Can antibodies with specificity for soluble antigens mimic the therapeutic effects of intravenous IgG in the treatment of autoimmune disease?

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Intravenous Ig (IVIg) mediates protection from the effects of immune thrombocytopenic purpura (ITP) as well as numerous other autoimmune states; however, the active antibodies within IVIg are unknown. There is some evidence that antibodies specific for a cell-associated antigen on erythrocytes are responsible, at least in part, for the therapeutic effect of IVIg in ITP. Yet whether an IVIg directed to a soluble antigen can likewise be beneficial in ITP or other autoimmune diseases is also unknown. A murine model of ITP was used to determine the effectiveness of IgG specific to soluble antigens in treating immune thrombocytopenic purpura. Mice experimentally treated with soluble OVA + anti-OVA versus mice treated with OVA conjugated to rbcs (OVA-rbcs) + anti-OVA were compared. In both situations, mice were protected from ITP. Both these experimental therapeutic regimes acted in a complement-independent fashion and both also blocked reticuloendothelial function. In contrast to OVA-rbcs + anti-OVA, soluble OVA + anti-OVA (as well as IVIg) did not have any effect on thrombocytopenia in mice lacking the inhibitory receptor FcγRIIB (*FcγRIIB*$^{-/-}$ mice). Similarly, antibodies reactive with the endogenous soluble antigens albumin and transferrin also ameliorated ITP in an FcγRIIB-dependent manner. Finally, broadening the significance of these experiments was the finding that anti-albumin was protective in a K/BxN serum–induced arthritis model. We conclude that IgG antibodies directed to soluble antigens ameliorated 2 disparate IVIg-treatable autoimmune diseases.

Introduction

Immune thrombocytopenic purpura (ITP) is an autoimmune disease characterized by platelet clearance mediated by pathogenic antiplatelet antibodies (1–3). It is thought that this platelet clearance is mediated by Fcγ receptor–bearing (FcγR-bearing) macrophages in the reticuloendothelial system (RES) (4). While intravenous Ig (IVIg) is widely used in the treatment of ITP and other autoimmune/inflammatory diseases, its mechanism of action has not been fully elucidated. In murine models of ITP, it has been demonstrated that IVIg ameliorates ITP by a mechanism dependent upon the expression of the inhibitory FcγR FcγRIIB (5, 6). In addition, IVIg induces RES blockade (4, 7, 8); this "competitive" RES blockade has long been considered to be the primary mechanism whereby IVIg increases platelet counts in patients with ITP (4, 9, 10). We have previously found that IVIg (11) and some monoclonal mimetics of IVIg (12) can block murine RES function.

IVIg can potentially bind to a number of different cell surface or soluble antigens (13–21), and antibody specificities within IVIg may be responsible for different therapeutic effects through a variety of mechanisms (22–29). We undertook the present study to establish whether antibodies to soluble antigens could ameliorate ITP. In particular, IgGs targeted to either a soluble or a cell-bound antigen were compared in murine ITP. OVA was selected as the primary target antigen because it can be used in its soluble form or can be coupled to syngeneic rbcs (OVA-rbcs), and the same anti-OVA antibody can be used with both OVA and OVA-rbcs.

We demonstrate that, like IVIg, antibodies to soluble antigens can ameliorate ITP in an FcγRIIB- dependent manner. In addition, anti-albumin was protective for K/BxN serum–induced inflammatory arthritis (30, 31). Taken together, these new data demonstrate that IgG reactive with soluble antigens can mimic the therapeutic effects of IVIg in treating these 2 different autoimmune diseases.

Results

IgG reactive with a soluble antigen can ameliorate ITP. CD1 mice were injected with 1 mg soluble OVA that had been preincubated with the indicated concentration of anti-OVA (Figure 1, gray bars), IVIg, or nothing 1 day prior to injection of antiplatelet antibody. After an additional 24 hours, all mice were bled for platelet counts. Mice that received anti-platelet antibody alone displayed ITP, compared with control mice (horizontal white bar). The OVA + anti-OVA preparation significantly prevented thrombocytopenia at dosages of 1.0 and 0.5 mg anti-OVA/mouse ($P < 0.001$) as assessed by platelet counts 24 hours after anti-platelet antibody injection. In addition, IVIg (50 mg/mouse) also significantly inhibited the onset of

Nonstandard abbreviations used: FcγR, Fcγ receptor; FcRn, neonatal Fc receptor; ITP, immune thrombocytopenic purpura; IVIg, intravenous Ig; RES, reticuloendothelial system.

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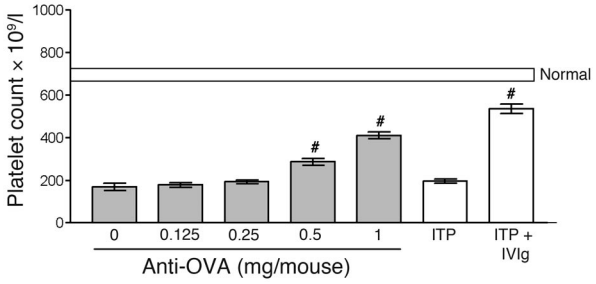

Figure 1
Pretreatment of mice with soluble OVA + anti-OVA ameliorates ITP. CD1 mice were pretreated by intravenous injection of 1 mg OVA that had been preincubated with the indicated dose of anti-OVA (gray bars), followed 24 hours later by injection of 2 μg anti-platelet antibody. The *x* axis denotes the treatment; *y* axis denotes platelet counts taken 24 hours after injection with anti-platelet antibody. Normal, unmanipulated mice (the white horizontal bar represents the mean platelet count ± 1 SEM); ITP, mice injected with antiplatelet antibody only; ITP + IVIg, mice pretreated with 50 mg IVIg followed by antiplatelet antibody; $n = 9$ mice for each group from 3 independent experiments. $^{\#}P < 0.001$ vs. ITP mice. Data are presented as mean ± SEM.

ITP. Individually, neither OVA (first column) nor anti-OVA (data not shown) alone affected the platelet count. Mice treated with OVA + control IgG were also not protected from the development of ITP (data not shown). In addition, we have also observed that a 50 μg/mouse dose of monoclonal anti-OVA in combination with 1 mg of soluble OVA was as successful at ameliorating ITP as was 50 mg IVIg (Supplemental Figure 2; supplemental material available online with this article; doi:10.1172/JCI200522753DS1).

IgG reactive with a cell-associated antigen can ameliorate ITP. CD1 mice were injected intravenously with 10^8 OVA-rbcs, OVA-rbcs + anti-OVA, or OVA-rbcs + control IgG or 50 mg IVIg or were left untreated. Twenty-four hours later, mice received an injection of antiplatelet antibody; after an additional 24 hours, all mice were bled. Mice treated with OVA-rbcs + anti-OVA (Figure 2) were significantly protected from the development of thrombocytopenia ($P < 0.001$) compared with mice receiving OVA-rbcs alone or the other control groups.

IgG reactive with a soluble or cell-associated antigen blocks RES function. To assess whether the regimes under study inhibited RES function, we analyzed the clearance rate of fluorescently labeled, antibody-sensitized, syngeneic rbcs. For the soluble antigen studies, mice were either untreated or injected with OVA + anti-OVA or OVA + control IgG or IVIg, followed 24 hours later by sensitized, fluorescent rbcs (Figure 3A). IVIg significantly inhibited rbc clearance, as we have previously shown (11). Mice pretreated with OVA + anti-OVA had a significantly impaired ability to clear sensitized rbcs ($P < 0.001$ at 30, 120 and 1,000 min). Mice pretreated with OVA + control IgG displayed the same clearance rate as otherwise untreated mice. For the cell-associated antigen studies, mice were untreated or injected with OVA-rbcs + anti-OVA or OVA-rbcs + control IgG or IVIg (Figure 3B). Mice pretreated with OVA-rbcs + anti-OVA had a significantly impaired ability to clear sensitized rbcs ($P < 0.01$ at 10 min; $P < 0.001$ at 30, 120 and 1,000 min) as compared with otherwise untreated mice.

FcγRIIB expression is required for protection with soluble but not cell-associated antigens. C57BL/6 (*FcγRIIB*$^{+/+}$) mice and *FcγRIIB*$^{-/-}$ mice were injected daily with antiplatelet antibody to induce stable thrombocytopenia. Mice were then treated with OVA + anti-OVA or OVA + control IgG or IVIg 2 days after ITP induction. Treatment of mice with OVA + anti-OVA or IVIg significantly reversed ITP in wild-type mice ($P < 0.001$ at days 3 and 4) (Figure 4A). However, we found that neither OVA + anti-OVA nor IVIg ameliorated ITP in *FcγRIIB*$^{-/-}$ mice (Figure 4B).

In sharp contrast to the results with the soluble OVA antigen system, both wild-type (Figure 5A) and *FcγRIIB*$^{-/-}$ mice (Figure 5B) showed significant increases in platelet counts after treatment with OVA-rbcs + anti-OVA ($P < 0.001$ at days 3 and 4). Although the genetic backgrounds of the *FcγRIIB*$^{-/-}$ mice and the *FcγRIIB*$^{+/+}$ mice are not identical, these data confirm earlier observations that FcγRIIB is required for IVIg action (5).

IgG reactive with preinjected or endogenous antigens can ameliorate ITP. To determine whether it is necessary to incubate antigen and antibody before injection to ameliorate the thrombocytopenia in our model, we preinjected mice with either 1 mg or 10 mg OVA followed by 1 mg anti-OVA after 4 hours. Significant reversal of ITP was achieved with anti-OVA in these mice (Figure 6A). To ascertain whether antibody to endogenous soluble antigens could also ameliorate ITP, thrombocytopenic mice were treated with 1 mg anti-albumin or 1 mg anti-transferrin on day 2 (Figure 6B). Anti-albumin significantly increased platelet counts of thrombocytopenic mice at days 3 ($P < 0.01$) and 4 ($P < 0.001$). Anti-transferrin also increased platelet counts at days 3 and 4 ($P < 0.01$ at day 4). IVIg, but not control IgG, successfully increased platelet counts at days 3 and 4.

IgG reactive with a soluble antigen can ameliorate inflammatory arthritis. To evaluate the therapeutic role of antibodies directed to a soluble antigen in the K/BxN serum–induced arthritis model, we injected C57BL/6 mice with 50 mg IVIg, 1mg anti-albumin, 1 mg control IgG, or nothing 4 hours prior to administering K/BxN serum. Mice in an additional control group were not injected with the K/BxN serum. Mice that received K/BxN serum alone, or K/BxN serum + control IgG, developed joint swelling, which reached a plateau by day 8 after injection (Figure 7, A and B). IVIg and the anti-albumin treatment significantly ameliorated the arthritis as assessed by ankle width measurements as well as by clinical score, as compared with mice that received K/BxN serum or K/BxN serum plus treatment with control IgG (Figure 7, A and B).

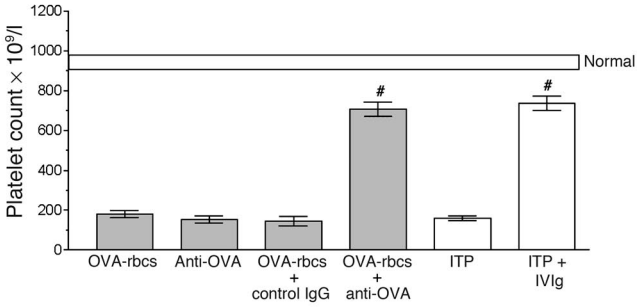

Figure 2
Pretreatment of mice with OVA-rbcs + anti-OVA ameliorates ITP. CD1 mice were injected intravenously with 10^8 OVA-rbcs + anti-OVA or OVA-rbcs + control IgG, or OVA-rbcs or anti-OVA only, as indicated on the *x* axis. Mice were then treated as described in Figure 1. $n = 9$ mice for each group from 3 independent experiments. $^{\#}P < 0.001$ vs. ITP mice. Data are presented as mean ± SEM.

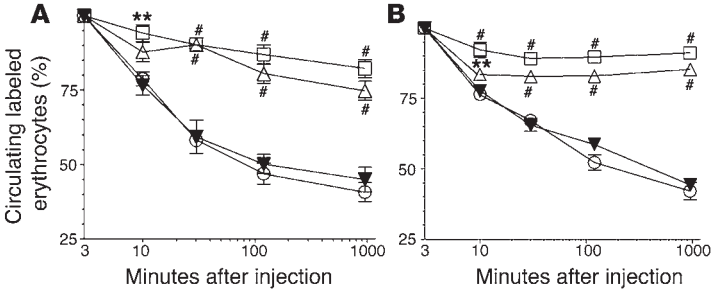

Figure 3
Antibody reactive with soluble OVA or OVA-rbcs inhibits RES function. (**A**) RES blockade using soluble OVA. CD1 mice were untreated (open circles), treated intraperitoneally with IVIg (open squares), or intravenously with OVA + anti-OVA (open triangles) or OVA + control IgG (filled triangles), followed 24 hours later by intravenous injection of fluorescently labeled, TER-119–opsonized syngeneic rbcs. Blood samples were taken at the times indicated on the *x* axis and the percentage of fluorescent rbcs in the circulation assessed by flow cytometry. (**B**) RES blockade using OVA-rbcs. Mice were not pretreated (open circles), pretreated intraperitoneally with 50 mg IVIg (open squares), or pretreated intravenously with 10^8 OVA-rbcs sensitized with 10 μg anti-OVA (open triangles) or OVA-rbcs pretreated with control IgG (filled triangles), followed 24 hours later by intravenous injection of fluorescently labeled TER-119–opsonized syngeneic rbcs. The percentage of fluorescent rbcs was assessed as in **A**. The percentage of fluorescent rbcs at 3 min was considered to be 100%; $n = 5$ mice for each group from 5 independent experiments. $^{**}P < 0.01$, $^{\#}P < 0.001$. Data are presented as mean ± SEM.

Discussion

We evaluated the ability of antibodies that target soluble antigens to ameliorate ITP and compared the mechanism of action of this therapeutic strategy to that of antibodies directed to a cell-associated antigen. OVA was used as the primary model antigen because it is absent in mice and can be prepared in either a soluble or cell-associated form (32), which allowed us to test the same anti-OVA antibody with both soluble and cell-associated OVA.

IVIg has been shown to require the presence of the inhibitory FcγRIIB to ameliorate murine ITP (5, 6). In wild-type mice, antibodies to both soluble and cell-associated OVA ameliorated ITP to the same extent as did IVIg. In *FcγRIIB*$^{-/-}$ mice, however, targeting soluble OVA did not ameliorate ITP. In contrast, targeting cell-associated OVA ameliorated ITP in *FcγRIIB*$^{-/-}$ mice. The findings suggest that antibodies to soluble antigens function via a mechanism similar to that of IVIg, whereas antibodies reactive to cell-associated antigens function by a different pathway. The observation that OVA-rbcs + anti-OVA is effective in the absence of FcγRIIB suggests that it may function by inhibiting activating Fcγ receptors on macrophages in the RES. All of the 3 therapeutic regimes that resolved ITP (soluble, particulate, IVIg) also blocked the ability of the RES to clear antibody-sensitized fluorescently labeled erythrocytes. RES blockade has long been considered to be a primary mechanism of action of both IVIg and a different IgG therapeutic product (anti-RhD) in the treatment of ITP (4, 7–10, 33). While our work does not directly examine the mechanism that each of these therapeutic regimes could use to inhibit RES function, the observation that IVIg and antibodies to soluble antigens (but not to OVA-rbcs) resolve ITP in an FcγRIIB-dependent manner argues that this mechanism may be more complex than originally thought. In particular, RES inhibition may occur via several distinct mechanisms, including (a) competition for activating FcγRs; (b) macrophage exhaustion (by engulfment and antigen processing of sensitized erythrocytes); or (c) inhibitory signaling via FcγRIIB (in the case of IVIg and/or antibodies to soluble antigens). The precise details of how each of these regimes inhibits the function of the RES remain to be determined, although blockade of the RES by antibodies to particulate antigens (i.e., that ameliorate ITP independent of FcγRIIB) suggests it may function by either of the first 2 mechanisms (a or b). In the case of antibodies to soluble antigens, we have observed that they ameliorated both murine ITP as well as inflammatory arthritis. Since the immunological mechanisms involved in both of these diseases are different with respect to the involvement of a functioning RES (i.e., phagocytosis of opsonized platelets in the spleen vs. inflammation and joint destruction), we do not believe that simple RES competitive blockade fully explains the therapeutic effects of anti–soluble antigen–mediated amelioration of autoimmune–disease.

In this study, mice were injected with OVA (or OVA-rbcs) that had been pretreated with anti-OVA in vitro, followed by the in vivo administration of this antibody-antigen mixture. Although this exogenous antigen strategy worked well in our model, it might not be feasible in humans with ITP. To determine whether IgG reactive with soluble endogenous antigens also could increase platelet counts in thrombocytopenic mice, mice were injected with anti-albumin or anti-transferrin. Both of these antibodies also ameliorated the ITP in control mice but were not successful at ameliorating ITP in *FcγRIIB*$^{-/-}$ mice as expected (Supplemental Figure 3), indicating that, similarly to OVA + anti-OVA, IgG reactive with endogenous soluble antigens also functions in an IVIg-like manner.

It has also been suggested that IVIg mediates its effect in inflammatory disease by affecting complement activation (34–38). We found that the therapeutic effect of all of the preparations tested

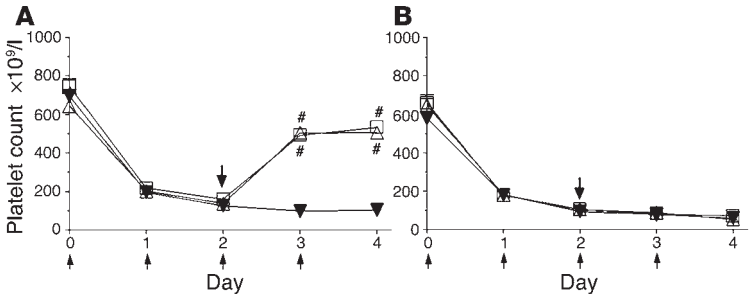

Figure 4
FcγRIIB expression is required for reversal of ITP by soluble OVA + anti-OVA. Control (C57BL/6) mice (**A**) or *FcγRIIB*$^{-/-}$ mice (**B**) were injected with 2 μg anti-platelet antibody on days 0 through 3 (upward-pointing arrows). On day 2 (downward-pointing arrows) mice were injected intraperitoneally with IVIg (open squares) or intravenously with OVA + anti-OVA (open triangles) or OVA + control IgG (filled triangles). Mice were bled daily for platelet counting; $n = 5$ mice for each group from 5 independent experiments. $^{\#}P < 0.001$ vs. control IgG. Data are presented as mean ± SEM.

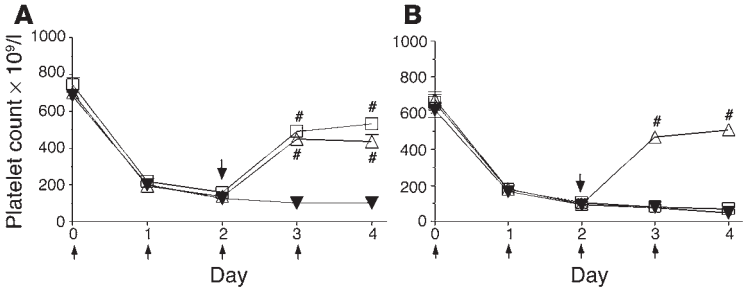

Figure 5
FcγRIIB expression is not required for reversal of ITP by OVA-rbcs + anti-OVA. Control (C57BL/6) mice (**A**) or *FcγRIIB*$^{-/-}$ mice (**B**) were injected with 2 μg anti-platelet antibody on days 0 through 3 (upward-pointing arrows). On day 2 (downward-pointing arrows) mice were injected intraperitoneally with 50 mg IVIg (open squares) or intravenously with OVA-rbcs + anti-OVA (open triangles) or OVA-rbcs + control IgG (filled triangles). Platelet counting was as described in Figure 4; $n = 5$ mice for each group from 5 independent experiments. $^{\#}P < 0.001$ vs. OVA-rbcs alone. Data are presented as mean ± SEM.

was, however, similar in normal mice and mice depleted of complement by cobra venom factor (Supplemental Figure 4). In addition, the fact that the monoclonal antibody directed to OVA was successful in ameliorating ITP under the conditions used (OVA was incubated with the monoclonal anti-OVA antibody at approximately 67:1 molar ratio; see Supplemental Figure 2) makes it unlikely that antibodies reactive with soluble antigens must form large immune complexes that activate complement as part of their therapeutic effectiveness. This is consistent with the work of Samuelsson et al., who observed that IVIg can inhibit ITP in mice genetically deficient in C3 (5).

A recent report by Akilesh and coworkers has demonstrated that the neonatal Fc receptor (FcRn) may have a role in the anti-inflammatory action of IVIg in the K/BxN serum–induced arthritis model (39). Work by this group and others suggests that IVIg occupies the FcRn, displacing the binding of the pathogenic antibodies, which leads to an increased catabolism of IgG. Since albumin can also bind to the FcRn, which prolongs its lifespan (40), a similar mechanism could be proposed for explaining the effects of the anti-albumin and perhaps the anti-OVA (+ OVA) in both the ITP and the K/BxN serum–induced arthritis models. One possibility is that the combination of IgG + albumin might more effectively compete for the IgG binding sites of FcRn, thus diverting higher levels of the pathogenic antibodies to degradation. The only caveat with this concept is that the fractional catabolic rate of transferrin does not appear to be directly related to its serum concentration, and, therefore, transferrin may not meaningfully interact with the FcRn (41, 42). Since anti-transferrin antibody ameliorated murine ITP almost as well as anti-albumin did, this last observation does not support this theory.

In conclusion, we demonstrate that antibodies to an otherwise immunologically inert soluble antigen can ameliorate both ITP and inflammatory arthritis. These observations suggest that antibodies to human proteins, such as albumin or transferrin, for example, could potentially augment or perhaps even replace current therapies such as IVIg in the treatment of some autoimmune diseases.

Methods

Mice. CD1 mice (6–10 wk of age) were purchased from Charles River Laboratories Inc. C57BL/6 and *FcγRIIB*$^{-/-}$ (B6;129S4-Fcγr2b^{tm1Rav}/J) mice were purchased from The Jackson Laboratory. All mice were housed in the St. Michael's Hospital Research Vivarium. All methods involving animals in this study have been approved by the Animal Care Committee of St. Michael's Hospital, Toronto, Ontario, Canada.

Reagents. The monoclonal antibody specific for integrin α_{IIb} (rat $IgG_{1\kappa}$, clone MWReg 30) was purchased from BD Biosciences — Pharmingen. OVA (grade V), rabbit IgG anti-OVA, 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDAC), and PKH26 Red Fluorescent Cell Linker Kit were purchased from Sigma-Aldrich. The IVIg (Gamimune N, 10%) was from Bayer Inc. FITC-conjugated $F(ab')_2$ anti-rabbit IgG and control (rabbit) IgG were purchased from Cedarlane Laboratories Ltd. Rabbit IgG anti-albumin and anti-transferrin were purchased from Research Diagnostics Inc. The monoclonal antibody anti–TER-119 specific for murine erythrocytes (clone TER-119, rat IgG_{2b}) was purchased from BD Biosciences — Pharmingen. Microdispenser tubes (250 μl) for blood collection were from VWR International.

Soluble and particulate antigen studies. OVA + anti-OVA: One milligram OVA was dissolved in PBS and was incubated with 1 mg anti-OVA (unless otherwise indicated) for 1 hour at 37°C. The solution was then injected intravenously in a 200-μl volume. Control groups included mice treated with OVA only, anti-OVA only, or OVA preincubated with control IgG.

OVA-rbcs + anti-OVA: OVA was coupled to rbcs by a modified version of that employed by Soldera et al. (32). Then rbcs were resuspended at 2.5×10^8/ml in 5 mg/ml OVA in saline, and 1.9 mg/ml EDAC was added. After a 1-hour incubation at 4°C, the cells were washed once with a 2 mg/ml solution of OVA in PBS followed by 1 wash in PBS. The presence of OVA on rbcs was established by flow cytometry (Supplemental Figure 1). For treatment of ITP, 10^8 OVA-rbcs were sensitized with 10 μg of anti-OVA for 1 hour at 22°C and injected intravenously in a volume of 200 μl. Control IgG was used as a control.

Induction and treatment of ITP. Mice were rendered thrombocytopenic by intraperitoneal injection of 2 μg anti–integrin α_{IIb} antibody in 200 μl PBS, pH 7.2, as previously described (11, 12). For experiments where the experimental therapeutic intervention preceded the induction of ITP (as shown,

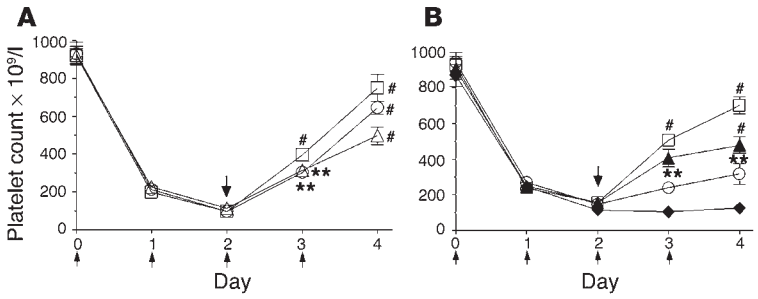

Figure 6
Antibodies to endogenous soluble antigens ameliorate ITP. Thrombocytopenia was induced and platelets counted as described in Figure 4. On day 2 (downward-pointing arrow), CD1 mice were treated (**A**) intraperitoneally with 50 mg IVIg only (open squares), 1 mg OVA (open circles), or 10 mg OVA (open triangles), followed 4 hours later by 1 mg anti-OVA intravenously; (**B**) intraperitoneally with 50 mg IVIg (open squares) or intravenously with 1 mg anti-albumin antibody (filled triangles), 1 mg anti-transferrin antibody (open circles), or 1 mg control IgG (filled diamonds); $n = 6$ mice for each group from 2 independent experiments. $^{\#}P < 0.001$, $^{**}P < 0.01$, treatment vs. control IgG. Data are presented as mean ± SEM.

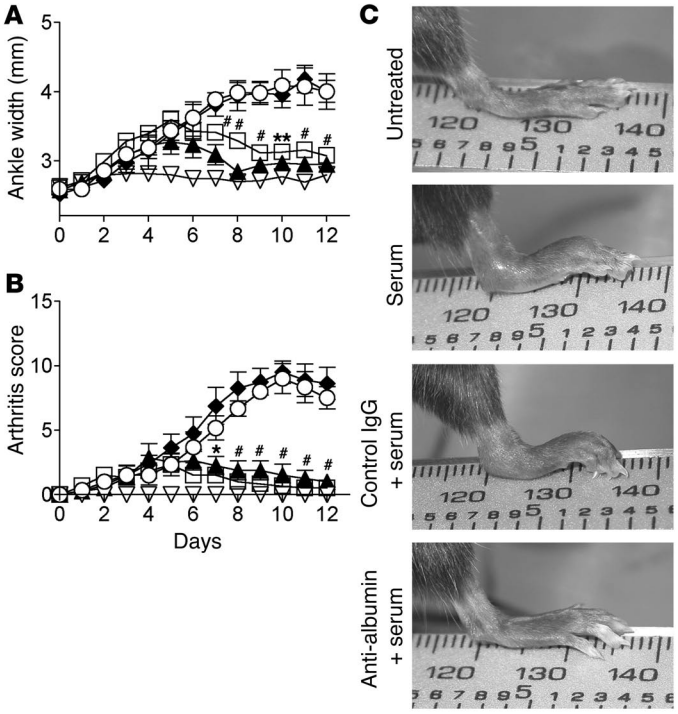

Figure 7
Antibodies to murine albumin ameliorate K/BxN serum–induced inflammatory arthritis. (**A**) Ankle width and (**B**) overall arthritis score following induction of arthritis by injection with K/BxN serum. C57BL/6 mice were injected on day 0 with K/BxN serum (open circles), IVIg + K/BxN serum (open squares), anti-albumin + K/BxN serum (filled triangles), or control IgG + K/BxN serum (filled diamonds) or left untreated (open triangles). $^{\#}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, anti-albumin vs. control IgG. Data represented as the mean ± SEM; $n = 3$ from 2 independent experiments (with serum and with IVIg + serum); $n = 4$ from 3 independent experiments (untreated and treated with control IgG + serum and anti-albumin + serum). (**C**) Hind limbs of representative C57BL/6 mice at day 9 after serum injection.

for example, in Figures 1 and 2), mice were first injected intravenously with OVA + anti-OVA or OVA-rbcs + anti-OVA followed at 24 hours by a single injection of antiplatelet antibody. Mice were bled for platelet counts after an additional 24 hours. For experiments where ITP preceded the therapeutic intervention (as shown, for example, in Figures 4–6), mice were injected daily with anti-platelet antibody and then with the test preparation on day 2. Mice were bled daily and platelets counted as described below.

In experiments where we wished to avoid the possibility of the formation of "pre-formed" immune complexes, mice were injected intraperitoneally with the indicated dose of soluble OVA and 4 hours later by 1 mg anti-OVA in a volume of 200 μl intravenously. Mice injected with anti-albumin or anti-transferrin alone received 1 mg of antibody intravenously in 200 μl PBS, pH 7.2, on day 2.

For all IVIg treatments, mice were injected intraperitoneally with 50 mg of IVIg (equivalent to 2 g/kg body weight). Platelets were counted as previously described (6, 11, 12), with the following minor modifications. Mouse blood (45 μl) was collected via bleeding from the saphenous vein into microdispenser tubes preloaded with 5 μl of 1% EDTA in PBS and then further diluted to a final dilution of 1:12,000 in 1% EDTA/PBS. Platelets were counted on a flow rate–calibrated FACScan Flow Cytometer (BD). Forward scatter versus side scatter was used to gate platelets as previously described (11, 12).

RES blockade. RES blockade was assessed as previously described (12). Briefly, rbcs were opsonized with 8 μg of anti–TER-119 antibody at 22°C for 30 minutes. The opsonized rbcs were then labeled with the PKH26 fluorescent marker according to the manufacturer's directions. These clear antibody-sensitized fluorescently labeled cells in a volume of 200 μl were injected into the mice via the tail vein. At the indicated times after injection of these rbcs, mice were bled, and the percentage of fluorescent rbcs remaining in circulation was ascertained by flow cytometry as described (12).

K/BxN serum–induced arthritis and arthritis scoring. For induction of arthritis, mice were given a single intraperitoneal injection of 600 μl of diluted serum (diluted to 50% strength with PBS), as described by Akilesh et al. (39). Mice in an additional control group were left untreated. Ankle width was measured laterally across the joint with a caliper (39). Arthritis was also clinically scored daily by an independent observer blinded to the treatment of the mice. Each paw was scored as follows: 0, unaffected; 1, slight swelling; 2, moderate swelling; 3, severe swelling involving the entire paw (foot, digits, ankle); and the overall score was calculated as the sum of individual scores for each paw as described by de Fougerolles et al. (43). Mice injected with anti-albumin or the IgG control received 1 mg of IgG intravenously in 200 μl PBS 4 hours prior to the induction of arthritis. Mice in the IVIg group were injected intraperitoneally with 50 mg of IVIg 4 hours prior to the induction of arthritis.

Statistical analysis. Data was analyzed using the Student's *t* test or by 1-way ANOVA, as appropriate. $P < 0.05$ was considered significant.

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