Pathogenesis of immune thrombocytopenia

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Summary

Immune thrombocytopenic purpura (ITP) is an autoimmune disorder characterized by antibody-mediated platelet destruction. The platelet, as an accessible target, has made ITP an attractive disorder in the study of autoimmunity. However, the pathogenesis of ITP has proven complex with diverse pre-existing challenges to the immune system in the form of infection, genetic predisposition, underlying autoimmunity repertoire, inhibition of platelet production, perturbations of cell mediated effector and effector pathways, sequestered harbors within lymphoid organs, and responsiveness to intervention. This chapter surveys key new insights into the pathogenesis of ITP and attempts to integrate them into a model that may serve as a template for future investigation.

Immune thrombocytopenia (ITP) is a syndrome characterized by antibody-mediated platelet destruction and variably reduced platelet production. The disease can be divided conceptually into primary ITP in which no predisposing condition is identified, and secondary forms that may occur in association with broader autoimmune conditions, inherited or acquired immune deficiency, or certain infections [1]. This chapter will focus on the pathogenesis of primary ITP in adults but will reference insights on pathogenesis gained from secondary forms of the disease. We will first review the relatively well-studied immune effector mechanisms that cause thrombocytopenia and then consider the more perplexing question of how autoantibodies to platelets develop.

Effector pathways

IgG antibody-mediated platelet destruction
ITP is caused most often by platelet-reactive IgG antibodies that accelerate platelet clearance and impair platelet production to a variable extent. Compelling supporting evidence for the first mechanism includes:
• rapid passive induction of acute transient thrombocytopenia in normal hosts by ITP plasma, and its IgG fraction, but not in recipients who have undergone splenectomy [2,3];
• shortened survival of autologous $^{111}$In-labeled platelets in essentially all patients with ITP, with rapid uptake in the spleen, liver or more diffusely;
• response within days to splenectomy, to intravenous anti-(RhD) and to other therapeutics that act by impeding IgG-Fcγ receptor-mediated peripheral clearance.

Platelet-reactive antibodies are most commonly of the IgG subclass alone or in combination with other IgG subclasses [4]. Complement-fixing IgM antibodies and rarely IgA antibodies in the absence of IgG antibodies have been described in a small subset of patients [5], but their impact on clinical course is uncertain. Platelet autoantibodies may be products of a limited number of B-cell clones [6]. Most ITP-related antibodies bind to high prevalence glycoproteins (GPs) or GP complexes (especially GPsIa/IIa, IIb/IIIa, and less commonly GPs Ia/IIa, IV or VI) [7,8]. Conformation-dependent binding sites have been delineated within the β-propeller domain of αIIb [9,10], but diverse determinants on β3 have also been identified [11,12]. However, other potentially important antigens have been implicated [13] that are not detected with existing commercial assays. Formation of antibodies to such highly prevalent GP antigens might increase the likelihood of clinical disease. However, antibodies to less prevalent or as yet unidentified antigens may also cause ITP in 30–40% of patients in whom antibodies are not detected. Such antibodies may precede and initiate overt disease, or they may influence the clinical course and response to therapy in ways yet to be determined. There is no straightforward explanation as to why patients develop autoantibodies to several structurally unrelated platelet surface proteins [7]. This situation appears to be quite different from warm autoimmune hemolytic anemia, with which ITP is often compared, where antibodies are typically confined to epitopes within the Rh locus. In patients with ITP, antibodies to cytoplasmic proteins are common [7,14], and are thought to arise in response to platelet destruction. It has been theorized that proteosomal degradation of antibody-coated platelets in antigen presenting cells (APCs) may generate novel immunogenic epitopes from normal platelet proteins [15], leading to epitope spread [16]. Alternative explanations, including somatic mutation of autoantibodies and cross-reactivity to unrecognized sharing of structural motifs, have not been excluded.

There is some experimental evidence [17,18] and clinical suggestion [19–21] that patients with anti-GPⅠb antibodies have more severe thrombocytopenia and their disease is less responsive to intravenous immunoglobulin (IVIG) and corticosteroids than those with antibodies restricted to GpⅡb/Ⅲa. In part, this difference has been attributed to suppression of megakaryopoiesis [22] (vide infra), but pathways of platelet injury that accelerate clearance independent of tissue macrophage Fcγ receptors have been identified in murine models [17,18], including desialylation of GPⅠb leading to platelet clearance through Ashwell–Morell and MAC-1 receptors on hepatocytes and on liver macrophages [23]. The extent to which these mechanisms operate in vivo and their contribution to refractory disease are unknown.

**Antibody-mediated inhibition of platelet production**

Physiologic platelet production is a complex and only partially understood process that can be subdivided into two sequential phases [24]. The first phase, which requires several days, involves the development of megakaryocytes (MK) from hematopoietic stem cells followed by nuclear endomitosis, establishment of an internal membrane system, formation of specific granules and other components of the maturation process. The second, more rapid, step results in the generation of platelets, and involves remodeling of the cytoplasm into proplatelets with extrusion into the vascular spaces within the bone marrow, reversible formation of preplatelets, and eventual irreversible scission into individual platelets. It is estimated that each MK releases about $10^4$ platelets and that a total of $10^3$ platelets are generated each day in healthy adults. Production capacity can increase approximately 10-fold upon demand in healthy individuals [24]. Platelet production is regulated primarily by TPO (TPO knockout mice have platelet counts ~20% of normal). TPO is synthesized constitutively, predominantly in the liver. Plasma TPO is regulated primarily by platelet clearance, to a lesser extent by binding to MKs and intraplatelet endocytosis and catalysis [25,26], and to an unknown extent by platelet-regulated production within the bone marrow (reviewed in [27]). TPO binds to its cognate high affinity receptor, cMPL, which is expressed on platelets, megakaryocytes and their progenitors. *In vitro*, binding of TPO and TPO mimetics to cMPL receptors on normal hematopoietic cells acts primarily to enhance self-renewal, promote migration of progenitors to osteoblastic and vascular niches [28], and stimulate generation, proliferation and the initial steps in the maturation of MK progenitors, with less effect or even inhibition of proplatelet formation and platelet shedding when present at high concentrations [29,30]. However, the relevance of these findings to in vivo development requires additional study. Moreover, platelet lifespan is also controlled in part by apoptosis [31], which may be accelerated by platelet antibodies [32], and requirements to support endothelial integrity [33], which becomes more apparent at very low platelet counts.

ITP MKs may lack granularity, show reduced ploidy, and in some instances, few platelets are seen budding from the plasma membranes [34]. ITP MKs may undergo apoptosis accompanied by increased expression of caspase 3 [35]. Fragments from degenerating MKs can be seen to have been engulfed by
Macrophages [36] or neutrophils [35]. Calculated platelet production is “inappropriately” within the normal range or overtly reduced (reviewed in [37]) in most patients, and plasma TPO levels are “inappropriately” normal or minimally elevated because the protein is cleared along with antibody-coated platelets.

There is increasing appreciation that these morphologic and kinetic changes result from immune injury to developing MKs. MKs express GPIb, GPIIIb/IIIa and likely other platelet antigens during their development, which makes them potential targets of ITP antibodies. Some autoantibodies might recognize MK antigens preferentially [38]. Approximately two-thirds of ITP plasmas (and IgG fractions) suppress MK development from TPO-induced CD34+ progenitors, reduce MK ploidy [39] and may induce apoptosis [35]. However, little is known about the effect of ITP antibodies on the latter steps in platelet production, which is an important gap in knowledge. Anti-cMPL antibodies have been described in a few patients [40]. Pharmacologic doses of thrombopoietin receptor agonists (TRAs) may attenuate antibody or T-cell mediated MK apoptosis or other developmental defects [41].

Notwithstanding in vitro data and the clinical success of TRAs, questions remain about the importance of impaired “effective” platelet poiesis in vivo. First, the finding of shortened platelet survival in all ITP patients complicates estimates of impaired platelet production. Second, any therapy that increases platelet mass without affecting antibody production must eventually reduce the amount of antibody per cell and thereby, decrease platelet clearance as well. Third, impaired platelet production seems on its surface to be incompatible with the long-held notion of compensated thrombopoiesis in ITP and suggests that macrothrombocytes and the often reported increased numbers of reticulated platelets must arise through a developmental process that is more complex than increased platelet turnover alone. Fourth, it is unclear how glucocorticoids increase apparent platelet production [42], unless they attenuate antibody-mediated intramedullary MK [35] or platelet destruction.

**Platelet function**

Infrequently, ITP antibodies induce clinically significant platelet dysfunction by directly blocking access of agonists to platelet GP receptors (reviewed in [43]) or by inducing a storage pool-like defect [44]. However, the far more common and unexplained phenomenon is the increased prevalence of arterial and venous thrombosis in patients with seemingly uncomplicated ITP [45–47]. This is generally attributed to co-existing anti-phospholipid antibodies [48,49], procoagulant microparticles [50], or unrelated predisposing conditions [51]. These findings are by no means well understood or universally accepted and there is little convincing data to date as to whether or how the platelet autoantibodies themselves contribute to thrombotic risk and whether this is enhanced by TRAs, splenectomy [52] or other modalities that succeed in raising the platelet count without suppressing antibody production. Some ITP antibodies cause GPIb to translocate into lipid rafts and initiate intracellular signaling through FcγRIIa or through the invariant chain γ [53]. On the other hand, platelet activation by at least one anti-CD9-like ITP antibody was inhibited by a monoclonal anti-FcγRIIa antibody [54]. Thus, to date a clear relationship between any specific pathways leading to platelet activation by ITP antibodies in vitro and the risk of thrombosis remains to be established.

**Platelet clearance**

Antibody-coated platelets are removed primarily in the spleen and liver by Fcγ receptors. The initial effectiveness of IVIG and anti-D and likely glucocorticoids, danazol, vinca alkaloids, colchicine and anti-CD20 is mediated by inhibiting antibody-mediated platelet clearance at the Fcγ receptor level. Several patients have shown partial response to a monoclonal anti-FcγRIIb antibody [55] and anti-(Rh)D appears to raise platelet counts by competing with IgG-coated platelets for FcγRIIa [56]. Whether IVIG works in ITP by sending an inhibitory signal through FcγRIb [57] or through entirely different mechanisms [58] awaits resolution [59]. Polymorphisms in FcγRIa and FcγRIIIA have been implicated in responsiveness to splenectomy, corticosteroids [60] and rituximab [61]. Remarkably little is known about how glucocorticoids and other agents that impair peripheral clearance affect FcγRs subclass expression or function [62].

There are additional pathways that might play a role in platelet clearance in vivo and contribute to variable responsiveness and occasional refractoriness to all interventions that interfere with peripheral clearance. For example, in a passive murine model of ITP, phagocytosis of antibody-coated platelets was downregulated through a suppressive signal generated by platelet CD47 via macrophage signal regulatory protein [63]. Some ITP anti-GPIb antibodies generate additional “eat me” signals on platelets, including GPIb clustering and expression of P-selectin and phosphatidylinerine [53]. In experimental systems, anti-GPIbα antibodies induce GPIbα desialylation, as mentioned [23]. In a murine passive model of ITP, the effectiveness of IVIG was reported to require an interaction between its terminal sialic acid and SIGN1 (specific ICAM3 grabbing non-integrin-related 1, analogous to human DC-Sign, dendritic cell-specific intercellular adhesion molecules-3-grabbing non-integrin) [64], whereas sialylation did not effect the efficacy of IVIG in a second model [65]. Lastly, some ITP antibodies fix C3b to platelets [66], although a role for C3b receptor-mediated clearance of ITP platelets or intravascular lysis has not been established.
Generation of platelet autoantibodies: lessons from secondary ITP

In theory, platelet autoantibodies might originate through either of two general mechanisms. First, platelet-reactive antibodies may arise as one of many autoantibodies having diverse specificities in patients who have an acquired or constitutive defect in the elimination of autoreactive clones. In support of the premise, the prevalence of ITP is higher in patients with defects in the immune system such as the autoimmune lymphoproliferative syndrome, Wiskott-Aldrich syndrome, common variable hypogammaglobulinemia, systemic lupus erythematosus and chronic lymphocytic leukemia than it is in the general population (reviewed in [1]).

Second, cross-reactive antibodies that develop in response to exogenous antigens caused, for example, by infection or inflammation may emerge serendipitously as a result of somatic mutation in otherwise immunologically “normal” individuals. In support of the second premise, few otherwise healthy patients with ITP have an overt underlying predisposition to develop autoantibodies. Only approximately 2% of children [67] and perhaps even fewer adults [68] report a family history of ITP. However, it is not uncommon to encounter family members with autoimmune thyroid disease, systemic lupus erythematosus, pernicious anemia or other autoimmune conditions. There is little consistent evidence of specific MHC class I or class II restrictions that span ethnic groups or geographic regions [69,70], although there may be alterations in the frequencies of certain MHC class II alleles among patients with H. pylori-associated ITP [71]. Skewing of single nucleotide polymorphisms in the regulatory regions of TNFα, IL-2, and certain cytokine receptors has been reported (e.g. [72–74]) and in some, but not in all studies, the FcγRIIa Val allele and FcγRIIB 232 T isoform are reported to contribute to disease severity, chronicity and perhaps responsiveness to eradication of H. pylori [60,72,75–78]. However, caution is indicated when interpreting these retrospective analyses of small cohorts within different ethnic groups; the results of ongoing GWAS studies are awaited.

Additional support for the second general mechanism comes from the finding that in children ITP is often a self-limited disease that is preceded by infection. This pattern is generally attributed to dissipation of cross-reacting antibodies that originated in response to infection. The cross-reactivity hypothesis is the development of transient autoantibody-mediated thrombocytopenia that occurs in 1–4/105 children within 6 weeks of MMR vaccination [79], although this is seen rarely if at all in adults [80]. In children with Varicella Zoster Virus (VZV) associated ITP, serum antibodies eluted from an affinity column conjugated with VZV glycoproteins [81] cross-reacted with normal platelets and, in contrast to chronic ITP, platelet-reactive T-cell activity was normal. These data are consistent with the high rate of spontaneous remission in children, i.e. as the antigens associated with the infectious agents are cleared; cross-reactive anti-platelet antibodies dissipate (figure 1).

Infections may exacerbate thrombocytopenia in patients with ITP without a change in antibody per se, as seen in a mouse model [82], by suppressing platelet production but also by accelerating platelet destruction. Early in infection, pathogens...
encounter Toll-like receptors (TLR) on ‘‘professional phagocytes’’ [83]. TLRs bind proteins with canonical molecular structures expressed on microbes, a critical first step in rapid stimulation of the innate immune response. Platelets express TLR1–9 [84–86]. Binding of lipopolysaccharide (LPS) to platelet TLR4 causes thrombocytopenia in vivo [84,86,87], and significantly enhances Fc-mediated platelet phagocytosis of antibody-coated platelets by mononuclear phagocytes [88]. Conversely, elimination of H. pylori as an example of an infectious agent associated with ITP reverses the downregulation of FcγRIIB caused by active infection, and thereby might reduce antibody production (see below).

As mentioned, there is compelling evidence for an association between chronic ITP and persistent infection in adults. The first common association identified was with HIV infection, where a marked increase in ITP among homosexual males was described even before the cause of AIDS was identified. Anti-retroviral therapy increases platelet counts in patients with HIV who do not have other causes for the thrombocytopenia and the incidence of HIV-associated ITP has fallen in regions where effective anti-viral therapy is in widespread use [89]. Multiple mechanisms contribute to ITP–HIV, including accelerated platelet clearance and suppressed platelet production. Sera contain antibodies against viral proteins ( nef, gag, env, pol, GP160, gp120 and p24), some of which cross-react with amino acids 49 to 66 of GPIIa [90,91]. Anti-GPIIb/IIIa antibodies fragment platelets by activating NADPH oxidase, generating peroxide and other reactive oxygen species through platelet phospholipase A2 and 12-lipoxygenase [92]. Fragmentation is inhibited by dexamethasone, which impedes enzyme translocation to and from the plasma membrane [93], although it is unclear how this effector pathway can be reconciled with the high response rate of affected patients to typical ITP therapy such anti-(Rh)D, IVIG and splenectomy, each of which acts to disrupt thrombopoiesis as well as platelet egress into the circulation [112].

**Environmental stress**

Oxidative modification of proteins has also been associated with the development of autoantibodies. Vanin-1, which antagonizes the anti-inflammatory actions of peroxisome proliferator-activated receptor-γ, has been implicated in ITP. Expression of the VNN1 gene is increased in children with acute ITP and those who progress to chronic disease [105,106]. An increased serum level of oxidants and decreased antioxidants was reported to correlate with platelet counts [107]. In mice, VNN1 is involved in the movement of T-cell precursors within the thymus [108] that may suggest a role in regulating T-cell repertoire. However, there is as yet no direct evidence that ITP platelets express such oxidatively modified protein antigens or that platelet autoantibodies recognize modified proteins on the platelet surface.

**T-cell effector mechanisms**

A non-antibody-mediated mechanism of immune thrombocytopenia has been suspected based on the finding that plasma from only 16 of 26 (62%) ITP patients caused thrombocytopenia when infused into healthy recipient, although this could be a matter of titre and pre-adsorption of high affinity antibody to donor platelets [15]. Also, 15–20% of patients do not respond to IVIG and in some studies anti-platelet antibodies are detected in only approximately 60% of patients with ITP, although again this outcome could reflect low antibody titre or undetected antigen specificities. In the latter setting, cytotoxic T-cells (CTL) with the ability to lyse platelets in vitrō have been identified in peripheral blood [109], results confirmed in both humans [110,111] and in a murine model of ‘‘ITP’’ [23–25]. Of note, T-cell mediated ITP in this model is insensitive to IVIG [25], which may suggest a novel mechanism mediating treatment resistance. CTLs may also accumulate in the bone marrow where they might attain an effector to target ratio sufficient to disrupt thrombopoiesis as well as platelet egress into the circulation [112].
Pathogenesis of platelet autoantibodies

The etiology of chronic ITP in adults is unknown. We previously proposed that ITP might best be described as a syndrome arising from defects in immune tolerance that can be classified descriptively into:

- central defects in clonal deletion in the thymus or bone marrow or in receptor editing;
- blocks in differentiation, leading to skewing of peripheral B-cell subsets;
- peripheral tolerance defects, e.g. arising from somatic mutation in the setting of immune stimulation [1].

The lymphocyte repertoire is monitored and purged of auto-reactive specificities that arise at different stages of development; a defect at any of these stages could in theory lead to the development of ITP. In its simplest formulation, chronic ITP could arise in:

- a host with a normal immune response to exogenous antigen that develops cross-reactive antibodies (molecular mimicry);
- a defect in B-cell differentiation, referred to here as the immune reconstitution syndrome;
- a central loss of tolerance that enables autoantibody, producing B-cell clones to emerge.

This classification has implications for diagnosis and management. First, failure to appreciate differences in the pathogenesis of ITP, e.g. in older and younger populations, might obscure clues from analysis of clinical, biochemical or genetic data. Second, the likelihood of durable remission may be greatest in those with a peripheral tolerance defect who will revert to a normal immune repertoire after antigen elimination and lowest in patients with a central defect in whom a substantial proportion of their primary repertoire is autoreactive, which reconstitutes rapidly post-therapy.

Molecular mimicry/antibody cross-reactivity

As mentioned, ITP may occur in the context of the host response to foreign antigens derived from H. Pylori, HIV, Hepatitis C, VZV or MMR vaccine in children, among others. Somatic mutation may lead to epitope spread, changing antibody specificity from foreign to self [98]. The inciting infection may be asymptomatic and the presence of the inciting antigen might dissipate by the time the patient presents with ITP. This would help to explain why some studies of patients with H. pylori have reported that the response of ITP to antibiotic eradication is more successful in patients with disease of more recent onset. The absence of a persistent defect in the host’s immune system allows spontaneous recovery once the inciting agent is deleted, as observed in most cases of childhood ITP. One inference from this proposed pathogenic pathway is that the distinction between primary and secondary ITP will continue to blur as other infections that predispose to the development of ITP are identified, e.g. through B-cell repertoire cloning using high-throughput screening of antibody libraries against peptides, which would then be mapped to similar epitope libraries derived from infectious agents.

Defects in B-cell differentiation/immune reconstitution

An ITP syndrome may occur after bone marrow or solid organ transplantation, that varies in severity from mild and transient to severe and persistent, presumably the result of microchimerism with alloreactivity by passenger host lymphocytes against recipient platelets not constrained by host T-cells (reviewed in [1]). Transient severe thrombocytopenia has also been reported in approximately 1–2% of patients who receive anti-CD52 for multiple sclerosis [113]. Development of ITP may appear paradoxical in this setting characterized by severe T- and B-cell depletion. However, the B-cell population, which may harbour “naturally-occurring” self-reactive anti-platelet antibodies, perhaps more prevalent at the onset of treatment in some patients with multiple sclerosis, might reconstitute before suppressor T-cell subsets regain full function. This sequence might lead to a temporary imbalance between B-effector and T-regulatory networks. This paradigm may extend to other clinical situations in which ITP occurs in the setting of immune suppression, including chronic lymphocytic leukemia, common variable immune deficiency (in which maintaining IgG levels may decrease autoantibody production), and potentially the increased propensity for ITP to develop in the elderly [1].

Central defects in tolerance

ITP is part of the more expansive immune diathesis that develops in patients with the autoimmune lymphoproliferative syndrome, and some patients with Evans syndrome, systemic lupus erythematosus and anti-phospholipid antibody syndrome. ITP is more likely to be chronic in these settings absent new approaches to restore the intrinsic abnormality in cell mediated immunity [1].

Pathogenesis of chronic ITP

The defects that lead to loss of tolerance in chronic primary ITP are unknown. Patients with active ITP typically have a cytokine profile that is skewed toward a CD4+, Th0/Th1 activation pattern with increased IL-2 and IFNγ, reduced IL-4 and IL-10, and decreased immunosuppressive transforming growth factor-β, which tends to normalize when patients are in remission [26–28]. This pattern of cytokines promotes differentiation of self-reactive B-cells into autoantibody, producing plasma cells and the development of CTLs when they exist [26–28], but fails to explain their ontogeny and platelet-specificity.

Recent development

Others and we have provided a comprehensive overview of the abnormalities in antigen presenting cells and lymphocyte subsets that have been identified in adults and children with ITP.
[114–116], to which the reader is referred. Here, we focus on recent studies involving two specific opposing pathways that modulate autoantibody production in other autoimmune disorders that have been the subject of considerable recent study, namely loss of CD4+CD25+FoxP3+ T-regulatory cells (Tregs) and activation of the Th17/IL-17/IL-17-receptor axis.

Tregs

Tregs dampen autoimmune responses and help maintain self-tolerance [117]. Genetic deficiencies of Tregs are associated with diverse autoimmune phenomena in humans (IPEX syndrome) [118] and in mice (Scurfy) [119]. In support of their role in ITP, one third of nude mice reconstituted with Treg-depleted CD4+CD25− T-cells from syngeneic BALB/c mice developed antibody-mediated thrombocytopenia [120]. DCs from patients with ITP showed increased capacity to present platelet antigens to T-cells and reduced capability to upregulate indoleamine 2,3-dioxygenase-1, which reduced the generation of Tregs [121,122]. Deficiencies in peripheral blood Treg number and function have been repeatedly observed in patients with active ITP (reviewed in [116]), but the same findings occur in other autoimmune disorders. As with skewing of the cytokine profile, deficiency in Tregs might contribute to a favourable environment for autoreactive B-cells to expand and differentiate once tolerance has been broken. Moreover, a deficiency in CD19+, CD24hi, CD38hi B-regulatory cells has been observed, which might impair the production of IL-10 and thereby, the recruitment of Tregs [123]. However, it is unclear how diverse therapies that increase platelet counts in ITP, including IVIG, rituximab, and TRAs, normalize Treg function and dampen the pro-inflammatory cytokine profile (reviewed in [116]). This might result from increasing the autoantigen load (high zone tolerance) or if platelets modulate T-cell responses, e.g. by releasing TGF. This is consistent with other properties that suggest a role for platelets in adaptive immunity, including expression of TLRs, CD40, CD40L and chemokines, such as CXCL5, CXCL3 CCL5, and IL-1α [124,125]. The fact that thrombocytopenia almost always recurs once therapy is stopped in the setting of seemingly restored peripheral Treg function heightens the need to identify platelet-specific Tregs in lymphoid organs where autoreactive T-cells are more likely to develop and persist, such as the thymus [126].

Th17 cells

Recent attention has also been focused on the Th17 subset of T-helper cells and the IL-17/IL-17-receptor (IL-R) axis [127]. Th17, like Tregs, differentiate from naive CD4+ cells. TGF-β upregulates Foxp3 expression in the absence of IL-6, which blocks the expression of RORα and RORγ and increases generation of Tregs. Conversely, when IL-6 is present, RORα and RORγ are expressed, which act in concert with TGF-β to initiate development of TH17 cells [127]. IL-17 augments production of IL-1, IL-6, IL-8, and IFNγ. Six IL-17 ligand (IL17A-F) and five receptors (IL17RA-E) have been identified. Overexpression of the IL-17 transcription factor RORγt in mice induced the development of anti-platelet antibodies [128]. Increased numbers of Th17 cells and elevated plasma levels of IL-17 have also reported in adults and children with several autoimmune disorders, including ITP in some studies [129–135]. In one study, ITP patients had a lower frequency of an IL-17F SNP, which was associated with the severity of thrombocytopenia [136]. Yet, seemingly paradoxically, CD16+ ITP monocytes were reported to inhibit proliferation of Th17 cells in vitro [137]. Differences in patient populations, treatment histories, methodologies and the inability to track platelet-specific responses complicate interpretation and design of definitive studies.

Lymphoid organs

Studies of peripheral blood APCs, lymphocyte subpopulation and cytokines in the peripheral blood might not mirror the organ-specific environments, such as in the spleen, which is a known site of anti-platelet antibody synthesis. T follicular helper cells and Tregs within the proliferative lymphoid nodules (PLNs) and germinal centers (GCs) of spleens from patients with ITP were reduced compared with spleens removed for other reasons [138]. The density of T follicular helper cells and Tregs was lower in the PLNs, but not GCs, which also contain GP IIb/IIa within IgM containing immune complexes tightly bound to follicular dendritic cells, closely approximated to proliferating B-cells. These studies suggest that PLNs are the sites of autoantigen stimulation related to a lack of T-cell control [138]. The ratio for Th1 cells to Tregs was reported to be increased in the spleens of patients who failed rituximab therapy despite depletion of peripheral blood B-cells [139]. It has also been suggested that rituximab may induce the differentiation of autoimmune plasma cells into long-lived resident-cells in the spleen of patients who failed treatment [140]. These studies serve to highlight potential differences between findings in specialized lymphoid organs and those made on components of peripheral blood.

Concluding comments

Current understanding of the pathogenesis of ITP is the result of integrating outcomes of basic research studies and clinical trials and observations. Each new advance in understanding has generated new questions and novel directions for investigation. The ability to identify and monitor platelet-reactive T-cell and B-cell clones would facilitate pivotal advances in diagnosis and the understanding of pathogenesis, clinical diversity, natural history and response to intervention in affected patients.

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