Brief report

A role for IL-1 receptor antagonist or other cytokines in the acute therapeutic effects of IVIg?

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The exact mechanism of action of IVIg in the amelioration of immune thrombocytopenic purpura (ITP) is still unclear. Studies have suggested that IVIg may function through the regulation of cytokines, including interleukin-1 receptor antagonist (IL-1Ra), an inhibitor of phagocytosis. Using a mouse model relevant to ITP, we confirm an increase in mouse serum levels of IL-1Ra after exposure to IVIg, yet a recombinant IL-1Ra did not ameliorate thrombocytopenia. IVIg has also been shown to affect the expression of other regulatory cytokines. We have also recently established that IVIg specifically targets activating FcγRs on CD11c$^+$ dendritic cells (DCs) as its primary mechanism of action in the amelioration of murine ITP. Herein, we show that IVIg functions therapeutically in mice lacking specific cytokines or their receptors that can potentially affect DC/macrophage function (IL-1 receptor, IL-4, IL-10, IL-12β, TNF-α, IFN-γ receptor, MIP-1α). This suggests that while IVIg may mediate the release of a variety of cytokines, the cytokines tested do not directly participate in the mechanism of IVIg action. (Blood. 2007;109:155-158)

Introduction

The exact mechanism of action of IVIg in the treatment of autoimmune diseases such as immune thrombocytopenic purpura (ITP) remains unresolved. Proposed modes of action include Fc-receptor blockade, inhibitory effects mediated by FcγRIIB, immunomodulation (reviewed in Bayry et al[1] and Crow and Lazarus[2]), and the recent finding that IVIg specifically targets activating FcγRs on CD11c$^+$ dendritic cells (DCs) in a mouse model of ITP.[3] Modified cytokine production may play a role in the pathogenesis of autoimmune diseases such as ITP (reviewed in Zhou et al[4]), and thus cytokine modulation by IVIg might contribute to the reversal of thrombocytopenia. Indeed, studies have demonstrated modulation of numerous proinflammatory and anti-inflammatory cytokines both in vivo and in vitro after exposure to IVIg.[5-7] Work by Aukrust et al[7] has demonstrated an increase in IL-6, -8, and TNF-α within 1 hour of IVIg administration.

Both IVIg[7,8] and anti-D[9] have been shown to induce the production of IL-1 receptor antagonist (IL-1Ra), a potent anti-inflammatory cytokine that can counteract the inflammatory effects of IL-1.[10] IVIg can also induce secretion of IL-1Ra in human monocytes,[11] and administration of IL-1Ra can successfully inhibit the phagocytosis of anti-D–opsonized red blood cells (RBCs).[12] In addition, IL-1Ra can also regulate the activated state of DCs.[13]

IVIg can also inhibit the differentiation/maturation of human DCs and abrogate their production of IL-12 while increasing production of the anti-inflammatory cytokine IL-10.[14] The exact role that cytokines play in the therapeutic action of IVIg, however, remains unresolved. Using a mouse model of ITP,[15] we have analyzed the therapeutic effect of IVIg (Gamimune) in mice lacking specific cytokines/receptors that can potentially affect DC and/or macrophage function (IL-1R, IL-4, IL-10, IL-12β, TNF-α, IFN-γR, MIP-1α), or in mice lacking the common cytokine receptor γ chain (required for signal transduction through the receptors for IL-2, -4, -7, -9, -15, and -21). We present data that suggest that while cytokine modulation may play some role clinically in the long-term effects of IVIg in humans with true ITP, the key cytokines tested were not required for the acute protective effect of IVIg in murine ITP.

Materials and methods

Reagents

The IVIg used was from Bayer (Elkhart, IN). The anti–integrin α_{IIb} antibody (clone MWReg30) was from PharMingen (Mississauga, ON, Canada). Recombinant IL-1Ra (anakinra) was from Amgen Canada (Mississauga, ON, Canada). The murine IL-1Ra enzyme-linked immunosorbent assay (ELISA) kit was from R&D Systems (Minneapolis, MN).

Mice

Mice deficient in IL-1R (B6.129S7-*Il1r1*^{tm1Imx}), IL-4 (B6.129P2-*Il4*^{tm1Cgn}), IL-10 (B10.129P2(B6)-*Il10*^{tm1Cgn}), IL-12β (B6.129S1-*Il12b*^{tm1Jm}), TNF-α (B6;129S6-*Tnf*^{tm1Gkl}), IFN-γR (B6.129S7-*Ifngr*^{tm1Agt}), MIP-1α (B6.129P2-*Ccl3*^{tm1Unc}), or common cytokine receptor γ chain (B6.129S4-*Il2rg*$^{tm1Wjl/J}$) and appropriate control mice were all purchased from The Jackson Laboratory (Bar Harbor, ME). CD1 mice were purchased from Charles River Laboratories (St Constant, PQ, Canada). Mice were housed in the St Michael's Hospital Research Vivarium.

Induction and reversal of murine ITP

ITP was induced and treated as previously described.[15] Briefly, mice were injected daily (days 0-3) with 2 μg antiplatelet antibody to induce

Submitted May 22, 2006; accepted August 14, 2006. Prepublished online as *Blood* First Edition Paper, September 5, 2006; DOI 10.1182/blood-2006-05-023796.

An Inside *Blood* analysis of this article appears at the front of this issue.

The online version of this article contains a data supplement.

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thrombocytopenia; platelets were enumerated by flow cytometry as described.[15] Mice received 50 mg IVIg (generally equivalent to 2 g/kg body weight) intraperitoneally on day 2.

Detection of serum IL-1Ra

C57BL/6 mice were bled for serum at the indicated times after injection of IVIg. Murine IL-1Ra was detected by ELISA, according to the manufacturer's directions.

Statistics

Statistics were calculated according to Student *t* test and are presented as mean ± SEM. Note, in some cases where the error bars are not apparent, this is due to a minimal SEM.

Results and discussion

To determine the potential role that IL-1Ra may play in the amelioration of murine ITP by IVIg, we first questioned if IVIg could increase the levels of IL-1Ra in mice. Analysis of mouse serum taken at 0, 1, 6, 24, and 48 hours after administration of IVIg revealed that IVIg did indeed induce the production of IL-1Ra, reaching peak levels at 24 hours, the time in which platelet recovery is readily observed in our model (Figure 1A). This finding is consistent with the possibility that IVIg-induced IL-1Ra might play a role in the amelioration of thrombocytopenia.

Due to the fact that deletion of the IL-1 receptor (IL-1R) gene is epistatic to IL-1Ra gene deletion,[16] we next questioned if IVIg would function in mice lacking the IL-1R, a ligand for IL-1Ra, by using IL-1R–deficient mice. IL-1R–deficient mice and control mice were rendered thrombocytopenic and treated with IVIg (Figure 1B). Platelet enumeration revealed that both wild-type mice and IL-1R–deficient mice responded to IVIg treatment, suggesting that IVIg function is not dependent on the presence of IL-1Ra. This result also implies that IVIg function is not reliant on the presence of IL-1, a potent proinflammatory cytokine that can also promote DC maturation.[17] In addition, we found that a monoclonal antibody that reacts with RBCs (TER-119) and mimics the action of anti-D in the amelioration of murine ITP[18,19] also induced the in vivo production of IL-1Ra in mice (Figure S1, available at the *Blood* website; see the Supplemental Figures link at the top of the online article). These data correlate with the IL-1Ra production observed in children with chronic ITP infused with anti-D.[9] However, similar to our findings with IVIg, this anti-RBC antibody also protected against thrombocytopenia in IL-1R–deficient mice (Figure S1). Thus, these findings do not support a role for IL-1Ra in the amelioration of murine ITP by either IVIg or TER-119.

In spite of these results, we nonetheless examined the ability of a recombinant IL-1Ra to reverse ITP or inhibit its induction. Initial experiments using a standard dose of IL-1Ra demonstrated that the administration of 50 μg of recombinant IL-1Ra to thrombocytopenic mice had no effect on thrombocytopenia (Figure 1C). Mice injected with a supraphysiologic dose (1 mg) of IL-1Ra on 2 consecutive days also showed no increase in the platelet count (Figure 1D). In addition, pretreatment of mice with 1 mg IL-1Ra did not prevent the induction of thrombocytopenia (Figure 1E). Thus there does not appear to be any direct role for IL-1Ra in the amelioration of murine thrombocytopenia. However, since IL-1Ra is produced during inflammatory responses,[10] the elevated IL-1Ra seen in mice after IVIg administration is hypothesized to be more of a marker of the inflammatory process rather than having a role in reversing thrombocytopenia.

Modulation of serum cytokines has been reported in patients exposed to IVIg.[7,20-22] Pertinent to ITP, a recent study has demonstrated increases in IL-10 and MCP-1 in ITP patients after IVIg treatment.[5] IVIg can also inhibit the differentiation/maturation of human DCs and abrogate their production of IL-12.[14] IVIg has also demonstrated the ability to inhibit TNF-α production by immature DCs.[14]

To address the potential role that selected cytokines may play in the action of IVIg, we analyzed the treatment of thrombocytopenia in mice lacking key proinflammatory regulators that could potentially affect DC or macrophage function (Figure 2A). Mice lacking

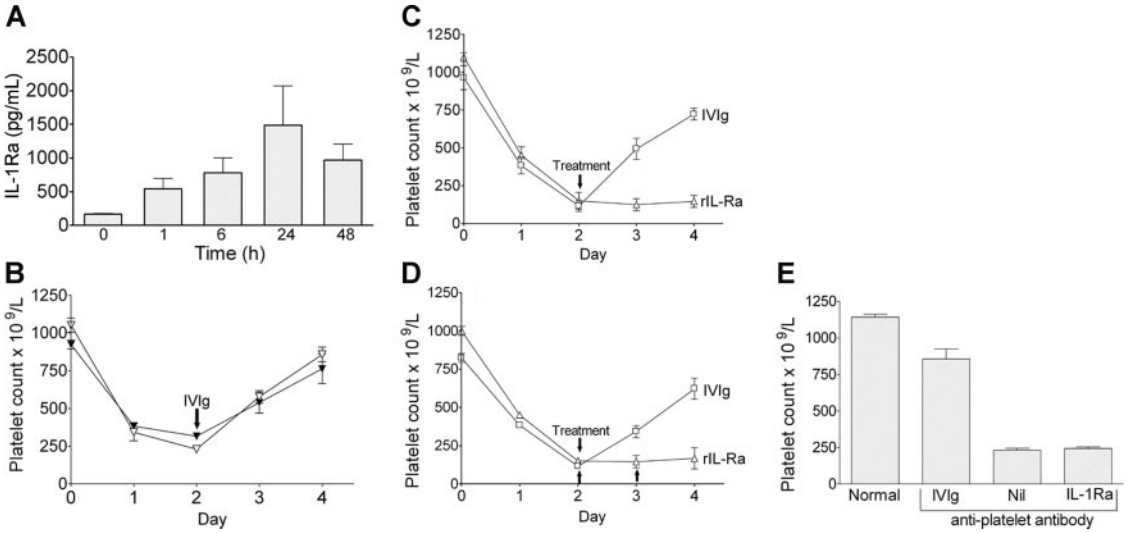

Figure 1. IL-1Ra does not play a role in the acute effects of IVIg. (A) C57BL/6 mice were injected with 50 mg IVIg and each mouse was bled at the times indicated on the x-axis. Sera were assessed for IL-1Ra by ELISA. Values are expressed in pg IL-1Ra/mL on the y-axis. Data are expressed as mean ± SEM; n = 4 mice from 2 separate experiments. (B) C57BL/6 mice and IL-1R–deficient mice were injected on days 0 to 3 with antiplatelet antibody to induce thrombocytopenia. On day 2, mice received 50 mg IVIg (arrow). Mice were bled daily for platelet enumeration by flow cytometry just prior to antiplatelet antibody injection. C57BL/6 mice (▼), IL-1R–deficient mice (▽); n = 6 mice per each group. (C) CD1 mice were injected with antiplatelet antibody and assessed as in panel B, and mice received either 50 mg IVIg on day 2 (□; arrow) or 50 μg rIL-1Ra on day 2 (△); n = 6 mice per each group. (D) Mice were injected with antiplatelet antibody and received either 50 mg IVIg on day 2 (□; down arrow) or 1 mg rIL-1Ra on days 2 and 3 (△; up arrows); n = 6 mice per each group. (E) CD1 mice were pretreated with 50 mg IVIg (column 2), nothing (column 3), or 1 mg rIL-1Ra (column 4) on day 0. On day 1, antiplatelet antibody was given as indicated. On day 2, all mice were bled for platelet counts; n = 6 mice per each group.

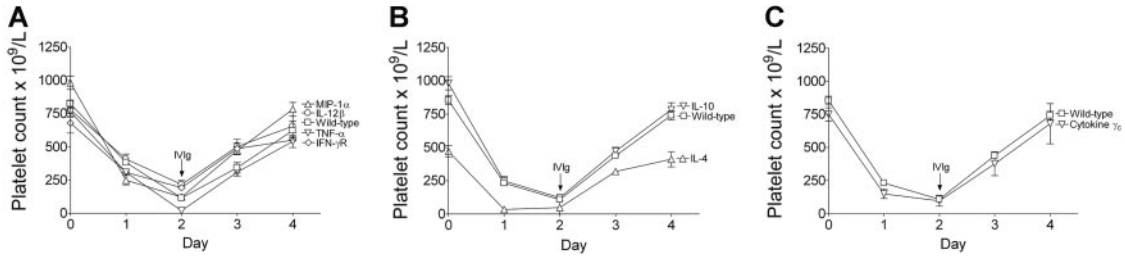

Figure 2. Mice lacking key inflammatory or anti-inflammatory cytokines respond normally to IVIg treatment of ITP. Control mice (wild-type) and mice lacking the indicated specific proinflammatory cytokines (A), anti-inflammatory cytokines (B), or mice genetically lacking the common cytokine receptor γ chain (C) were rendered thrombocytopenic by daily injection (days 0-3) of antiplatelet antibody as in Figure 1B. On day 2, all mice received 50 mg IVIg (arrow). Mice were bled daily just prior to antiplatelet antibody injection and platelets enumerated as in Figure 1B. The y-axis represents platelet counts; the x-axis, the length of the experiment in days; n = 6 mice per each strain.

TNF-α, IL-12β, MIP-1α, or IFN-γR were rendered thrombocytopenic as in Figure 1B, and all mice fully responded to IVIg treatment despite the lack of individual expression of these cytokines.

The anti-inflammatory cytokine IL-10 can play an important role in dampening macrophage activation,[23] and its production by DCs is up-regulated in response to IVIg in vitro.[14] The anti-inflammatory cytokine IL-4 can induce the production of IL-1Ra[24] and up-regulate expression of the inhibitory IgG receptor FcγRIIB,[25] which is absolutely required for IVIg to ameliorate murine models of ITP.[26,27] We found, however, that IVIg did function therapeutically in IL-10–deficient mice as well as in IL-4–deficient mice (Figure 2B).

Mice deficient for the common cytokine receptor γ chain, which is required for signal transduction through the receptors for IL-2, -4, -7, -9, -15, and -21, responded to IVIg treatment (Figure 2C), which suggests that IVIg function is not reliant upon the expression of these cytokines.

The immunoregulatory compound nitric oxide (NO) can be induced by IL-1,[28] and NO's production in murine macrophages by IFN-γ can be inhibited by IL-10.[29] NO can also be positively and negatively regulated by IVIg.[30,31] Based upon this, we also analyzed IVIg-mediated amelioration of thrombocytopenia in mice that were subjected to aminoguanidine, a specific inhibitor of iNOS, or L-NAME, a multi-spectrum NOS inhibitor (Figure S2). We found that after inhibition of iNOS (aminoguanidine), or of iNOS, eNOS, and nNOS (L-NAME), IVIg successfully ameliorated murine ITP, suggesting that NO is not required for IVIg to reverse murine ITP.

IVIg does indeed induce/suppress the production of multiple cytokines, and these cytokines may, for example, affect the synthesis of the antiplatelet antibody in humans with true ITP, an event not captured in our acute murine model. Thus while we demonstrate that the acute effects of IVIg do not require the presence of the individual cytokines tested, some of these cytokines may nevertheless play a role in the long-term effects of IVIg in humans with ITP.

Acknowledgments

We thank Mr Davor Brinc, Mr Hoang Le-Tien, and Dr Vinayakumar Siragam for assistance and helpful discussion, and the St Michael's Hospital Research Vivarium staff.

This work was supported by the Bayer–Canadian Blood Services–Héma Québec Partnership Fund.

Authorship

Contribution: A.R.C. designed research, performed research, analyzed data, and wrote the paper; S.S. performed research and analyzed data; J.W.S. designed research and edited the manuscript; J.F. contributed analytical tools and edited the manuscript; and A.H.L. designed research, analyzed data, and wrote the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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