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Immunomodulation by Intravenous Immunoglobulin: Role of Regulatory T Cells

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Abstract An altered immune homeostasis as a result of deficiency or defective function of $CD4^+CD25^+FoxP3^+$ regulatory T cells (Tregs) is common in several autoimmune diseases. Hence, therapeutic strategies to render Tregs functionally competent are being investigated. Intravenous immunoglobulin (IVIG) is being increasingly used for the treatment of a wide range of autoimmune and inflammatory diseases. Recent studies have demonstrated that IVIG induces the expansion of Tregs and enhances their suppressive functions. These effects of IVIG on Tregs correlate with the beneficial effects of IVIG in patients with autoimmune diseases. Thus, modulation of Tregs by IVIG represents a novel mode of action that explains the therapeutic effects of IVIG in T cell-mediated autoimmune diseases. However, the molecular mechanisms involved in IVIG-mediated modulation of Tregs are unclear and need further investigation.

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Introduction

Intravenous immunoglobulin (IVIG) is an established therapeutic for a wide range of autoimmune and immune-mediated inflammatory diseases [1, 2]. IVIG is a polyspecific immunoglobulin preparation obtained from pooled plasma of several thousand healthy donors [3, 4]. The beneficial effects of IVIG are attributed to multiple, mutually nonexclusive mechanisms that include modulation of Fc receptor expression and function, interference with activation of complement and the cytokine network, regulation of cell growth, and the effects on the activation and effector functions of dendritic cells, macrophages, natural killer (NK) cells, and T and B cells [1, 4].

Interestingly, recent reports have demonstrated a prominent role of $CD4^+CD25^+$ regulatory T cells (Tregs) in IVIG-mediated beneficial effects in autoimmune diseases [5]. Tregs play a critical role in the maintenance of immunological unresponsiveness to self-antigens and in the prevention of immune aggression and autoimmune diseases [6, 7]. Thus, the expansion of Tregs with an enhanced suppressive function represents a novel therapeutic approach in the treatment of autoimmune pathologies [8]. Recent studies have demonstrated that IVIG induces the expansion of Tregs and enhances their suppressive functions. Interestingly, these effects also correlate with the beneficial effects of IVIG in patients with autoimmune diseases. Thus, modulation of Tregs by IVIG represents a novel mode of action that explains the therapeutic effects of IVIG in T cell-mediated autoimmune diseases.

Phenotypic and Functional Features of Tregs

Tregs constitute a key player in the maintenance of self-tolerance in the peripheral tissues and in the prevention of deleterious immune responses. Recent evidence from experimental and clinical studies have clearly demonstrated that the deficiency of Tregs due to either genetic consequences or deliberate depletion results in exaggerated immune responses that lead to autoimmune diseases and inflammation [7].

Tregs are identified as either natural Tregs (nTregs) that emerge from the thymus or adaptive or induced Tregs (iTregs) that develop in the periphery from naïve $CD4^+$ T cells [8]. Tregs express characteristic markers such as CD25 (IL-2Rα), cytotoxic lymphocyte antigen 4 (CTLA-4), glucocorticoid-induced tumor necrosis receptor (GITR), chemokine receptor 4 (CCR4), lymph node homing receptor (CD62L), and forkhead box protein (FoxP3), the lineage-specific transcription factor [9]. Continuous expression of FoxP3 is critical for the suppressive function of Tregs. Interestingly, nTregs and iTregs share similar phenotypic markers, although iTregs are functionally unstable and distinct from nTregs. nTregs display a diverse T-cell receptor (TCR) specific for self-antigens. However, IL-2 and transforming growth factor-β (TGF-β) are required for the maintenance, survival and functioning of both nTregs and iTregs [10].

The cellular targets of Treg-mediated suppressor functions include $CD4^+$, $CD8^+$ T cells, dendritic cells (DCs), B cells, macrophages, monocytes, mast cells, natural killer (NK) cells, and NKT cells [9, 11]. Several mutually nonexclusive mechanisms have been proposed to explain the Treg suppressive function on these cells, which are mediated by both soluble factors and cell-associated molecules [7]. Although Tregs are antigen-specific, they can suppress responder T cells upon activation, irrespective of their antigen specificity [8]. Tregs can directly suppress responder T cells by secreting suppressor cytokines (TGF-β, IL-10, and IL-35), by depriving IL-2, and by causing granzyme- and perforin-mediated cytolysis leading to cell cycle arrest and apoptosis [6, 7, 9]. Furthermore, Treg-induced intracellular cyclic adenosine monophosphate (AMP) by CD39 and CD73 leads to inhibition of T cell proliferation and IL-2 production [9]. Tregs also inhibit the interaction of effector T cells with DCs, thereby interfering with T cell activation [12]. Thus, Tregs suppress the proliferation of naïve T cells and their differentiation from effector cells. In addition, the development of Tregs from naïve T cells is linked to Th17 differentiation. Under a steady state, Tregs can block the development of Th17 cells, which is mediated by inhibition of RORC expression by FoxP3 in a STAT3-dependent manner [13].

Treg interaction with DCs mediated by CTLA-4, LAG-3, and suppressor cytokines has been shown to downregulate the expression of co-stimulatory molecules CD80 (B7-1), CD86 (B7-2), and CD40 and the MHC–peptide complexes while upregulating the inhibitory B7-H3 molecules that lead to an impaired T cell stimulatory function of DCs [14–16]. Treg-modulated DCs also produce significantly lower levels of inflammatory cytokines IL-12, IL-1β, IL-6, and IL-8 and higher amounts of anti-inflammatory cytokine IL-10. Furthermore, CTLA-4-induced indoleamine 2,3-dioxygenase in DCs converts tryptophan into kynurenines, which act as potent immunosuppressive metabolites and can also induce de novo generation of Tregs. By a granzyme/perforin pathway, Tregs exert CD18/CD54 interaction-dependent cytotoxicity against both immature and mature DCs. Treg-expressed CD39 degrades adenosine triphosphate (ATP) to AMP and blocks the ATP-mediated activation of DCs [6, 7, 9, 11]. In addition, Tregs modulate the cross-talk between DCs and NK cells by controlling the mature NK cell number in the lymphoid organs [11].

In line with DCs, Tregs exert direct suppressive effects on monocytes and macrophages by downregulating the expression of MHC class II, CD40, CD80, and CD86 and the secretion of inflammatory cytokines (IL-1β, IL-6, IL-8, tumor necrosis factor [TNF], and macrophage inflammatory protein 1α [MIP-1α]), while favoring high expression of B7-H4 and anti-inflammatory cytokine IL-10. Thus, Treg-modulated monocytes and macrophages are poor stimulators of T cells. Tregs also enhance Fas/FasL-mediated apoptosis of lipopolysaccharide-treated monocytes [11].

Tregs also modulate the functions of B cells in several ways. Tregs reduce autoantibody production, inhibit T cell-dependent B cell responses by cell surface TGF-β1, and induce apoptosis of antigen-specific B cells via perforin and granyzymes.

Despite an established role for Tregs in the maintenance of self-tolerance and prevention of immune-mediated pathologies, Tregs fail to control persistent and chronic inflammation. Therefore, therapeutic strategies aimed at expanding Tregs and rendering them functionally competent are being explored [11]. In this context, IVIG is considered to be a promising therapeutic that can induce functionally competent Tregs in autoimmune and inflammatory conditions.

Modulation of Tregs by IVIG: a Novel Mechanism of Action

The established therapeutic efficacy of IVIG in T cell-mediated diseases such as Guillain–Barré syndrome, chronic inflammatory demyelinating polyneuropathy, and relapsing–

remitting multiple sclerosis (MS) was supported by recent findings of the expansion and enhanced suppressive function of human and murine Tregs following IVIG treatment. Thus, immunomodulation by IVIG through Tregs represents a novel mechanism of action.

Expansion of Tregs by IVIG

Using murine experimental autoimmune encephalomyelitis (EAE), an accepted model for MS, we have demonstrated the critical involvement of Tregs in IVIG-mediated protection against this disease. Interestingly, IVIG-induced protection was associated with an early and sustained peripheral expansion of antigen-specific $CD4^+CD25^+FoxP3^+$ Tregs in spleen and lymph nodes. Furthermore, depletion of Tregs using monoclonal antibody PC61 (anti-CD25) prior to EAE induction and treatment abolished the protective effects of IVIG [17]. Thus, it can be concluded that the beneficial effect of IVIG in relapsing–remitting MS might be related to the reestablishment of the Treg compartment.

The mechanism of IVIG-mediated enhancement of Treg numbers in lymphoid organs was investigated by employing adoptive transfer of TCR transgenic T cells specific for influenza hemagglutinin. We found that an increase in Treg numbers in the spleen following IVIG treatment was due to an expansion of the existing Treg population rather than their de novo generation [17]. Furthermore, in wild-type mice protected from EAE, IVIG did not enhance the secretion of TGF-β, a cytokine that favors the differentiation of $CD4^+$ T cells into iTregs.

Analogous to our in vivo results, De Groot and colleagues [18] demonstrated the expansion of iTregs in human peripheral blood mononuclear cells (PBMCs) by Treg-activating regions (referred to as Tregitopes) derived from the Fc portion of IgG molecules. Thus, co-incubation of PBMCs with antigens and Tregitopes enhanced the expression of cell surface markers such as $CD25^{high}$, CTLA-4, and GITR. Furthermore, they supported their observation with in vivo studies by showing that the administration of the murine homologue of the Fc region Tregitope resulted in the suppression of an immune response to a known immunogen. The authors hypothesized that the tolerizing effects of IVIG is related to Tregitope-mediated activation of Tregs [18].

In consensus with the experimental evidence, IVIG therapy enhanced the number of peripheral Tregs in patients with acute-stage Guillain–Barré syndrome and Kawasaki disease. The increased Treg numbers from IVIG was also correlated with an improvement of clinical parameters and symptoms [19, 20]. Similarly, following IVIG therapy in patients with systemic lupus erythematosus, an increase in $CD4^+CD25^+CD45RO^+$ T cell frequency was observed with progressive clinical improvement [21].

Enhancement of the Suppressive Function of Tregs by IVIG

Accumulating evidence from recent in vivo and in vitro studies clearly support the significance of enhanced suppressive function of Tregs in the therapeutic benefits of IVIG. In an EAE model, we demonstrated that adoptive transfer of Tregs from IVIG-treated mice to naïve mice followed by immunization with myelin oligodendrocyte protein (MOG) resulted in milder EAE compared with Tregs from untreated mice. Furthermore, Tregs from IVIG-treated mice were more efficient in suppressing the in vitro proliferation of TCR-stimulated $CD4^+FoxP3^-$ T cells compared with Tregs from untreated mice. In addition, IVIG-modulated Tregs efficiently prevented CNS damage in MOG-immunized mice by restricting encephalitogenic T cell infiltration and reducing the IFN-γ secretion. Thus, IVIG-expanded Tregs inhibit effector T cell development in the peripheral lymphoid organs, instead of targeting their function in the intended organ [17].

Analogous to the in vivo experimental model, IVIG treatment also enhances the suppressive function of human Tregs. Kessel and colleagues [22] demonstrated an increase in the expression of TGF-β, IL-10, and FoxP3 in $CD4^+CD25^{high}$Tregs following IVIG exposure. Furthermore, IVIG-modulated Tregs efficiently decreased the TNF-α production by $CD4^+CD25^-$ effector T cells [22]. Similarly, an activation of nTregs with a twofold increase in FoxP3 expression was observed following in vitro culture of PBMCs in the presence of Tregitopes derived from IgG [18]. Interestingly, Tregitope-induced Tregs significantly reduced the IL-5 production and converted Th2 cells to iTregs in the cultures of PBMCs obtained from donors allergic to birch pollen [18]. Thus, IVIG-expanded Tregs are functionally efficient in controlling the exaggerated immune responses.

Mechanisms of Modulation of Tregs by IVIG

The possible mechanisms involved in the IVIG-mediated modulation of Treg functions are depicted in Fig. 1. IVIG has been demonstrated to interact directly with the $CD4^+CD25^+$Tregs and conventional $CD4^+CD25^-$T cell surface molecules in mice. However, binding of IVIG to Tregs was higher than conventional T cells. Interestingly, IVIG enhanced the proliferation of murine Tregs in vitro without inducing TGF-β and IL-10 [17]. Furthermore, IVIG contains natural autoantibodies reactive to self molecules and might influence the Treg activation and expansion via direct interaction of natural autoantibodies with cell surface molecules such as CD4, CD5, CD95, TCR, and MHC [1]. However, in view of the multiple factors that influence Treg development and function, and

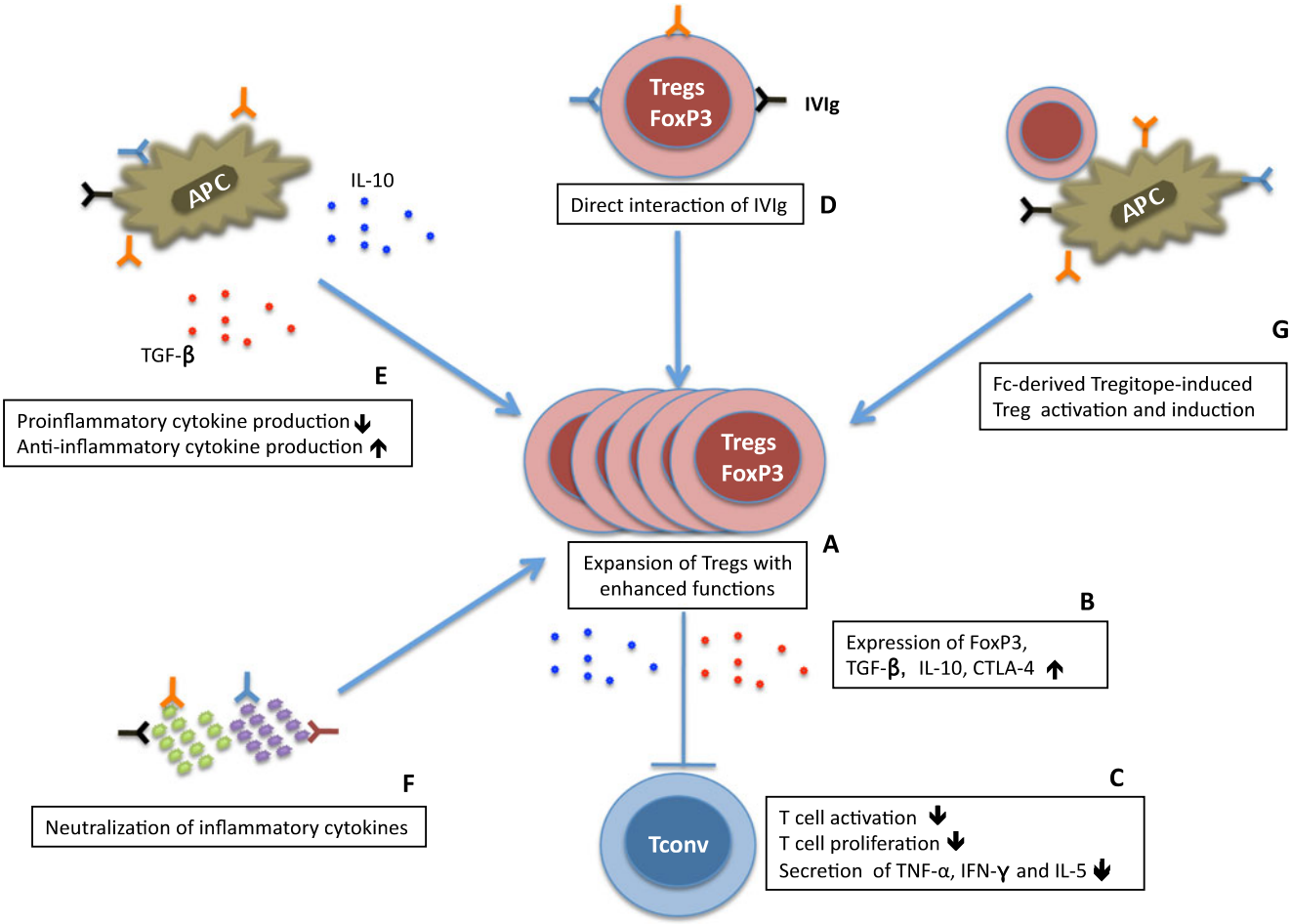

Fig. 1 The proposed mechanisms involved in the modulation of $CD4^+CD25^+FoxP3^+$ regulatory T cells (Tregs) by IVIG. Exposure of Tregs to IVIG leads to expansion and enhanced suppressive function (**a**). These IVIG-modulated Tregs exhibit an increased expression of FoxP3, TGF-β, and IL-10, which are the mediators of suppressive functions of Tregs (**b**). IVIG-modulated Tregs are efficient inhibitors of conventional T cell (Tconv) activation, proliferation, and cytokine secretion (**c**). These effects of IVIG on Tregs might be mediated by direct interaction of self-reactive natural autoantibodies with T cell surface molecules (**d**). In addition, IVIG-mediated modulation of cytokine network as a result of altered cytokine production from antigen-presenting cells (*APC*) (**e**) and neutralization of inflammatory cytokines (**f**) can create a microenvironment favorable for Treg expansion that also enhances their suppressive function. Finally, the conserved T cell epitopes derived from the Fc region (Tregitopes) can induce and activate Tregs (**g**)

the diverse targets of IVIG, involvement of other cellular compartment and their cytokines in the modulation of Tregs by IVIG cannot be excluded [1, 8]. Accordingly, De Groot and colleagues [18] proposed a model where IgG-derived Tregitopes presented on MHC II+ Ag-presenting cells activate Tregs, leading to downregulation of effector cell activation and function via regulatory cytokine or contact-dependent signalling, or both. However, the model failed to explain the antigen dependence for activation/expansion of antigen-specific Tregs.

IVIG interacts with different innate immune cells like macrophages, DCs, NK cells, monocytes, and neutrophils to inhibit the production of proinflammatory cytokines (IL-1β, IL-6, IL-12, TNF-α), while favoring anti-inflammatory cytokines (IL-1RA, TGF-β, IL-10) [5]. IVIG may also contain anti-inflammatory cytokines like TGF-β, which can favor Treg induction. In addition, IVIG also contains neutralizing antibodies to several inflammatory cytokines [1]. Recently, monoclonal antibodies to inflammatory cytokines such as TNF-α and IL-15 have been successfully used in the treatment of several autoimmune diseases. Such therapy was associated with the induction of Tregs and the restoration of its functions [23]. Therefore, by modulating the inflammatory environment, IVIG may facilitate the expansion and enhanced functioning of Tregs.

The distinction between effects on nTregs versus iTregs (in humans, $CD4^+CD25^{high}$ cells are a mixture of both) and between the expansion of pre-existing $FoxP3^+$ cells versus their de novo conversion from conventional T cells is not always clear due to limitations of the experimental setup and the complexities of the human system. It is speculated that the interaction of IVIG with iTregs is also important in

humans in view of their potential role in regulating the pathogenesis of autoimmune diseases [24]. In the EAE model, $F(ab)_2$ and Fc preparations of IVIG did not differ in their protective effect and Treg induction [17]. In contrast, De Groot and colleagues [18] implicated Fc-derived Tregitopes in the activation and expansion of Tregs. Thus, the mechanisms underlying the IVIG-mediated modulation of Tregs might implicate multiple mechanisms depending on the pathology.

Conclusion

Tregs play an indispensable role in the maintenance of immune homeostasis and in the prevention of an autoimmune disease. Hence, the deficiency of Tregs or their functions lead to deleterious immune aggression that results in autoimmune and inflammatory diseases. IVIG, a widely used therapeutic preparation in several immune-mediated diseases, exerts immunomodulatory effects by targeting various soluble and cellular compartments of the immune system. Emerging research evidence has revealed the role of modulation of Tregs in the therapeutic effects of IVIG and represents a novel mechanism of action in T cell-mediated diseases. In view of rational therapeutic strategies that aim to enhance or restore Treg functions in the treatment of autoimmune diseases, IVIG proves to be a promising tool. However, despite the demonstration of expansion and enhanced suppressive functions of Tregs by IVIG, the underlying mechanisms are unclear. Thus, deciphering the active components of IVIG that mediate the interaction between Tregs and the molecular events involved is a priority in understanding the mechanisms of action of IVIG.

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