leaf area. Forests have potentially faster growing species and leaf areas that are several times greater, but ANPP responses are abiotically constrained because such biomes typically do not experience much precipitation variability. Or, if variability is substantial, a water surplus [estimated as the ratio of annual precipitation to potential evapotranspiration (PPT/PET)] typically exists (Fig. 4). Thus, most forests have sufficient moisture to meet evapotranspirational demands even during years with below-average precipitation; and during wet years, other resources such as light or nutrients limit production responses in forests. It is in herbaceous-dominated ecosystems, such as grasslands and old fields of the central United States, that the production potential of the vegetation is high, the PPT/ PET ratio is near or below one, and substantial precipitation variability occurs (Fig. 4). These biomes display the greatest interannual variability in ANPP under current precipitation patterns (Fig. 1) and may be the most responsive to future climatic changes.

Our results have two implications. First, assessing temporal variability in primary production is important for quantifying energetic constraints on organisms, population dynamics, and community structure across a range of terrestrial biomes (21). Second, the inherently high climatic variability of the central United States has been described as detrimental for detecting climate change in this region (22). Certainly, there is evidence that longterm climate changes have caused dramatic shifts in species distributions and biome boundaries across North America (23). However, initial biotic responses to global changes will not be manifest as biome shifts but rather as more subtle changes in ecosystem states and processes. Our results provide a basis for predicting which biomes will respond most rapidly and strongly to global change phenomena, particularly to alterations in precipitation. If the sensitivity of ANPP in grasslands to precipitation variability portends responses to alterations in other resources, then these biomes may be especially valuable as ecological bellwethers of global change.

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- We also assessed growing season precipitation and evapotranspiration (24). Over the range of productivity encompassed by these sites, annual precipitation was the strongest predictor of variation in ANPP

(Fig. 2) $\{r^2 = 0.36 \text{ for the relationship between AET and ANPP [as in (1)]}\}$.

- 11. The LTER network began in 1980 (25). Each LTER site included in this analysis used unique methods for estimating ANPP (see individual site Web pages at http://lternet.edu). When multiple communities were sampled, only a representative community type was selected from each site (Table 1). We attempted to include the longest and highest quality data available from each site. Although the length of record of ANPP varied (from 6 to 23 years), there was no relationship between the length of the data set and the means or CVs for ANPP or precipitation. We also constructed data sets of average length (12 years) by random sampling of the longest data sets (Konza Prairie, Cedar Creek, and Shortgrass Steppe). Means and variances calculated for these shorter term data did not differ significantly from those from the longterm data (26).
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- 27. We thank the principal investigators, information managers, and scientists responsible for measuring ANPP at the LTER sites and J. Aber, W. Bowman, D. Schimel, G. Shaver, R. Ruess, T. Fahey, and W. Lauenroth for providing comments on earlier versions of the manuscript. Research was supported and data were provided by the NSF Long-Term Ecological Research Program, the VEMAP data group and sponsors (S. Aulenbach, NASA, the Electric Power Research Institute, and the U.S. Department of Agriculture's Forest Service), and Kansas State University. This paper is dedicated to the memory of Dr. James T. Callahan, whose foresight and support of the LTER program for over 20 years enabled these data to be collected.

10 July 2000; accepted 7 December 2000

Anti-inflammatory Activity of IVIG Mediated Through the Inhibitory Fc Receptor

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The molecular basis for the anti-inflammatory property of intravenous gamma globulin (IVIG) was investigated in a murine model of immune thrombocytopenia. Administration of clinically protective doses of intact antibody or monomeric Fc fragments to wild-type or Fc γ receptor-humanized mice prevented platelet consumption triggered by a pathogenic autoantibody. The inhibitory Fc receptor, Fc γ RIIB, was required for protection, because disruption either by genetic deletion or with a blocking monoclonal antibody reversed the therapeutic effect of IVIG. Protection was associated with the ability of IVIG administration to induce surface expression of Fc γ RIIB on splenic macrophages. Modulation of inhibitory signaling is thus a potent therapeutic strategy for attenuating autoantibody-triggered inflammatory diseases.

Although first introduced for the treatment of hypogammaglobulinemia, IVIG has since been shown to have broad therapeutic applications in the treatment of infectious and inflammatory diseases (1). The polyclonal specificities found in these preparations have been demonstrated to be responsible for some of the biological effects of IVIG. For example, IVIG has been used as prophylaxis against infectious agents and in the treatment of necrotizing dermatitis (2). Independent of these antigen-specific effects, IVIG has well-recognized anti-inflammatory activities, generally attributed to the immunoglobulin G (IgG) Fc domains. These activities, first applied for the treatment of immune thrombocytopenia (ITP) (3, 4), have been extended to the treatment of a variety of immune mediated inflammatory disorders including autoimmune cytopenias, Guillain-Barré syndrome, myasthenia gravis, anti-Factor VIII autoimmune disease, dermatomyositis, vasculitis, and uveitis (5-10). A variety of explanations have been put forward to account for these activities, including Fc receptor blockade, attenuation of complement-mediated tissue damage, neutralization of autoantibodies by antibodies to idiotype, neutralization of superantigens, modulation of cytokine production, and down-regulation of B cell responses (11-14). However, the importance of any of these mechanisms to the in vivo activity of IVIG has not been established.

To investigate the mechanism by which

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IVIG mediated its anti-inflammatory effects, we used a murine model of ITP. As described previously, the murine IgG2a anti-platelet monoclonal antibody (mAb) 6A6 triggers a rapid consumption of platelets when injected intravenously into wild-type mice (15, 16), mimicking the effect of a pathogenic autoantibody. As in human ITP, IVIG, administered at 1.0 g/kg, was protective (Fig. 1A). This protective effect was dose-dependent, with 50% protection observed at 0.5 g/kg, decreasing to a negligible effect at 0.1 g/kg (17). Administration of equimolar concentrations of Fab or Fc fragments, followed by 6A6, revealed that only the Fc fragment conferred the protective effect of IVIG (Fig. 1B). The pathogenic effect of 6A6 was dependent on the presence of the low-affinity activating IgG Fc receptor, FcyRIII. In its absence (Fig. 1C), mice were also protected from the ITP induced by 6A6. In contrast, deleting the high-affinity activating FcyR, FcyRI, was not protective (17). To further define the mechanisms of IVIG protection, we developed a transgenic model in which the endogenous murine FcvRIII gene was replaced by a human transgene encoding the FcyIIIA protein, expressed on natural killer (NK) cells, macrophages, and mast cells (18). These FcyR-humanized mice were fully susceptible to the pathogenic effect of this murine IgG2a antibody (Fig. 1D), despite the lack of FcyRIII expression on neutrophils. Thus, the requirement for neutrophils in the pathogenicity of this form of ITP was minimal. In contrast, splenectomy was partially protective (17), implicating splenic effector cells, such as macrophages, in the FcR-dependent pathogenicity of ITP. Protection in these FcR-humanized mice was observed either upon IVIG administration or by blocking the human IIIA with the monoclonal antibody to human FcyRIII, 3G8 (Fig. 1D). However as defined below, the mechanisms underlying these two treatments are distinct.

Previous studies have demonstrated that the responses triggered by FcRyIII are counterbalanced by the inhibitory receptor FcyRIIB, under conditions when the effector cell coexpresses both molecules. This coupling has been seen in murine models of glomerulonephritis (19), in syngenic and xenograft tumor protection studies with antitumor antibodies, and in immune complextriggered inflammatory responses in the skin and lung (20-22). However, in those models, deletion of RIIB enhanced sensitivity to cytotoxic IgGs and immune complexes. In the model of ITP described here, RIIB-deficient mice were as sensitive to the pathogenic effects of 6A6 as their wildtype counterparts (Fig. 2). This was the case even at limiting concentrations of autoantibody, indicating that the effector cells mediating ITP are not substantially modulated by RIIB. However, in contrast to wild-type mice, RIIB-deficient mice were defective in their protective responses through IVIG administration (Fig. 2A).



Fig. 1. (A) IVIG protects against experimental immune thrombocytopenia. Wild-type BALB/c mice were injected with IVIG (1.0 g/kg) before administration of anti-platelet mAb 6A6 (*15*, *16*). Mean platelet counts from untreated (blue circles) and IVIG-treated (red circles) mice are presented. (**B**) The Fc portion of IVIG protects against ITP. Equimolar concentrations of IVIG (open triangles), Fab (yellow triangles), or Fc fragments (red triangles) were administered 1 hour before 6A6 and compared with 6A6 alone (black triangles). (**C**) ITP is mediated by activation Fc receptors. ITP was induced in wild-type (wt; blue circles) and FcR γ -chain–deficient mice (green circles). (**D**) ITP in hRIIIA^{Tg}/RIII^{-/-} mice. Nadir platelet counts after 6A6 injection are depicted after administration of mAb 3G8 or IVIG (*26–28*).



Fig. 2. The protective effect of IVIG is mediated through the inhibitory receptor RIIB. (**A**) Mean platelet counts after 6A6 injection into RIIB^{-/-} mice are shown for IVIG-treated (1.0 g/kg; yellow circles) and untreated (blue circles) groups (29). Red circles indicate IVIG + 6A6 in wild-type controls. The panel is representative of four different experiments. (**B**) RIIB blocking was performed in wild-type mice with anti-Ly17.2 (30). Mean platelet counts are presented for mice pretreated with IVIG (1.0 g/kg; red circles) or IVIG + anti-Ly17.2 mAb (6 µg/g; yellow circles) before 6A6 injection.

Fig. 3. (A) In vivo induction of spleen RIIB⁺ cells by IVIG. Wild-type mice were injected with IVIG (1.0 g/kg). After 4 hours, splenocytes were analyzed by FACS for mac-1 and RIIB expression. (B) Representative FACS dot plots on mac-1– and Ly17.2-stained B220-negative splenocytes from one control and one IVIG-treated mouse.



Similarly, blocking RIIB with a specific mAb (anti-Ly17.2) in wild-type mice treated with IVIG negated its protective effect (Fig. 2B). These data demonstrated a clear requirement for FcyRIIB in the mechanism of IVIG protection, suggesting that IVIG might modulate RIIB expression or function, in a manner that counteracts the activation responses triggered by FcRIII engagement. Staining of splenic macrophages before and after treatment with IVIG revealed a 60% increase in expressing high levels of FcyRIIB after IVIG treatment (Fig. 3), supporting a model in which enhanced surface expression of RIIB is responsible for mediating the protective effect of IVIG. Because FcyRIIB mediates its inhibitory effect by the recruitment of the SH2-containing inositol phosphatase SHIP, an intracellular signaling molecule with pleiotropic effects on phosphatidylinositol (3,4,5)triphosphate (PIP3)-dependent sig-



Fig. 4. Effects of IVIG on 6A6-induced thrombocytopenia in wild-type, $Fc\gamma RI$ -, C3-, complement receptor 1/2 (CRI/II)–, and RIB-deficient mice. The panel shows mean nadir platelet counts reached after injection with IVIG (1.0 g/kg) followed by mAb 6A6 (0.3 $\mu g/g$; red) or 6A6 alone (blue).

naling cascades (23, 24), modulating RIIB expression could result in greatly amplified inhibitory effects. However, additional effects of IVIG on SHIP-dependent signaling cannot be excluded.

Protection is seen with the Fc fragment of IVIG alone (Fig. 1B), and we therefore investigated whether the inductive response was dependent on the high-affinity Fc receptor, FcRI, which is capable of binding this molecule. FcyRI-deficient mice were susceptible to the pathogenic effects of 6A6 and were fully protected by IVIG administration (Fig. 4). Similar results were observed with complement-deficient mice (Fig. 4), indicating that the mechanism by which Fc fragments of IVIG induce protection through RIIB does not require these classical antibody-binding components. These data support the conclusion that IVIG mediates its protective effect by its ability to induce the expression of the inhibitory FcyRIIB receptor on effector cells that would otherwise trigger clearance of the opsonized platelets. A pronounced effect of even a modest induction of RIIB can be predicted in cells where activation through FcyRIII is not previously balanced by an inhibitory receptor. ITP can also be treated by blocking the activation receptor, FcRIII, as shown in Fig. 1D. This blocking effect on activation receptors may explain the therapeutic effect of anti-D in the treatment of ITP (25), where complexes of anti-D-opsonized red blood cells are likely to compete for FcRIII occupancy with complexes of autoantibody-opsonized platelets.

The present report demonstrates that manipulation of the inhibitory FcR pathway is a practical therapeutic means for controlling autoantibody-mediated inflammation. IVIG is thus a demonstrated therapeutic whose mechanism of action is targeted to this inhibitory pathway. The emergence of additional molecules of this class can be anticipated on the basis of the insights provided by this mechanism of IVIG action.

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- 27. ITP was induced by intravenous injection of mouse IgG2a mAb 6A6 (0.3µg/g). At 2, 4, 6, and 24 hours, blood (20 µl) was collected with Unopette kits (Becton Dickinson), and platelets were counted under a phase-contrast microscope. In protection experiments, mice were injected with IVIG (10%, Bayer Corporation; 1.0 g/kg), Fab and Fc fragments of IVIG (0.3 g/kg), mAb 3G8 (0.5 µg/g) for blocking hFcγRIII, or anti-Ly17.2 (6 µg/g) for blocking FcγRII, 1 hour before 6A6.
- Digestion of human IVIG was performed with papain (1 mg per 100 mg of IVIG). The resulting fragments were purified with ProteinA HiTrap and Sephacryl S-200 columns (Amersham-Pharmacia Biotech).
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 We thank M. Hogarth, T. Takai, and M. Carroll for knockout mice; R. Good for 6A6 mAb; and U. Hämmerling for anti-Ly17.2 mAb. IVIG used in these studies was a generous gift from the Bayer Corporation. We thank D. White and V. Lappi for excellent technical assistance. These studies were supported by grants from the NIH and the Bayer Corporation. A.S. is a fellow of the Gunnar and Lillian Nicholson Exchange Fund and the Swedish Society for Medical Research. T.L.T is a fellow of the Cancer Research Institute.

31 October 2000; accepted 12 December 2000