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SHORT COMMUNICATION

Intravenous immunoglobulin-mediated expansion of regulatory T cells in autoimmune patients is associated with increased prostaglandin E2 levels in the circulation

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$CD4^+FoxP3^+$ regulatory T cells (Tregs) are immunosuppressive cells that are critical for immune tolerance. Several studies have demonstrated that one of the anti-inflammatory mechanisms of action of intravenous immunoglobulin (IVIg) involves the expansion of Tregs. Recently, we demonstrated that IVIg-mediated Treg expansion involves the cyclooxygenase-2 (COX-2)-dependent induction of prostaglandin E2 (PGE_2) in human dendritic cells (DCs). However, the validity of these findings in autoimmune patients is lacking. In this report, we demonstrate that the IVIg-mediated expansion of Tregs in autoimmune patients is associated with increased levels of circulatory PGE_2. Due to its immunomodulatory effects on various immune cells, this increase in PGE_2 represents one of the pathways by which IVIg exerts anti-inflammatory effects.

IVIg is a therapeutic preparation of normal circulating IgG obtained from the pooled plasma of many healthy donors. Currently, high-dose IVIg therapy (1–2 g/kg body weight) is used in the treatment of diverse autoimmune and inflammatory conditions, such as Guillain–Barré syndrome (GBS), Kawasaki disease, idiopathic thrombocytopenic purpura and inflammatory myositis.[1] The beneficial effects of IVIg are mediated by numerous mutually non-exclusive cellular and molecular mechanisms. These mechanisms indirectly suggest the functions of circulating IgG in the maintenance of immune homeostasis. The proposed mechanisms of IVIg include the following: inhibiting the activation of innate immune cells, such as DCs, macrophages, monocytes and neutrophils; suppressing pathogenic Th1 and Th17 subsets and reciprocally expanding $CD4^+FoxP3^+$ Tregs; inhibiting inflammatory cytokines and enhancing anti-inflammatory molecules; blocking complement activation; modulating B-cell functions; and neutralizing pathogenic autoantibodies.[2,3]

Several studies have demonstrated that IVIg expands Tregs in both humans and experimental models.[4,5] These findings have also been confirmed in IVIg-treated autoimmune patients.[6,7] Recently, we demonstrated that IVIg-mediated Treg expansion requires the induction COX-2-dependent PGE_2 in human DCs.[8] The inhibition of COX-2 activity in DCs led to the abrogation of IVIg-mediated Treg expansion both *in vitro* and *in vivo* in an experimental autoimmune encephalomyelitis model. However, the effect of IVIg therapy on PGE_2 levels in autoimmune patients has not yet been investigated. IVIg is recommended as a first-line therapy for patients with GBS. Therefore, we aimed to explore whether the IVIg-mediated expansion of Tregs in five patients with GBS was associated with increased levels of circulatory PGE_2.

Heparinized blood samples were obtained from five GBS patients (three men and two women, with ages ranging from 57 to 82 years) before and 1 week following IVIg therapy. The patients were naive to IVIg therapy and had not been under any immunosuppressive or immunomodulatory treatments in the previous three months. Relevant ethical committee approval

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(84-2012-08, CHU Limoges, France) was obtained for this study. The muscular weakness of the patients was determined by the Medical Research Council grading system, and the modified Rankin scale was used for motor function disability analysis. Three patients with severe disease received IVIg (Privigen) at the rate of 0.4 g/kg for five consecutive days. Two patients with less severe disease received IVIg at 0.4 g/kg for three consecutive days.

Peripheral blood mononuclear cells were obtained from the patients using Ficoll-Paque (1.077 g/ml) density centrifugation. The Treg population was analyzed by flow cytometry (LSR II; BD Biosciences, Le Pont de Claix Cedex, France) by labeling peripheral blood mononuclear cells with fluorochrome-conjugated anti-CD4 and anti-FoxP3 antibodies (Figure 1a). Up to 50 000 cells were acquired for each sample on FACSDiva software (BD Biosciences), and the data were analyzed using Flowjo Tree software (Flowjo, Ashland, OR, USA).

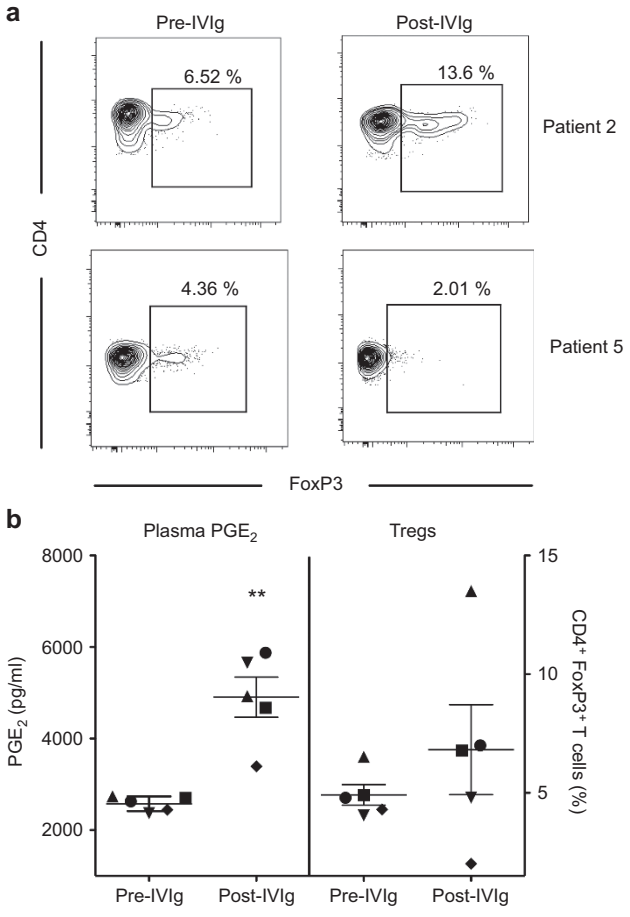

Figure 1 IVIg-mediated expansion of Tregs in autoimmune patients is associated with increased levels of circulatory PGE_2. (**a**) Representative analysis of $CD4^+FoxP3^+$ Tregs in the peripheral blood mononuclear cells of two GBS patients before (Pre-IVIg) and 1 week following IVIg therapy (Post-IVIg). (**b**) Temporal changes in the amounts of PGE_2 (pg/ml of plasma) and frequencies of $CD4^+FoxP3^+$ Tregs in the circulation of five GBS patients before (Pre-IVIg) and 1 week following IVIg therapy (Post-IVIg). Each symbol represents an individual patient, and the lines represent mean±s.e.m. values. $^{**}P<0.001$, two-tailed Student's *t*-test. GBS, Guillain–Barré syndrome; IVIg, intravenous immunoglobulin; PGE2, prostaglandin E2; Treg, regulatory T cell.

The amount of PGE_2 in the plasma samples of GBS patients was estimated by ELISA.[8] Briefly, ELISA plates were incubated with plasma samples and serially diluted standards overnight at 4 °C. Following three washes with PBS-Tween (PBST) buffer, the plates were blocked with 1% BSA-PBST for 1 h at 37 °C. Then, the wells were incubated with anti-PGE_2 antibodies at 1:200 dilution (Sigma-Aldrich, St.Louis, MO, USA) for 6 h at 37 °C, followed by washing with PBST. Next, the plates were incubated with HRP-labeled anti-rabbit secondary antibodies at 1:2000 dilution (Jackson ImmunoResearch, West Grove, PA, USA) for 2 h at 37 °C, followed by the addition of 3,3′,5,5′-tetramethylbenzidine (Sigma-Aldrich) as a substrate. The absorbance values were measured at 492 nm.

Notably, the incidence of GBS is 0.6–4.0 per year per 100 000 people, thus explaining the difficulties in obtaining treatment-naïve patients for clinical studies. To avoid the influence of previous IVIg exposure or other immunomodulatory therapies on the Treg population, we restricted our study to only treatment-naive patients. We found that IVIg therapy led to increased frequencies of Tregs in four of the five GBS patients (Figure 1). The pre-IVIg levels of Tregs in these patients were 5.07%±0.52% (mean±s.e.m.) and increased to 8.02%±1.89% following IVIg therapy. However, the fifth patient failed to display such an increase in Treg frequency following IVIg therapy (Figure 1).

Then, we explored whether the IVIg-mediated increase in Treg frequencies was associated with augmented PGE_2 levels in the circulation. In fact, the four GBS patients who showed increased Treg frequencies following IVIg therapy also presented with significant increases in the plasma levels of PGE_2. The pre-IVIg level of PGE_2 in the circulation was 2609.5±82.3 pg/ml (mean±s.e.m.), and it increased to 5282.8±287.5 pg/ml (Figure 1b). Thus, these results corroborate our previous data indicating a role for DC-secreted PGE_2 in IVIg-mediated Treg expansion and, hence, validate the therapeutic relevance of our report.[8] Notably, the up-regulation of PGE_2 was also minimal in a patient who did not show Treg enhancement (Figure 1b). Therefore, these results indicate that Treg expansion by IVIg is directly linked to the amount of absolute increase in PGE_2. Currently, the cellular source that contributes to IVIg-induced PGE_2 in autoimmune patients is not known. Based on previous reports, we propose that DCs and monocytes might be the primary contributors.[8,9]

Currently, IVIg therapy is an established treatment of choice for GBS (level A recommendation). The available clinical data demonstrate that GBS patients who receive IVIg therapy within 2 weeks after the onset of this disease exhibit accelerated recovery. IVIg might benefit GBS patients *via* several mutually nonexclusive mechanisms, including the neutralization of neuromuscular-blocking antibodies by anti-idiotype antibodies.[2,3] Our results indicate that the beneficial effects of IVIg on GBS patients are associated with the peripheral expansion of Tregs.

Various cells, including epithelial cells, fibroblasts and innate immune cells, produce PGE_2. COX enzymes such as COX-1 and COX-2 are critical for converting arachidonic acid into PGE_2. COX-1 is constitutively expressed, whereas COX-2 is inducible. Previous studies have demonstrated that PGE_2 inhibits the functions of NK cells and granulocytes, restricts the phagocytic functions and pathogen-killing activities of macrophages, and blocks the induction of antigen-specific immune responses. The exposure of DCs to PGE_2 leads to the impairment of their ability to stimulate Th1 and cytotoxic T-cell responses. In addition, PGE_2 mediates the generation and expansion of Tregs and enhances their immunosuppressive functions.[10] Thus, PGE_2 exerts modulatory effects on several immune cells. Of interest, IVIg suppresses the activation of innate immune cells and pathogenic T-cell subsets and induces Treg expansion. Therefore, our report indicates that increased levels of PGE_2 following IVIg therapy might be partly responsible for the immunomodulatory effects of IVIg on various immune cells. In support of this finding, a recent report demonstrated that monocyte-derived PGE_2 plays a role in the IVIg-mediated inhibition of IFNα production by Toll-like receptor-stimulated plasmacytoid DCs.[9]

CONFLICT OF INTEREST

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