IVIg-mediated amelioration of murine ITP via FcγRIIB is independent of SHIP1, SHP-1, and Btk activity

Andrew R. Crow, Seng Song, John Freedman, Cheryl D. Helgason, R. Keith Humphries, Katherine A. Siminovitch, and Alan H. Lazarus

It has been established that amelioration of murine immune thrombocytopenia purpura (ITP) by IVIg is dependent on the inhibitory receptor FcγRIIB. Co-cross-linking of the FcγRIIB with the B-cell receptor complex or with FceRI in mast cells results in cell inhibition, which is mediated by recruitment of the inositol phosphatase SHIP1 to the cytoplasmic tail of the FcγR. The FcγRIIB can also associate with protein tyrosine phosphatase SHP-1 as a potential secondary target of the receptor. Alternatively, homooaggregation of FcγRIIB can induce a proapoptotic state in B cells that is dependent on the presence of Bruton tyrosine kinase (Btk), a kinase also expressed in monocytes. We sought to determine if these signaling pathways may direct IVIg-mediated FcγRIIB-dependent regulation of in vivo monocyte function in a murine model of ITP in which IVIg functions in an FcγRIIB-dependent manner. We demonstrate that mice deficient in SHIP1, SHP-1, and Btk respond to the ameliorating effects of IVIg with the same kinetics as control mice. We conclude that IVIg-mediated inhibitory pathways operating via monocyte FcγRIIB may involve a transmembrane signaling pathway different from that of B cells. (Blood. 2003;102:558-560)

Introduction

The inhibitory low-affinity receptor for immunoglobulin G (IgG), FcγRIIB, is widely expressed by hematopoietic cells such as B cells, mast cells, and monocytes. Two main FcγRIIB-mediated inhibitory signaling pathways in B cells have been identified.

The first pathway is initiated by co-cross-linking of the FcγRIIB with the B-cell receptor complex, which leads to a dominant-negative signal, resulting in cell inactivation. This event delivers a signal through a motif in the cytoplasmic tail of FcγRIIB, the immunoreceptor tyrosine-based inhibitory motif (ITIM; for a review, see Ravetch and Lanier1). SHIP1 binds directly, via its SH2 domain, to the phosphorylated receptor ITIM of FcγRIIB. This same inhibitory pathway also blocks antigen-induced mast cell degranulation by recruitment of SHIP1 to the ITIM via co-cross-linking of FcγRIIB with the activating high-affinity IgE receptor, FcεRI.2,3 Evidence also indicates that ITIM-dependent FcγRIIB-mediated immune suppression could involve the tyrosine phosphatase SHP-1.4,5

The second major inhibitory pathway mediated by FcγRIIB in B cells is ITIM independent and involves immune complex–mediated homooaggregation of FcγRIIB.6 This event leads to delivery of a proapoptotic signal to the B cell. It has been shown that signaling via FcγRIIB homooaggregation is dependent on the presence of Bruton tyrosine kinase (Btk), a B-cell kinase also expressed in monocytes.9

We10 and others11,12 have shown that intravenous immunoglobulin (IVIg) can successfully ameliorate thrombocytopenia in a murine model of immune thrombocytopenia purpura (ITP), a disease state that is mediated by monocyte phagocytosis of opsonized platelets.13,14 Samuelsson et al15 recently demonstrated that the acute activity of IVIg in preventing murine ITP requires the expression of FcγRIIB, and we have shown that the activity of IVIg in treating murine ITP is not dependent on the presence of B or T cells, nor anti-idiotype reactivity of IVIg.10 To determine if the FcγRIIB-dependent activity of IVIg uses the ITIM-dependent inhibitory pathway (SHIP1/SHP-1) versus the homooaggregation (Btk)–dependent signaling pathway, we used mice genetically deficient for these key signaling molecules. We found that FcγRIIB knockout (KO) mice rendered thrombocytopenic were refractory to IVIg treatment. Mice lacking FcγRIIB-dependent signaling mediators including SHIP1, SHP-1, and Btk, however, all responded to IVIg treatment, indicating that either the known FcγRIIB signaling pathways are not used by IVIg for its effect or that monocytes use another inhibitory pathway via FcγRIIB.

Study design

Mice

Wild-type, heterozygous, and homozygous SHIP1 KO mice were as previously described15 and bred at the British Columbia Cancer Agency (Vancouver, BC, Canada). SHP-1–deficient motheaten (me/me) mice and...
control littermates were bred at the Mt Sinai Hospital Samuel Lunenfeld Research Institute (Toronto, ON, Canada). Btk KO mice, FcγRIIB KO mice, and C57BL/6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME).

Induction and reversal of ITP

Thrombocytopenia was induced by intraperitoneal injection of 2 μg rat antimouse integrin αIIb antibody (PharMingen, Mississauga, ON, Canada) as previously described.10 The following day, mice were injected with 2 g/kg human serum albumin (HSA) control protein, followed by 2 g/kg IVlg (Bayer, Elkhart, IN) on day 2. Platelet counts were assessed as previously described.10

Results and discussion

It is well accepted that inhibition of cell function through the inhibitory receptor FcγRIIB requires recruitment of the inositol phosphatase SHIP1 to the receptor ITIM in both B cells1,3,16,18 and mast cells.2,4 The expression of FcγRIIB on monocytes can lead to the down-regulation of phagocytosis,20 although the pathway by which FcγRIIB down-modulates monocyte function remains unclear. To better understand this inhibitory event in vivo, we examined IVlg-mediated amelioration of ITP, which uses FcγRIIB to alleviate thrombocytopenia.11 ITP was the first autoimmune disease successfully treated with IVlg and IVlg is currently used to treat many autoimmune diseases.21-23 We have shown that IVlg can successfully ameliorate a murine model of antibody-induced thrombocytopenia with similar doses and kinetics as in humans treated with ITP.24 We investigated the signaling mediators used by IVlg through analyzing IVlg treatment of ITP in mice genetically deficient for primary signaling molecules juxtaposed to FcγRIIB.

Mice injected with antplatelet antibody became thrombocytopenic by day 1 after injection (Figure 1A). Treatment of mice with 2 g/kg HSA (control protein) did not affect ITP. However, treatment of mice with 2 g/kg IVlg successfully reversed the disease in wild-type mice (Figure 1A). In separate experiments, thrombocytopenic wild-type mice treated with HSA alone for the duration of the experiment displayed no increase in platelet count (data not shown). In contrast to wild-type mice, IVlg had no effect in FcγRIIB KO mice (Figure 1A); platelet counts in these mice remained low throughout the course of the experiment. These findings confirm that in our model system, the activity of IVlg in treating ITP is completely dependent on FcγRIIB expression. In addition, the complete lack of any elevation in platelet counts in

Figure 1. IVlg requires expression of FcγRIIB but not SHP1 to ameliorate murine ITP. (A) FcγRIIB KO (○) or control wild-type (●) mice (C57BL/6) were injected with 2 μg anti-integrin αIIb antibody on days 0, 1, 2, and 3. The arrow (↑) denotes injection of anti-integrin αIIb antibody. All mice received an injection of 2 g/kg HSA control protein on day 1, followed by 2 g/kg IVlg on day 2 (●●●). denotes injection of HSA or IVlg. Mice were bled daily for platelet enumeration. (B) SHP-1 heterozygous KO (○) and homozygous KO (●) mice and littermate control (●●●) mice were treated as described in panel A. Data are expressed as mean ± SEM; n = 10 mice/data point.

FCγRIIB KO mice following IVlg treatment (Figure 1A, day 2 versus days 3 and 4) suggests that “competitive” reticuloendothelial system (RES) blockade per se does not significantly contribute to IVlg-mediated amelioration of thrombocytopenia.

The inositol phosphatase SHIP1 preferentially binds the FcγRIIB ITIM and it has been established that SHIP1 is the primary negative signaling pathway used by FcγRIIB in B cells1,3,16,18 and mast cells.2,4 SHIP1 can negatively regulate FcγR-mediated phagocytosis24 and overexpression of SHIP1 in murine macrophages can inhibit phagocytosis.25 Thus, we surmised that SHIP1 expression might play a key role in monocyte FcγRIIB-dependent inhibition by IVlg and hypothesized that SHIP1 KO mice would be unresponsive to IVlg treatment. We found, however, that IVlg was able to exert its effects in both homozygous and heterozygous SHIP1 KO mice to the same extent as littermate controls (Figure 1B). Thus the FcγRIIB-dependent activity of IVlg in immune thrombocytopenia is not dependent on the expression or activity of SHIP1.

The tyrosine phosphatase SHP-1 can dephosphorylate multiple immunoreceptor-regulated substrates, leading to cell inactivation.5,6 SHP-1 has been shown to bind FcγRIIB under extreme conditions such as receptor superclustering2 or hyperphosphorylation.7 We examined whether this enzyme is a possible mediator in the pathway by which IVlg exerts its protective effect. Administration of IVlg to thrombocytopenic SHP-1–deficient mice resulted in an increase in platelet counts, indistinguishable from that of control littermates (Figure 2A). This suggests that like SHIP1, SHP-1 is not required for the FcγRIIB-mediated activity of IVlg. The FcγRIIB ITIM contains 2 distinct, albeit overlapping, binding sites for SHIPs and SHPs17; thus there exists the possibility that a redundancy may exist in the SHIP/SHP families. However, evidence suggests that FcγRIIB ITIM binds to SHIP proteins, but not SHP-1 or SHP-2 in vivo.26

Because these major known ITIM-dependent signaling intermediates did not appear to be required for activity of IVlg, we questioned whether IVlg therapy resulted in ITIM-independent FcγRIIB-mediated inhibition. This inhibitory pathway focuses on delivery of a proapoptotic signal generated through FcγRIIB homoaggregation27 and is dependent on the presence and expression of the Tec family kinase Btk8,9 a B-cell kinase also expressed in monocytes.9 IVlg has been shown to induce apoptosis in multiple cell types, including monocytes,28 IVlg ameliorated thrombocytopenia in both Btk KO mice and control mice (Figure 2B), indicating that the activity of IVlg is not dependent on a Btk-dependent proapoptotic event.

We demonstrate that FcγRIIB-dependent IVlg activity is not reliant on the established signaling pathways downstream of FcγRIIB. Mice lacking the normal FcγRIIB downstream signaling
mediators, SHIP1, SHP-1, or Btk, all responded to IVlg treatment of ITP as successfully as control mice. This suggests that inhibitory signaling through FcγRIIB on monocytes may use a different pathway than B cells. Indeed, a recent report has demonstrated that SHIP-dependent inhibition of monocyte function can occur independently of FcγRIIB, a mechanism distinct from that used by B-cell expressed FcγRIIB. It may be that inhibition of monocyte function by IVlg occurs via an FcγRIIB signaling pathway unique to monocytes.

References


Acknowledgments

We thank Ms Alison F Starkey, Mr Hoang Le-Tien, Mr Davor Brinc, and Dr Vinayakumar Saragam for assistance and helpful discussion; Dr John W Semple for critical review of the manuscript; and Ms Carolyn Bateman of the British Columbia Cancer Agency Joint Animal Facility, Ms Michele Deverill of the Samuel Lunenfeld Research Institute, and the St Michael’s Hospital Research Vivaria staff.