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Immune Dysregulation in Immune Thrombocytopenia (ITP)

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Abstract

Immune thrombocytopenia (ITP) is a bleeding disorder characterized by low platelet counts due to decreased platelet production as well as increased platelet destruction by autoimmune mechanisms. A shift toward Th1 and possibly Th17 cells together with impaired regulatory compartment, including Tregs and Bregs, have been reported, suggesting a generalized immune dysregulation in ITP. Interestingly, several treatments including the use of thrombopoietic agents appear to be associated with improvement in the regulatory compartment. Understanding how Th1/Th17/Treg differentiation and expansion are controlled is central to uncovering how autoimmunity may be sustained in chronic ITP and reversed following response to therapy. In this review, we will summarize the recent findings on the state of the Breg and Treg compartments in ITP, the role of monocyte subsets in the control of Th/Treg expansion and our working model of how the regulatory compartment may impact response to treatment and the means by which this information may guide therapy in ITP patients in the future.

Introduction

Immune thrombocytopenia (ITP) is a bleeding autoimmune disease due to decreased platelet production as well as accelerated platelet destruction mediated in part by autoantibody-based destruction mechanisms.[1] Most autoantibodies in ITP are isotype switched and harbor somatic mutations,[2] 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.[3–7] More recently, increased Th17 cells or IL-17 cytokine were reported in ITP patients,[8–11] implicating a possible role for Th17 cells in ITP immunopathology, although some reports did not detect any difference.[12;13] Moreover, a role for cytotoxic T cells in direct lysis of platelets and megakaryocytes in the bone marrow has been proposed.[14–16] 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, we and others have described a deficiency in generation and/or defective functions of ITP regulatory T cell (Treg)[17–22] and regulatory B cells (Bregs)[23] which will be discussed in more detail in this review.

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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.[24] The exact mechanism that accounts for inefficient Treg compartment in ITP remains to be defined. 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.[25] 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.[21] 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.[21] We therefore proposed that 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.[21] Nevertheless, the observed polyclonal Treg dysregulation[21] 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[26] may help clarify why loss of tolerance is toward platelets rather than other tissues.

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,[27] which in turn regulates Th polarization, pro-inflammatory differentiation of other antigen presenting cells (APCs) and autoimmune responses.[28] The alteration that we have detected in ITP patients is both at the phenotypic and functionality of B cells.[23] Specifically, frequency of previously described as Breg population,[29] characterized as $CD19^{+}CD24^{hi}CD38^{hi}$ cells, was decreased in non-splenectomized ITP patients off treatment. We found that ITP B cells have impaired IL-10 response after stimulation and a reduced ability to dampen of monocyte activation.[23] In mouse models, IL-10 secreting regulatory B cells promote differentiation of Tregs[30] or their recruitment,[31] 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.[17–22] 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[29] does not explain the antigen-specific autoreactivity, but rather is consistent with a perturbed immune reactive state.

Monocyte control of Th/Treg imbalance in ITP

The data so far suggests that a generalized immune dysregulation exists in ITP with an imbalance between the regulatory (Tregs) and effector T cells (Th1/Th17) that we predict drives pathogenic T and B cell effector responses at least against platelets. Understanding how Th1/Th17/Treg differentiation and expansion are controlled is likely to provide an explanation of how autoreactivity may be sustained in chronic ITP. Human monocytes

which are generally regarded as precursors of tissue macrophages and dendritic cells (DCs),[32] are increasingly recognized for their ability to trigger and polarize Th responses[33;34] as well as to both stimulate and suppress Tcell responses during infection and in autoimmune diseases.[34;35] They can be phenotypically divided based on surface expression of CD14 (LPS receptor) and CD16 (low affinity Fcγ receptor III) expression into subsets, each with distinct functional activities. The major monocyte subpopulation characterized by high CD14 but no CD16 expression ($CD14^{hi}CD16^{-}$), also referred to as classical monocytes, have higher phagocytic activity.[36] The minor CD16+ cells produce higher tumor necrosis factor (TNF) after stimulation and expand under infectious or inflammatory conditions.[37;38] With regards to the control of Th development, we have found that monocyte subsets in ITP patients are functionally altered. Specifically, we found that their numbers which expand under infectious or inflammatory conditions,[37;38] are positively associated with higher Th1 but lower Treg numbers in ITP patients.[39] Furthermore, depletion of CD16+ monocytes increased Treg and Th17 proliferation, but inhibited Th1 expansion, whereas addition of CD16+ cells to sorted cocultures of T cells and $CD14^{hi}CD16^{-}$ monocytes depressed Treg and Th17 proliferation, but increased Th1 expansion. Using transwell studies, we showed that ITP monocyte-mediated modulation of Treg/Th proliferation is dependent on secretion of soluble factors following contact with monocytes. Antibody blocking with anti-IL-12 reversed monocyte-mediated effects on Treg/Th proliferation. These studies are consistent with a model in which ITP CD16+ monocytes are polarized to secrete the Th1 cytokine IL-12, promoting Th1 responses while concomitantly inhibiting Treg and Th17 development.

Immuno-modulatory effects of treatment with TPO agents

Amongst the treatment options available to ITP patients, the recently licensed thrombopoietic agents have yielded overall durable responses in patients with chronic and refractory ITP while on treatment by increasing platelet production.[40] Interestingly, improved Treg[41] and Breg[23] function in ITP patients was associated with increased platelet counts following use of these agents, despite their apparent lack of immunomodulatory activity. Similarly, improved Treg compartment was reported in ITP patients with a platelet response to rituximab,[20] and treatment with high dose dexamethasone was shown to increase the frequency of circulating Tregs [19] as well as decrease Th1 cells.[13] As a working model, we hypothesize that a burst in platelet numbers correlates with increased TGF-β levels, which in turn increases/improves the regulatory compartment. Indeed, in a cross-sectional study,[41] we found increased TGF-β levels in addition to improved Treg activity in responders but not non-responders. In addition, the frequency of $CD19^{+}CD24^{hi}CD38^{hi}$ subpopulation, previously described as Bregs,[29] was increased in non-splenectomized ITP responders to TPO treatment and in 4 patients with elevated platelet counts, B cell-mediated monocyte suppressive activity was improved.[23] Although it remains to be proven, our hypothesis is that increased frequency of $CD19^{+}CD24^{hi}CD38^{hi}$ subpopulation in ITP patients with higher platelet counts can lead to improvement in B cell-mediated immunoregulation, which in turn may contribute to improved Treg activity. Because CD40L is expressed on and in platelets,[42] and B cell regulatory activity in vitro requires activation through CD40 engagement,[29] one may speculate that platelets may directly stimulate Breg activity in vivo in ITP patients with increased platelet counts through upregulation of CD40L, although this may imply that these platelets are activated.[43] Future studies are needed to determine the status of the Breg compartment in ITP patients on treatment with non-TPO agents and whether platelets interact directly with Bregs. With respect to treatment with rituximab, which also depletes Bregs, clinical remission using this therapy in patients with lupus and rheumatoid arthritis is thought to be associated with preferential repopulation of Breg compartment relative to pathogenic B cells.[44] We therefore hypothesize that in ITP patients, an early and persistent repopulation of Bregs will lead to recovery. Longitudinal

studies analyzing the Breg compartment in patients treated with rituximab are needed to test this.

In general, a better understanding of the immune etiology of response/non-response to treatment may lead to identification of biomarkers for predicting in advance which patients can benefit from such therapies. Despite the efficacy of TPO agents, some ITP patients do not respond to treatments with these agents and little is known about the mechanism of non-response. We recently showed in a cross-sectional study that CD16+ monocytes are expanded in nonresponders to TPO treatment whereas their numbers are normalized in TPO treatment responders.[45] Given that CD16+ monocytes inhibit Treg development, while promoting Th1 responses, we hypothesize that an expansion of CD16+ monocytes in non-responders leads to inappropriate regulation of pathogenic Th1 responses against platelets and megakaryocytes. Longitudinal studies are needed to establish an association between quantitative alterations in monocyte subsets and response/non-response to TPO treatment. Given the high monetary cost associated with these drugs,[46] this information may lead to identification of biomarkers to TPO treatment not only in patients with ITP but possibly in other immune mediated disorders.[47]

Summary and future perspectives

A number of studies suggest that a generalized dysregulation in immune regulatory networks may be present in ITP as in other autoimmune diseases.[17–23] However, based on the current data we cannot distinguish whether the dysregulation is the trigger for autoimmunity or is secondary to the ITP disease process. Longitudinal studies that monitor patients as the disease progresses are needed to address the in vivo relevance of Breg and Treg dysregulation in ITP. Nevertheless, the observation that these regulatory compartments have the potential to be normalized following therapy raises the possibility that their analysis could serve as cellular biomarkers that might guide therapy in ITP patients in the future. Understanding mechanisms of homeostatic control of these regulatory cells is paramount in deciphering why they may be altered in disease and in response to therapy. Our data showing that specific monocyte subsets can control of Th/Treg development highlights the importance of the innate immune system in this process and suggest that specific monocyte subsets may be a critical target in ITP to control inflammatory responses. The challenge is now to understand how antigen non-specific mechanisms that control potentially pathogenic Th responses cells are specifically directed at platelets, but not other cell types, in ITP.

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