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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

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/Sh 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/Sh 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 and...

*Institut Pasteur, Unité d’Allergologie Moléculaire et Cellulaire, Département d’Immunologie, F-75015 Paris, France; †INSERM, U760, F-75015 Paris, France; ‡Institut Pasteur, Unité de Recherche et d’Expertise Histotéchnologie et Pathologie, Département de Pathologie, F-75015 Paris, France; and §Department of Molecular Cell Biology, Vrije Universiteit Medical Center, Amsterdam, The Netherlands

D.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 provided expertise; N.V.R. provided reagents; P.B., M.D., F.J., and D.A.M. analyzed and discussed results; P.B., M.D., and F.J. wrote the manuscript; and P.B. designed and supervised the research.

Address correspondence and reprint requests to Dr. Pierre Bruhns, Unité d’Allergologie Moléculaire et Cellulaire, Département d’Immunologie, Institut Pasteur, 45 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: FcRn, neonatal Fc receptor; GPI, glucose-6-phosphate-isomerase; K/BxN-PA, K/BxN serum-induced passive arthritis; RA, rheumatoid arthritis; wt, wild-type.

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www.jimmunol.org/cgi/doi/10.4049/jimmunol.1003642
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 from The Jackson Laboratory (Bar Harbor, ME). KRN mice were described previously (4). hFcRn mice were obtained from D. C. Roopenian (The Jackson Laboratory) and crossed to SKO mice to obtain 6KO and hFcRn tg mice. FcRIIIA (Bar Harbor, ME). Fcγ on the C57BL/6J background (6th–12th generation backcross), except W/W v mice. All mice were used between 7 and 9 wk of age. All mouse protocols were approved by the Animal Care and Use Committees of Paris, Ile de France, Medical School, Boston, MA), and the Institut de Génétique et de Biologie Mole´culaire et Cellulaire (Strasbourg, France). Mice used in experiments were not otherwise specified, arthritis was induced by an i.v. injection of 150 µl K/BxN serum. Anti-FcγRII (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 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).

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 IgG subclasses were from Southern Biotechnology Associates; and GPI and 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 × 10⁶ Chinese hamster ovary-K1 transfectants expressing mFcγRs (4) was incubated for 30 min at 4°C with indicated mAbs before flow cytometry analysis.
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 induced, but they are not abolished in FcγRIIIA−/− mice (5, 14). The clinical score was higher in double-deficient FcγRIIIB/IIIA−/− mice that also lack inhibitory FcγRIIb compared with the clinical score of FcγRIIIA−/− mice (Fig. 1A). FcγRIIIB deficiency has indeed been reported to increase the susceptibility to K/BxN-PA (17), as well as to other arthritids 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. 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 FcγRIIIB/IIIA−/− FcγRII/II−/− (5KO) mice (4) to demonstrate the role of FcγRIV in K/BxN-PA. These mice express no activating IgG FcγR, 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, Supplementary 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.

Arthritic symptoms are indeed reduced, but they are not abolished in FcγRIIIA−/− mice (5, 17). Together these observations suggest the involvement of activating FcγR other than FcγRIIIA (i.e., FcγRI and/or FcγRIV) in K/BxN-PA.
the difference in susceptibility to K/BxN-PA between these two strains (Supplemental Fig. 2B, 2D). Higher neutrophil (15) and/or monocyte numbers in Wm than in W/Wm 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/Wm mice (24). Lower numbers of neutrophils may therefore protect W/Wm 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 FcγRI 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-GP 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-GP 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 protects from degradation (30). It has no affinity for mouse human FcRn has an affinity for mouse IgG2 (29) that it can protect from degradation (30). It has no affinity for mouse human FcRn has an affinity for mouse IgG2 (29) that it can protect from degradation (30). It has no affinity for mouse human FcRn has an affinity for mouse IgG2 (29) that it can protect from degradation (30). It has no affinity for mouse human FcRn has an affinity for mouse IgG2 (29) that...


