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Imbalanced immune homeostasis in immune thrombocytopenia

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ABSTRACT

Immune thrombocytopenia (ITP) is an autoimmune bleeding disorder resulting from low platelet counts caused by inadequate production as well as increased destruction by autoimmune mechanisms. As with other autoimmune disorders, chronic ITP is characterized by perturbations of immune homeostasis with hyperactivated effector cells as well as defective regulatory arm of the adaptive immune system, which will be reviewed here. Interestingly, some ITP treatments are associated with restoring the regulatory imbalance, although it remains unclear whether the immune system is redirected to a state of tolerance once treatment is discontinued. Understanding the mechanisms that result in breakdown of immune homeostasis in ITP will help to identify novel pathways for restoring tolerance and inhibiting effector cell responses. This information can then be translated into developing therapies for averting autoimmunity not only in ITP but also many autoimmune disorders.

1. Introduction

Immune thrombocytopenia (ITP) is an autoimmune bleeding disorder resulting from immune destruction of platelets and insufficient platelet production. Autoreactive antibodies to platelet antigens, mainly platelet glycoprotein IIb/IIIa complex, and/or GPIb/IX complex are considered responsible for accelerated destruction of platelets by the reticuloendothelial system and probably for inhibition of megakaryopoiesis [1]. The observation that these autoantibodies are isotype-switched and harbor somatic mutations [2] strongly supports the involvement of $CD4^+$ helper T cells in ITP pathogenesis. Indeed, initial studies demonstrated that patients with ITP possess activated platelet-autoreactive T cells and cytokine imbalance with increasing shift toward type 1 cytokine interleukin (IL)-2 and interferon (IFN)-γ [3]. More recently, altered T-helper type 2 (T_H2) signature in ITP has also been identified [4]. In addition, studies indicate increased T_H17 cells or IL-17 cytokine in ITP patients [5–7], implicating a possible role for T_H17 cells in ITP immunopathology, although two reports did not detect any difference [8,9]. Similarly, elevated levels of $CD4^+$ T-cell subset, T_H22 cells [10], and their associated cytokines IL-22 and tumor necrosis factor (TNF)-α, were reported in ITP [9,11]. With respect to pathogenic effector T cells that drive autoantibody production, both splenic and peripheral of T-follicular helper (T_{FH}) cell frequencies were elevated in ITP [12]. This $CD4^+$ T-cell subset, characterized by expression of the surface expression of chemokine (C-X-C motif) receptor 5 (CXCR5) and production of B-cell–promoting cytokine IL-21, provides help to B cells to generate the initial wave of antibody response as well as in promoting B-cell differentiation into high-affinity antibody-producing cells and long-lasting IgG antibody [13]. In ITP patients, the frequency of splenic T_{FH} cells correlated with both with germinal center and plasma cell percentages in the spleens of ITP patients [12]. Furthermore, in vitro, simulation of T_{FH} signaling through provision of IL-21 and CD40 engagement led to the differentiation of splenic B cells into plasma cells and to the secretion of antiplatelet antibodies in ITP patients [12]. Altogether, these data suggest the involvement of T_{FH} in ITP pathogenesis.

Failure of peripheral immune regulation and suppression may be one explanation for the “hyperactivated” platelet-specific effector T and B cells in ITP. This is supported by studies showing that unlike ITP patients, healthy individuals harbor platelet-specific autoreactive T cells that are tolerized in the periphery [14]. Altered regulatory T-cell (Treg) and B-cell (Breg) numbers and function have been reported in patients with ITP in several studies including ours, suggesting that these patients may have deficiency in generation and/or defective functions of regulatory arm of the adaptive immune system. Understanding the mechanism by which effector and regulatory cells of the adaptive immune system are dysregulated in ITP can help guide the design of novel, focused, and improved therapeutic strategies for these patients. Some of the recent studies that have examined the immune dysregulation are discussed below.

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2. Dysregulated innate immune response in ITP

The innate immune system is a major driver of the adaptive immunity. By upregulating costimulatory markers and producing cytokines, dendritic cells (DCs) are critical in triggering the effector arm of the adaptive immune system to clear infections. Because DCs maintain self-tolerance in steady state, any dysregulated DC immune response to pathogens can lead to a chronic inflammatory state. As such, DCs play a pivotal role in promoting or inhibiting autoimmunity. In ITP patients, DCs derived from peripheral $CD14^+$ monocyte subset ($CD14^+$ moDCs) were reported to have increased levels of costimulatory (CD80 and CD86) molecules and express higher levels of IL-12p70 [15]. Furthermore, decreased levels and activity of immunomosuppressive enzyme indoleamine 2,3-dioxygenase 1, were found in ITP DCs, consistent with their reduced ability to induce Tregs [16,17]. In humans, circulating monocytes, regarded as precursors of tissue macrophages and DCs [18]. can be phenotypically divided based on surface expression of CD14 (LPS receptor) and CD16 (low affinity Fcγ receptor III) expression with the major monocyte subpopulation characterized by high CD14 but no CD16 expression ($CD14^{hi}CD16^-$), also referred to as classical monocytes, and a minor $CD16^+$ subset that expand under infectious or inflammatory conditions [19,20]. To explore the mechanism of T-cell dysregulation in ITP, we have examined the role of peripheral monocytes rather than monocyte-derived DCs in inducing effector T-cell responses [21]. Using a simple in vitro culture, we have been examining the functional consequence of culturing monocytes, ex vivo, from patients with ITP and controls, with T cells, enabling us to examine the direct functional consequences of the action of monocytes on T cell function [21,22]. Using this assay system, we have found that ITP $CD16^+$ monocytes are polarized to secrete IL-12, promoting type 1 $CD4^+$ T-cell responses while concomitantly inhibiting Treg development [21]. In addition to functional alteration in $CD16^+$ monocytes, we have also found increased frequency of this monocyte subset in ITP patients [21]. We therefore postulate that the altered $CD16^+$ monocyte molecular signature may contribute to Treg dysregulation in ITP patients. These data, however, do not exclude the involvement of other innate immune cell types or inflammatory cytokines in driving the defects in the Treg compartment in ITP patients.

3. Role of inflammatory cytokines in T-cell dysregulation in ITP

In chronic ITP, the ongoing inflammation may further amplify the regulatory defect, preventing them from suppressing effectively. For example, pro-inflammatory cytokines, in particular TNF-α, that are secreted by activated monocytes and macrophages can negate the functions of Tregs and render them defective as was reported in patients with lupus and rheumatoid arthritis, whereas TNF-α blockade in patients with rheumatoid arthritis appears to be associated with rescue of Treg function [23]. Signaling through TNF receptor type II (TNFRII), which is constitutively expressed by Tregs and is upregulated by TNF-α, can mediate this inhibition [24]. We recently reported higher expression levels of TNFRII on ITP Tregs, raising the possibility that impaired Treg compartment in ITP patients may be due to heightened sensitivity of ITP Tregs to TNF-α–mediated inhibition [25]. Indeed, higher levels of TNF-α are present in plasma/serum of ITP patients [26–28]. and we found that non-Treg $CD4^+$ T cells from ITP patients expressed higher TNF-α levels. Treatment with TNF-α blockers has only been reported in a small group of patients with ITP, although it was efficacious in all four treated patients [29,30]. Using our T cell–monocyte coculture assay, we found that in vitro TNF-α blockade in samples from ITP patients can induce much stronger Treg expansion. These findings raise the possibility that TNF-α blockers through their ability to increase Treg subset proliferation may be efficacious in ITP patients.

4. Immunomodulatory treatments in ITP

Therapies that redirect the immune system to a state of tolerance are likely to be the most effective for the treatment of autoimmune diseases including ITP. Because of the critical role of Tregs in the maintenance of immune tolerance, inducing functional regulatory T cells can be considered as a tolerogenic strategy for treatment of ITP. Improved Treg frequency and/or activity has been reported to be associated with use of rituximab and intravenous immunoglobulin (IVIG) [3,31]. Importantly, patients with improved Treg function following treatment with rituximab appeared to have better prognosis [31]. Treatment with high-dose dexamethasone was also shown to increase the frequency of circulating Tregs [32], as well as decrease in Th1 cells [9]. These data are consistent with the generally accepted immunomodulatory activities of these treatments, although the exact mechanism for improved Treg frequency/function following treatment is unclear.

4.1. Thrombopoietin agents for continuous treatment

The recently licensed thrombopoietic agents for treatment of ITP, by increasing platelet production, have yielded overall durable responses in a high proportion of patients previously refractory to ITP [33]. Interestingly, we have found improved Treg [34] and Breg [35] function in chronic ITP patients associated with increased platelet counts following use of these agents, despite apparent lack of immunomodulatory activity associated with such agents. Furthermore, a direct correlation between circulating TGF-β levels, a known immunomodulatory cytokine, and platelet counts was observed [34]. Using a mouse model, Nishimoto, et al [36] recently demonstrated complete suppression of both antiplatelet autoantibody production and T-cell responses to platelet autoantigens following treatment with thrombopoietin. In their model, treatment with thrombopoietin also promoted the peripheral induction of Foxp3($^+$) Tregs in conjunction with elevated circulating TGF-β levels. Indeed, levels of TGF-β, which are lower in ITP patients with active disease [37–39], were reported to increase following treatment with a number of ITP treatments [37–39]. These data have raised the possibility that platelet-derived TGF-β may be responsible for improvement of the regulatory compartment. Possible mechanisms include the generation of Treg subsets including $CD62L^{neg}Foxp3^+$ Tregs [40], increasing thymic turnover of Tregs [41], or improving Treg survival [42]. Nevertheless, questions remain as to whether the restored Treg activity in these patients can dampen autoreactive responses once treatment stops. Similarly, it remains to be determined whether the underlying immune dysregulation in ITP may explain why some ITP patients do not respond to treatments with various ITP treatments including thrombopoietic agents despite the efficacy of such drugs. Given the high monetary cost associated with these latter drugs [43], it is essential to better understand the immune etiology of non-responsiveness to such agents as a means to identify biomarkers for predicting in advance response/non-response to treatment. It has been shown that platelets can modulate DC maturation especially in (immature) DCs that have differentiated from monocytes. Specifically, monocyte-derived DCs matured in presence of activated, but not resting platelets, expressed higher levels of IL-12 [44–46]. We have previously shown that IL-12 not only promotes T_H1 responses, but can also inhibit Treg subset development [22]. Platelets are activated in ITP patients [47]. raising the possibility