Cutting Edge: The Murine High-Affinity IgG Receptor Fc γRIV Is Sufficient for Autoantibody-Induced Arthritis

David A. Mancardi, Friederike Jönsson, Bruno Iannascoli, Huot Khun, Nico Van Rooijen, Michel Huerre, Marc Daëron and Pierre Bruhns

J Immunol published online 19 January 2011
http://www.jimmunol.org/content/early/2011/01/19/jimmunol.1003642

Supplementary Material
http://www.jimmunol.org/content/suppl/2011/01/19/jimmunol.1003642.DC1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Cutting Edge: The Murine High-Affinity IgG Receptor FcγRIV Is Sufficient for Autoantibody-Induced Arthritis

David A. Mancardi,*†,1 Friederike Jönsson,*‡,1 Bruno Iannascoli,*† Huot Khun,‡ Nico Van Rooijen,§ Michel Huerre,‡ Marc Daëron,*† and Pierre Bruhns,*‡

K/BxN serum-induced passive arthritis was reported to depend on the activation of mast cells, triggered by the activating IgG receptor FcγRIIIA, when engaged by IgG1 autoantibodies present in K/BxN serum. This view is challenged by the fact that FcγRIIIA-deficient mice still develop K/BxN arthritis and because FcγRIIIA is the only activating IgG receptor expressed by mast cells. We investigated the contribution of IgG receptors, IgG subclasses, and cells in K/BxN arthritis. We found that the activating IgG2 receptor FcγRIV, expressed only by monocytes/macrophages and neutrophils, was sufficient to induce disease. K/BxN arthritis occurred not only in mast cell-deficient W/Wv mice, but also in mice whose mast cells express no activating IgG receptors. We propose that at least two autoantibody isotypes, IgG1 and IgG2, and two activating IgG receptors, FcγRIIIA and FcγRIV, contribute to K/BxN arthritis, which requires at least two cell types other than mast cells, monocytes/macrophages, and neutrophils. The Journal of Immunology, 2011, 186: 000–000.

Rheumatoid arthritis (RA) is a systemic autoimmune disease due to autoantibodies in the serum of patients (e.g., anti–glucose-6-phosphate-isomerase [GPI] autoantibodies) (1). IgG anti–GPI autoantibodies are present in the serum of offspring of KRN-transgenic C57BL/6 crossed to NOD mice (2) (K/BxN serum), which develop spontaneously an RA-like disease. Normal mice injected with K/BxN serum develop passive arthritis (K/BxN-PA). IgG1 autoantibodies represent the majority of IgG anti–GPI autoantibodies, and they are therefore thought to account for K/BxN-PA. K/BxN serum, however, also contains IgG2 anti–GPI autoantibodies (3).

Among the three murine activating IgG receptors (FcγR), FcγRIIA can be engaged by IgG1 and IgG2, whereas FcγRI and FcγRIV can be engaged by IgG2 only (4). K/BxN-PA is abrogated in mice lacking the FcγRI subunit (5, 6) that is required for the expression and function of these FcγRs (7). Interestingly, K/BxN-PA is reduced, but not abolished, in FcγRIIA−/− mice (5, 8), suggesting that FcγRI and/or FcγRIV contribute to disease. K/BxN-PA is, however, unaffected in mice lacking FcγRI (5, 6). The role of FcγRIV has not been investigated.

FcγRIV is expressed by monocytes/macrophages and neutrophils, and FcγRIIIA by monocytes/macrophages, neutrophils, and mast cells. These three cell types have been reported to be mandatory for K/BxN-PA. K/BxN-PA is indeed, abrogated in wild-type (wt) mice depleted of monocytes/macrophages (9), depleted of neutrophils (10), or lacking mature neutrophils (11). Based on experiments performed in mast cell-deficient W/Wv mice, mast cells are considered necessary for K/BxN-PA (12). A requirement for mast cells in K/BxN-PA appears, however, in contradiction with the fact that K/BxN-PA is not abolished in FcγRIIIA−/− mice, because FcγRIIIA is the only activating FcγR expressed by mast cells. W/Wv mice display many abnormalities other than mast cell deficiency that may explain these contradictory results (13–15).

We therefore investigated the role of FcγRIV, as well as the requirement for mast cells, in K/BxN-PA. We found that mast cell-deficient W/Wv mice and mice expressing no FcγR but FcγRIV developed K/BxN-PA. FcγRIV is indeed sufficient to induce arthritis in the absence of other activating IgG receptors. FcγRIV-dependent arthritis was induced by IgG2 autoantibodies and required both monocytes/macrophages and FcγRIIIA-expressing monocytes/macrophages.

Received for publication November 4, 2010. Accepted for publication December 20, 2010.

This work was supported by Institut Pasteur, INSERM, Agence Nationale de la Recherche (Grants 05-JCJC-026-01 and 09-GENO-0314-01), and funding under the Sixth Research Framework Program of the European Union (Project MUGEN, LSHG-CT-2005-005203). D.A.M. was financially supported by the Fondation pour la Recherche Médicale and is currently a recipient of a fellowship from the Institut Pasteur. Attitude of the University of Paris, 25 Rue du Docteur Roux, 75015 Paris, France. E-mail address: bruhns@pasteur.fr

The online version of this article contains supplemental material.

Abbreviations used in this article: FcRa, neonatal Fc receptor; GPI, glucose-6-phosphate-isomerase; K/BxN-PA, K/BxN serum-induced passive arthritis; RA, rheumatoid arthritis; wt, wild-type.

Copyright © 2011 by The American Association of Immunologists, Inc. 0022-1767/11/$16.00
and neutrophils. FcγRIV also contributed to arthritis in wt mice. Importantly, we identified FcγRIIIA and FcγRIV as the IgG receptors responsible for K/BxN-PA in wt mice.

Materials and Methods

Mice

The wt mice were purchased from Charles River Laboratories and FcγRIIB−/−, FcγRIIIA−/−, FcγRI, and FcγRIIa mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and crossed to 5KO mice to obtain 6KO and hFcRn tg (4). hFcRn tg mice (Bar Harbor, ME) were obtained from D. Mathis, C. Benoist (Harvard Medical School, Boston, MA) and the Institut de Génétique et de Biologie Moléculaire et Cellulaire (Strasbourg, France). Mice used in experiments were on the C57BL/6J background (6th–12th generation backcross), except W/W v mice (Ile de France). Mice used in experiments were approved by the Animal Care and Use Committees of Paris, Ile de France, Paris, France.

Reagents

Anti-mFcγRIIA (275033) and anti-mFcγRI (293322) were from R&D Systems; anti-FcγRIIB/IIIA (2.4G2), CD11b, CD11c, Gr1, SiglecF, CD117, DX5, and IgE were from BD Biosciences; HRP-coupled anti-mouse IgG subclasses were from Southern Biotechnology Associates; and GPI and control mAbs (6A6) were from Dr. R. Good (University of South Florida College of Medicine, Tampa, FL). Purified anti-mFcγRII (2178, 2179) was provided by M.P. Hogarth (ARI, Heidelberg, Victoria, Australia) and anti-FcγRs (4) was incubated for 30 min at 4°C with indicated mAbs before flow cytometry analysis.

Cell counts and flow cytometry analysis

Cell counts in whole blood from 7–9-wk-old mice were determined using an ABC Vet automatic blood analyzer (Horiba ABX). Ex vivo cells were defined as follows by flow cytometry: monocytes/macrophages (blood/peritoneum: CD11b+/Gr1−); bronchoalveolar lavage: CD11c+/Gr1+); neutrophils (Gr1+/SiglecF−); basophils (IgE+/DX5+); and mast cells (IgE+/CD117+). A total of 2 × 10^6 Chinese hamster ovary-K1 transfectants expressing mFcγRs (4) was incubated for 30 min at 4°C with indicated mAbs before flow cytometry analysis.

FIGURE 1. FcγR IV is sufficient to induce K/BxN-PA, and K/BxN-PA occurs in the absence of mast cells. A, K/BxN-PA in indicated mice (n = 3). Statistical differences between wt and 5KO mice are indicated for each time point if significant. B and C, K/BxN-PA in 5KO mice injected with indicated mAbs (n = 4) (B) or with indicated liposomes (n = 6) or mAbs (n = 4) (C). H&E stainings of sections of paraffin-embedded decalcified ankle joints on day 6. Original magnification X20. D, Mice were injected with K/BxN serum on day 0 and i.p. with luminol on the indicated day. Images show color-coded maps of photon flux superimposed on black and white photographs. Bioluminescence is expressed as average radiance of all four joints (n = 5). E, K/BxN-PA in indicated mice injected with 50 μl K/BxN serum (n = 6). Statistical differences between wt and W/W v mice are indicated for each time point if significant. F, Percentage of basophils among leukocytes in blood from indicated mice acquired by flow cytometry (n = 8). G, K/BxN-PA in mice injected with 40 μl K/BxN serum and indicated mAbs (n = 4). Data are representative from at least two independent experiments and presented as mean ± SEM (A–E, G). NS, p > 0.05, *p < 0.05, **p < 0.01, ***p < 0.001.
Statistical analyses

Data were analyzed using one-way ANOVA with Bonferroni posttest (Fig. 1F; Supplemental Fig. 2C) or Student t test (all other data). Statistical significance is indicated.

Results and Discussion

Classically, K/BxN-PA is thought to depend on FcγRIIIA. Arthritic symptoms are indeed reduced, but they are not abolished in FcγRIIIA−/− mice (5) (Fig. 1A). The clinical score was higher in double-deficient FcγRIIIB/III A−/− mice that also lack inhibitory FcγRIIB compared with the clinical score of FcγRIIIA−/− mice (Fig. 1A). FcγRIIB deficiency has indeed been reported to increase the susceptibility to K/BxN-PA (17), as well as to other arthritis models (18, 19). FcγRIIIA-independent K/BxN-PA is therefore negatively regulated by FcγRIIB. No K/BxN-PA could be induced in FcγRI−/− mice, as reported (5, 6, 17). Together these observations suggest the involvement of activating FcγR other than FcγRIIIA (i.e., FcγRI and/or FcγRIV) in K/BxN-PA.

Although the deletion of FcγRI prevented arthritis in an active model of RA (20), FcγRI−/− mice developed K/BxN-PA as wt mice (5, 6) (data not shown). Supporting the latter finding, this receptor was reported not to be expressed in naive mice on blood monocytes, tissue macrophages, or neutrophils, but only on dendritic cells and elicited or derived macrophages (21). Indeed, we observed FcγRI on macrophage cell lines (Supplemental Fig. 1A) and on bone marrow-derived macrophages (Supplemental Fig. 1B), as expected (20, 21), but not on freshly isolated monocytes, macrophages, neutrophils, or other blood, splenic, or peritoneal cells from naive C57BL/6J mice (Supplemental Fig. 1B) when stained with specific (Supplemental Fig. 1C), nonblocking (Supplemental Fig. 1D), anti-FcγRI mAbs. In addition, FcγRI expression was undetectable on Mac-1+ cells in blood or peritoneum even following K/BxN serum injection (Supplemental Fig. 1E). These data do not support a contribution of FcγRI to K/BxN-PA (5, 6), and we therefore investigated the possible contribution of FcγRIV.

Supporting this possibility, K/BxN-PA was reported to be reduced but not abolished in double-deficient FcγRIIIB/III A−/− mice (8). We used FcγRIIIB/III A−/− FcγRII/IIB−/− (5KO) mice (4) to demonstrate the role of FcγRIV in K/BxN-PA. These mice express no activating IgG FcR, but FcγRIV could induce K/BxN-PA without FcγRI and FcγRII deficiency (8). We used FcγRIIIB/III A−/− mice (5, 6) (data not shown). Supporting the latter finding, this receptor was reported not to be expressed in naive mice on blood monocytes, tissue macrophages, or neutrophils, but only on dendritic cells and elicited or derived macrophages (21). Indeed, we observed FcγRI on macrophage cell lines (Supplemental Fig. 1A) and on bone marrow-derived macrophages (Supplemental Fig. 1B), as expected (20, 21), but not on freshly isolated monocytes, macrophages, neutrophils, or other blood, splenic, or peritoneal cells from naive C57BL/6J mice (Supplemental Fig. 1B) when stained with specific (Supplemental Fig. 1C), nonblocking (Supplemental Fig. 1D), anti-FcγRI mAbs. In addition, FcγRI expression was undetectable on Mac-1+ cells in blood or peritoneum even following K/BxN serum injection (Supplemental Fig. 1E). These data do not support a contribution of FcγRI to K/BxN-PA (5, 6), and we therefore investigated the possible contribution of FcγRIV.

Supporting this possibility, K/BxN-PA was reported to be reduced but not abolished in double-deficient FcγRIIIB/III A−/− mice (8). We used FcγRIIIB/III A−/− FcγRII/IIB−/− (5KO) mice (4) to demonstrate the role of FcγRIV in K/BxN-PA. These mice express no activating IgG FcR, but FcγRIV could induce K/BxN-PA without FcγRI and FcγRII deficiency (8).

These mice express no activating IgG FcR, but FcγRIV could induce K/BxN-PA without FcγRI and FcγRII deficiency (8). We used FcγRIIIB/III A−/− FcγRII/IIB−/− (5KO) mice (4) to demonstrate the role of FcγRIV in K/BxN-PA. These mice express no activating IgG FcR, but FcγRIV could induce K/BxN-PA without FcγRI and FcγRII deficiency (8).

These mice express no activating IgG FcR, but FcγRIV could induce K/BxN-PA without FcγRI and FcγRII deficiency (8). We used FcγRIIIB/III A−/− FcγRII/IIB−/− (5KO) mice (4) to demonstrate the role of FcγRIV in K/BxN-PA. These mice express no activating IgG FcR, but FcγRIV could induce K/BxN-PA without FcγRI and FcγRII deficiency (8).
the difference in susceptibility to K/BxN-PA between these two strains (Supplemental Fig. 2B, 2D). Higher neutrophil (15) and/or monocyte numbers in W5th in W/Wv mice (Supplemental Fig. 2B) may explain this difference. Supporting this hypothesis, a transfer of neutrophils was recently reported to be sufficient to restore K/BxN-PA in W/Wv mice (24). Lower numbers of neutrophils may therefore protect W/Wv mice from K/BxN-PA. Altogether, these results suggest that mast cells may not be mandatory for K/BxN-PA. This conclusion is strengthened by our results showing that mice that do not express activating FcγR on their mast cells, and basophils still develop K/BxN-PA (i.e., FcγRIIAlow−/− mice, FcγRIIBIIA−/− mice, and 5KO mice). Mast cells may, however, participate in K/BxN-PA (12, 25) and human RA, as high numbers of degranulated mast cells can be observed in the joints of patients (26).

K/BxN-PA was reported to depend primarily on IgG1 autoantibodies (3). FcγRIV, however, has no affinity for IgG1 (27). The finding that K/BxN serum could induce arthritis in 5KO mice can be explained by the presence of IgG2 anti-GPI Abs (3) in K/BxN serum (Fig. 2A). Indeed, IgG1 purified from K/BxN serum induced arthritis in wt mice, but not in 5KO mice, whereas purified IgG2 induced arthritis similarly in both mice (Fig. 2B). Endogenous IgG2 is protected from degradation by FcRn in 5KO, but not in 5KO mice lacking FcRn (6KO mice) (Fig. 2C, left panel). In accordance with these data, FcγRIV-dependent arthritis was strongly inhibited in K/BxN mice following K/BxN serum injection (Fig. 2D), probably due to the lower in vivo persistence of anti-GPI IgG2b Abs in 6KO than in 5KO mice (Fig. 2C, right panel) as expected (28). K/BxN-PA was restored when 6KO mice were transgenic for human FcRn (Fig. 2D). Indeed, human FcRn has an affinity for mouse IgG2 (29) that it can protect from degradation (30). It has no affinity for mouse IgG1. Incidentally, the low affinity of human FcRn for mouse IgG2 had not been previously reported to be sufficient for in vivo function. Taken together, these results demonstrate that IgG2 autoantibodies are sufficient to induce K/BxN-PA not only in 5KO mice but also in wt mice. Importantly, blocking FcγRIV reduced K/BxN-PA in wt mice (Fig. 2E). These results indicate that FcγRIV, and therefore IgG2 autoantibodies, contribute to K/BxN-PA in normal mice. Supporting this conclusion, blocking FcγRIIAlow markedly reduced but blocking both receptors was required to abolish arthritis in wt mice (Fig. 2E). Similar results were obtained in FcγRIIBIIA−/− (Supplemental Fig. 2E) or in FcγRIIIA−/− mice (Supplemental Fig. 2A), in which FcγRI accounted for arthritis. FcγRIV therefore contributes significantly to arthritis together with FcγRIIA in wt mice, even though the contribution of FcγRIIA is predominant.

In conclusion, genetically modified mice, expressing a restricted set of activating IgG receptors, enabled us to demonstrate that the high-affinity IgG receptor FcγRIV was sufficient to induce K/BxN-PA (i.e., in the absence of FcγRI, FcγRIIB, and FcγRIIIA). IgG2 autoantibodies contained in K/BxN serum can, by themselves, induce and significantly contribute to K/BxN-PA. Our results also demonstrate that, together, FcγRIIAlow and FcγRIV are responsible for K/BxN-PA symptoms in wt mice. In addition, our results provide two independent pieces of evidence demonstrating that mast cells are not mandatory for K/BxN-PA. Indeed, K/BxN-PA was unaltered in mast cell-deficient W5th mice, and mice for which mast cells could not be activated directly by autoantibodies from K/BxN serum developed K/BxN-PA. Finally, our results demonstrate that activation of neutrophils and monocytes/macrophages is sufficient to induce autoantibody-induced arthritis.

Acknowledgments
We thank S. Darvellte, F. Nato (Plate-forme de Production de Protéines Recombinantes et d’Anticorps, Institut Pasteur, Paris, France) for generation, cloning, and characterization of anti-GP1 mAbs, A.-M. Nicola (Plate-Forme d’Imagerie Dynamique, Institut Pasteur, Paris, France) for help with the bioluminescence experiments, and C. Dechepare for administrative help. We also thank our colleagues for generous gifts: S. Verbeek, J.-P. Kinet, M. Lamers, D.C. Roopenian, D. Mathis, and C. Benoist for mice and J.V. Ravetch, H. Karayuama, R. Coffman, R. Good, and M.P. Hogarth for Abs. Cl3MDP was a gift from Roche Diagnostics GmbH.

Disclosures
The authors have no financial conflicts of interest.

References


