette VS. Immune thrombocytopenic purpura. N Engl J Med 2002;346:995–1008)

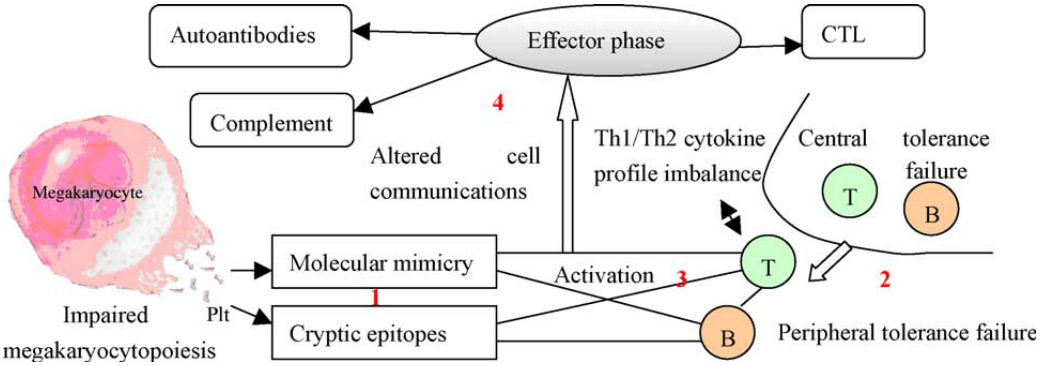

Figure 6. Multi-step disorders in ITP.
(1) Self-antigen generation, (2) T, B central and peripheral tolerance failure, (3) activation of T–B cells and (4) effector phase that lead to platelet clearance. (#) Single dysfunction does not always lead to clinical symptom, for the final stage of antibody production usually needs 1, 2 and 3 (Zhou B, Zhao H, Yang RC, Han ZC. Multi-dysfunctional pathophysiology in ITP. Crit Rev Oncol Hematol. 2005; 54(2):107-16).

Conclusion and future perspectives

ITP is a complex, chronic, often cell-specific, autoimmune disease that is still not fully understood. The improved understanding of the innate and adaptive immune systems however is allowing us to understand and appreciate some of the complex interactions between platelets, the immune system, and the development of ITP.

Immune-mediated platelet destruction in ITP occurs by a complex process involving multiple components of the immune system. The initiating event for the dysregulation remains unclear, although recent evidence helps to explain the processes by which the disorder may perpetuate itself.

Rational therapy available for ITP awaits a more thorough understanding of the relative contribution of each item in the different steps in the maintenance of normal immune status and how the relative contribution of dysfunction of each could be accurately quantified in the treatment of ITP.

REFERENCES

1. **George JN, Woolf SH, Raskob GE.** ITP: Practice guideline developed by explicit methods for the American Society of Hematology. Blood 1996; 88:3–40.
2. **Buchanan GR, Adix L.** Outcome measures and treatment end points other than platelet count in childhood ITP. Semin Thromb Hemost 2001;27:277–285.
3. **Cines DB, Bussel JB, Liebman HA, Luning Prak ET.** The ITP syndrome: pathogenic and clinical diversity. Blood. 2009; 113(26):6511-6521.
4. **Lazarusa AH, Semplea JW, Cines DG.** Innate and adaptive immunity in ITP. Semin Hematol. 2013 January; 50(1): S68–S70.
5. **Cines DB, Blanchette VS.** Immune thrombocytopenic purpura. N Engl J Med 2002;346:995–1008.
6. **Blanchette V, Bolton-Maggs P.** Childhood immune thrombocytopenic purpura: diagnosis and management. Hematol Oncol Clin North Am. 2010; 24(1):249-73.
7. **Kuwana M, Ikeda Y.** The role of autoreactive T-cells in the pathogenesis of idiopathic thrombocytopenic purpura. Int J Hematol 2005;81:106 –112.
8. **Gernsheimer T, Stratton J, Ballem PJ, Slichter SJ.** Mechanisms of response to treatment in autoimmune thrombocytopenic purpura. N Engl J Med 1989; 320:974 –980.
9. **Gernsheimer T.** Chronic idiopathic thrombocytopenic purpura: mechanisms of pathogenesis. Oncologist. 2009; 14:12–21 .
10. **Roark JH, Bussel JB, Cines DB, Siegel DL.** Genetic analysis of autoantibodies in idiopathic thrombocytopenic purpura reveals evidence of clonal expansion and somatic mutation. Blood. 2002; 100:1388–1398 .
11. **Ogawara H, Handa H, Morita K, Hayakawa M, Kojima J, Amagai H, et al.** High Th1/Th2 ratio in patients with chronic idiopathic thrombocytopenic purpura. Eur J Haematol. 2003; 71:283–288 .
12. **Wang JD, Chang TK, Lin HK, Huang FL, Wang CJ, Lee HJ.** Reduced expression of transforming growth factor-β1 and correlated elevation of interleukin-17 and interferon-γ in pediatric patients with chronic primary immune thrombocytopenia (ITP). Pediatr Blood Cancer. 2011 Oct;57(4):636-40.
13. **Li S, Wang L, Zhao C, Li L, Peng J, Hou M.** CD8+ T cells suppress autologous megakaryocyte apoptosis in idiopathic thrombocytopenic purpura. Br J Haematol. 2007; 139(4):605-11.

14. **Audia S, Samson M, Guy J, Janikashvili N, Fraszczak J, Trad M, et al.** Immunologic effects of rituximab on the human spleen in immune thrombocytopenia. Blood. 2011 Oct 20;118(16):4394-400.
15. **Li X, Zhong H, Bao W, Boulad N, Evangelista J, Haider MA, et al.** Defective regulatory B-cell compartment in patients with immune thrombocytopenia. Blood. 2012 Oct 18;120(16):3318-25.
16. **Cines DB, Liebman H, Stasi R.** Pathobiology of secondary immune thrombocytopenia. Semin Hematol. Jan; 2009 46(1 Suppl 2):S2–14.
17. **O'Leary ST, Glanz JM, McClure DL, Akhtar A, Daley MF, Nakasato C, et al.** The risk of immune thrombocytopenic purpura after vaccination in children and adolescents. Pediatrics. Feb; 2012 129(2):248–55 .
18. **Shoenfeld Y, Agmon-Levin N.** 'ASIA' - autoimmune/inflammatory syndrome induced by adjuvants. J Autoimmun. Feb; 2011 36(1):4–8 .
19. **Johnsen J.** Pathogenesis in immune thrombocytopenia: new insights. Hematology Am Soc Hematol Educ Program. 2012;2012:306-12.
20. **Neunert C, Lim W, Crowther M.** The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. Blood. 2011;117(16):4190-4207.
21. **Kaznelson P.** Verschwinden die Hamorrhagischen Diathese beieinem Falle von essentielle Thrombopenie (Frank) nach Milz extirpation. Wiener Klinische Wochenschrift 1916; 29: 1451–1454.
22. **Doan CA, Bouroncle BA & Wiseman BK.** Idiopathic and secondary thrombocytopenic purpura: clinical study and evaluation of 381 cases over a period of 28 years. Annals of Internal Medicine, 1960; 53: 861 .
23. **Wadenvik H, Jacobsson S, Kutti J, Syrjala M.** In vitro and in vivo behavior of 111In-labelled platelets: an experimental study of healthy male volunteers. Eur J Haematol 1987; 38:415–425
24. **Olsson B, Ridell B, Jernås M, Wadenvik H.** Increased number of B-cells in the red pulp of the spleen in ITP. Ann Hematol. 2012 Feb;91(2):271-7.
25. **Vianelli N, Galli M, de Vivo A, Intermesoli T, Giannini B, Mazzucconi MG, et al.** Efficacy and safety of splenectomy in immune thrombocytopenic purpura: long-term results of 402 cases. Haematologica. 2005; 90(1):72-7.
26. **Kojouri K, Vesely SK, Terrell DR, George JN.** Splenectomy for adult patients with idiopathic thrombocytopenic purpura: a systematic review to assess long-term platelet count responses, prediction of response, and surgical complications. Blood. 2004; 104:2623–2634
27. **Peerschke EIB, Andemariam B, Yin W, Bussel JB.** Complement activation on platelets correlates with a decrease in circulating immature platelets in patients with immune thrombocytopenic purpura. Br J Haematol. 2010;148(4):638-645.
28. **Najaoui A1, Bakchoul T, Stoy J, Bein G, Rummel MJ, Santoso S, Sachs UJ.** Autoantibody-mediated complement activation on platelets is a common finding in patients with immune thrombocytopenic purpura (ITP). Eur J Haematol. 2012 Feb;88(2):167-74.
29. **Hed J.** Role of complement in immune or idiopathic thrombocytopenic purpura. Acta Paediatrica Supplementum, 1998; 424:37–40.
30. **Cooper N, Bussel J.** The pathogenesis of immune thrombocytopaenic purpura. Br J Haematol. 2006; 133(4):364-74.
31. **Andersson PO, Wadenvik H.** Chronic idiopathic thrombocytopenic purpura (ITP): molecular mechanisms and implications for therapy. Expert Reviews in Molecular Medicine, 2004; 6: 1–17.
32. **Andersson PO, Olsson A, Wadenvik H.** Reduced transforming growth factor-beta1 production by mononuclear cells from patients with active chronic idiopathic thrombocytopenic purpura. British Journal of Haematology 2002; 116:862–867.
33. **Mouzaki A, Theodoropoulou M, Gianakopoulos I, Vlaha V, Kyrtsonis MC, Maniatis A.** Expression patterns of Th1 and Th2 cytokine genes in childhood idiopathic thrombocytopenic purpura (ITP) at presentation and their modulation by intravenous immunoglobulin G (IVIg) treatment: their role in prognosis. Blood. 2002; 100(5):1774-9.
34. **Panitsas FP, Theodoropoulou M, Kouraklis A, Karakantza M, Theodorou GL, Zoumbos NC, et al.** Adult chronic idiopathic thrombocytopenic purpura (ITP) is the manifestation of a type-1 polarized immune response. Blood, 2004; 103: 2645–2647.
35. **Abboud RM, Laver J, Xu F, Weksler B, Bussel JB.** Serum levels of GM-CSF are elevated in patients with thrombocytopenia. British Journal of Haematology 1996; 92:486–488.
36. **Chanock S.** The etiology of childhood immune thrombocytopenic purpura: how complex is it? Journal of Pediatric Hematology/Oncology, 2003; 25(Suppl. 1), S7–10.
37. **Olsson S, Cagnoni F, Dignetti P, Melioli G, Canonica GW.** Low concentrations of cytokines produced by allergen-stimulated peripheral blood mononuclear cells have potent effects on nasal polyp-derived fibroblasts. Clin Exp Immunol. 2003; 132(2):254-60.
38. **Harrington WJ, Minnich V, Hollingsworth JW, Moore CV.** Demonstration of a thrombocytopenic factor in the blood of patients with thrombocytopenic purpura. J Lab Clin Med. 1951; 38: 1–10.

39. **Wang T, Zhao H, Ren H, Guo J, Xu M, Yang R, et al.** Type 1 and type 2 T-cell profiles in idiopathic thrombocytopenic purpura. Haematologica. 2005; 90(7):914-23.
40. **Panitsas FP, Theodoropoulou M, Kouraklis A, Karakantza M, Theodorou GL, Zoumbos NC, et al.** Adult chronic idiopathic thrombocytopenic purpura (ITP) is the manifestation of a type-1 polarized immune response. Blood. 2004; 103(7):2645-7 .
41. **Sakakura M, Wada H, Tawara I, Nobori T, Sugiyama T, Sagawa N, et al.** Reduced Cd4+Cd25+ T cells in patients with idiopathic thrombocytopenic purpura. Thromb Res. 2007;120(2):187-93.
42. **Hu Y, Ma DX, Shan NN, Zhu YY, Liu XG, Zhang L, et al.** Increased number of Tc17 and correlation with Th17 cells in patients with immune thrombocytopenia. PLoS One. 2011; 6(10):e26522 .
43. **Zhu X, Ma D, Zhang J, Peng J, Qu X, Ji C, et al.** Elevated interleukin-21 correlated to Th17 and Th1 cells in patients with immune thrombocytopenia. J Clin Immunol. 2010;30(2):253-9 .
44. **Hu Y, Li H, Zhang L, Shan B, Xu X, Li H, et al.** Elevated profiles of Th22 cells and correlations with Th17 cells in patients with immune thrombocytopenia. Hum Immunol. 2012; 73(6):629-35 .
45. **Semple JW.** Immune pathophysiology of autoimmune thrombocytopenic purpura. Blood Rev 2002;16:9–12.
46. **Gruel Y, Boizard B, Daffos F, Forestier F, Caen J, Wautier JL.** Determination of platelet antigens and glycoproteins in the human fetus. Blood. 1986;68:488–92.
47. **Filion MC, Proulx C, Bradley AJ, Devine DV, Sékaly RP, Décary F, et al.** Presence in peripheral blood of healthy individuals of autoreactive T cells to a membrane antigen present on bone marrow-derived cells. Blood. 1996 Sep 15;88(6):2144-50.
48. **Lo D, Freedman J, Hesse S, Brinster RL, Sherman L.** Peripheral tolerance in transgenic mice: tolerance to class II MHC and non- MHC transgene antigens. Immunol Rev 1991;122:87–102.
49. **Jonuleit H, Schmitt E.** The regulatory T cell family: distinct subsets and their interrelations. J Immunol 2003;171:6323–7.
50. **Zhou B, Zhao H, Yang RC, Han ZC.** Multi-dysfunctional pathophysiology in ITP. Crit Rev Oncol Hematol. 2005; 54(2):107-16.
51. **Sakaguchi S, Miyara M, Costantino CM, Hafler DA.** FOXP3+ regulatory T cells in the human immune system. Nat. Rev. Immunol. 2010; 10:490–500.
52. **Buckner JH.** Mechanisms of impaired regulation by CD4(+)CD25(+)FOXP3(+) regulatory T cells in human autoimmune diseases. Nat Rev Immunol. 2010; 10:849–859.
53. **Yu J, Heck S, Patel V, Levan J, Yu Y, Bussel JB, et al.** Defective circulating CD25 regulatory T cells in patients with chronic immune thrombocytopenic purpura. Blood. 2008; 112:1325–1328.
54. **Zhang XL, Peng J, Sun JZ, Liu JJ, Guo CS, Wang ZG, et al.** De novo induction of platelet-specific CD4(+)CD25(+) regulatory T cells from CD4(+)CD25(−) cells in patients with idiopathic thrombocytopenic purpura. Blood. 2009; 113:2568–2577.
55. **McMillan R.** Autoantibodies and autoantigens in chronic immune thrombocytopenic purpura. Semin Hematol. 2000;37:239 –248.
56. **He R, Reid DM, Jones CE, Shulman NR.** Spectrum of Ig classes, specificities, and titers of serum antiglycoproteins in chronic idiopathic thrombocytopenic purpura. Blood. 1994;83:1024 –1032.
57. **Kravitz MS, Shoenfeld Y.** Thrombocytopenic conditions—autoimmunity and hypercoagulability: Commonalities and differences in ITP, TTP, HIT, and APS. Am J Hematol. 2005; 80:232–242.
58. **Kiefel V, Freitag E, Kroll H, Santoso S, Mueller-Eckhardt C.** Platelet autoantibodies (IgG, IgM, IgA) against glycoproteins IIb/IIIa and Ib/IX in patients with thrombocytopenia. Ann Hematol. 1996;72:280 –285.
59. **Fabris F, Scandellari R, Ruzzon E, Randi ML, Luzzatto G, Girolami A.** Platelet-associated autoantibodies as detected by a solid-phase modified antigen capture ELISA test (MACE) are a useful prognostic factor in idiopathic thrombocytopenic purpura. Blood. 2004;103:4562– 4564.
60. **Fillatreau S, Sweenie CH, McGeachy MJ, Gray D, Anderton SM.** B cells regulate autoimmunity by provision of IL-10. Nat Immunol. 2002; 3:944–950
61. **Moulin V, Andris F, Thielemans K, Maliszewski C, Urbain J, Moser M.** B lymphocytes regulate dendritic cell (DC) function in vivo: increased interleukin 12 production by DCs from B cell-deficient mice results in T helper cell type 1 deviation. J Exp Med. 2000; 192:475–482
62. **Blair PA, Noreña LY, Flores-Borja F, Rawlings DJ, Isenberg DA, Ehrenstein MR, et al.** CD19(+)CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic Lupus Erythematosus patients. Immunity. 2010; 32:129–140.

63. **Carter NA, Vasconcellos R, Rosser EC, Tulone C, Muñoz-Suano A, Kamanaka M, et al.** Mice lacking endogenous IL-10-producing regulatory B cells develop exacerbated disease and present with an increased frequency of Th1/Th17 but a decrease in regulatory T cells. J Immunol. 2011; 186:5569–5579

64. **Amu S, Saunders SP, Kronenberg M, Mangan NE, Atzberger A, Fallon PG.** Regulatory B cells prevent and reverse allergic airway inflammation via FoxP3-positive T regulatory cells in a murine model. J Allergy Clin Immunol. 2010; 125:1114–1124.

65. **Chang M, Nakagawa PA, Williams SA, Schwartz MR, Imfeld KL, Buzby JS, et al.** Immune thrombocytopenic purpura (ITP) plasma and purified ITP monoclonal autoantibodies inhibit megakaryocytopoiesis in vitro. Blood. 2003;102:887– 895.

66. **Jessica HR, James BB, Douglas BC, Don LS.** Genetic analysis of autoantibodies in idiopathic thrombocytopenic purpura reveals evidence of clonal expansion and somatic mutation. Blood. 2002;100:1388–98.

67. **Takai T.** Roles of Fc receptors in autoimmunity. Nat Rev Immunol 2002;2:580–92.

68. **Roth R, Nakamura T, Mamula MJ.** B7 costimulation and autoantigen specificity enable B cells to activate autoreactive T cells. J Immunol 1996;157:2924–31.

69. **Shevach EM.** Organ-specific autoimmunity. In: Paul WE, editor. Fundamental immunology. 4th ed. Philadelphia, PA: Lippincott-Raven Publishers; 1999. p. 1089–125.

70. **Cines DB, McKenzie SE, Siegel DL.** Mechanisms of action of therapeutics in idiopathic thrombocytopenic purpura. J Pediatr Hematol Oncol. 2003;25:S52–6.

71. **Louwes H, Zeinali Lathori OA, Vellenga E, de Wolf JT.** Platelet kinetic studies in patients with idiopathic thrombocytopenic purpura. Am J Med. 1999;106:430–4.

72. **Takahashi R, Sekine N, Nakatake T.** Influence of monoclonal antiplatelet glycoprotein antibodies on in vitro human megakaryocyte colony formation and proplatelet formation. Blood. 1999;93:1951–8.

73. **Cremer M, Schulze H, Linthorst G, et al.** Serum levels of thrombopoietin, IL-11, and IL-6 in pediatric thrombocytopenias. Ann Hematol. 1999;78:401–7.