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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 Wsh 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 spontane-
ously an RA-like disease. Normal mice injected with K/BxN-serum develop passive arthritis (K/BxN-PA). IgG1 autoanti-
obodies 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 FcγR subunit (5, 6) that is required for the expression and function of these FcγR (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, un-
affected 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, abro-
gated 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/W mice, mast cells are considered neces-
sary 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/W 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/W 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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Abbreviations used in this article: FcRs, neonatal Fc receptor; GPI, glucose-6-
phosphate-isomerase; K/BxN-PA, K/BxN serum-induced passive arthritis; RA, rheuma-
toid arthritis; wt, wild-type.

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and neutrophils. FcγRIV also contributed to arthritis in wt mice. Importantly, we identified FcγRIIIA and FcγRIII 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 FcγRIIIA /−/−, FcγRIIB /−/−, FcγRγ /−/−, FcγRγ /+−, FcγRγ /−−, and W/W v mice from The Jackson Laboratory (Bar Harbor, ME). FcγRγ /−/− and 5KO mice were described previously (4). HfeRγ /−/− mice were obtained from D. C. Roopenian (The Jackson Laboratory) and crossed to 5KO mice to obtain 6KO and hFcRn tg (Bar Harbor, ME). Fcg on the C57BL/6J background (6th–12th generation backcross), except W/W v Mole´culaire et Cellulaire (Strasbourg, France). Mice used in experiments were Medical School, Boston, MA), and the Institut de Ge´ne´tique et de Biologie York, NY), anti-Gr1 mAb (RB6-8C5) by R. Coffman (DNAX, Palo Alto, CA), and anti-platelet mAb (6A6) by Dr. R. Good (University of South Florida College of Medicine, Tampa, FL). Purified anti-mFcγRI (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 Biotechnologies). 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. 66A (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 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−), eosinophils (CD11b+/SiglecE−), basophils (IgE+/DX5−), and mast cells (IgE+/CD117+).

A total of 2 × 10⁷ Chinese hamster ovary-K1 transfectants expressing mFcγRs (4) was incubated for 30 min at 4°C with indicated mAbs before flow cytometry analysis.

Reagents

Anti-mFcγRIIIA (275003) and anti-mFcγRI (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 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 Biotechnologies). 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.

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Statistical analysis

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<sup>−/−</sup> mice (5) (Fig. 1A). The clinical score was higher in double-deficient FcγRIIB/IIIA<sup>−/−</sup> mice that also lack inhibitory FcγRIIB compared with the clinical score of FcγRIIIA<sup>−/−</sup> 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<sup>−/−</sup> 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<sup>−/−</sup> 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<sup>+</sup> 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/IIIA<sup>−/−</sup> mice (8). We used FcγRIIB/IIIA<sup>−/−</sup> FcγRII/II<sup>i</sup> (5KO) mice (4) to demonstrate the role of FcγRIV in K/BxN-PA. These mice express no activating IgG FcR, but FcγRIV can bind FcγRI immobilized on a solid phase, as seen for human IgG2a in the presence of FcγRIIb (with a lower affinity than FcγRI) (22). FcγRIVB<sup>−/−</sup> mice do not develop K/BxN-PA, and 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/W<sup>v</sup> and W<sup>sh</sup>, to further investigate the role of mast cells in K/BxN-PA. We found, as previously described (12, 17), that most W/W<sup>v</sup> mice did not develop K/BxN-PA. Importantly, all W<sup>sh</sup> 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/W<sup>v</sup> and W<sup>sh</sup> mice have many other abnormalities (14, 15) (Supplemental Fig. 2B). Noticeably, we found that W/W<sup>v</sup> mice display basopenia, whereas W<sup>sh</sup> mice display basophilia (Fig. 1F). Basophil depletion, however, did not prevent K/BxN-PA in W<sup>sh</sup> mice (Fig. 1G, Supplemental Fig. 2C). Basophils could therefore not replace mast cells and explain why W<sup>sh</sup>, but not W/W<sup>v</sup> mice, developed arthritis. Similarly, depletion of platelets did not abolish K/BxN-PA in W<sup>sh</sup> mice, indicating that the higher number of platelets found in W<sup>sh</sup> compared with W/W<sup>v</sup> mice could not explain the differences in arthritis development between the two strains. Basophils could not prevent K/BxN-PA in W<sup>sh</sup> mice (Fig. 1G, Supplemental Fig. 2C). Basophils could therefore not replace mast cells and explain why W<sup>sh</sup>, but not W/W<sup>v</sup> mice, developed arthritis. Similarly, depletion of platelets did not abolish K/BxN-PA in W<sup>sh</sup> mice, indicating that the higher number of platelets found in W<sup>sh</sup> compared with W/W<sup>v</sup> mice could not explain the differences in arthritis development between the two strains.
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/W mice 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/+ 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 FcγRs on their mast cells, and basophils still develop K/BxN-PA (i.e., FcγRIIIA–/– 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-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 6KO 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 (29). Human FcRn has an affinity for mouse IgG2 (29) that it can protect from degradation (30). It has no affinity for mouse IgG1 (29). It has no affinity for mouse IgG1 (29). It has no affinity for mouse IgG1 (29). It has no affinity for mouse IgG1 (29). It has no affinity for mouse IgG1 (29). It has no affinity for mouse IgG1 (29).

Supporting this conclusion, blocking FcγRIIIA markedly reduced but blocking both receptors was required to abolish arthritis in wt mice (Fig. 2E). Similar results were obtained in FcγRIIB/IIIA−/− (Supplemental Fig. 2E) or in FcγRIIIA−/– mice (Supplemental Fig. 2D), in which FcγRIIIA accounted for arthritis. FcγRII therefore contributes significantly to arthritis together with FcγRIIIA in wt mice, even though the contribution of FcγRIIIA 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, 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/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.

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Disclosures

The authors have no financial conflicts of interest.

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