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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

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

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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), FcγRIIIA 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 FcγRIIIA−/− 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 FcR 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,

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1D.A.M. and F.J. performed experiments and designed part of the research; B.I. genotyped mice and produced essential reagents; H.K. and M.H. performed histology and discussed results; P.B., M.D., and F.J. wrote the manuscript; and P.B. designed and supervised the research.

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The online version of this article contains supplemental material.

Abbreviations used in this article: FcR, neonatal Fc receptor; GPI, glucose-6-phosphate-isomerase; K/BxN-PA, K/BxN serum-induced passive arthritis; RA, rheumatoid arthritis; wt, wild-type.
and neutrophils. FcγRIV also contributed to arthritis in wt mice. Importantly, we identified FcγRIIIA and FcγRIIV 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−/−, FcγRIIA−/−, FcγRIII−/−, FcγRI−/−, FcγRI-2a−/−, FcγRI-2b−/−, FcγRIIb−/−, FcγRIIc−/−, FcγRIV−/−, and 5KO mice were purchased 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 approved by the Animal Care and Use Committees of Paris, Ile de France, and the University of Florida College of Medicine, Tampa, FL. Purified anti-mFcγR mAbs (9E9) were provided by J.V. Ravetch (The Rockefeller University, New York, NY), anti-Gr1 mAb (RB6-8C5) by R. Coffman (DNAX, Palo Alto, CA), and anti-platelet mAb (6α6b) by Dr. R. Good (University of South Florida College of Medicine, Tampa, FL). Purified anti-FcγRII (2178, 2179) was provided by J.J. Pagano (New York Blood Center, New York). Hybridomas producing anti-mFcγRRIIb, IIb/IIIa (2.4G2), CD11b, CD11c, Gr1, SiglecF, CD117, DX5, and IgE were from BD Biosciences; HRP-coupled anti-mouse IgM, CD117, osteopontin, and osteonectin mAbs were from R&D Systems; anti-FcγRIIIA (275003) and anti-FcγRI (290322) 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 and IgG subclasses were from Southern Biotechnology Associates; and GPI and laminin were from Sigma-Aldrich. Hybridomas producing anti-mFcγR mAb (9E9) were provided by J.V. Ravetch (The Rockefeller University, New York, NY), anti-Gr1 mAb (RB6-8C5) by R. Coffman (DNAX, Palo Alto, CA), and anti-platelet mAb (6α6b) by 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-CD200R3 mAb (Ba103) by H. Karasuyama (Tokyo Medical and Dental University, Tokyo, Japan). PBS-liposomes and clodronate-liposomes were prepared as published (16).

Anti-GPI IgG were purified from K/BxN serum using protein G, polyclonal IgG1, and IgG2 fractions using anti-mIgG1 or anti-mIgG2 Sepharose beads (Nordic Immunology). IgG subclasses were determined by ELISA using HRP-coupled anti-mouse IgG1, anti-mouse IgG2a plus anti-mouse IgG2c, or anti-mouse IgG2b (Southern Biotechnology). Specific anti-GPI levels were measured by anti-subclass ELISA on GPI-coated plates using IgG1, IgG2a, and IgG2b anti-GPI mAbs (obtained in collaboration with the Ab Production Platform, Institut Pasteur, Paris, France) as internal standards.

K/BxN-PA

K/BxN serum was generated, and arthritis was scored as described (6). When not otherwise specified, arthritis was induced by an i.v. injection of 150 μl K/BxN serum. Anti-FcγRIV (200 μg/mouse) and anti-FcγRIIIA (100 μg/mouse) blocking mAbs were injected i.v. 30 min before K/BxN serum and on days +1/+2/+4. Liposomes (2.1 mg/mouse) and anti-CD200R3 (100 μg/mouse) were injected i.v. 24 h before K/BxN serum. 6A6 (50 μg/mouse) or anti-Gr1 (500 μg/mouse) mAbs were injected i.v. on days −1/+1/+3/+5. Sections of paraffin-embedded ankle joints were stained with hematoxylin and eosin (H&E). Bioluminescence from mice injected with 10 mg/mouse luminol i.p. was acquired on an IVIS-100 (Caliper LifeSciences).

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 × 10⁵ Chinese hamster ovary-K1 transfectants expressing FcγRIV (4) was incubated for 30 min at 4°C with indicated mAbs before flow cytometry analysis.
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 FcγRIIB/III/II/II-/-/- 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), FcγRIIIA-/-/-/ 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, or neutrophils, or other blood, splenic, or peritoneal cells from naive C57BL/6J mice (Supplemental Fig. 1A) 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 FcγRIIIA-/-/- mice (8). We used FcγRIIB/III/II/II-/-/- (5KO) mice (4) to demonstrate the role of FcγRIV in K/BxN-PA. These mice express no activating IgG FcR, but FcγRIV. We found that K/BxN serum (Fig. 1A, red curve) or IgG purified from K/BxN serum (data not shown) induced arthritis in 5KO mice. Clinical scores and histological inflammation induced under these conditions were, however, milder than in wt mice (Supplemental Fig. 1F). Blocking of FcγRIV with anti-FcγRIV mAbs prevented the development of arthritis (Fig. 1B, Supplemental Fig. 1G) in 5KO mice. FcγRIV is therefore sufficient for K/BxN-PA. FcγRIV-dependent arthritis was abolished when monocytes/macrophages or neutrophils were depleted in 5KO mice (Fig. 1C), as reported for wt mice (9, 10). A recent report demonstrating that K/BxN-PA could be restored in neutrophil-deficient mice by transferring wt or FcγRIIIA-/-/- bone marrow, but not by transferring FcγRI-/-/- bone marrow (11), supports the role of FcγRIV on neutrophils in K/BxN-PA. Activated neutrophils and inflammatory monocytes/macrophages can release myeloperoxidase. Myeloperoxidase can oxidize luminol, leading to photon emission that can be detected by bioluminescence imaging (22). Following luminol injection, luminescence was detected in the joint areas, as early as 1 d after K/BxN serum injection in both wt and 5KO mice (Fig. 1D). Whereas K/BxN-PA was reported to require mast cell activation (23), FcγRIV is not expressed on mouse mast cells. We therefore investigated how FcγRIV could induce K/BxN-PA without being expressed on mast cells and whether FcγRIV contributes to K/BxN-PA in wt mice.

We used mast cell-deficient mouse strains, W/Wv and Wsh, to further investigate the role of mast cells in K/BxN-PA. We found, as previously described (12, 17), that most W/Wv mice did not develop K/BxN-PA. Importantly, all Wsh mice developed arthritis when injected i.v. (Fig. 1E) or i.p. (Supplemental Fig. 1H) with K/BxN serum (in agreement with unpublished data discussed in Ref. 23). Similar results were reported for these two mouse strains in another passive model of arthritis (15). In addition to their mast cell deficiency (Supplemental Fig. 2A), W/Wv and Wsh mice have many other abnormalities (14, 15) (Supplemental Fig. 2B). Noticeably, we found that W/Wv mice display basopenia, whereas Wsh mice display basophilia (Fig. 1F). Basophil depletion, however, did not prevent K/BxN-PA in Wsh mice (Fig. 1G, Supplemental Fig. 2C). Basophils could therefore not replace mast cells and explain why Wsh, but not W/Wv mice, developed arthritis. Similarly, depletion of platelets did not abolish K/BxN-PA in Wsh mice, indicating that the higher number of platelets found in Wsh compared with W/Wv mice could not explain why Wsh, but not W/Wv mice, developed arthritis.

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.

**FIGURE 2.** FcγRIV and IgG2 autoantibodies contribute to K/BxN-PA in wt mice. A. Total Ig (Total), IgG1 (1), IgG2a/c (2a/c), and IgG2b (2b) anti-GPI content in unfractionated K/BxN serum or in purified IgG1 or IgG2 fractions from K/BxN serum. B. Arthritis induced by 80 μg K/BxN IgG1 or K/BxN IgG2 (n = 3). C. Left panel, Relative endogenous levels of indicated IgG subclasses in the serum of naive 5KO (n = 4) and 6KO (n = 5) mice as measured by ELISA. Right panel, Anti-GPI IgG2b concentration in the serum of 5KO and 6KO mice (n = 4) injected with 500 μg anti-GPI IgG2b mAb at t = 0, collected at indicated times. D, K/BxN-PA in 5KO (n = 5), 6KO (n = 5), and hFcRn666KO mice (n = 3). Statistical differences between 6KO and hFcRn666KO mice are indicated for each time point if significant. E, K/BxN-PA in mice injected with indicated mAbs (n = 4). Data are representative from two independent experiments (A, C–E) and represented as mean ± SEM (B–E). NS, p > 0.05, *p < 0.05, **p < 0.01, ***p < 0.001.
the difference in susceptibility to K/BxN-PA between these two strains (Supplemental Fig. 2B, 2D). Higher neutrophil (15) and/or monocyte numbers in W° than in W/W° 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/W° mice (24). Lower numbers of neutrophils may therefore protect W/W° 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., FcγRIIA-/- mice, FcγRIIB/IIIA-/- 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–IgG1 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 6KO mice following K/BxN serum injection (Fig. 2D), probably due to the lower in vivo persistence of anti–IgG1 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 FcγRn has an affinity for mouse IgG2 (29) that it can protect from degradation (30). It has no affinity for mouse FcγRIIA (31). FcγRIIA is predominant.

In conclusion, genetically modified mice, expressing a re-stricted set of activating IgG receptors, enabled us to demon-strate 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, FcγRIIIA 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 W° 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. Dartevelle, F. Nato (Plate-forme de Production de Proteines Recombinantes et d'Anticorps, Institut Pasteur, Paris, France) for generation, cloning, and characterization of anti–IgG1 mAbs, A.-M. Nicola (Plate-Forme d'Imagerie Dynamique, Institut Pasteur, Paris, France) for help with the bioluminescence experiments, and C. Detchpare 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. Karayumyan, R. Coffman, R. Good, and M.P. Hogarth for Abs. Cl2MDP was a gift from Roche Diagnostics GmbH.

Disclosures

The authors have no financial conflicts of interest.

References


Supplemental Figure 1. (A-C) Clone 290322 is a non-blocking anti-FcγRI specific mAb. (A) FcγRI-expressing macrophage cell line. Histograms show the binding of indicated mAbs to RAW cells. (B) FcγRI is not expressed in naive C57BL/6 mice. Representative expression of FcγRI on bone marrow-derived macrophages (BMdM) differentiated in the presence of murine M-CSF for 8 days, and in the presence or not of IFNγ or LPS for the last 24 hours before analysis, or on monocytes/macrophages or neutrophils from indicated tissues. (C) Representative staining of FLAG-tagged mouse FcγRs CHO-K1 transfectants by indicated mAbs analyzed by flow cytometry. (D) Density plots show the binding of monomeric murine IgG2a or anti-mouse FcγRI when incubated with mFcγRI-expressing CHO-K1 transfectants in the indicated order. (E) K/BxN serum injection does not induce FcγRI expression in wt mice. Representative expression of FcγRI on Mac-1+ cells from blood or peritoneum of untreated or K/BxN serum-injected wt mice (day 6 post-injection). (F) Milder arthritic symptoms in 5KO than in wt mice. Representative hematoxilin-eosin staining of sections of paraffin-embedded decalcified ankle joints from mice from Fig.1A on day 6 injected with K/BxN serum (acute inflammation with focal recruitment of polymorphonuclear cells and monocytes) or not injected (no signs of inflammation). Magnification: 20x. (G) FcγRIV is responsible for K/BxN-PA in 5KO mice. Representative paraffin-embedded ankle sections of 5KO mice stained with hematoxilin-eosin, on day 6. Magnification: 200x. (H) K/BxN PA develops in mast cell-deficient Wsh mice. Representative paraffin-embedded ankle sections of Wsh mice injected with K/BxN serum i.v. or i.p. (n=3). (A-H) Data are representative of at least two independent experiments and (G,H) represented as mean +/-SEM.
Supplemental Figure 2. (A) W/Wv and Wsh mice lack peritoneal mast cells. Percentages of IgE+/CD117+ mast cells among peritoneal cells in indicated mice. (B) Blood cell population abnormalities in W/Wv and Wsh mice. Cell counts in the blood of indicated naive mice were acquired on an automatic blood analyzer, and the percentage of neutrophils among WBC was determined by flow cytometry following CD11b and Gr-1 staining (n=8). (C) Efficient basophil depletion in Wsh mice. Representative percentages of IgE+/DX5+ basophils among blood leukocytes in Wsh mice from Fig.1G. (D) High platelet numbers are not mandatory for K/BxN-PA in Wsh mice. K/BxN-PA in mice injected i.v. with 80μL K/BxN serum and indicated mAbs (Statistical differences are indicated for each time point if significant), and platelet counts from these mice acquired on an automatic blood analyzer (n=4). (E,F) FcγRII is responsible for FcγRIIIA-independent K/BxN-PA. K/BxN-PA in indicated mice injected with indicated mAbs (n=4). (A-E) Data are representative of at least two independent experiments and (D-F) represented as mean +/-SEM.