

Cutting Edge: Intravenous Ig Inhibits Invariant NKT Cell-Mediated Allergic Airway Inflammation through FcγRIIIA-Dependent Mechanisms

This information is current as of March 3, 2011

Luiza M. Araujo, Angélique Chauvineau, Ren Zhu, Séverine Diem, Elvire A. Bourgeois, Anaïs Levescot, Michel Huerre, Jean-Marc Gombert, Jagadeesh Bayry, Marc Daëron, Pierre Bruhns, Srinivasa V. Kaveri and André Herbelin

J Immunol 2011;186:3289-3293; Prepublished online 11 February 2011;
doi:10.4049/jimmunol.1003076
<http://www.jimmunol.org/content/186/6/3289>

Supplementary Data	http://www.jimmunol.org/content/suppl/2011/02/11/jimmunol.1003076.DC1.html
References	This article cites 36 articles , 4 of which can be accessed free at: http://www.jimmunol.org/content/186/6/3289.full.html#ref-list-1
Subscriptions	Information about subscribing to <i>The Journal of Immunology</i> is online at http://www.jimmunol.org/subscriptions
Permissions	Submit copyright permission requests at http://www.aai.org/ji/copyright.html
Email Alerts	Receive free email-alerts when new articles cite this article. Sign up at http://www.jimmunol.org/etoc/subscriptions.shtml/

Cutting Edge: Intravenous Ig Inhibits Invariant NKT Cell-Mediated Allergic Airway Inflammation through FcγRIIIA-Dependent Mechanisms

Luiza M. Araujo,^{*,†} Angélique Chauvineau,^{‡,§,¶} Ren Zhu,^{*,†} Séverine Diem,^{*,†} Elvire A. Bourgeois,^{*,§,||} Anaïs Levescot,^{*,§,||} Michel Huerre,[#] Jean-Marc Gombert,^{‡,§,¶} Jagadeesh Bayry,^{†,*,††,‡‡,§§,¶¶} Marc Daëron,^{§§,¶¶} Pierre Bruhns,^{§§,¶¶} Srinivasa V. Kaveri,^{†,*,††,‡‡,§§,¶¶} and André Herbelin^{*,§,||,1}

Despite their increasing use in autoimmune, inflammatory, and allergic conditions, the mechanism of action of i.v. Igs (IVIg) is poorly understood. On the basis of the critical role of invariant NKT (iNKT) cells in allergic airway inflammation (AAI) and their constitutive expression of the low-affinity IgG receptor FcγRIIIA, we surmised that IVIg targets iNKT cells to exert their anti-inflammatory effect. We found that IVIg treatment significantly inhibited AAI in OVA-sensitized C57BL/6 mice and downregulated α-galactosylceramide-induced iNKT cell activation and cytokine production. Allergic responses were restored in iNKT cell-deficient mice by transferring iNKT cells from PBS- but not from IVIg-treated mice, suggesting that IVIg acts directly on activated iNKT cells that have a critical role in AAI. The inhibitory effects of IVIg on both iNKT cell activation/function and OVA-driven AAI were lost in FcγRIIIA^{-/-} mice. Our data unravel an FcγRIIIA-dependent inhibitory effect of IVIg on activated iNKT cells that confers protection in AAI. *The Journal of Immunology*, 2011, 186: 3289–3293.

Intravenous Ig (IVIg) is a therapeutic molecule prepared from pools of plasma obtained from several thousand healthy blood donors. In addition to its use in the treatment of primary and secondary immune deficiencies, IVIg has been used as a therapeutic option in a large number of auto-

immune, allergic, and inflammatory conditions including idiopathic thrombocytopenic purpura, Guillain-Barré syndrome, myasthenia gravis, and Kawasaki disease (1–6). Considering studies conducted in mouse models, several mutually non-exclusive mechanisms may account for the therapeutic effects of IVIg (1–3, 7–11). It is believed that the beneficial effect of IVIg may be related to its content of natural polyreactive Abs reactive with a large number of immunologically relevant molecules (12–14). Notably, anti-inflammatory activities of IVIg are mediated through both FcγRIIB- and/or FcγRIIIA-dependent and FcγR-independent mechanisms. Thus, IVIg has been shown to exert a regulatory role on both the humoral and cellular compartment of the immune system.

Invariant NKT (iNKT) cells are innate lymphocytes that recognize glycolipids presented by the nonclassical MHC class Ib family member CD1d (15–17). iNKT cells release massive amounts of IFN-γ following activation through their semi-invariant TCR, but they are also unique in their ability to secrete high amounts of IL-4 in addition to other Th2 cytokines such as IL-13 (16, 18–21). iNKT cells are therefore implicated in the physiopathology of several autoimmune and inflammatory diseases (19, 22, 23), including allergic airway inflammation (AAI), in asthma (18, 24, 25).

Allergic asthma is a chronic inflammatory disease of lung, the incidence and morbidity of which has increased dramatically in the past few decades, especially in industrialized countries (26, 27). Asthma is a heterogeneous and multifactorial disease and is characterized by an airway hyperreactivity in response to

^{*}Unité Mixte de Recherche 8147, Centre National de la Recherche Scientifique, Hôpital Necker, Paris 75783, France; [†]Université Paris Descartes, Paris, France; [‡]Laboratoire d'Immunologie-Immunopathologie, Centre Hospitalier Universitaire La Milétrie, Poitiers 86000, France; [§]INSERM U935, Hôpital Paul Brousse, Villejuif 94802, France; [¶]University of Poitiers, 86000 Poitiers Cedex, France; ^{||}Université Paris-Sud XI, 91405 Orsay Cedex, France; [#]Unité de Recherche et d'Expertise Histotechnologie et Pathologie, Institut Pasteur, Paris 75015, France; ^{**}Unité 872, INSERM, Paris 75006, France; ^{††}Centre de Recherche des Cordeliers, Equipe 16-Immunopathologie and Thérapeutique Immunointervention, Université Pierre et Marie Curie-Paris 6, Unité Mixte de Recherche S 872, Paris 75006, France; ^{‡‡}International Associated Laboratory Immunopathology and Thérapeutique Immunointervention in Immunohematological Diseases, Institut National de la Santé et de la Recherche Médicale, France-Indian Council of Medical Research, Mumbai 400 012, India; ^{§§}Institut Pasteur, Unité d'Allergologie Moléculaire et Cellulaire, Département d'Immunologie, Paris 75015, France; and ^{¶¶}INSERM, U760, Paris 75015, France

¹S.V.K. and A.H. shared senior authorship of this work.

Received for publication September 14, 2010. Accepted for publication January 7, 2011.

This work was supported by INSERM, the Centre National de la Recherche Scientifique, Chancellerie des Universités de Paris (Legs Poix), Université Pierre et Marie Curie

and Université Paris Descartes, Agence Nationale de Recherches (ANR-09-GENO-014-1), Ministère de la Recherche and Institut Pasteur, and European Community's Seventh Framework Programme (FP7/2007-2013) under Grant Agreement HEALTH-F2-2010-260338-ALLFUN (to J.B.).

Address correspondence and reprint requests to Dr. André Herbelin or Dr. Srinivasa V. Kaveri, INSERM U935, Campus CNRS, Bâtiment A, Rez-de Chaussée, 7 Rue Guy Moquet, Villejuif 94802, France (A.H.) or INSERM U872, Equipe 16, Centre de Recherche des Cordeliers, 15 Rue de l'Ecole de Médecine, Paris, 75006, France (S.V.K.). E-mail addresses: andre.herbelin@inserm.fr (A.H.) or srini.kaveri@crc.jussieu.fr (S.V.K.).

The online version of this article contains supplemental material.

Abbreviations used in this article: AAI, allergic airway inflammation; BALF, bronchoalveolar lavage fluid; α-GC, α-galactosylceramide; iNKT, invariant NKT; IVIg, i.v. Ig; MFI, mean fluorescence intensity; WT, wild-type.

Copyright © 2011 by The American Association of Immunologists, Inc. 0022-1767/11/\$16.00

a variety of specific and nonspecific stimuli, mucus hypersecretion, pulmonary eosinophilia, Th2 cytokine production, hyperplasia and hypertrophy of smooth muscle, and elevated concentrations of serum IgE (28). IVIg has been considered as one of the therapeutic options in the treatment of asthma, although the underlying mechanisms have not been explored (29, 30). Given the critical role of iNKT cells in AAI, we surmised that IVIg targets this cellular population to exert its anti-inflammatory effect.

Materials and Methods

Mice

Six- to 8-wk-old male C57BL/6 wild-type (WT) and $J\alpha 18^{-/-}$ mice were bred and maintained in our animal facility under specific pathogen-free conditions. $Fc\gamma RIIIA^{-/-}$ mice (N7 C57BL/6J) were purchased from The Jackson Laboratory. $J\alpha 18^{-/-}$ mice were backcrossed for 13 generations onto a C57BL/6 background. Animal experiments were performed according to the French Institutional Committee.

Induction of AAI and IVIg treatment

Mice were sensitized with OVA (100 μ g, i.p., per mouse; Sigma-Aldrich) absorbed to 1.6 mg aluminum hydroxide gel (Merck). After 7 d, mice were exposed to intranasal Ag (50 mg/d for OVA) or PBS on 3 consecutive d. Inhibition of AAI by IVIg in OVA-sensitized mice was assessed by injecting IVIg (10 mg, i.p., per mouse) 1 h prior to the first of the three consecutive challenges with OVA. In some experiments, OVA-sensitized WT or $J\alpha 18^{-/-}$ mice were adoptively transferred with total splenocytes (2.5×10^4 iNKT cell equivalents) or sorted iNKT cells (2.5×10^4) from WT mice that were pretreated with PBS or IVIg 1 h prior to sacrifice and transfer. Bronchoalveolar lavage fluid (BALF) and lungs were recovered 24 h after the last challenge. Total and differential cell counts were determined in the BALF, and histological analysis of lung sections was evaluated in a blinded fashion by a histopathologist (M.H.) as previously described (25). Peribronchial and perivascular inflammation was graded on a subjective scale of 0, 1, 2, and 3 corresponding to no, mild, moderate, or severe inflammation, respectively, with an increment of 0.5 if the inflammation fell between two integers.

Analysis of iNKT cells

The proportion of iNKT cells as well as their surface expression of CD69 was determined in splenocytes by flow cytometry using fluorochrome-conjugated anti-TCR- α (clone H57-597), anti-CD5 (clone 55-7.3), and anti-CD69 (clone H1.2F3) from BD Biosciences (Le Pont de Claix, France) and an allophycocyanin-conjugated α -galactosylceramide (α -GC)-loaded CD1d tetramer (CD1d: α -GC TT) provided by the National Institutes of Health Tetramer Core Facility. To assess iNKT cell activation in vivo, mice received an injection of α -GC (Kirin Brewery; 1 μ g/dose, i.v.). The in vivo modulatory effect of IVIg on iNKT cells was examined by injecting IVIg 1 h before α -GC challenge. Sera were recovered 2 h later. To compare iNKT cell activity between WT and $Fc\gamma RIIIA^{-/-}$ mice, FACS-sorted iNKT cells as CD1d: α -GC TT $^{+}$ CD5 $^{+}$ cells (2.5×10^4 /well) were stimulated with α -GC-pulsed dendritic cells (DC) for 48 h (20).

Levels of cytokines in the BALF, homogenate lung, serum, and cell-free culture supernatants were assessed using ELISA DuoSet kits (R&D Systems).

Statistical analysis

Data are expressed as the mean values $\pm$ SEM for each group of mice. We analyzed statistical comparisons with two-tailed unpaired Student *t* test and Mann-Whitney *U* test. We considered a *p* value <0.05 significant.

Results and Discussion

IVIg inhibits airway inflammation in OVA-sensitized and challenged mice

We first examined the protective effect of IVIg on the evolution of AAI in OVA-sensitized C57BL/6 mice. As shown in Fig. 1, IVIg treatment inhibited the recruitment of inflammatory cells in the airway passage. Thus, the total number of infiltrating cells (Fig. 1A) and, in particular, the percentage (Fig. 1B) and number (Fig. 1C) of eosinophils, one of the major mediators of allergic responses, was significantly down-

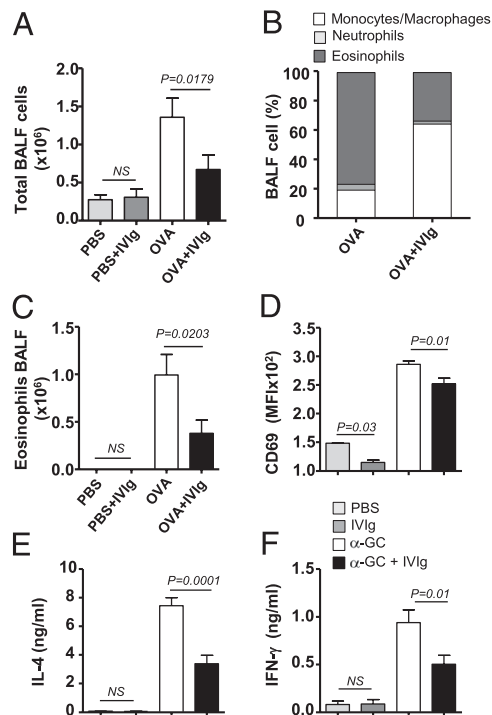


FIGURE 1. IVIg inhibits airway inflammation in OVA-sensitized and challenged WT mice and modulates the activation and function of iNKT cells. *A* and *B*, WT mice were sensitized with OVA and treated with IVIg or PBS 1 h prior to the first challenge with OVA or PBS. Total number of infiltrating cells (*A*), percentage (*B*), and number (*C*) of eosinophils in the BALF were determined 24 h after the last OVA challenge. The data shown are the pooled results of three independent experiments. *n* = 4 or 5 mice per group per experiment. Data represent means $\pm$ SEM (*D–F*). WT mice were injected with PBS or IVIg before administration of α -GC. After 2 h, the animals were bled and sacrificed. *D*, MFI of the early activation marker CD69 on splenic iNKT cells. IL-4 (*E*) and IFN- γ (*F*) levels in the serum. Data are pooled results from two independent experiments. *n* = 5 mice per group per experiment. Data represent means $\pm$ SEM.

regulated following IVIg treatment. Despite the notion that C57BL/6 strain develops poor airway hyperreactivity compared with other strains (24, 25), IVIg treatment of OVA-sensitized C57BL/6 mice led to a significant decrease of this cardinal feature of asthma (Supplemental Fig. 1*A*). However, the production of pro-Th2 cytokines was unaffected by IVIg (Supplemental Fig. 1*B*). These results thus document the therapeutic benefit of IVIg in severe childhood asthma (4, 29, 30).

IVIg downregulates the activation and function of iNKT cells

Activated iNKT cells have been shown to play a crucial role in development of allergic asthma following OVA sensitization and challenge (18, 24, 25). Absence of iNKT cells indeed abolishes experimental AAI. In view of the observed protective effect of IVIg in AAI, we asked whether IVIg treatment has repercussion on the activation and cytokine production by iNKT cells. Following injection of naive or α -GC-pretreated mice with IVIg, we assessed the expression level of the activation marker CD69 on iNKT cells. As shown in Fig. 1*D*, IVIg treatment significantly downregulated the mean fluorescence intensity (MFI) of CD69 on iNKT cells both in naive or α -GC-pretreated mice. Furthermore, IVIg treatment also reduced significantly the serum levels of IL-4 (Fig. 1*E*) and IFN- γ (Fig. 1*F*) following in vivo activation of iNKT cells by α -GC. Moreover, in vitro addition of IVIg alleviates

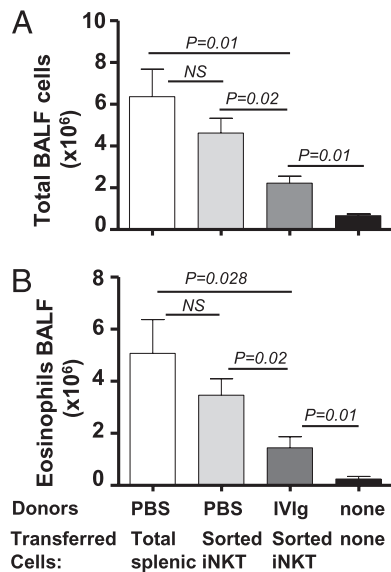


FIGURE 2. IVIg treatment blocks the capacity of iNKT cells to restore AAI in $J\alpha 18^{-/-}$ mice. Groups of OVA-sensitized $J\alpha 18^{-/-}$ mice were adoptively transferred with sorted iNKT cells from PBS or IVIg-treated WT mice 1 h prior to the first challenge with OVA. Total infiltrating cells (A) and eosinophils (B) were determined in the BALF in recipient $J\alpha 18^{-/-}$ mice 24 h after the last OVA challenge. Results represent means $\pm$ SEM from a representative experiment out of two. $n = 3$ –5 mice per group. Mice that did not receive iNKT cells (none) did not respond to OVA challenge.

production of IL-4 by sorted iNKT cells that were activated by α -GC-loaded DC (Supplemental Fig. 2). These data indicate that IVIg modulates the activation and function of iNKT cells.

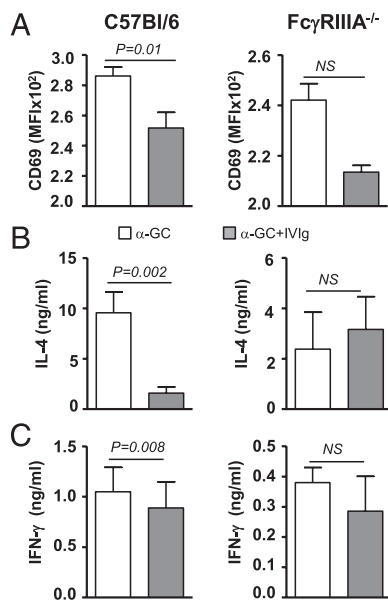


FIGURE 3. Inhibitory effect of IVIg on iNKT cells is Fc γ RIIIA dependent. WT and $Fc\gamma RIII A^{-/-}$ mice were injected with PBS or IVIg before administration of α -GC. After 2 h, the animals were bled and sacrificed. A, MFI of the early activation marker CD69 on splenic iNKT cells. IL-4 (B) and IFN- γ (C) levels were determined in the serum. Data are pooled results from two independent experiments. $n = 4$ mice per group per experiment. Data represent means $\pm$ SEM.

IVIg treatment blocks the capacity of iNKT cells to restore AAI

$J\alpha 18^{-/-}$ mice lack iNKT cells and are resistant to AAI (18, 24). To evaluate whether IVIg-mediated protection in AAI is due to its direct effect on pathogenic effector iNKT cells, we adoptively transferred iNKT cells from PBS- or IVIg-treated WT mice into iNKT cell-deficient $J\alpha 18^{-/-}$ mice. As expected, OVA-prensensitized $J\alpha 18^{-/-}$ mice that did not receive iNKT cells did not experience AAI upon Ag challenge. OVA-prensensitized $J\alpha 18^{-/-}$ mice that received total splenocytes or purified iNKT cells from PBS-treated WT mice developed airway inflammation as evidenced by massive lung infiltration by inflammatory cells, including eosinophils (Fig. 2A, 2B). Interestingly, animals that were transferred with iNKT cells from IVIg-treated WT animals showed >50% reduction in total cell infiltration and eosinophilia upon Ag challenge (Fig. 2A, 2B). This result demonstrates that the inhibitory effect of IVIg on AAI can be transferred by iNKT cells and that iNKT cells are therefore less pathogenic upon IVIg treatment.

Once pharmacologically activated by their ligand, iNKT cells are protective rather than pathogenic during AAI (31, 32). For this reason, we also examined whether IVIg-preconditioned iNKT cells were able to transfer protection to normal naive mice upon challenge with OVA. To address this issue, we transferred IVIg-preconditioned iNKT cells from naive mice to OVA-prensensitized recipient animals. Neither PBS- nor IVIg-preconditioned iNKT cells from naive mice were able to confer protection to recipient mice upon

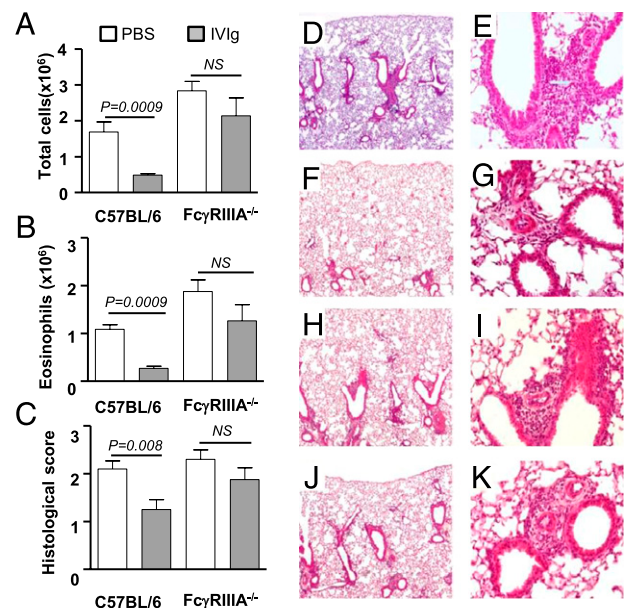


FIGURE 4. Inhibitory effect of IVIg on airway inflammation is mediated by Fc γ RIIIA. WT and $Fc\gamma RIII A^{-/-}$ mice were sensitized with OVA and treated with IVIg or PBS 1 h prior to the first challenge with OVA or PBS. Infiltrating total cells (A) or eosinophils (B) in the BALF and histological analysis (C–K) were determined 24 h after the last challenge. Peribronchial scores for PBS- or IVIg-treated OVA-sensitized/challenged WT and $Fc\gamma RIII A^{-/-}$ groups of mice are represented in C. Results represent means $\pm$ SEM from a representative experiment out of two. WT mice: $n = 3$ or 5; $Fc\gamma RIII A^{-/-}$ mice: $n = 5$ or 6. Periodic acid-Schiff staining of lung sections did not display robust presence of mucus in both WT and $Fc\gamma RIII A^{-/-}$ (not shown). Representative H&E-stained tissue sections of lungs from each group of mice are shown: WT mice treated with PBS (D, E) or IVIg (F, G), and $Fc\gamma RIII A^{-/-}$ mice treated with PBS (H, I) or IVIg (J, K). D, F, H, J, original magnification $\times 20$. E, G, I, K, original magnification $\times 200$.

challenge with OVA (Supplemental Fig. 3). These results demonstrate that IVIg has a suppressive effect on effector iNKT cells but is unable to modulate these cells and render them protective.

The inhibitory effect of IVIg on activated iNKT cells and AAI is FcγRIIIA dependent

The blockade of cell-surface FcγR is a possible mechanism for the anti-inflammatory properties of IVIg. In view of the fact that iNKT cells constitutively express surface FcγRIIIA but not FcγRI, -IIb, or -IV (33), we examined the anti-inflammatory effect of IVIg on iNKT cells in FcγRIIIA^{-/-} mice. iNKT cell counts were normal in FcγRIIIA^{-/-} mice compared with WT mice (Supplemental Fig. 4A, 4B). In addition, iNKT cells from both strains produced similar levels of IL-4 after in vitro stimulation with α-GC-pulsed DC, suggesting normal functioning of iNKT cells in FcγRIIIA^{-/-} mice (Supplemental Fig. 4C).

To determine whether FcγRIIIA are involved in the inhibitory effect of IVIg on iNKT cells, FcγRIIIA^{-/-} and WT mice were injected with IVIg 1 h before α-GC treatment. Interestingly, the inhibitory effect of IVIg on iNKT cell activation as measured by the expression of CD69 (Fig. 3A) and by the production of IL-4 (Fig. 3B) and IFN-γ (Fig. 3C) was lost in FcγRIIIA^{-/-} mice as compared with PBS-treated mice. These findings indicate that the inhibitory effect of IVIg on iNKT cells requires FcγRIIIA.

These findings led us to determine whether IVIg inhibits iNKT cell-mediated AAI through FcγRIIIA-dependent mechanisms. Consistent with this view, we found that FcγRIIIA deficiency led to a quasicomplete loss of the anti-inflammatory effect of IVIg treatment in OVA-sensitized and challenged animals (Fig. 4). Indeed, the total number of infiltrating cells (Fig. 4A) and eosinophils (Fig. 4B), as well as peribronchial and perivascular inflammation (Fig. 4C–K), was significantly decreased following IVIg treatment in WT mice but not in FcγRIIIA^{-/-} mice. It is of note that WT and FcγRIIIA^{-/-} mice had a similar capacity to develop AAI in response to OVA immunization and challenge. Together, our data demonstrate an FcγRIIIA-dependent inhibitory effect exerted by IVIg on activated iNKT cells that confers protection in AAI. Together, these data allow us to infer that the beneficial effect of IVIg on iNKT cell-mediated allergic airway inflammation implicates FcγRIIIA, although we do not provide evidence of a direct effect of IVIg on FcγRIIIA expressed by iNKT cells in vivo in our model.

A well-accepted paradigm is that the inhibitory functions of IgG are mediated by FcγRIIB, which contains a cytoplasmic immune tyrosine-based inhibitory motif that recruits phosphatases, leading to suppression of intracellular signal transduction (34). By contrast, FcγRIIIA is established as an activating receptor that is required for multiple Ab-immune complex-dependent inflammatory responses in vitro and in vivo. However, Park-Min et al. (35) have shown that inhibition of IFN-γ responses by immune complexes is independent of FcγRIIB. These authors revealed that immune complexes that are contained in IVIg inhibit IFN-γ signaling in macrophages by mechanisms dependent on FcγRIIIA, although being an activating receptor, and suggest that inhibition of IFN-γ signaling contributes to the therapeutic efficacy of IVIg (35, 36). Consistent with these data, our

results demonstrate an inhibitory role for FcγRIIIA in IVIg-mediated suppression of iNKT cell activation. This suppressive effect on iNKT cells contributes to the anti-inflammatory effects of IVIg in airway inflammation. Interestingly, the NK cell-dependent effect of IVIg has also been suggested to be of relevance in the prevention of recurrent pregnancy loss (5). Strategies aimed at functional inhibition of iNKT cells by targeting through IVIg alone or in combination with other appropriate therapeutics should help in paving the way for fighting allergic conditions and in maintaining a critical balance between tumor immunity and self-tolerance.

Acknowledgments

We thank Kirin Brewery for KRN7000 (α-GC), the National Institutes of Health Tetramer Core Facility for the CD1d:α-GC tetramer, and M. Taniguchi for the Jα18^{-/-} mice.

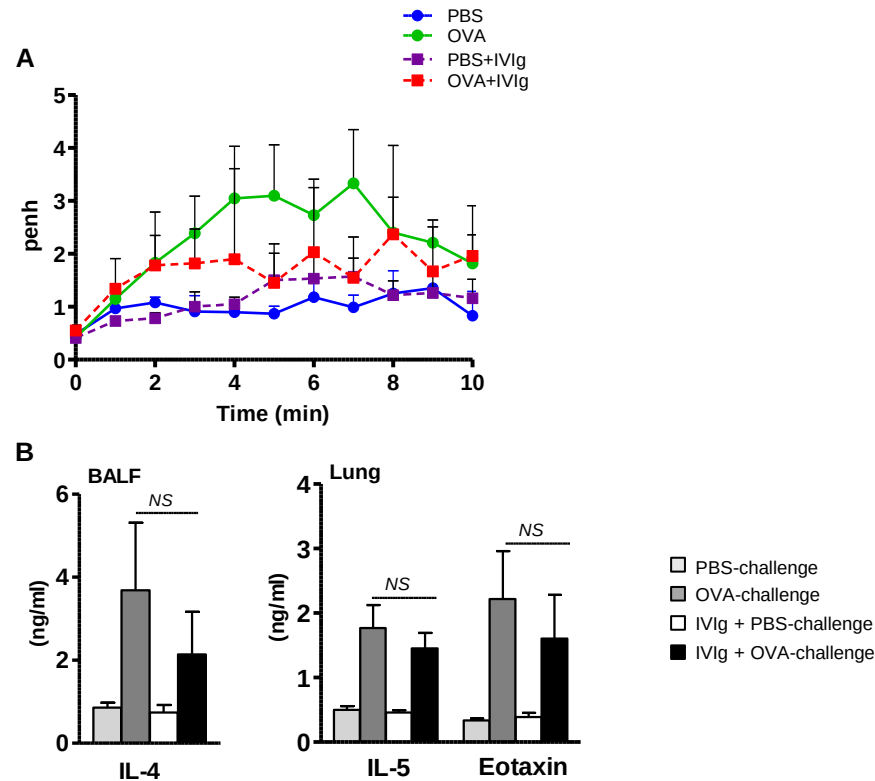
Disclosures

The authors have no financial conflicts of interest.

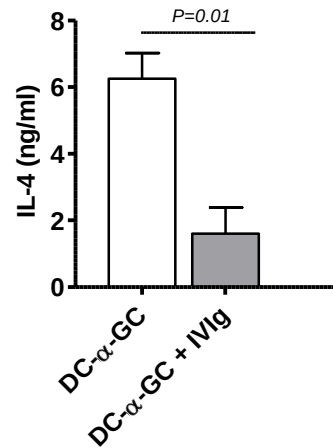
References

1. Nimmerjahn, F., and J. V. Ravetch. 2008. Anti-inflammatory actions of intravenous immunoglobulin. *Annu. Rev. Immunol.* 26: 513–533.
2. Tha-In, T., J. Bayry, H. J. Metselaar, S. V. Kaveri, and J. Kwekkeboom. 2008. Modulation of the cellular immune system by intravenous immunoglobulin. *Trends Immunol.* 29: 608–615.
3. Kazatchkine, M. D., and S. V. Kaveri. 2001. Immunomodulation of autoimmune and inflammatory diseases with intravenous immune globulin. *N. Engl. J. Med.* 345: 747–755.
4. Mazer, B. D., and E. W. Gelfand. 1991. An open-label study of high-dose intravenous immunoglobulin in severe childhood asthma. *J. Allergy Clin. Immunol.* 87: 976–983.
5. Sapir, T., M. Blank, and Y. Shoenfeld. 2005. Immunomodulatory effects of intravenous immunoglobulins as a treatment for autoimmune diseases, cancer, and recurrent pregnancy loss. *Ann. N. Y. Acad. Sci.* 1051: 743–778.
6. Gold, R., M. Stangel, and M. C. Dalakas. 2007. Drug Insight: the use of intravenous immunoglobulin in neurology—therapeutic considerations and practical issues. *Nat. Clin. Pract. Neurol.* 3: 36–44.
7. Sherer, Y., Y. Levy, F. Fabrizzi, and Y. Shoenfeld. 1999. Immunomodulation of various autoimmune diseases by intravenous immunoglobulin. *Drugs Today (Barc)* 35: 513–518.
8. Bruhns, P., A. Samuelsson, J. W. Pollard, and J. V. Ravetch. 2003. Colony-stimulating factor-1-dependent macrophages are responsible for IVIg protection in antibody-induced autoimmune disease. *Immunity* 18: 573–581.
9. Akilesh, S., S. Petkova, T. J. Sproule, D. J. Shaffer, G. J. Christianson, and D. Roopenian. 2004. The MHC class I-like Fc receptor promotes humoral mediated autoimmune disease. *J. Clin. Invest.* 113: 1328–1333.
10. Siragam, V., A. R. Crow, D. Brinc, S. Song, J. Freedman, and A. H. Lazarus. 2006. Intravenous immunoglobulin ameliorates ITP via activating Fc gamma receptors on dendritic cells. *Nat. Med.* 12: 688–692.
11. Paquin Proulx, D., E. Aubin, R. Lemieux, and R. Bazin. 2010. Inhibition of B cell-mediated antigen presentation by intravenous immunoglobulins (IVIg). *Clin. Immunol.* 135: 422–429.
12. von Gunten, S., and H. U. Simon. 2008. Natural anti-Siglec autoantibodies mediate potential immunoregulatory mechanisms: implications for the clinical use of intravenous immunoglobulins (IVIg). *Autoimmun. Rev.* 7: 453–456.
13. Vani, J., S. Elluru, V. S. Negi, S. Lacroix-Desmazes, M. D. Kazatchkine, J. Bayary, and S. V. Kaveri. 2008. Role of natural antibodies in immune homeostasis: IVIg perspective. *Autoimmun. Rev.* 7: 440–444.
14. Seite, J. F., Y. Shoenfeld, P. Youinou, and S. Hillion. 2008. What is the contents of the magic draft IVIg? *Autoimmun. Rev.* 7: 435–439.
15. Kawano, T., J. Cui, Y. Koezuka, I. Toura, Y. Kaneko, K. Motoki, H. Ueno, R. Nakagawa, H. Sato, E. Kondo, et al. 1997. CD1d-restricted and TCR-mediated activation of valpha14 NKT cells by glycosylceramides. *Science* 278: 1626–1629.
16. Bendelac, A., P. B. Savage, and L. Teyton. 2007. The biology of NKT cells. *Annu. Rev. Immunol.* 25: 297–336.
17. Mattner, J., K. L. DeBord, N. Ismail, R. D. Goff, C. Cantu, III, D. Zhou, P. Saint-Mezard, V. Wang, Y. Gao, N. Yin, et al. 2005. Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. *Nature* 434: 525–529.
18. Akbari, O., P. Stock, E. Meyer, M. Kronenberg, S. Sidobre, T. Nakayama, M. Taniguchi, M. J. Grusby, R. H. DeKruyff, and D. T. Umetsu. 2003. Essential role of NKT cells producing IL-4 and IL-13 in the development of allergen-induced airway hyperactivity. *Nat. Med.* 9: 582–588.
19. Zhu, R., S. Diem, L. M. Araujo, A. Aumeunier, J. Denizeau, E. Philadelphie, D. Damotte, M. Samson, P. Gourdy, M. Dy, et al. 2007. The Pro-Th1 cytokine IL-12 enhances IL-4

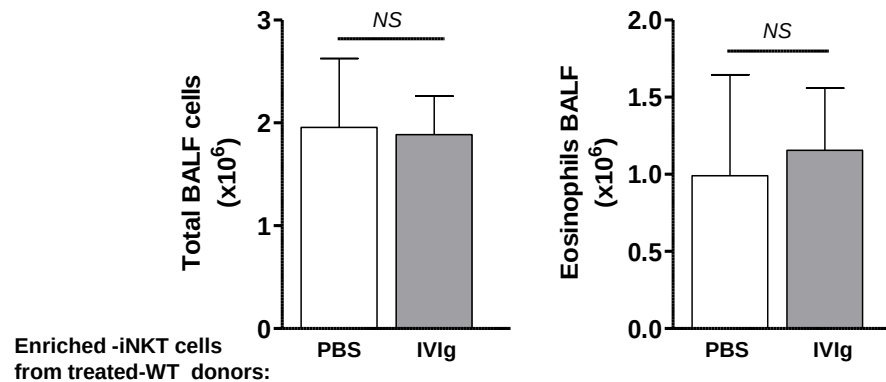
- production by invariant NKT cells: relevance for T cell-mediated hepatitis. *J. Immunol.* 178: 5435–5442.
20. Bourgeois, E., L. P. Van, M. Samson, S. Diem, A. Barra, S. Roga, J. M. Gombert, E. Schneider, M. Dy, P. Gourdy, et al. 2009. The pro-Th2 cytokine IL-33 directly interacts with invariant NKT and NK cells to induce IFN- γ production. *Eur. J. Immunol.* 39: 1046–1055.
 21. Lisbonne, M., and M. C. Leite-de-Moraes. 2003. Invariant Valpha14 NKT lymphocytes: a double-edged immuno-regulatory T cell population. *Eur. Cytokine Netw.* 14: 4–14.
 22. Wilson, S. B., and T. L. Delovitch. 2003. Janus-like role of regulatory iNKT cells in autoimmune disease and tumour immunity. *Nat. Rev. Immunol.* 3: 211–222.
 23. Mars, L. T., J. Novak, R. S. Liblau, and A. Lehuen. 2004. Therapeutic manipulation of iNKT cells in autoimmunity: modes of action and potential risks. *Trends Immunol.* 25: 471–476.
 24. Lisbonne, M., S. Diem, A. de Castro Keller, J. Lefort, L. M. Araujo, P. Hachem, J. M. Fourneau, S. Sidobre, M. Kronenberg, M. Taniguchi, et al. 2003. Cutting edge: invariant V alpha 14 NKT cells are required for allergen-induced airway inflammation and hyperactivity in an experimental asthma model. *J. Immunol.* 171: 1637–1641.
 25. Araujo, L. M., J. Lefort, M. A. Nahori, S. Diem, R. Zhu, M. Dy, M. C. Leite-de-Moraes, J. F. Bach, B. B. Vargaftig, and A. Herbelin. 2004. Exacerbated Th2-mediated airway inflammation and hyperresponsiveness in autoimmune diabetes-prone NOD mice: a critical role for CD1d-dependent NKT cells. *Eur. J. Immunol.* 34: 327–335.
 26. Beasley, R. 2002. The burden of asthma with specific reference to the United States. *J. Allergy Clin. Immunol.* 109(5, Suppl): S482–S489.
 27. Weiss, K. B., and S. D. Sullivan. 2001. The health economics of asthma and rhinitis. I. Assessing the economic impact. *J. Allergy Clin. Immunol.* 107: 3–8.
 28. Wills-Karp, M. 1999. Immunologic basis of antigen-induced airway hyper-responsiveness. *Annu. Rev. Immunol.* 17: 255–281.
 29. Orange, J. S., E. M. Hossny, C. R. Weiler, M. Ballou, M. Berger, F. A. Bonilla, R. Buckley, J. Chinen, Y. El-Gamal, B. D. Mazer, et al; Primary Immunodeficiency Committee of the American Academy of Allergy, Asthma and Immunology. 2006. Use of intravenous immunoglobulin in human disease: a review of evidence by members of the Primary Immunodeficiency Committee of the American Academy of Allergy, Asthma and Immunology. [Published erratum appears in 2006. *J. Allergy Clin. Immunol.* 117: 1483.] *J. Allergy Clin. Immunol.* 117(4, Suppl): S525–S553.
 30. Schwartz, H. J., R. W. Hostoffer, E. R. McFadden, Jr., and M. Berger. 2006. The response to intravenous immunoglobulin replacement therapy in patients with asthma with specific antibody deficiency. *Allergy Asthma Proc.* 27: 53–58.
 31. Hachem, P., M. Lisbonne, M. L. Michel, S. Diem, S. Roongapinun, J. Lefort, G. Marchal, A. Herbelin, P. W. Askenase, M. Dy, and M. C. Leite-de-Moraes. 2005. Alpha-galactosylceramide-induced iNKT cells suppress experimental allergic asthma in sensitized mice: role of IFN- γ . *Eur. J. Immunol.* 35: 2793–2802.
 32. Morishima, Y., Y. Ishii, T. Kimura, A. Shibuya, K. Shibuya, A. E. Hegab, T. Iizuka, T. Kiwamoto, Y. Matsuno, T. Sakamoto, et al. 2005. Suppression of eosinophilic airway inflammation by treatment with alpha-galactosylceramide. *Eur. J. Immunol.* 35: 2803–2814.
 33. Kim, H. Y., S. Kim, and D. H. Chung. 2006. Fc γ RIII engagement provides activating signals to NKT cells in antibody-induced joint inflammation. *J. Clin. Invest.* 116: 2484–2492.
 34. Amigorena, S., C. Bonnerot, J. R. Drake, D. Choquet, W. Hunziker, J. G. Guillet, P. Webster, C. Sautes, I. Mellman, and W. H. Fridman. 1992. Cytoplasmic domain heterogeneity and functions of IgG Fc receptors in B lymphocytes. *Science* 256: 1808–1812.
 35. Park-Min, K. H., N. V. Serbina, W. Yang, X. Ma, G. Krystal, B. G. Neel, S. L. Nutt, X. Hu, and L. B. Ivashkiv. 2007. Fc γ RIII-dependent inhibition of interferon- γ responses mediates suppressive effects of intravenous immune globulin. *Immunity* 26: 67–78.
 36. Clynes, R. 2007. IVIG therapy: interfering with interferon- γ . *Immunity* 26: 4–6.



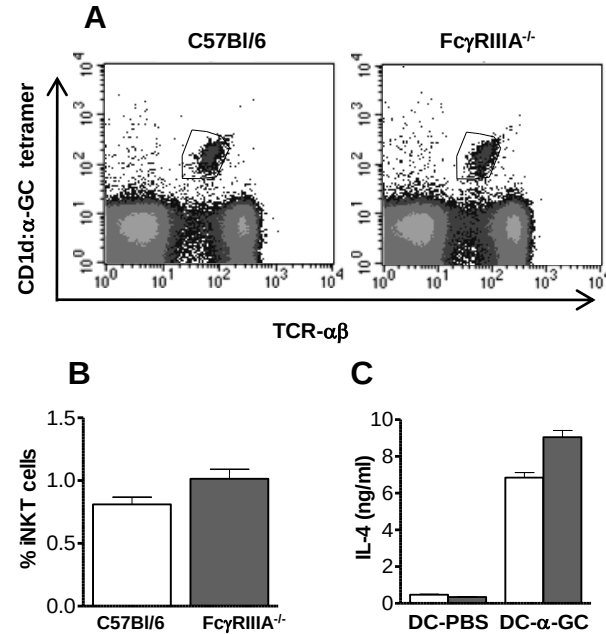
Supplementary Fig. 1: IVIg-treatment inhibits bronchopulmonary hyperreactivity but not local Th2-type cytokine production in OVA-sensitized and -challenged mice. WT mice were sensitized with OVA and treated with IVIg or PBS 1h prior to the first challenge with OVA or PBS. (A) Bronchopulmonary hyperreactivity to metacholine was measured 1d after the last challenge. Data represent the mean $\pm$ s.e.m. Penh values of a representative experiment out of two ($n=4-6$ mice per group). The difference between OVA-sensitized and -challenged mice treated with IVIg and OVA-sensitized and -challenged mice treated with PBS is significant ($P=0.006$, ANOVA repeated measure). (B) The levels (means $\pm$ s.e.m) of IL-4 in BALF and IL-5 and eotaxin in the lung homogenates were determined 24h after the last challenge. Data are pooled results from two independent experiments. $n=6$ to 11 mice per group. NS: not significant.



Supplementary Fig.2: *In vitro* inhibitory effect of IVIg on IL-4 production by iNKT cells. Freshly sorted iNKT cells as CD1d:α-GC T^T⁺ CD5⁺ cells (2.5x10⁴ cells/well) from spleen from WT mice were cultured for 48h with α-GC-pulsed DC (DC α-GC) in presence or absence of IVIg (8mg/ml). IL-4 levels in cell-free culture supernatants were measured. Data are pooled results from three independent experiments. Data represent means±s.e.m.



Supplementary Fig. 3: IVIg-preconditioned iNKT cells from naïve mice do not confer protection to WT recipients. OVA-sensitized WT mice were transferred with iNKT-enriched cells (2×10^6 /mouse) from PBS- or IVIg-treated mice 1h prior to the first challenge with OVA or PBS. Infiltrating total (A) cells and eosinophils (B) in the BALF were determined 24h after the last challenge. Data are pooled results from two independent experiments. $n=9$ mice per group. Data represent means $\pm$ s.e.m. NS: not significant.



Supplementary Fig. 4: Analysis of iNKT cells in FcγRIIIA^{-/-} mice. Representative flow cytometry plots defining the iNKT cell population as α-GC:CD1d TT⁺ CD5⁺ cells (A). Percentage of iNKT cells in the spleen of naïve WT or FcγRIIIA^{-/-} mice (B). Data are expressed as mean s.e.m. of a representative experiment out of two (*n*=4 mice per group). FACS-sorted splenic iNKT cells from WT or FcγRIIIA^{-/-} mice were stimulated with α-GC-pulsed DC for 48h. IL-4 levels in cell-free culture supernatants were measured by ELISA (C). Data are expressed as mean s.e.m. of a representative experiment out of two (*n*=3 mice per group).