A highly purified form of staphylococcal protein A alleviates murine immune thrombocytopenia (ITP)

Immune thrombocytopenia (ITP) is an autoimmune disorder where anti-platelet antibodies and/or T cells specifically target platelets, leading to peripheral platelet destruction and decreased bone marrow platelet production by megakaryocytes (Semple *et al*, 2011; Zufferey *et al*, 2017). Although the pathogenesis is highly complex, incompletely understood and multifactorial (Zufferey *et al*, 2017), one of the key-mechanisms is phagocytosis of immunoglobulin (Ig)G coated-platelets by Fcγ receptor (FcγR)-bearing macrophages in the spleen, resulting in thrombocytopenia and clinical complications of bruising and bleeding (Semple *et al*, 2011; Zufferey *et al*, 2017). First line treatment for ITP is aimed at the inhibition of the anti-platelet antibodies and breakdown of platelets. If ineffective, other treatments can be considered, such as immune suppression or splenectomy or treatments stimulating platelet production by megakaryocytes (Provan *et al*, 2010; Zufferey *et al*, 2017). Intravenous immunoglobulin (IVIg) is an effective first-line treatment for ITP (Provan *et al*, 2010), but it is associated with high cost, limited supply and risk of adverse effects, such as headaches, febrile reactions and haemolysis. Staphylococcal protein A (SpA) is an immune-modulating virulence protein produced by many strains of *Staphylococcus aureus* (Foster, 2005). It affects a number of immune processes and binds with high affinity to the IgG Fc region and forms discrete immune complexes *in vitro* (Okano *et al*, 2015). PRTX-100 is a highly purified form of SpA that is prepared from *S. aureus* Strain A676 using Good Manufacturing Practices. Early-phase clinical studies in patients with rheumatoid arthritis, an autoimmune disease predominantly affecting joints, showed that PRTX-100 had positive effects on measures of disease activity (Bernton *et al*, 2014). ITP is also an autoimmune disorder involving platelets and it was suggested that PRTX-100 may perhaps be able to inhibit platelet phagocytosis by macrophages *in vitro* (Bernton *et al*, 2014). If PRTX-100 is able to rescue ITP *in vivo* remains unstudied.

We therefore investigated the efficacy of PRTX-100 *in vivo* using a well-established murine model of ITP, which harbours both the antibody-mediated as well as the T cell component (Chow *et al*, 2010). In this active model of ITP, splenocytes from CD61-knockout (CD61-KO) mice immunized against $CD61^+$ platelets were transferred into severe combined immunodeficient (SCID, $CD61^+$) mice. Platelet counts were determined before splenocyte transfer and weekly thereafter. Groups of SCID mice were treated twice weekly with either phosphate-buffered saline (PBS), vehicle control, IVIg (2 g/kg injected intraperitoneally [ip]) or by intravenous (iv) infusion of PRTX-100 (250 μg/kg). Blood was collected weekly via the saphenous vein into PBS:citrate-phosphate-dextrose-adenine (CPDA) solution and platelet counts were measured by a Beckman Coulter Counter-LH750 haematology analyser (Brea, CA). Four weeks after splenocyte transfer, the efficacy of IVIg and PRTX-100 in raising platelet counts was evaluated.

We found that, compared with control SCID mice engrafted with naïve splenocytes (Fig 1, column A, platelet counts 886 ± standard deviation [SD] 242 × 10^9/l), mice receiving splenocytes from immune CD61-KO mice suffered from significant thrombocytopenia at 2, 3 and 4 weeks after splenocyte transfer (Fig 1, column B, platelet counts 173 ± SD 141, 150 ± SD 136 and 185 ± SD 149 × 10^9/l, at 2, 3 and 4 weeks after splenocyte transfer, respectively), confirming the induction of thrombocytopenia in our model. Twice weekly treatment with IVIg 2 g/kg in mice receiving CD61-KO splenocytes rescued platelet counts at 2, 3 and 4 weeks after splenocyte transfer (Fig 1, column C, platelet counts 470 ± SD 246, 595 ± SD 229 and 739 ± SD 273 × 10^9/l, at 2, 3 and 4 weeks after splenocyte transfer, respectively). Weekly treatment with PRTX-100 with 250 μg/kg also significantly rescued platelet counts at 3 and 4 weeks after splenocyte transfer (Fig 1, column D, 436 ± SD 175 and 619 ± SD 202 × 10^9/l, at 3 and 4 weeks after splenocyte transfer, respectively). No adverse effects were observed in the IVIg or PRTX-100 treated mice.

The mechanisms by which IVIg prevents platelet clearance in ITP are not fully understood but several modes of action have been suggested (Galeotti *et al*, 2017). PRTX-100, which alleviated murine ITP similarly to IVIg, has a number of immunomodulatory effects. Its ability to form discrete immune complexes with endogenous IgG may allow it to serve as an IVIg-mimetic in the treatment of ITP. Of interest, recent work has suggested that SpA may actually disrupt immunity by interfering with long-lived plasma cells (Keener *et al*, 2017). The exact mechanism by which PRTX-100 is ameliorating ITP in our murine ITP model is unknown but we are currently studying this.

In summary, PRTX-100 effectively raised platelet counts in a murine model of ITP. In addition, PRTX-100 has demonstrated an acceptable safety profile in initial cohorts of two dose escalation clinical trials (3 patients with persistent/chronic ITP in each study), with a platelet response

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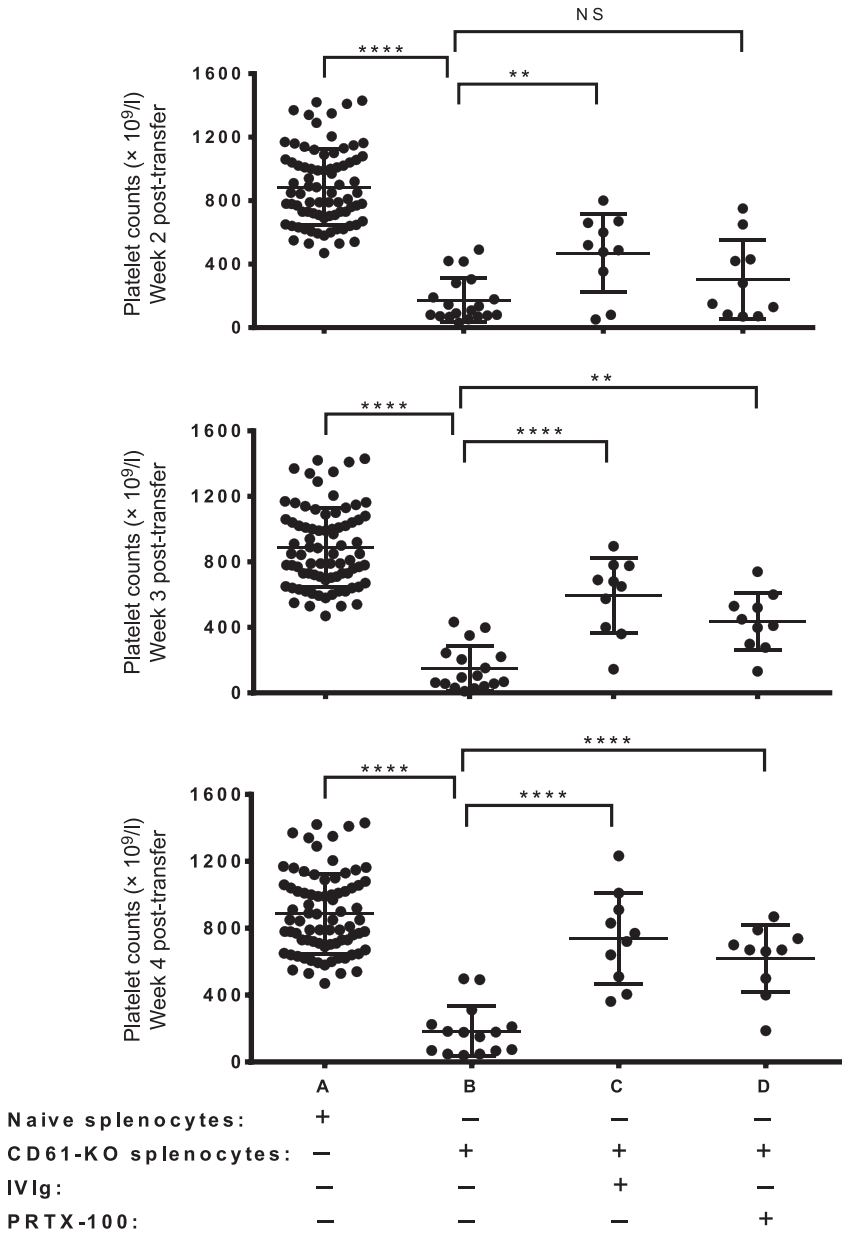

Fig 1. PRTX-100 and IVIg ameliorate platelet counts in an active murine ITP model. Thrombocytopenia in irradiated SCID mice transferred with 4×10^4 splenocytes from non-immune CD61-KO mice (column A, $n = 79$) or SCID mice transferred with 4×10^4 immune splenocytes from CD61-KO mice immunized against wild type BALB/c platelets (column B, $n = 19, 17, 15$ for 2, 3, 4 weeks after splenocyte transfer, respectively) with treatment with twice weekly IVIg 2 g/kg intraperitoneally (column C, $n = 10$) or PRTX-100 250 μg/kg intravenously (column D, $n = 10$) (Fig 1). Platelet counts were evaluated 2 (top panel), 3 (middle panel) and 4 (lower panel) weeks after splenocyte transfer. Statistical comparisons were made using one-way analysis of variance with Dunnett's multiple comparison test. $^{**}P < 0{\cdot}01$, $^{****}P < 0{\cdot}0001$, NS, not significant. Error bars represent standard deviations (SD).

observed in a patient treated with a low dose of PRTX-100 (Bussel *et al*, 2016). Further testing of PRTX-100 with higher dose cohorts in these clinical trials is currently ongoing. PRTX-100 has high potential to be effective in treating human ITP.

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Author contributions

R.K. and M.D.C. designed research, analysed and interpreted data, performed statistical analyses, made the figure, wrote and edited the paper; R.A., E.R.S. performed experiments and collected data; R.F.F. designed research, interpreted data and edited the manuscript; J.W.S. provided financial resources, designed research, analysed and interpreted data and edited the manuscript.

Conflicts of interest

M.D.C. and R.F.F. are paid consultants of Protalex Inc. The remaining authors declare no financial conflicts of interest.

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