Clinical & Experimental Immunology
The Journal of Translational Immunology

Clinical and Experimental Immunology ORIGINAL ARTICLE doi: 10.1111/cei.13580

IVIg increases interleukin-11 levels, which in turn contribute to increased platelets, VWF and FVIII in mice and humans

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Accepted for publication 11 January 2021
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Summary

The mechanisms of action of intravenous immunoglobulins (IVIg) in autoimmune diseases are not fully understood. The fixed duration of efficacy and noncumulative effects of IVIg in immune thrombocytopenia (ITP) and acquired von Willebrand disease (AVWD) suggest other mechanisms besides immunological ones. Additionally to the peripheral destruction of platelets in ITP, their medullary hypoproduction emerged as a new paradigm with rescue of thrombopoietin receptor agonists (TPO-RA). In an ITP mouse model, interleukin (IL)-11 blood levels increase following IVIg. IL-11 stimulates the production of platelets and other haemostasis factors; recombinant IL-11 (rIL-11) is thus used as a growth factor in post-chemotherapy thrombocytopenia. We therefore hypothesized that IVIg induces IL-11 over-production, which increases platelets, VWF and factor VIII (FVIII) levels in humans and mice. First, in an ITP mouse model, we show that IVIg or rIL-11 induces a rapid increase (72 h) in platelets, FVIII and VWF levels, whereas anti-IL-11 antibody greatly decreased this effect. Secondly, we quantify for the first time in patients with ITP, AVWD, inflammatory myopathies or Guillain–Barré syndrome the dramatic IL-11 increase following IVIg, regardless of the disease. As observed in mice, platelets, VWF and FVIII levels increased following IVIg. The late evolution (4 weeks) of post-IVIg IL-11 levels overlapped with those of VWF and platelets. These data may explain thrombotic events following IVIg and open perspectives to monitor post-IVIg IL-11/thrombopoietin ratios, and to assess rIL-11 use with or without TPO-RA as megakaryopoiesis co-stimulating factors to overcome the relative hypoproduction of platelets or VWF in corresponding autoimmune diseases, besides immunosuppressant.

Keywords: acquired von Willebrand disease, Factor VIII, immune thrombocytopenia, interleukin-11, intravenous immunoglobulin

Introduction

Intravenous immunoglobulin (IVIg) is used in the treatment of several autoimmune and inflammatory diseases, including immune thrombocytopenia (ITP), Guillain–Barré syndrome (GBS), chronic inflammatory demyelinating polyradiculoneuropathy, multi-focal motor or demyelinating neuropathies, inflammatory myopathies (IM) and acquired von Willebrand disease (AVWD) [1]. However, the mechanisms underlying IVIg efficacy remain a matter of debate, and several possible intricate modes of action are postulated. Indeed, anti-idiotypic, complement-scavenging, neonatal Fc receptor saturation and/or immunomodulatory and anti-inflammatory effects, through direct action on the activation of innate immunity, B or T cells or their cytokine production, are the main recognized mechanisms of IVIg efficacy [1]. However, the fixed

duration and non-cumulative effects of successive IVIg efficacy, requiring unlimited reinfusions every 3–4 weeks in the treatment of ITP and AVWD, are uncommon with usual immunomodulatory mechanisms, apart from that of an anti-idiotypic effect that is assumed among several others. Therefore, pure immunomodulatory actions of IVIg may not represent the leading mechanisms involved in ITP and AVWD.

Indeed, in addition to peripheral platelet destruction, a new paradigm of medullary-associated hypoproduction or insufficient increase in production of platelets has also emerged in the pathogenesis of ITP [2–5]. This notion argues for the involvement of an absolute or relative deficit in some of the stimulating/growth factors during megakaryopoiesis in ITP [6]. Therefore, the use of treatments that stimulate megakaryopoiesis, including thrombopoietin receptor agonists (TPO-RA), has recently joined the therapeutic arsenal for ITP [7].

Interleukin (IL)-11 is a pleiotropic stromal protein belonging to the IL-6 family. IL-11 can be expressed in a wide variety of tissues, including the central nervous system, testis, mucosa, liver and haematopoietic organs [8–10]. IL-11 is not detected in the blood of healthy humans or mice at steady state. Nevertheless, several studies demonstrate that IL-11 over-production is induced by IVIg in experimental mouse models of autoimmune encephalitis and ITP [11–14]. IL-11 exhibits anti-inflammatory effects and even exerts a beneficial effect on tissue myelin repair in mice [9,15,16].

Interestingly, IL-11 and its recombinant form have been shown to increase the levels of platelets, von Willebrand factor (VWF) and/or factor VIII (FVIII) in mice, healthy humans and patients with inherited von Willebrand disease or post-chemotherapy thrombocytopenia [17–20]. Indeed, IL-11 improves megakaryopoiesis and haematopoiesis by acting as a co-factor of TPO and in synergy with other haematopoietic cytokines, such as erythropoietin and granulocyte/macrophage colony-stimulating factor [21–23].

Therefore, we hypothesized that IVIg exerts beneficial effects in haemostasis and human autoimmune and/or inflammatory disorders, at least in part, via increasing IL-11 levels which, in turn, act as a co-stimulatory and/or growth factor to increase several haemostatic factors. Thus, in this experimental and observational study using a murine ITP model, we first aimed at validating the observation that IVIg increases the blood levels of platelets via IL-11 action. In addition, we explored the effects of IVIG and IL-11 in haemostasis parameters such as FVIII and VWF in the same ITP model. Secondly, we also demonstrate and, for the first time, to our knowledge, quantify in humans that IVIg increases blood levels of IL-11 as in mice, together with a secondary increase in the aforementioned haemostatic factors.

Materials and methods

ITP mouse model

A dose escalation mouse model of passive ITP induced by the platelet-depleting antibody MWReg30 (anti-CD41 against a surface glycoprotein IIb of platelets, clone MWReg30, rat IgG1λ; BD Biosciences, Franklin Lakes, NJ, USA, catalogue no. 553847) was established as previously reported [12,24]. Experiments were performed following approval by the relevant ethical committee and the French Ministry of Agriculture (reference APAFIS no. 17201-2018102215024574 v3), and were conducted in accordance with the recommendations for the care and use of laboratory animals following all regulations.

Eight-week-old female C57BL/6 mice were purchased from Envigo Rms Sarl (Jackson Laboratory, Bar Harbor, ME, USA). We chose to work on only female mice to limit interindividual variability due to gender, and for the practical reason that female mice allow easier blood collection because of their higher weight. We worked with groups of five mice each, after calculating the minimum number of animals to use. The group size (n) required 80% power and a two-tailed significance level ($\alpha = 0{\cdot}05$) to gain a significant effect, using effect size (E/S) = $k \times \delta$, where t-value at degrees of freedom (d.f.) = $n$total–2, where $n$total = n1 + n2, δ is the non-centrality parameter and $k = (1/n1 + 1/n2)1/2$. MWreg30 was injected into the mice by the intraperitoneal (i.p.) route at a dose of 68 μg/kg for the first 2 days (days 0 and 1), followed by increasing doses of an additional 34 μg/kg for each subsequent day (i.e. 102 μg/kg on day 2 and 136 μg/kg on day 3). With this protocol, the platelet count nadir is reached on day 2 and is maintained as long as MWreg30 administration is continued [12]. The experiments were terminated on day 4.

Treatment of mice with human IVIg, mouse IL-11 or mouse IL-11 antibody

For ITP treatment, we defined four groups of five mice each: (1) IVIg (Gamunex® 100 mg/ml; Grifols Deutschland, Frankfurt, Germany, CIP code no. 34009 301 695 99) were injected i.p. at a dose of 2 g/kg 2 h after MWreg30 administration on day 2. (2) Recombinant mouse IL-11 (R&D Systems, Minneapolis, MN, USA, catalogue no. 418-ML) was injected at a dose of 10 μg/mouse on day 2 following the same timeline. Each group was composed of five mice. (3) To neutralize IL-11, a mouse anti-IL-11 antibody (monoclonal rat IgG2A, clone no. 188520; R&D Systems, catalogue number MAB418) was injected at a dose of 100 μg/mouse i.p. at the same time as IVIg injection. (4) Control mice with ITP received phosphate-buffered saline (PBS) ×1 i.p. (OmniPur®; Calbiochem, San Diego, CA, USA, catalogue no. 6505-OP).

Blood collection and platelet count analysis in mice

Blood was drawn in citrated tubes under general anaesthesia (isoflurane 3·5%) from the mice by the retro-orbital route on days 0, 2 and 4. Part of the blood was used for automated haemogram analysis, while the rest of the blood was centrifuged at 300 ***g*** for 20 min at 4°C before harvesting the plasma on ice. Citrated plasma was stored at −20°C until use for assays.

The Scil Vet abc machine (scil Animal Care Company, Viernheim, Germany) was used to analyze platelet counts (giga/L, PLT).

IM, AVWD, ITP and GBS patients treated with IVIg

In this retrospective study from several diseases and hospitals we used one of the two possible available blood samples, either serum in ITP and GBS patients and plasma in IM, GBS and AVWD patients. We used an available patient cohort from Caen Hospital with AVWD [four patients, female/male = 3/1; median age 60 years (range = 54–64)] and a patient cohort from AP-HM Marseille with ITP [six patients, all female; median age 57 years (range = 17–80 years)]. Platelet count and plasma levels of IL-11 and haemostatic factors were analysed before and 72 h post-IVIg. We also obtained plasma samples (before and 72 h post-IVIG) from the patient cohort from the Internal Medicine Department of Pitié-Salpêtrière Hospital (Paris) with IM [12 patients, female/male = 8/4; eight with polymyositis and four with dermatomyositis; median age 56 years (range = 26–73 years)] and a cohort from Limoges Hospital (pre-, 1 and 4 weeks post-IVIg therapy) to investigate GBS patients [five patients, female/male = 3/2; median age 38 (range = 29–48 years)]. This observational study received approval from the local institutional review board of Caen Normandy University (CLERS no. 1686, 20/07/2020). In all patients, IVIg (Octagam®; Octapharma, Boulogne Billancourt, France, CIP code no. 562 114 9 or Tegeline®; LFB-Biomedicaments, Les Ulis, France, CIP code 5598982) was administered as follows: 1 g/kg on days 1, 2 or 3 (in nine patients) or 0·5 g/kg/day from days 1 to 4 due to renal insufficiency (three patients).

Enzyme-linked immunosorbent assay (ELISA) for mouse and human FVIII

Flat-bottomed 96-well ELISA microplates were coated overnight with human IgG monoclonal BO2C11 antibodies that recognize the anti-C2 domain of human FVIII and cross-react with mouse Factor VIII (FVIII) with the same detection profile (1 μg/ml, 50 μl/well). After the usual saturation step, plasma samples were serially diluted in dilution buffer starting from 1/10 and continuing until 1/640. The detection process used a mouse biotinylated secondary antibody (GMA8015, catalogue no. GMA-8015) that recognizes the anti-domain A2 of human FVIIII and mouse FVIII. The final step used a streptavidin–horseradish peroxidase (HRP) conjugate (R&D Systems, catalogue no. DY998) and 3′,5,5′-tetramethylbenzidine (TMB) substrate (Thermo Fisher Scientific, Fremont, CA, USA) solution. The reaction was stopped by the addition of 2 N HCl. Optical density was recorded (absorbance 450 nm) using a Tecan infinite M200 PRO microplate reader (Thermo Fisher Scientific).

Quantification of IL-11 and VWF in mouse and human plasma

IL-11 levels in patient plasmas were quantified using ELISA kits specific for the species (R&D Systems, catalogue no. DY218). Assays were performed using 1 : 2 diluted plasma samples, as per the manufacturer's instructions. The sensitivities of the ELISA kits were 31·3 pg/ml for human IL-11 and 125 pg/ml for mouse IL-11.

Human VWF antigen was quantified by using a kit from R&D Systems, catalogue no. DY2764-05, and mouse VWF was measured with a kit from Abcam, Cambridge, MA, USA, catalogue no. ab208980.

Statistical analysis

Quantitative variables are expressed as means ± standard error of the mean (s.e.m.). All statistical analyses were performed using GraphPad Prism version 6.0. After confirmation of normal distribution by skewness and kurtosis tests, two-way analysis of variance (ANOVA) with Tukey's multiple comparison test or Student's *t*-test were used to determine statistical significance of the data. P-value < 0.05 was considered statistically significant.

Ethical approval information

Ethical approval for this study was granted by the French Ministry of Agriculture approval (reference APAFIS, no. 17201-2018102215024574 v3) and the local institutional review board of Caen Normandy University (CLERS no. 1686-20/07/2020).

Results

Protective effects of IVIg in the ITP mouse model are mediated in part via increased IL-11 levels in plasma

As shown in Fig. 1, platelet levels were clearly decreased from days 0 to 2 in all four mice groups following MWReg30 infusion (Fig. 1a); platelet count nadir was maintained in control PBS-treated mice, IVIG or IL-11 injection on day 2 led to increased platelet levels, as analyzed on day 4 (Fig. 1b). Neutralization of IL-11 by

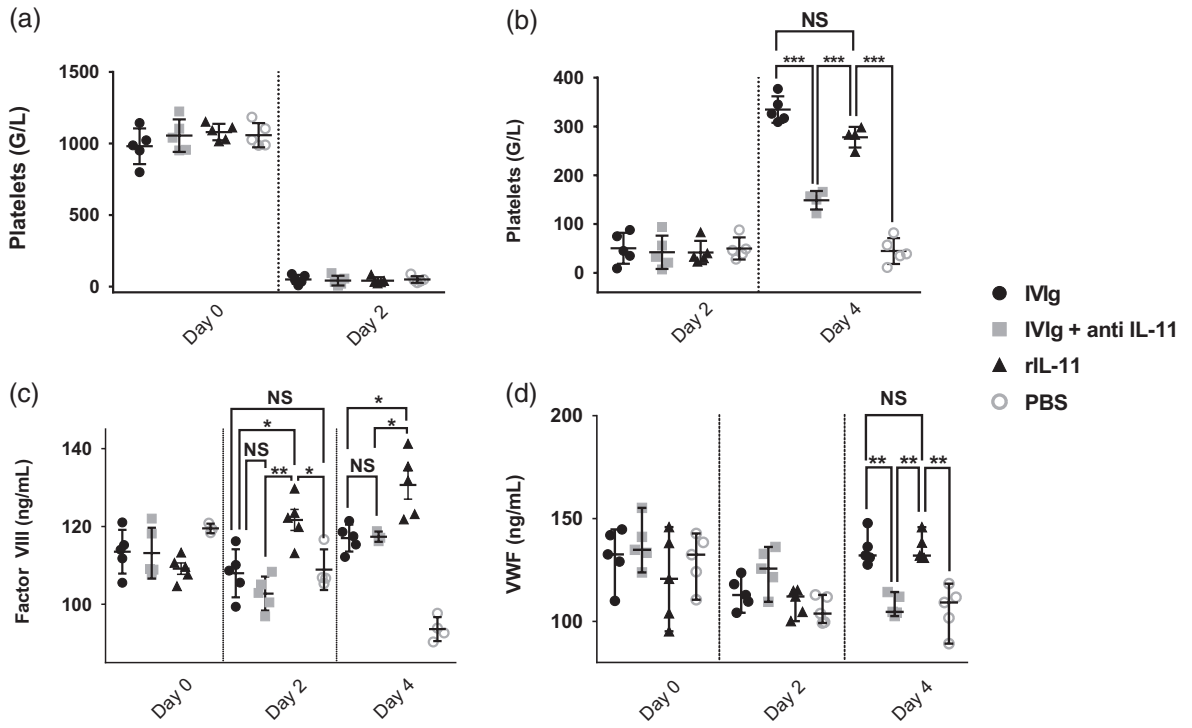

Fig. 1. Evolution of platelet, Factor VIII (FVIII) and von Willebrand factor (VWF) levels in a mouse model of immune thrombocytopenic purpura (ITP). A dose escalation mouse model of passive ITP was induced in C57BL/6 mice by daily injection of a platelet-depleting antibody, MWReg30, until the end of the experiment. The mice were divided into four groups of five mice each that received one of the four following additional interventions at day 2, 6 h before blood sample collection: (1) intravenous immunoglobulin (IVIg) (2 g/kg), (2) IVIg plus mouse anti-interleukin (IL)-11 antibody (100 μg/mouse), (3) recombinant mouse IL-11 (rIL-11) (10 μg/mouse) or (4) phosphate-buffered saline (PBS) (equivalent volume of IVIg). (a) Platelet count (G/L) [mean ± standard error of the mean (s.e.m.), $n = 5$ mice/group) on day 0 (before) and day 2 (after) MWReg30 injection, uniformly and dramatically decreased at day 2. (b) Platelet levels (mean ± s.e.m., $n = 5$ mice/group) measured on day 4 in various treatment groups. Whereas platelet levels remained low at day 4 compared to day 2 in PBS and IVIg plus mouse anti-IL-11 monoclonal antibody groups, their levels significantly and comparably increased in both IVIg and mouse rIL-11 groups. (c) FVIII levels in the plasma was measured by enzyme-linked immunosorbent assay (ELISA) (mean ± s.e.m., $n = 5$ mice/group) on days 0, 2 and 4. The levels of FVIII significantly increased in the corresponding groups compared to levels measured in PBS, IVIg and IVIg plus mouse anti-IL-11 antibody groups, remaining significant on day 4. However, there was no significant difference in FVIII levels in IVIg *versus* IVIg plus mouse anti-IL-11 antibody groups at days 0, 2 and 4, these levels being already initially normal and remaining above the lower limit. (d) VWF levels in the plasma were measured by ELISA (mean ± s.e.m., $n = 5$ mice/group) on days 0, 2 and 4. The levels of VWF showed a slight decrease from days 0 to 2 in all four groups. At day 4, the levels of VWF were significantly and similarly increased only in rIL-11 and IVIg groups. Of note, neutralization of IL-11 completely abrogated the effect of IVIg on VWF. $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$; n.s. = not significant by two-way analysis of variance (ANOVA).

the administration of anti-IL-11 monoclonal antibodies together with IVIg severely impaired the ability of IVIg to enhance platelet levels. Thus, both IVIg and IL-11 exerted similar effects on platelet levels in this model, and the infusion of IL-11 neutralizing monoclonal antibodies prevented most of the protective effect of IVIg. Together, these data showed that IL-11 was directly responsible, at least in part, for the increased platelet levels in response to IVIg in an ITP mouse model.

IVIg and IL-11 induce increased FVIII plasma levels in an ITP mouse model

Mouse plasma samples from all groups were tested for FVIII levels by ELISA at various time-points (days 0, 2 and 4; Fig. 1c). Following the induction of ITP, in the PBS group FVIII levels were gradually reduced over time (Fig. 1c, PBS group, $P < 0{\cdot}01$). However, in the IVIg group FVIII levels were unchanged from days 0 to 4. This trend indicates the capacity of IVIg to rescue ITP mice from the loss of FVIII. Conversely, rIL-11 therapy clearly increased the plasma levels of FVIII (day 4), which were significantly higher than those observed in PBS ($P < 0{\cdot}05$) and IVIg ($P < 0{\cdot}05$) groups.

VWF plasma levels increase following IVIg in an ITP mouse model is mediated in part by IL-11

Similar to FVIII, VWF gradually declined in blood following induction of ITP and remained lower in the PBS group

(Fig. 1d, $P < 0{\cdot}01$). Regardless of the treatments, VWF levels were always low on day 2. However, both IVIg and IL-11 significantly enhanced VWF levels on day 4 compared to the PBS and IVIg + anti-IL-11 groups ($P < 0{\cdot}01$). Additionally, administration of anti-IL-11 abrogated the effect of IVIg on VWF levels ($P < 0{\cdot}01$). Together, these data show that both IVIg and IL-11 exhibit similar effects on VWF levels and the positive effects of IVIg on VWF levels are mediated mainly through IL-11.

IL-11 is undetectable in IVIg-naive patients, whereas it is detectable at low levels at 4 weeks in previously treated patients then dramatically increases in all patients following IVIg

We then explored if induction of IL-11 following IVIg therapy is restricted only to haematological disorders or is a universal feature, irrespective of disease. Among the 27 patients tested, all 25 patients who had not received IVIg before blood collection had no detectable IL-11 in their serum ($n = 11$) or plasma ($n = 14$) samples (Fig. 2). In contrast, the two AVWD patients who were previously treated with IVIg a month previously exhibited low but detectable IL-11 levels. Once all 27 patients had been treated with IVIg, IL-11 had become detectable ($P < 0{\cdot}001$) at different levels according to the type of blood sample and time of collection. Indeed, IL-11 levels at 72 h were higher in plasma (199·1 pg/m; 3·3–1114) than in serum (76·98 pg/ml; 32·5–363·3), regardless of the disease involved.

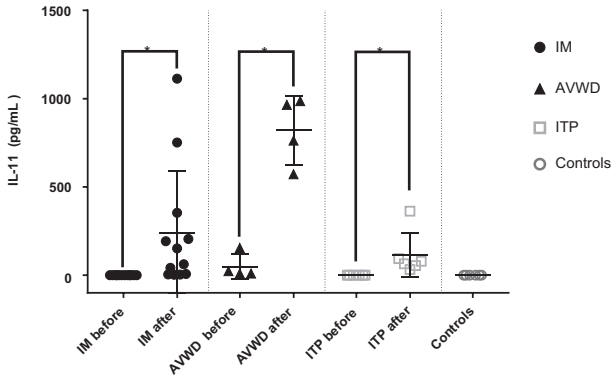

Fig. 2. Induction of interleukin (IL)-11 and enhancement of haemostatic factors levels following intravenous immunoglobulin (IVIg) is a universal phenomenon. IL-11 was measured via enzyme-linked immunosorbent assay (ELISA) in the plasma of inflammatory myopathies (IM, $n = 12$) and acquired von Willebrand disease (AVWD, $n = 4$) patients and healthy controls (controls, $n = 6$), or in the serum of immune thrombocytopenia (ITP, $n = 6$) patients. The median IL-11 levels increased in all patients from the steady state to 72 h following IVIg, regardless of the underlying disease. The lower increase in ITP patients was observed in serum. $^{*}P < 0{\cdot}05$; by Student's *t*-test.

FVIII and VWF levels increase in parallel with IL-11 levels in humans after IVIg

As IVIg induced IL-11 in patients similarly to the mouse model, this raised the prospect that IVIg could enhance VWF and FVIII in treated patients via IL-11. In addition to the increased IL-11 levels in plasma or serum (Fig. 2), IVIg significantly increased ($P < 0{\cdot}05$) plasma levels of both VWF (panel A, Fig. 3) and FVIII (panel B, Fig. 3) in AVWD and IM patients.

Monitoring of platelet, VWF and IL-11 levels after IVIg in AVWD and ITP patients

Platelet and IL-11 levels were undetectable and low, respectively, in all six IVIg-naive ITP patients before IVIg; then, both levels increased in parallel at 72 h after treatment (panel A, Fig. 4). The same trend was seen for IL-11 levels in the two IVIg-naive AVWD patients, while platelet levels remained within normal values before and after IVIg. Moreover, VWF levels also increased following IVIg significantly in both AVWD and ITP patients (panel B, Fig. 4).

IL-11 levels decrease over time following IVIg and increase again after new infusion

We analysed whether the increase in IL-11 following IVIg is a transient phenomenon or would persist beyond the half-life of infused IgG, i.e. more than 21 days. In Fig. 5, serial assessments of IL-11 performed in GBS patients showed detectable levels at 1 week following IVIg, which subsequently decreased to barely detectable levels at 4 weeks. Furthermore, in both AVWD patients regularly treated every 4 weeks with IVIg, IL-11 levels were readily detectable and increased again soon after (72 h) the new infusion (Fig. 4. Monitoring of VWF levels showed a similar evolution to that of IL-11 in GBS patients (Fig. 5).

Discussion

Our experimental and observational study showed, first, that IVIg increases the blood levels of platelets, VWF and/or FVIII both in an ITP mouse model and in ITP patients; secondly, that these effects were mediated at least in part by IL-11 increase in mice. Additionally, this study described and for the first time, to our knowledge, aimed at quantifying in humans, that IVIg co-ordinately increased IL-11, FVIII and VWF levels in various diseases: ITP, AVWD, IM and GBS. To corroborate the direct beneficial effects of IL-11 in an ITP mouse model, we demonstrated that specific neutralization of IL-11 abrogated IVIg-induced increase in platelet and VWF levels. Moreover, IL-11 injection in mice elicited an even stronger effect than IVIg alone on FVIII levels.

However, the lack of a clear difference between plasma FVIII levels following IVIg alone and IVIg combined with anti-IL-11 antibody in the mouse ITP model (Fig. 1c)

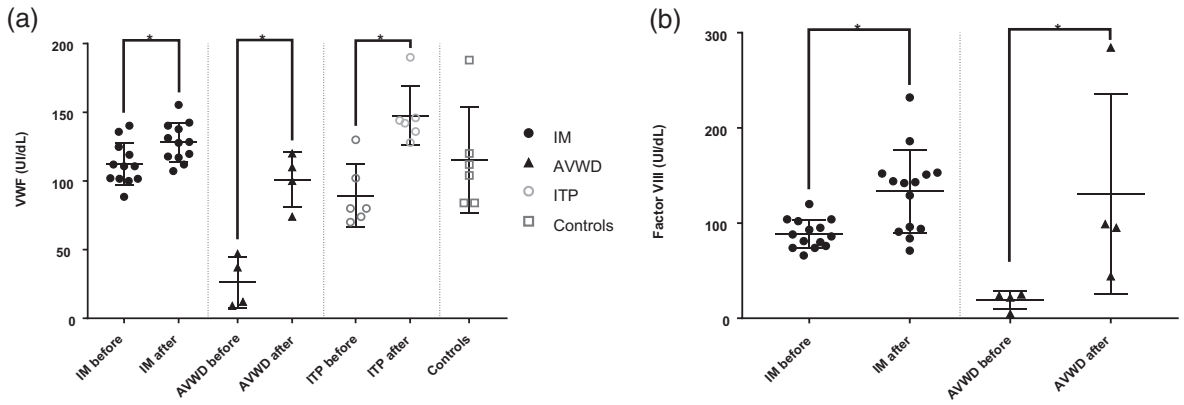

Fig. 3. Increase in the levels of von Willebrand factor (VWF) before and 72 h after intravenous immunoglobulin (IVIg) in inflammatory myopathies (IM, $n = 12$), immune thrombocytopenic purpura (ITP, $n = 6$), and acquired von Willebrand disease (AVWD, $n = 4$) patients and healthy controls (controls, $n = 6$). VWF and factor VIII (FVIII) levels were measured in the plasma of AVWD patients while VWF was measured in the serum of ITP patients by enzyme-linked immunosorbent assay (ELISA) before and 72 h following IVIg. Therefore, FVIII could not be measured in serum samples. In addition, VWF was measured in the plasma of six healthy donors. IVIg significantly increased the median levels of VWF (a) and FVIII (b), regardless of the underlying disease. $^{*}P < 0{\cdot}05$; $^{***}P < 0{\cdot}001$; by Student's *t*-test.

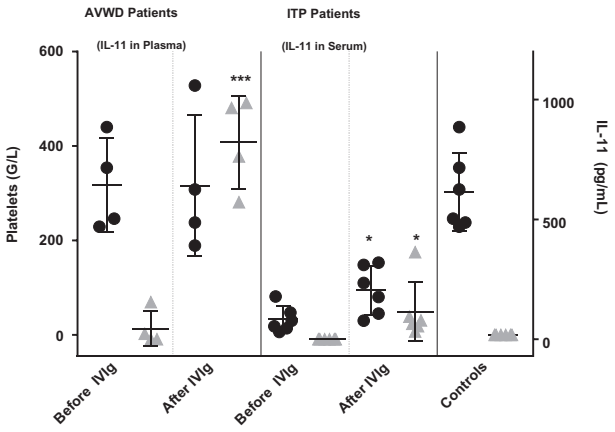

Fig. 4. Increase in the levels of von Willebrand factor (VWF), platelets and IL-11 in patients with acquired VWF or immune thrombocytopenia following intravenous immunoglobulin (IVIg). IL-11, VWF and platelet levels were measured in acquired VWF (AVWD, $n = 4$) and immune thrombocytopenia (ITP, $n = 6$) before and 72 h post-IVIg therapy. (a) IVIg significantly increased the levels of IL-11 in both AVWD and ITP patients and also the levels of platelets in ITP patients, whereas platelet levels did not exhibit significant changes in AVWD patients. (b) Similarly, both IL-11 and VWF levels significantly increased following IVIg in both patient groups. $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$; by Student's *t*-test.

may have several non-exclusive explanations: the amount of anti-IL-11 antibody required to completely abrogate the increase of FVIII may be higher than those required for platelets and VWF; the lesser the considered factor initial level, the lesser this last increases following IVIg; thus, as the FVIII level is not decreased in ITP, contrary to VWF, that has a partial platelet origin, FVIII-level enhancement following IVIg should be less pronounced than those of platelets and VWF whose level and activity are impaired in ITP. Finally, post-IVIg levels of IL-11 and VWF exhibited parallel evolution profiles in GBS patients with clear increases and decreases at 4 weeks, similar to what has been shown for platelets and VWF, respectively, in ITP and AVWD patients. A slow decrease in VWF level in GBS patients over time has several possible explanations: the normality of VWF levels and production before IVIg may allow only a mild enhancement in their increase following IVIg; consequently, VWF clearance is not increased in GBS. Conversely, in AVWD, their levels also may exhibit a slower decrease to their previous physiological values. Altogether, these results suggest that, regardless of the underlying disease, IVIg universally induces IL-11 and haemostatic factors increase in humans and mice. IL-11 increase following IVIg in humans was evoked but not quantified in the preliminary data of Lewis *et al.* [13].

IVIg is associated with an increased frequency of thrombosis [25]. This adverse effect may be explained by our results showing that IVIg increases the levels of platelet, VWF and FVIII via an increase in IL-11 levels. Of note, recombinant IL-11 (Oprevelkin), which is licensed in the United States for the prevention of severe thrombocytopenia following myeloablative chemotherapy, results in a twofold increase in plasma fibrinogen and an increase in the VWF concentration with a normal multimer pattern, among possible side effects in healthy subjects [26,27]. As found in our study and others, IL-11 is undetectable in healthy humans at steady state

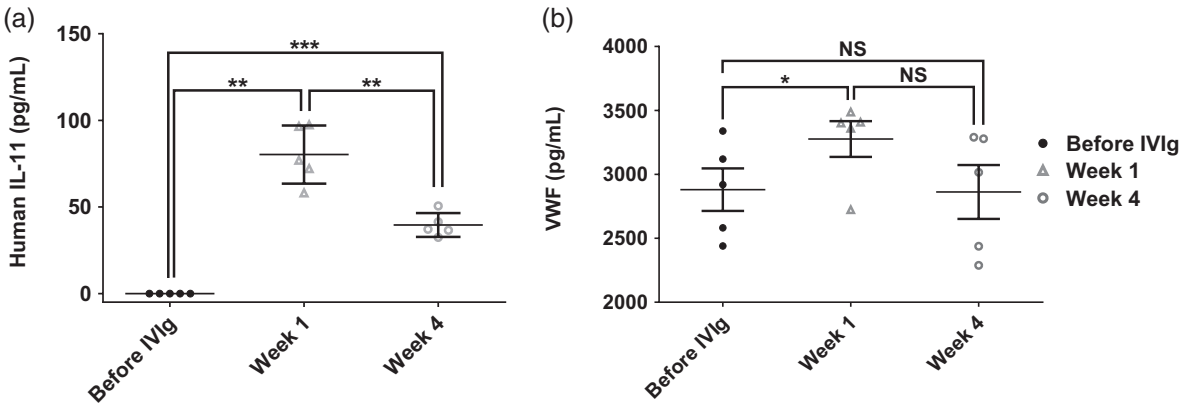

Fig. 5. Parallel kinetics of interleukin (IL)-11 and von Willebrand factor (VWF) levels following intravenous immunoglobulin (IVIg): assessment before and beyond the half-life of infused IgG in serum of Guillain–Barré syndrome (GBS) patients. IL-11 was quantified in the serum of five GBS patients before at 1 and 4 weeks following IVIg. (a) IL-11 from GBS patients was undetectable in serum before IVIg, then its levels significantly increased at week 1 and significantly decreased at week 4, remaining at a detectable level. (b) VWF levels exhibited an evolution parallel to those of IL-11. Initial normal levels of VWF at baseline increased following IVIg, then decreased to baseline values 4 weeks later. $^{*}P < 0{\cdot}05$; n.s. = not significant; by Student's *t*-test.

[8,9] and in IVIg-naive patients. Therefore, as IVIg is purified from the blood of healthy human volunteers, the resulting products are not expected to contain significant amounts of IL-11 and suggest the production of IL-11 following IVIg.

The immunomodulatory effects of IVIg probably result from several non-exclusive and co-ordinated mechanisms [28,29]. In the various inflammatory conditions treated with IVIg, including IM and GBS, the immunomodulatory actions gradually increase, with cumulative therapeutic effects from successive infusions, modifying the natural history of the disease. Conversely, in ITP or AVWD, IVIg is only suspensive, requiring indefinite infusions every 3–4 weeks [28,30]. We hypothesize that such variability in the strength of IL-11-mediated increase in VWF or platelet levels following IVIg probably depends upon TPO levels in low platelet count situations or pre-IVIg levels for VWF [9]. Indeed, Dams-Kozlowska *et al*., based on their data and those of Neben *et al*. and Turner *et al*., concluded that IL-11, which is not detectable at baseline in serum, acts synergistically with TPO to increase/enhance the number and the maturation of megakaryocytic progenitors [31–33].

The main limitation of our study is related to its retrospective design, resulting in missing late blood parameters data to assess the duration of IVIg effects. Moreover, the comparative use of knock-out mouse models for IL-11 and IL-11Rα should help to confirm these data. Furthermore, the intimate molecular/cellular mechanisms of the effects of IVIg on IL-11 production, as well as those of IL-11 on the increase in VWF and FVIII levels, remain to be elucidated. Indeed, it would be interesting to study the mechanism of IL-11 production in cell cultures, including mesenchymal stem cells. Moreover, for VWF and FVIII, either over-production or a simple increase in endothelial/platelet release are possible, especially considering that the former has a protective effect on the blood level of the latter. Of note, in our study the levels of IL-11 were more elevated in plasma than in serum, implying some heterogeneity that should be considered in future studies.

Reports have demonstrated isolated Fc-fragment show efficacy in some ITP patients and mouse models [34]. Therefore, future studies should investigate if this treatment could mediate IL-11 increase in mice and humans.

In conclusion, in addition to the multiple immunomodulatory mechanisms of IVIg, this study shows in mice and, for the first time, aimed at quantifying in humans, the increase in IL-11 blood levels following IVIg, which acts secondarily as a stimulating/growth factor to increase the levels of platelets, VWF and, to a lesser extent, FVIII. These preliminary results shed new light on IL-11 as a mechanistic missing link between IVIg and the timing of the stereotypical increase/decrease in these haemostatic factors. Therefore, rIL-11 may represent a promising alternative to IVIg, especially in the context of the worldwide shortage of IVIg products, to overcome the insufficient compensatory production of platelets or VWF in the corresponding autoimmune bleeding disorders, in addition to immunosuppressant when required. Moreover, IVIg use to treat inflammatory rheumatisms, associated or not with ITP and/or anti-phospholipid syndrome, could include risk due to the combining of several prothrombotic factors. Therefore, this study opens perspectives to monitor IL-11/TPO blood ratios concomitantly with levels of haemostatic factors in ITP and AVWD patients treated with IVIg.

Finally, confirmation of these results should lead to interventional trials assessing rIL-11, with and without TPO-RA in ITP, or in AVWD.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors. We are grateful to the patients and physicians from the Department of Internal Medicine and Clinical Immunology in Timone University Hospital, Pitié-Salpétrière Hospital, Hôpital Universitaire Limoges and Caen University Hospital who contributed to patient material. We also thank the staff of Centre d'Explorations Fonctionnelles (CEF) at Centre de Recherche des Cordeliers for their help.

Disclosures

There are no competing interests for any author.

Author contributions

A. A. conceived the overall design of the study and directed the human portion of the study; J. B. conceived and directed the animal portion of the study; A. N., Y. R. and A. K. performed biological assays in humans; A. N., J. B. and A. A. drafted the manuscript and the manuscript was approved by all co-authors; A. N., S. D. S. L.-D. and J. B. co-ordinated mouse experiments and engineered biological testing for FVIII ELISA; M. E., Y. A., O. B., J. M. V. and L. M. provided data and samples from patients with immune thrombocytopenic purpura, inflammatory myopathies and GBS; Y. R., S. D from Caen, G. M. and H. d. B provided data and samples from patients with acquired von Willebrand disease and contributed to draft optimization and use of statistical tests.

Data Availability Statement

All data relevant to the study are included in the article or uploaded as Supporting information. Some data are available upon reasonable request. Some data may be obtained from a third party and are not publicly available.

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