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Human Fc γ RIIA induces anaphylactic and allergic reactions

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IgE and IgE receptors (Fc ϵ RI) are well-known inducers of allergy. We recently found in mice that active systemic anaphylaxis depends on IgG and IgG receptors (Fc γ RIIA and Fc γ RIV) expressed by neutrophils, rather than on IgE and Fc ϵ RI expressed by mast cells and basophils. In humans, neutrophils, mast cells, basophils, and eosinophils do not express Fc γ RIIA or Fc γ RIV, but Fc γ RIIA. We therefore investigated the possible role of

Fc γ RIIA in allergy by generating novel Fc γ RIIA-transgenic mice, in which various models of allergic reactions induced by IgG could be studied. In mice, Fc γ RIIA was sufficient to trigger active and passive anaphylaxis, and airway inflammation in vivo. Blocking Fc γ RIIA in vivo abolished these reactions. We identified mast cells to be responsible for Fc γ RIIA-dependent passive cutaneous anaphylaxis, and monocytes/macrophages and

neutrophils to be responsible for Fc γ RIIA-dependent passive systemic anaphylaxis. Supporting these findings, human mast cells, monocytes and neutrophils produced anaphylactogenic mediators after Fc γ RIIA engagement. IgG and Fc γ RIIA may therefore contribute to allergic and anaphylactic reactions in humans. (*Blood*. 2012;119(11):2533-2544)

Introduction

We recently reported that neutrophils are sufficient to induce active systemic anaphylaxis (ASA) in mice.¹ Not only mouse neutrophils, but also human neutrophils, could indeed restore ASA when transferred into mice that are resistant to ASA because they lack activating IgG receptors (Fc γ R). Mouse neutrophils express 2 Fc γ Rs, Fc γ RIIA and Fc γ RIV, which accounted for ASA induction.¹ However, human neutrophils express neither Fc γ RIIA nor Fc γ RIV. They express 2 other Fc γ Rs, Fc γ RIIA and Fc γ RIIIB, which do not exist in mice.² Noticeably, Fc γ RIIA, but not Fc γ RIIIB, can bind mouse IgG.¹ Fc γ RIIA may therefore be responsible for inducing human neutrophil activation when transferred into ASA-resistant mice.

Anaphylaxis is a systemic hyperacute allergic reaction that develops within minutes after antigen/allergen exposure in humans. It can be reproduced experimentally by injecting antigen in animals immunized with the same antigen (active anaphylaxis), or in mice preinjected with antigen-specific IgE or IgG antibodies (passive anaphylaxis). Not only systemic anaphylaxis leading to hypothermia, hypotension, and respiratory distress, but also local anaphylaxis leading to extravasation and inflammation, can be induced in mice depending on the route used for antigen challenge. Different models were found to depend on different mechanisms. IgE-induced and IgG1-induced passive cutaneous anaphylaxis (PCA) required mast cells.^{3,4} IgE-induced passive systemic anaphylaxis (PSA) also required mast cells.^{5,6} However, IgG1-induced PSA was reported to require basophils,⁷ whereas IgG2-induced PSA required neutrophils.¹ Mast cells⁵ and basophils^{7,8} were not

required for ASA that depended on monocytes/macrophages,⁹ or on neutrophils¹ depending on the experimental model. Therefore, each of these 4 cell types contribute to a specific model of anaphylaxis, but their respective contribution in humans remains to be determined.

In mice, mast cells, basophils, neutrophils, and monocytes/macrophages express activating FcRs that require the association of the ITAM-containing Fc γ -subunit to be expressed and functional at the cell membrane. Importantly, Fc γ ^{-/-} mice developed neither PCA, nor PSA or ASA, indicating that activating FcRs are mandatory for the induction of these reactions. Mast cells and basophils express specifically the murine high-affinity IgE receptor Fc ϵ RI, and neutrophils and monocytes/macrophages express specifically the murine high-affinity IgG receptor Fc γ RIV.¹⁰ However, all of these cells express the low-affinity IgG receptor Fc γ RIIA. Passive anaphylaxis models have demonstrated that Fc ϵ RI is mandatory for IgE-induced PCA and PSA,¹¹ Fc γ RIIA for IgG1-induced PCA¹² and PSA,⁶ and Fc γ RIV for IgG2-induced PSA.¹ Fc γ RIIA and Fc γ RIV,¹ but not Fc ϵ RI,⁶ contributed detectably to ASA models.

Human neutrophils do not express Fc γ RIIA, and Fc γ RIV does not exist in humans.¹⁰ Instead human neutrophils express the low-affinity activating IgG receptor Fc γ RIIA. Fc γ RIIA possesses its own ITAM in its intracytoplasmic domain, and is not associated with the Fc γ -subunit.² The Fc γ RIIA ITAM, however, is noncanonical and has been described to be less potent in inducing cell activation in vitro than the Fc γ ITAM.^{13,14} Fc γ RIIA binds all

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4 human IgG subclasses,¹⁵ as well as mouse IgG1, IgG2a, and IgG2b subclasses.¹ Polymorphisms in the gene encoding FcγRIIA have been reported to be linked to bronchial asthma and allergic rhinitis,¹⁶ suggesting a role for FcγRIIA in allergic reactions. Mice transgenic for the *Fcgr2a* gene have been generated that recapitulate the expression of FcγRIIA in humans.¹⁷ These FcγRIIA^{tg} mice spontaneously developed autoimmune diseases on a wild-type (WT) background (ie, pneumonitis, glomerulonephritis, and rheumatoid arthritis).¹⁸ FcγRIIA, expressed on the FcRγ^{-/-} background, was sufficient to induce experimental models of thrombocytopenia¹⁹ and rheumatoid arthritis.²⁰ The ability of FcγRIIA to induce allergic reactions has not been investigated.

FcγRIIA is the most widely expressed FcR in humans,¹⁸ and remarkably the only activating IgG receptor constitutively expressed by mast cells, basophils, neutrophils, and eosinophils. Mast cells, basophils, and eosinophils are well-known effectors of allergic reactions, and our recent work suggests that neutrophils might be effectors of anaphylaxis.¹ We therefore studied the ability of human FcγRIIA to induce passive and active anaphylaxis, and models of allergic inflammation in skin and airways. To this aim, we used FcγRIIA-transgenic mice on backgrounds deficient for endogenous FcRs. We found that FcγRIIA was sufficient to induce mast cell and macrophage activation in vitro, and mast cell-dependent PCA and lung inflammation in vivo. FcγRIIA-induced PSA was dependent on monocytes/macrophages and neutrophils, but not on mast cells and basophils. Noticeably, FcγRIIA was sufficient to induce fatal ASA. Finally, human mast cells, monocytes, and neutrophils produced anaphylactogenic mediators after FcγRIIA engagement. FcγRIIA may therefore be the major activating IgG receptor contributing to allergic reactions and anaphylaxis in humans.

Methods

Mice

C57BL/6J FcγRIIA^{tg} mice were provided by M. P. Reilly (Jefferson Medical College, Philadelphia, PA), FcγRIIIB/IIIA triple-deficient (3KO) mice (N6 C57BL/6J) by S. Verbeek (Leiden University Medical Center, Leiden, The Netherlands), KRN^{tg} mice by D. Mathis and C. Benoist (Harvard Medical School, Boston, MA) and IGBMC (Strasbourg, France). WT C57BL/6J mice were purchased from Charles River, W^{sh}/W^{sh} and FcRγ^{-/-} C57BL/6J mice from The Jackson Laboratory. FcγRIIIB/IIIA^{-/-} FcεRI^{-/-} FcεRII^{-/-} (5KO; N6 C57BL/6J) mice were previously described.²¹ FcγRIIA^{tg} mice were intercrossed with 3KO, 5KO, FcRγ^{-/-}, and/or W^{sh}/W^{sh} mice to obtain 3KOIIA, 5KOIIA, FcRγ^{-/-}IIA, and W^{sh}3KOIIA-transgenic mice, respectively. All mice carrying the hFcγRIIA transgene were used as heterozygous animals. Nontransgenic littermates served as controls. Mice in all experiments were 6–10 weeks old. All mouse protocols were approved by the Animal Care and Use Committees of Paris, Ile de France, France.

Active systemic anaphylaxis

Mice were injected intraperitoneally on day 0 with 200 μg BSA, either in CFA or in alum, and boosted intraperitoneally on day 14 with 200 μg BSA in IFA or in alum, respectively. BSA-specific IgG1 and IgG2a/b/c antibodies in serum were titrated by ELISA on day 17 as described.¹ Mice with comparable antibody titers were challenged intravenously with 500 μg BSA, 8 days after the last immunization. Central temperature was monitored using a digital thermometer (YSI).

Passive systemic anaphylaxis

Immune complexes made of 1 mg OVA and 1 mg anti-OVA mAb (clone OVA-14), or 20 μL K/BxN serum and 50 μg GPI in 200 μL physiologic

solution were preformed at 37°C and injected intravenously. Alternatively, 50 or 150 μg of mAb IV.3 were injected intravenously. Central body temperature was recorded.

Passive cutaneous anaphylaxis

ICs were preformed by incubating OVA and OVA-14 in a 1:1 ratio for 15 minutes at 37°C. Indicated amounts of these ICs, or heat-aggregated (1 hour at 63°C) purified human IgG or anti-FcγRIIA mAb IV.3 were injected intradermally in 20 μL total volume, immediately followed by an intravenous (IV) injection of 100 μL PBS containing 2% Evans Blue. Thirty minutes later, Evans blue was extracted from 1-cm-diameter skin pieces using formamide at 63°C and quantified by absorbance (620 nm).

Airway inflammation

Mice were injected intranasally with 50 μL of rabbit anti-OVA antiserum and 500 μg of OVA intravenously. After indicated periods of time, mice were lethally anesthetized, and 4 broncho-alveolar lavages of, respectively, 0.5, 1, 1, and 1 mL PBS were performed. The supernatant of the first lavage was used to quantify MPO and keratinocyte-derived chemokine (KC) content. The cells from all lavages were pooled for cell count analysis. Hemorrhage score was determined on pooled lavages and ranged from 0 (no blood present), 1 (detectable hemorrhage), to 3 (strong hemorrhage).

Alveolar macrophages, recovered by 3 consecutive 1 mL broncho-alveolar lavages from individual mice, were exposed for 3 hours to 3 different conditions: (1) no stimulant, (2) plate-bound ICs made of 100 μg/mL OVA and 30 μg/mL rabbit anti-OVA, and (3) plate-bound anti-FcγRIIA mAbs (100 μg/mL). Supernatants were assayed for KC and MIP-1α by ELISA (R&D Systems).

In vivo blocking and depletion

Anti-FcγRIIA mAb (50 μg/mouse) was injected intravenously either once (24 hours), or twice (24 hours and 12 hours) before the experiment. 300 μL/mouse PBS- or clodronate-liposomes (at 7 mg/mL), 300 μg/mouse anti-Gr1, 300 μg/mouse anti-Ly-6G, 10 μg/mouse anti-CD200R3 mAbs, or 1 mg/mouse GdCl₃ were injected 24 hours before the experiment. Depletion of specific populations was ascertained using flow cytometry on blood samples taken during or after the experiment (examples are shown in supplemental Figure 3B–E, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).

Please refer to supplemental Methods for information on antibodies, reagents, and cells; flow cytometry analysis; lung histology; statistical analyses; and in vitro cell activation.

Results

FcγRIIA can trigger active systemic anaphylaxis

Active systemic anaphylaxis was induced by an IV antigen challenge in mice immunized with the same antigen. This protocol induced a body temperature decrease and mortality in WT mice, but not in FcRγ^{-/-} mice, immunized with antigen in Alum (supplemental Figure 1A) or in Freund adjuvant (supplemental Figure 1B). Immunizations in either adjuvant lead to the production of IgG1 and IgE antibodies, but only immunizations in Freund's adjuvant lead to the production of IgG2 antibodies in both mouse strains (data not shown). Human neutrophils express both FcγRIIA and FcγRIIIB (Figure 1A), but only FcγRIIA and not FcγRIIIB can bind mouse IgG (Figure 1B). To analyze the capacity of FcγRIIA to induce ASA, we developed transgenic mouse models expressing human FcγRIIA under the control of its own promoter, and deficient for endogenous FcRs. FcγRIIA^{tg} mice express FcγRIIA not only on neutrophils, but also on eosinophils, monocytes, macrophages, and weakly on basophils (Figure 1C). FcγRIIA^{tg} mice therefore reproduce the expression pattern found in

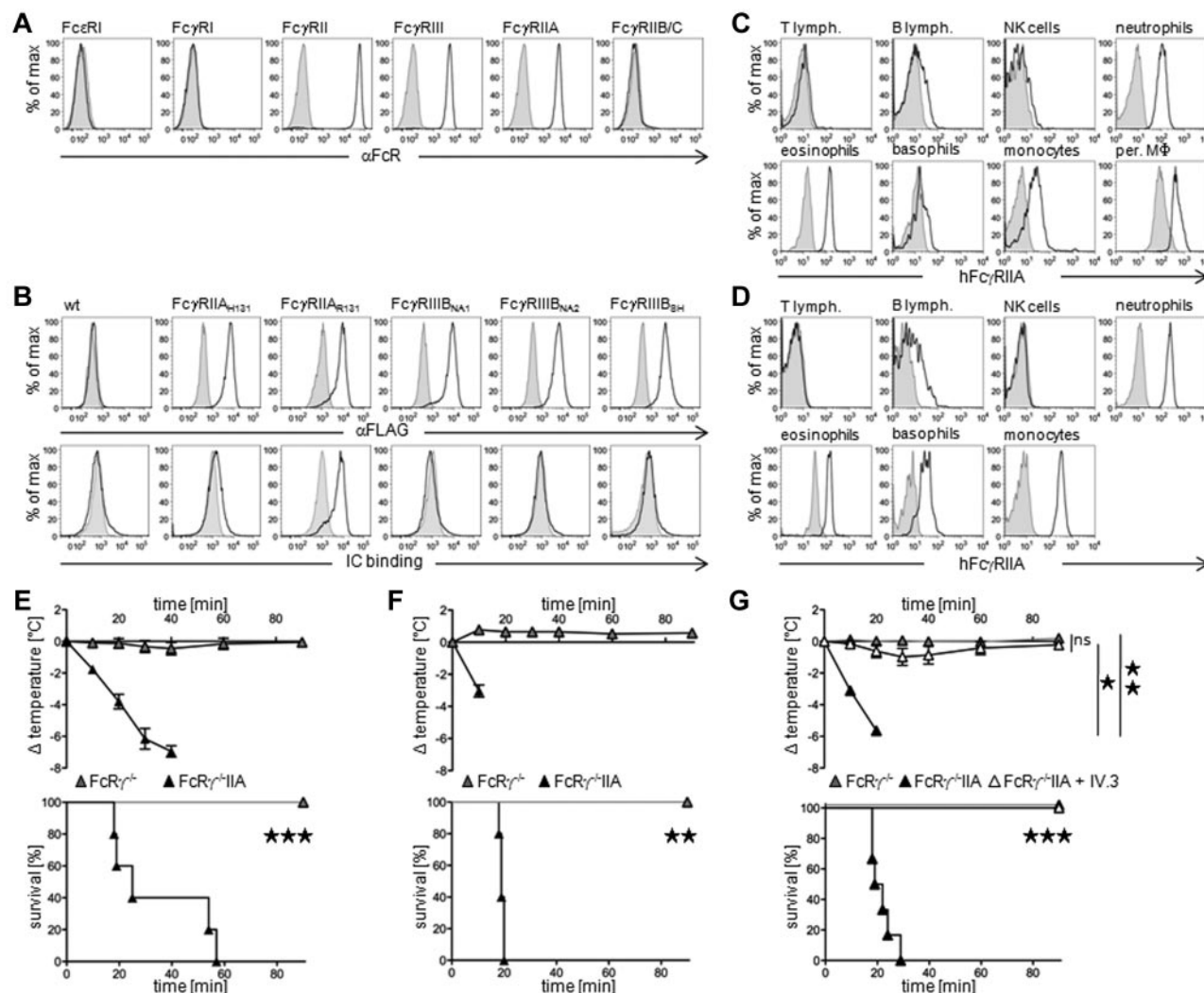


Figure 1. Fc γ RIIA can induce active systemic anaphylaxis. (A) Representative histogram plots of human FcR expression on human blood neutrophils. (B) Representative histogram plots of anti-FLAG mAb (top panel) or preformed mouse polyclonal IgG-immune complexes (bottom panel) binding to CHO transfectants expressing the indicated FLAG-tagged human polymorphic variants of Fc γ RIIA (H₁₃₁ or R₁₃₁) and Fc γ RIIIB (NA1, NA2, or SH). (C) Representative expression of Fc γ RIIA on blood and peritoneal cells from 3KOIIA mice (open histograms) or nontransgenic 3KO littermate controls (filled histograms): T cells (CD3⁺), B cells (CD19⁺), NK cells (DX5⁺/NK1.1⁺), neutrophils (Gr1^{hi}/CD11b⁺), eosinophils (Gr1^{int}/SiglecF⁺), basophils (IgE⁺/DX5⁺), and monocytes/macrophages (CD11b⁺/Gr1⁻). (D) Representative expression of Fc γ RIIA (open histograms) on human blood cells: T cells (CD3⁺), B cells (CD19⁺), NK cells (CD56⁺), neutrophils (CD24⁺), eosinophils (CCR3⁺/CDw125⁺), basophils (FceRI⁺/CD203c⁺), and monocytes (CD14⁺); or isotype control (closed histograms). (E-G) Indicated mice were immunized with BSA, (E) in Freund's adjuvant, or (F-G) in Alum, challenged with BSA and central temperatures and survival rates were monitored. (E) ASA in FcR γ ^{-/-} (n = 7) and FcR γ ^{-/-}IIA mice (n = 5). (F) ASA in FcR γ ^{-/-} (n = 4) and FcR γ ^{-/-}IIA (n = 5) mice. (G) ASA in FcR γ ^{-/-}IIA mice injected twice with anti-Fc γ RIIA mAb IV.3 (n = 5) or not (n = 6), before BSA-challenge. FcR γ ^{-/-} mice were used as controls (n = 7). (E-G) Data are represented as mean $\pm$ SEM. (A-G) Data are representative of at least 2 independent experiments (**P* < .05; ***P* < .01; ****P* < .001).

humans (Figure 1D). Noticeably, whereas human neutrophils and basophils express Fc γ RIIA (supplemental Figure 1C), mouse neutrophils express Fc γ RIIA and Fc γ RIV, and mouse basophils only Fc γ RIIA,¹ as ITAM-bearing activating Fc γ R.

After antigen challenge, ASA developed in FcR γ ^{-/-}IIA mice, but not in nontransgenic FcR γ ^{-/-} littermates, immunized with antigen in Freund's adjuvant (Figure 1E) or in Alum (Figure 1F) leading to a severe temperature drop and 100% mortality. IV injections of anti-Fc γ RIIA blocking mAbs abolished ASA-induced temperature drop and mortality in FcR γ ^{-/-}IIA mice immunized in Freund's adjuvant (data not shown) or in Alum (Figure 1G). Fc γ RIIA is therefore sufficient to trigger fatal active systemic anaphylaxis.

Fc γ RIIA can trigger passive systemic anaphylaxis

To investigate the potential of Fc γ RIIA to induce PSA, we used divalent (anti-Fc γ RIIA mAbs) or multivalent (IgG-immune

complexes) ligands. An IV injection of 150 μ g, but not of 50 μ g, anti-Fc γ RIIA mAb IV.3 induced a modest temperature drop in FcR γ ^{-/-}IIA mice, but not in FcR γ ^{-/-} mice (Figure 2A). Noticeably, FcR γ ^{-/-} mice lack all activating Fc γ Rs but express inhibitory Fc γ RIIB, which has been reported to negatively regulate PSA induced by mouse-activating Fc γ Rs.²² Fc γ RIIB binds, like human Fc γ RIIA, mouse IgG1 and IgG2 subclasses. IgG-immune complexes may therefore coaggregate human Fc γ RIIA with mouse Fc γ RIIB, leading to the inhibition of Fc γ RIIA-dependent activation,²³ and consequently of Fc γ RIIA-induced PSA. We therefore crossed Fc γ RIIA^{tg} mice to Fc γ RI/Fc γ RIIB/Fc γ RIIA^{-/-} (3KO) mice or to Fc γ RI/Fc γ RIIB/Fc γ RIIA^{-/-} FceRI/FceRII^{-/-} (5KO) mice (Figure 2B). 3KO and 5KO mice lack all IgG receptors except the activating IgG2 receptor Fc γ RIV,²¹ whereas FcR γ ^{-/-} mice lack all IgG receptors except the inhibitory IgG1/IgG2 receptor Fc γ RIIB. An injection of 150 μ g mAb IV.3 induced a significant temperature

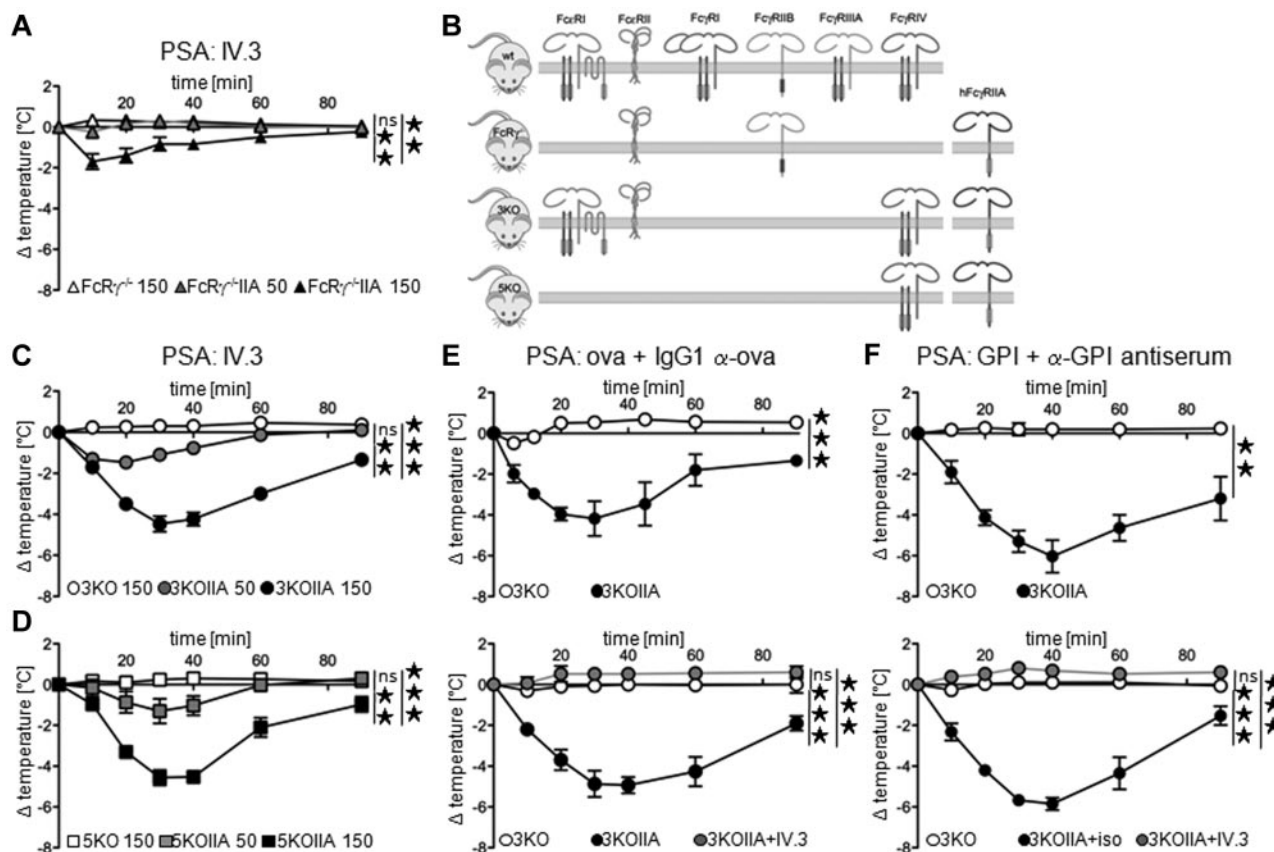


Figure 2. In vivo aggregation of Fc γ RIIA induces passive systemic anaphylaxis. (A, C–D) Indicated Fc γ RIIA-transgenic mice were injected with 50 μ g (gray symbols) or 150 μ g (black symbols) of mAb IV.3, and central temperatures were monitored ($n = 3$). Nontransgenic littermates injected with 150 μ g mAb IV.3 were used as controls (open symbols, $n = 3$). (A) Fc γ R^{-/-}, (C) 3KO, (D) 5KO backgrounds. (B) Schematic representation of Fc receptors expressed in the different mouse models used in this study. (E–F) Mice were injected with indicated preformed mouse IC and central temperatures were monitored. Gray symbols indicate mice injected with 50 μ g of mAb IV.3 24 hours before challenge ($n = 4$). Top panel (E) $n = 5$, (F) $n = 4$. Bottom panel (E) 3KO or 3KOIIA $n = 3$, (F) 3KO $n = 3$, 3KOIIA+iso $n = 4$. (A, C–F) Data are represented as mean $\pm$ SEM and are representative of at least 2 independent experiments (** $P < .01$; *** $P < .001$).

drop in 3KOIIA mice (Figure 2C) and in 5KOIIA mice (Figure 2D). Because 50 μ g mAb IV.3 induced no or a very weak temperature drop, we used this dose to block Fc γ RIIA in 3KOIIA and 5KOIIA mice.

Likewise, an IV injection of monoclonal IgG1- (Figure 2E) or polyclonal IgG-immune complexes (Figure 2F) induced a significant temperature drop in 3KOIIA mice, but not in 3KO mice. Pretreatment with anti-Fc γ RIIA mAb IV.3 abolished these temperature drops in 3KOIIA mice (Figure 2E–F bottom panels). Fc γ RIIA is therefore sufficient to trigger monoclonal IgG1-induced PSA and polyclonal IgG-induced PSA.

Neutrophils and monocytes/macrophages mediate Fc γ RIIA-dependent PSA

All cell types that express Fc γ RIIA, ie, neutrophils, monocytes/macrophages, basophils, and eosinophils (Figure 1C) can potentially contribute to PSA. We found recently that neutrophils are responsible for polyclonal IgG-IC-induced PSA,¹ whereas basophils were reported to be responsible for monoclonal IgG1-IC-induced PSA.⁷ Polyclonal IgG-IC-induced PSA was reduced by neutrophil depletion after injection of anti-Gr1 (Figure 3A) or anti-Ly-6G (Figure 3B) mAbs in 3KOIIA mice. Neutrophils therefore contribute to Fc γ RIIA-dependent PSA. Surprisingly, basophil depletion did not affect PSA (Figure 3C). To investigate whether Fc γ RIIA-triggered PSA depends on mast cells, 3KOIIA mice, and as negative controls 3KO mice were crossed with *W^{sh}/W^{sh}* mice. *W^{sh}* 3KOIIA mice developed unaltered Fc γ RIIA-triggered PSA, that is, in the absence of mast cells (Figure 3D).

However, depletion of monocytes/macrophages induced by toxic liposomes, but not by control liposomes, reduced PSA (Figure 3E). Supporting this result, PSA was reduced in 3KOIIA mice injected with gadolinium, which inhibits monocytes/macrophage function (Figure 3F). Monocytes/macrophages therefore also contribute to Fc γ RIIA-dependent PSA. Depletion of a single cell population reduced PSA, but depletion of both neutrophils and monocytes/macrophages abrogated PSA in 3KOIIA mice (Figure 3G). In line with these results, monocytes/macrophage depletion and neutrophil depletion also inhibited anti-Fc γ RIIA mAb-induced PSA (Figure 3H). Collectively, these data demonstrate that neutrophils and monocytes/macrophages, together, account for Fc γ RIIA-dependent passive systemic anaphylaxis.

To investigate if neutrophils and monocytes may also contribute to anaphylactic reactions in humans, we investigated whether these Fc γ RIIA-expressing cell types produce anaphylactogenic mediators after Fc γ RIIA engagement. Monocytes and neutrophils purified from the blood of normal donors could be activated in vitro by anti-Fc γ RIIA mAb- or human IgG-heat aggregates, as revealed by their decreased CD62L expression (Figure 4A). In the same conditions, both human monocytes and human neutrophils produced platelet activating factor (PAF; Figure 4B),²⁴ whereas monocytes, but not neutrophils, produced leukotriene B₄ (LTB₄; Figure 4C) and LTC₄ (Figure 4D). Human monocytes and neutrophils therefore produce anaphylactogenic mediators (ie, PAF and leukotrienes) after Fc γ RIIA engagement, supporting a role for Fc γ RIIA in human allergic reactions.

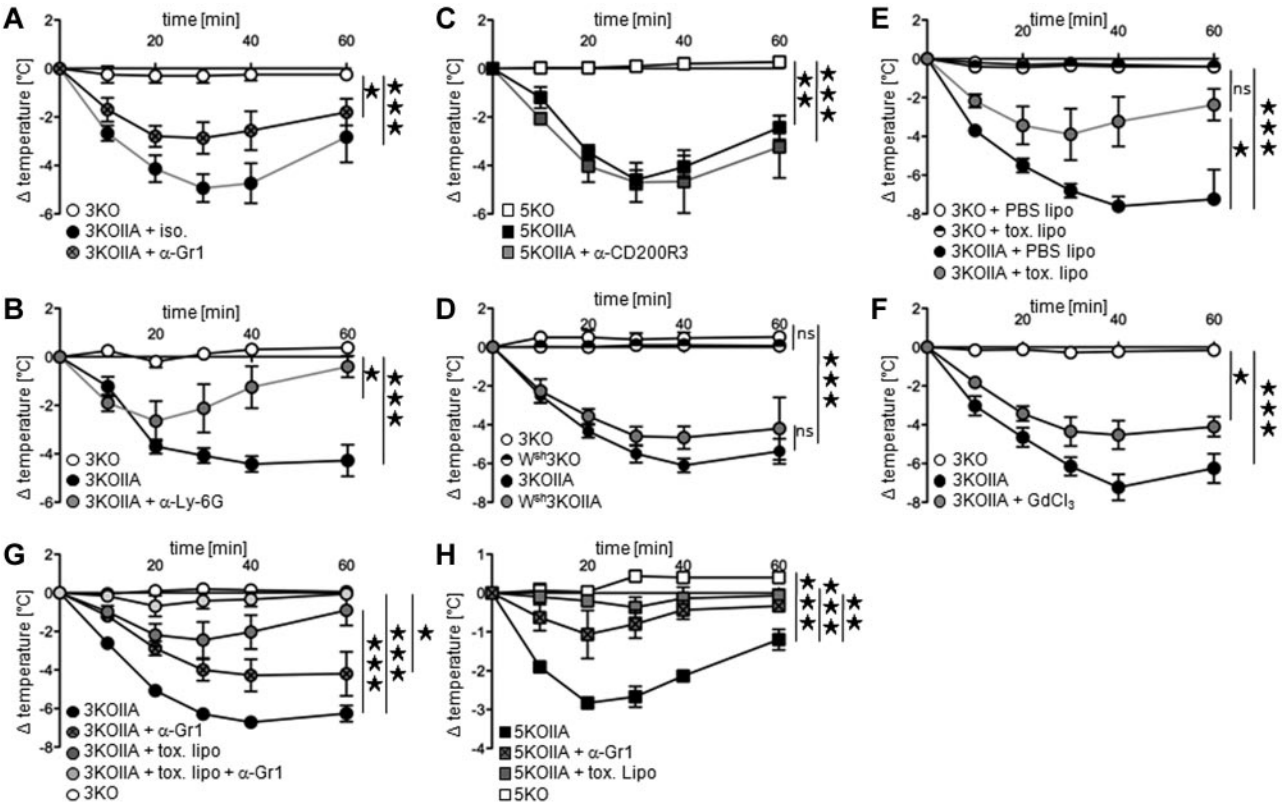


Figure 3. Neutrophils and monocytes/macrophages are necessary for Fc γ RIIA-dependent PSA. (A-G) Indicated mice were injected with preformed polyclonal IgG-IC (mouse anti-GPI antiserum plus GPI), and central temperatures were monitored. PSA in Fc γ RIIA transgenic mice injected with (A) anti-Gr1 mAbs (n = 8) or isotype (ISO) control (n = 3), (B) anti-Ly-6G mAbs (n = 4) or untreated control (n = 4), (C) anti-CD200R3 mAbs (n = 3) or untreated control (n = 3). Nontransgenic littermates were used as controls (A, n = 2; B, n = 4; C, n = 3). (D) PSA in W^{sh}3KOIIA mice and 3KOIIA mice (n = 3). Nontransgenic littermate controls 3KO (n = 3) and W^{sh}3KO mice (n = 4) were used as controls. (E-F) PSA in 3KOIIA mice injected with (E) PBS liposomes (PBS lipo) or clodronate liposomes (toxic lipo; n = 3), (F) Gadolinium chloride (GdCl₃) or not (n = 4). Nontransgenic littermates were used as controls (E, n = 2; F, n = 4). (G) PSA in 3KOIIA mice left untreated (n = 7), or injected with anti-Gr1 mAbs (n = 5), toxic liposomes (n = 6) or anti-Gr1 mAbs plus toxic liposomes (n = 6). Data are a compilation of 2 experiments. 3KO served as negative control (n = 2). Statistical significances are indicated among 3KOIIA groups. (H) mAb IV.3-induced PSA in indicated mice injected with anti-Gr1 mAbs, toxic liposomes, or left untreated (n = 3). (A-H) Data are represented as mean $\pm$ SEM, and (A-F,H) are representative of at least 2 independent experiments (* P < .05; ** P < .01; *** P < .001).

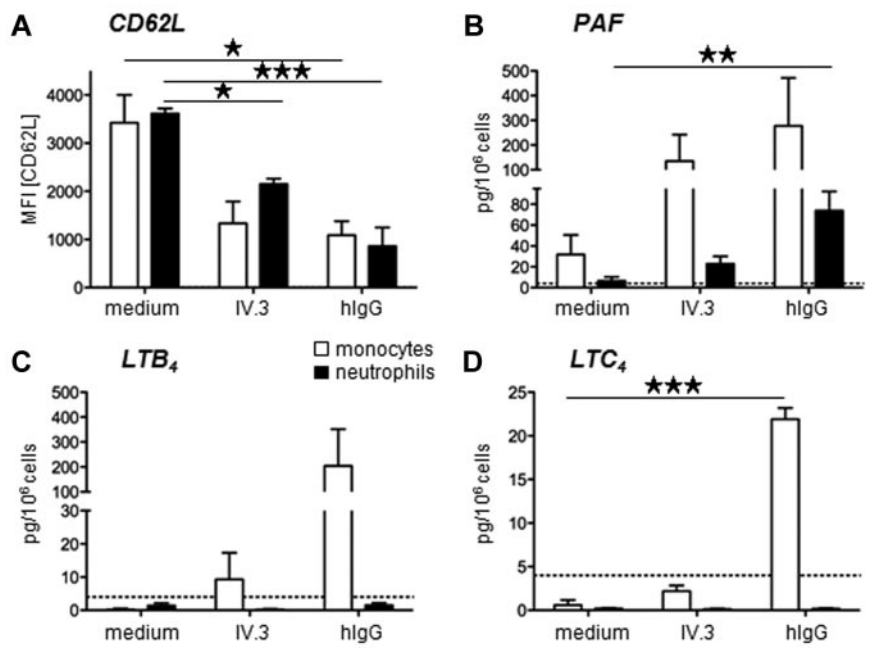


Figure 4. Human neutrophils and monocytes produce anaphylactogenic mediators. (A-D) Purified human monocytes or neutrophils were incubated in vitro with heat-aggregated human IgG or anti-Fc γ RIIA mAb IV.3, and (A) CD62L expression, (B) PAF, (C) LTB₄, and (D) LTC₄ production are represented. Mean results from the analysis of 3 normal donors are represented (* P < .05; ** P < .01; *** P < .001).

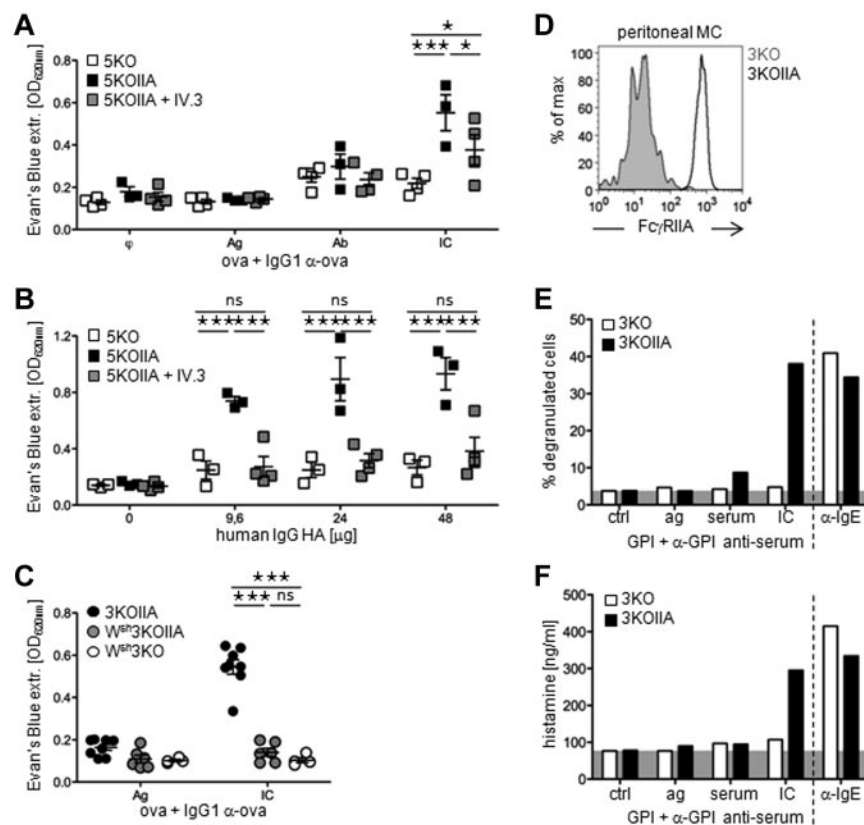


Figure 5. Mast cells are mandatory for FcγRIIA-dependent passive cutaneous anaphylaxis. (A-C) Mice were injected intradermally with indicated reagents and intravenously with Evans blue. Quantification of Evans blue extracted from skin tissue is represented. (A-B) PCA in 5KO (open symbols: A, n = 4; B, n = 3), 5KOIIA (black symbols: A, n = 3, B, n = 3), or 5KOIIA mice preinjected (A) once or (B) twice with 50 μg mAb IV.3 (gray symbols, n = 4). (C) PCA in 3KOIIA mice (black symbols, n = 4), in W^{sh}3KOIIA (gray symbols, n = 3), and as controls in W^{sh}3KO (open symbols, n = 2). NB: 2 points are represented per mouse, as each mouse was injected on 2 different sites with Ag and with IC. (D) Representative expression of FcγRIIA on peritoneal mast cells (c-kit⁺/IgE⁺) from 3KOIIA mice (open histograms) or 3KO littermate controls (filled histograms). (E-F) Peritoneal cells from 3KO (white bars) and 3KOIIA (black bars) mice were stimulated with indicated reagents. (E) The percentage of degranulated mast cells (a minimum of 200 cells were counted per experimental point), and (F) histamine content in the supernatant of each experimental point are represented. (A-F) Data are representative of at least 2 independent experiments (*P < .05; ***P < .001).

FcγRIIA triggers mast cell-dependent passive cutaneous anaphylaxis

Mast cells are responsible for IgE-induced PSA⁶ and for PCA.²⁵ IgG1-induced PCA, which depends on mouse FcγRIIA, was also reported to depend on mast cells using mast cell-deficient W/W^v or W^{sh}/W^{sh} mice.²⁶ Mast cells may also be responsible for cutaneous anaphylaxis in humans because degranulated mast cells are found in skin biopsies from allergic patients, and because mast cell-specific mediator levels correlate with the severity of allergic skin inflammation. As human mast cells express FcγRIIA,²⁷ we investigated whether FcγRIIA could trigger IgG-induced PCA. Intradermal injections of increasing doses of IgG1-IC (supplemental Figure 2A) or of mAb IV.3 (supplemental Figure 2B) induced cutaneous anaphylaxis in 5KOIIA mice, but not in 5KO mice, as assessed by Evans blue extravasation. Intradermal injections of IgG1-IC, but not of antigen or Ab alone, induced PCA in 5KOIIA mice that was inhibited by an IV pretreatment with anti-FcγRIIA mAb (Figure 5A, supplemental Figure 2C). Similarly, intradermal injections of heat-aggregated (HA) human polyclonal IgG induced cutaneous reactions in 5KOIIA mice, but not in 5KO mice, that were abolished by anti-FcγRIIA mAb pretreatment (Figure 5B, supplemental Figure 2D). FcγRIIA can therefore trigger mouse and human IgG-induced PCA.

Mouse IgG1-induced FcγRIIA-dependent PCA is abrogated in W/W^v and W^{sh}/W^{sh} mice (data not shown). We investigated whether FcγRIIA-triggered PCA also depends on mast cells by comparing 3KOIIA mice with W^{sh}3KOIIA mice. As expected, 3KOIIA mice developed mouse IgG1-induced PCA. W^{sh}3KOIIA mice, however, as well as W^{sh}3KO mice used as negative controls, were protected from mouse IgG1-induced PCA (Figure 5C, supplemental Figure 3E). Mast cells are therefore mandatory for FcγRIIA-triggered IgG-induced PCA.

FcγRIIA aggregation induces mast cell degranulation in vitro and ex vivo

Because IgG-induced mast cell-dependent PCA occurs in 3KOIIA mice, mast cells are expected to express FcγRIIA. Ex vivo peritoneal mast cells from 3KOIIA mice indeed express FcγRIIA (Figure 5D), but no other FcγR (not shown). IgG-IC induced ex vivo peritoneal mast cells from 3KOIIA mice, but not from 3KO mice to degranulate (Figure 5E) and release histamine (Figure 5F). Anti-IgE antibodies, however, induced IgE-bearing peritoneal mast cells from both naive strains to degranulate and release histamine. FcγRIIA aggregation can therefore activate mast cells.

Like peritoneal mast cells from 3KOIIA mice, in vitro cultured peritoneal cell-derived mast cells (PCMCs),²⁸ fetal skin-derived mast cells (FSMCs), and bone marrow-derived mast cells (BMCs) from the same mice all expressed FcγRIIA (Figure 6A). PCMCs from 3KOIIA mice, but not from 3KO mice, bound monoclonal IgG1-IC and polyclonal IgG-IC (Figure 6B). Incubation of 3KOIIA PCMCs with either of these immune complexes induced mast-cell activation as revealed by Ca²⁺ fluxes (Figure 6C) and intracellular protein phosphorylation (Figure 6D). FcγRIIA aggregation by IgG-IC induced consistent syk, PLCγ1, Akt, IκB, and ERK1/2 phosphorylation, but a barely detectable LAT phosphorylation compared with that induced by the aggregation of FcεRI by IgE plus antigen on the same cells (Figure 6D). FcγRIIA aggregation, similar to FcεRI aggregation, induced a sustained phosphorylation of SHP1, as previously reported in monocytes.²⁹ This SH2-containing inositol-5-phosphatase negatively regulates signaling by Fcγ-chain associated FcRs, and is mandatory for FcγRIIB-dependent negative regulation of mast cell activation.^{30,31}

FcγRIIA aggregation also induced PCMC degranulation, as revealed by β-hexosaminidase and histamine release (Figure 6E), supporting our results obtained with ex vivo peritoneal mast cells

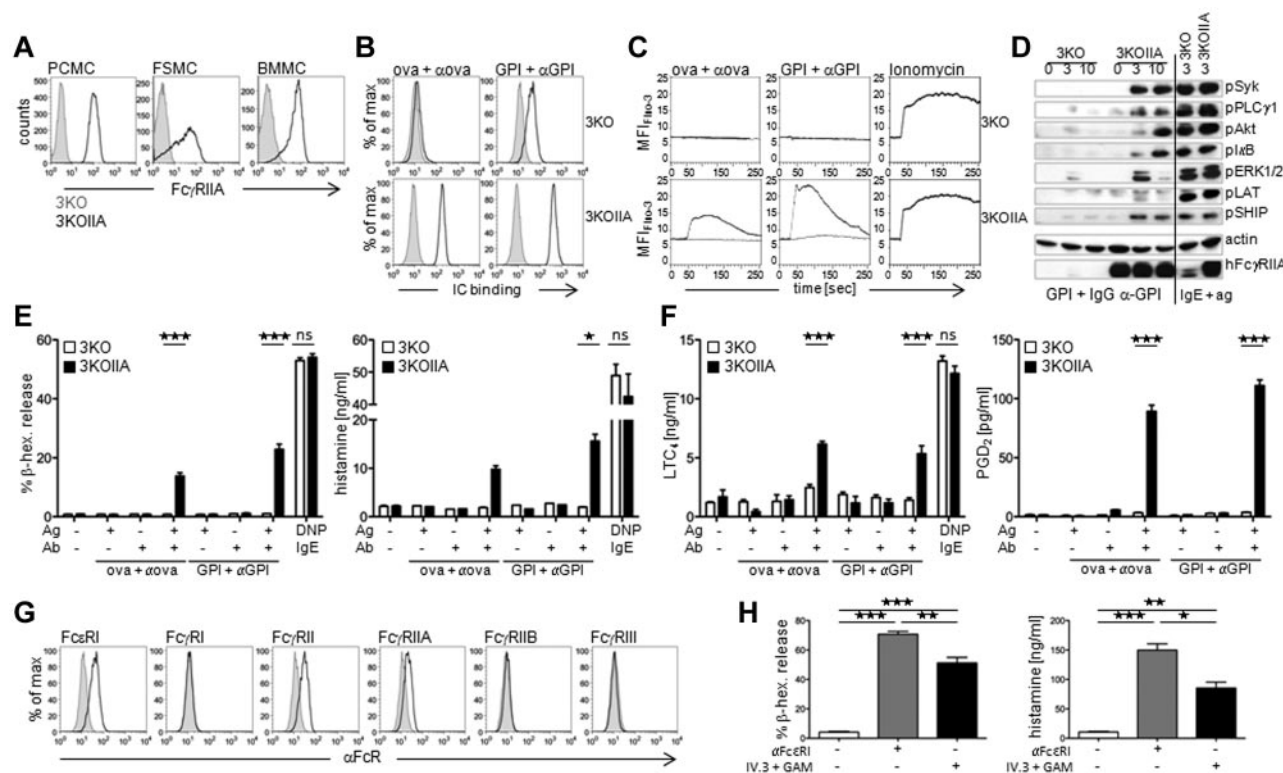


Figure 6. FcγRIIA activates mouse and human mast cells in vitro. (A) Representative expression of FcγRIIA on PCMCs, FSMCs, and BMMCs from 3KOIIA (open histogram) and 3KO mice (filled histogram). (B) PCMCs from indicated mice were incubated with indicated preformed mouse IgG-IC (αOVA: anti-OVA mAb; αGPI: polyclonal anti-GPI antiserum; open histograms) or not (filled histograms). Binding of ICs was detected by staining with F(ab')₂ GAM-PE. (C) Calcium fluxes in PCMCs from 3KO or 3KOIIA mice incubated with indicated IC (black curves) or Ag alone (gray curves). Ionomycin was used as control. (D) Western blot analysis of PCMC lysates after stimulation with indicated reagents for different periods of time. PCMCs sensitized overnight with IgE anti-DNP and challenged with DNP-HSA for 3 minutes served as positive controls. Actin was used as a loading control. FcγRIIA was used as a genotype control (reprobe after pERK1/2 staining). (E-F) Mediator release by PCMCs from 3KO (open bars) and 3KOIIA (black bars) mice challenged with indicated reagents. PCMCs sensitized overnight with IgE anti-DNP and challenged with DNP-HSA served as positive controls. NB: GPI+αGPI correspond to ICs made of GPI and polyclonal anti-GPI antiserum in panel E, and to ICs made of GPI and IgG purified from anti-GPI antiserum in panel F. (G) Representative histogram plots of human FcR expression on human SMCs. (H) Percentage of β-hexosaminidase release and quantification of histamine release by human SMCs incubated with anti-FcεRI mAb or with preformed complexes of mAb IV.3 and GAM. (E-F,H) Data are represented as mean ± SEM. (A-H) Data are representative from at least 2 independent experiments (**P* < .05; ***P* < .01; ****P* < .001).

(Figure 5E-F). In addition, FcγRIIA aggregation induced lipid mediator production, that is, LTC₄ and prostaglandin D₂ (PGD₂) by PCMCs (Figure 6F). Thus, IgG-immune complexes induced FcγRIIA-triggered activation of mast cells leading to the release of mediators involved in vascular permeability (eg, histamine, LTC₄, PGD₂), which is consistent with FcγRIIA's ability to induce PCA.

We therefore analyzed the expression of Fc receptors on human skin-derived mast cells (SMC), and the ability of FcγRIIA to induce human skin mast cell activation. Human SMC constitutively express the high-affinity IgE receptor FcεRI, as expected, and a single IgG receptor, FcγRIIA (Figure 6G), as described.²⁷ FcγRIIA aggregation by anti-FcγRIIA mAb induced β-hexosaminidase and histamine release by human SMC (Figure 6H). FcεRI aggregation was used as a positive control. FcγRIIA is therefore sufficient to activate human mast cells, and may be involved in the induction of mast cell-dependent inflammation and allergic reactions in humans.

FcγRIIA enables passive airway inflammation

Constriction of smooth muscles and subsequent granulocyte infiltration in the airways during asthmatic inflammation is thought to result from histamine and leukotriene release from mast cells after allergen inhalation. Thus, we wondered whether FcγRIIA may induce airway inflammation as it is expressed on mast cells and granulocytes. FcγRIIA is expressed in human lung tissue (Figure

7A), but also in lung sections (Figure 7B), and on alveolar macrophages (Figure 7C) from 3KOIIA mice. Unlike human asthma, most asthma models in mice are independent of antibody production by B cells,^{32,33} and consequently do not require FcRs. We therefore used a model of airway inflammation that depends on IgG and on FcγRs,³⁴ and that consists of an IV injection of OVA and of an intranasal injection of anti-OVA rabbit serum, presumably forming ICs in vivo. Preformed OVA-anti-OVA rabbit serum ICs could bind to CHO cells expressing FcγRIIA, but not to untransfected CHO cells (Figure 7D). CD11c⁺/Gr1⁺ alveolar macrophages represent more than 90% of the cells present in the alveolar space, as detected in broncho-alveolar lavages (BAL) of FcγR^{-/-}, FcγR^{-/-}IIA, and WT mice (Figure 7E). Concomitant intranasal instillation of anti-OVA rabbit serum and intravenous injection of OVA induced a massive infiltration of CD11c⁺/Gr1⁺ cells (> 80% of BAL content) in WT and in FcγR^{-/-}IIA mice, but not in FcγR^{-/-} mice (~5% CD11c⁺/Gr1⁺ granulocytes; Figure 7F). FcγRIIA therefore induces granulocytes recruitment to the lung, and can replace endogenous Fcγ-associated activating FcRs. Total cell numbers in the BAL were unchanged at t = 3 hours after challenge, but increased starting t = 6 hours and reached 5 times the background value at t = 16 hours in FcγR^{-/-}IIA mice, but not in FcγR^{-/-} mice (Figure 7G). Granulocyte numbers in BAL represented most of this increase, whereas alveolar macrophage numbers did not vary statistically along the time course.

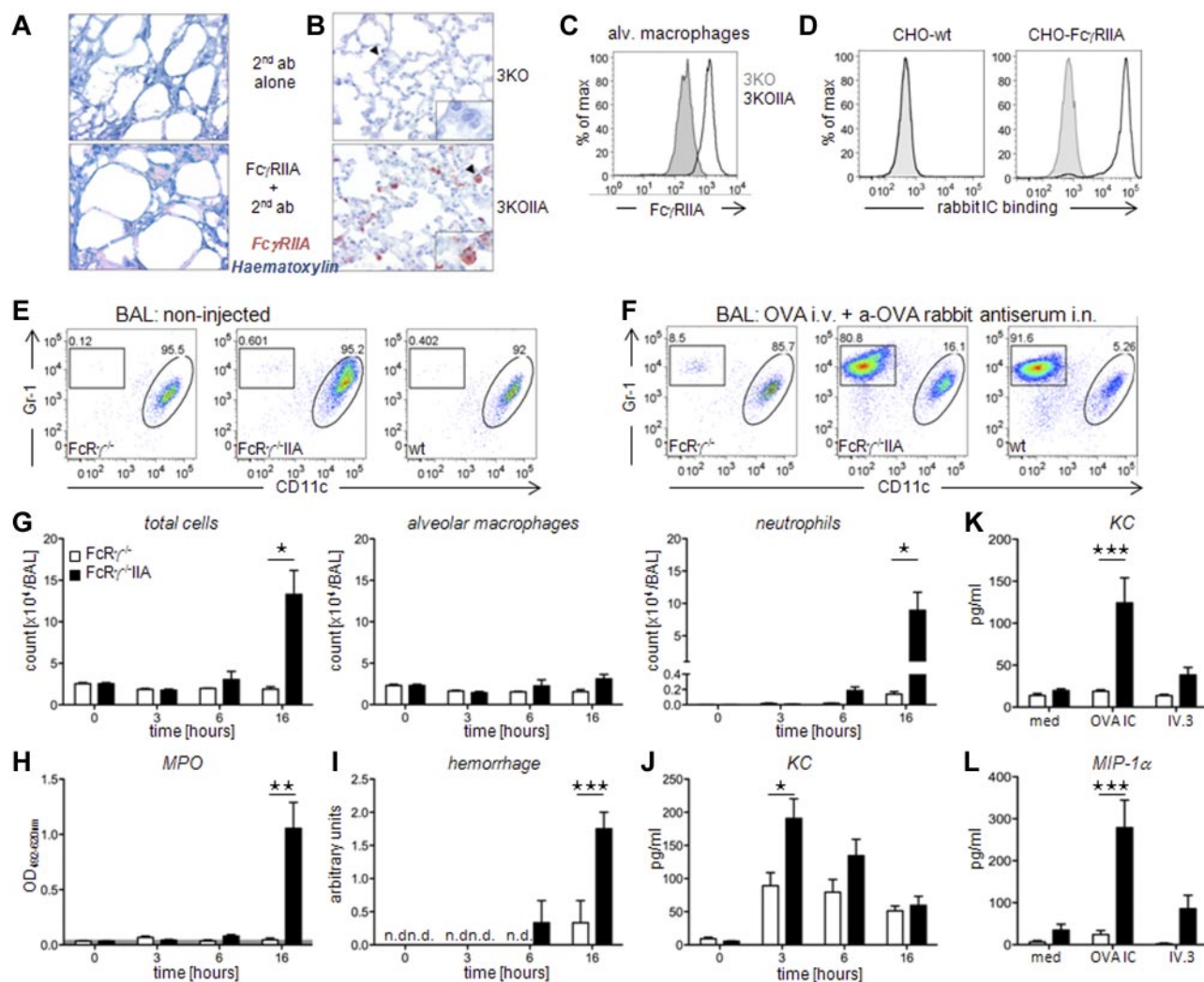


Figure 7. FcγRIIA can induce acute airway inflammation. (A-B) Sections of (A) human lung or (B) lung from 3KO or 3KOIIA mice, stained with Hematoxylin (blue) and anti-FcγRIIA rabbit antiserum (red). (C) Representative expression of FcγRIIA on mouse alveolar macrophages (CD11c⁺/Gr1⁻) from 3KOIIA (open histogram) and 3KO (filled histogram) mice. (D) F(ab')₂ DAR-FITC staining of WT or FcγRIIA-expressing CHO transfectants incubated with preformed ICs made of OVA and rabbit anti-OVA antiserum (open histograms) or not (filled histograms). (E-F) Representative density plots of CD45⁺ BAL cells from indicated mice (E) left untreated or (F) injected with antigen intravenously and antiserum intranasally. Cell types were discriminated as alveolar macrophages (CD11c⁺/Gr1^{int}, oval gate) and neutrophils (CD11c⁺/Gr1^{hi}, rectangular gate). (G-J) Time course of (G) cell counts, (H) MPO level, (I) hemorrhage score, and (J) KC levels in BAL from indicated mice after injection with antigen intravenously and antiserum intranasally (n ≥ 3). (K) KC secretion or (L) MIP-1α secretion by purified alveolar macrophages from indicated mice incubated ex vivo on plate-bound rabbit IgG-ICs (OVA-anti-OVA) or IV.3 mAb. (G-L) Data are represented as mean ± SEM. (A-L) Data are representative from at least 2 independent experiments, and (J) data are a compilation of 2 experiments (*P < .05; **P < .01; ***P < .001).

Myeloperoxidase, which is mainly produced by neutrophils and by inflammatory macrophages in vivo,^{35,36} was detected at t = 16 hours postchallenge in FcγRII^{-/-} mice, but not in FcγRI^{-/-} mice. (Figure 7H). Similar results were obtained when analyzing the hemorrhage score that reflects lung tissue damage (Figure 7I). KC, a chemokine produced by macrophages that can attract neutrophils to the site of inflammation, was found in BAL fluid of FcγRII^{-/-} mice and to a lesser extent in FcγRI^{-/-} mice, as early as 3 hours after inoculation of antibody and antigen (Figure 7J). This result suggests that alveolar macrophages are activated after FcγRIIA aggregation by IgG-immune complexes, and release KC before neutrophil accumulation in the broncho-alveolar space, in agreement with the dependency on alveolar macrophages reported for this disease model.³⁷ Supporting this hypothesis, purified alveolar macrophages from FcγRII^{-/-} mice, but not from FcγRI^{-/-} mice, secreted KC ex vivo after IgG-IC or anti-FcγRIIA mAb stimulation (Figure 7K). Similar results were obtained when analyzing MIP-1α secretion (Figure 7L), suggesting that FcγRIIA-triggered alveolar

macrophages contribute to chemokine-induced granulocyte recruitment to the lung. FcγRIIA can therefore induce airway inflammation characterized by granulocyte infiltration in a passive antibody-dependent mouse model.

Discussion

In this report, we provide evidence that human FcγRIIA contributes to IgG-mediated allergic reactions. Indeed, we demonstrate here for the first time that human FcγRIIA is sufficient to induce active and passive systemic anaphylaxis, cutaneous anaphylaxis, and lung inflammation in FcγRIIA-transgenic mice. Mast cells could be activated upon FcγRIIA engagement in vitro and were necessary for FcγRIIA-dependent PCA. Neither mast cells nor basophils, however, were mandatory for FcγRIIA-dependent PSA, which was induced by neutrophils and monocytes/macrophages.

Finally, targeting Fc γ RIIA with specific blocking mAbs abolished passive and active anaphylaxis.

Human Fc γ RIIA has been described to contribute to several models of autoimmune diseases and inflammatory reactions in transgenic mice. When expressed on a WT background, ie, as an additional activating IgG receptor, Fc γ RIIA was reported to increase the severity of experimental thrombocytopenia,¹⁷ and to increase the incidence of autoimmune arthritis, pneumonitis, and glomerulonephritis at older age.¹⁸ These reports suggested that Fc γ RIIA may also induce inflammatory diseases in the absence of other FcRs. Indeed, when expressed on a mouse FcR $\gamma^{-/-}$ background, that is, in the absence of other activating IgG receptors, Fc γ RIIA was reported to induce experimental thrombocytopenia¹⁷ and hemolytic anaemia,³⁸ rheumatoid arthritis,³⁹ nephritis, and Arthus reaction.⁴⁰ Along the same line, we show here that Fc γ RIIA induced IgG-dependent airway inflammation when expressed in FcR $\gamma^{-/-}$ mice. Inflammation was characterized by neutrophil infiltration of the broncho-alveolar space after KC (and MIP-1 α) secretion, probably by Fc γ RIIA-triggered alveolar macrophages. In this passive model of airway inflammation, we also observed a trend toward increased metacholine-induced bronchial resistance as measured by plethysmography, in FcR $\gamma^{-/-}$ IIA but not FcR $\gamma^{-/-}$ mice (data not shown). In addition to its contribution to autoimmune disorders, Fc γ RIIA may therefore also contribute to allergic reactions. Polymorphisms in the gene encoding Fc γ RIIA have indeed been identified as risk factors for bronchial asthma and allergic rhinitis.¹⁶ Further supporting a role for Fc γ RIIA in allergic reactions, we show here that Fc γ RIIA restored immediate hypersensitivity reactions in resistant mice: (1) Fc γ RIIA was indeed sufficient to induce fatal ASA following 2 different immunization protocols; (2) Fc γ RIIA engagement by intravenously injected divalent or multivalent agonists induced PSA; and (3) Fc γ RIIA induced PCA when human IgG aggregates, mouse immune complexes or anti-Fc γ RIIA mAb IV.3 were injected intradermally. Fc γ RIIA can therefore reproduce by itself in a transgenic mouse model the allergic/anaphylactic pathologies reported to be triggered by the endogenous activating mouse Fc γ Rs (Fc γ RI, Fc γ RIIA, and Fc γ RIV), with similar severities and kinetics. Altogether these results, obtained in Fc γ RIIA-transgenic mice suggest that Fc γ RIIA might be a major player in allergic, autoimmune, and inflammatory pathologies mediated by IgG in humans.

Passive mouse models of inflammatory and allergic diseases have been reported to depend on specific cell types. Indeed, mast cells are required for PCA³ and IgE-PSA,⁵ macrophages for ITP,⁴¹ lung inflammation³⁷ and passive rheumatoid arthritis,⁴² basophils for IgG1-PSA,⁷ and neutrophils for passive rheumatoid arthritis,⁴³ ASA, and polyclonal IgG-PSA.¹ All these myeloid cells express human Fc γ RIIA in transgenic mice (this paper and McKenzie et al¹⁷). Noticeably, Fc γ RIIA is the only activating (ITAM-bearing) IgG receptor expressed on these cells in humans, ie, on mast cells, neutrophils, eosinophils, and basophils. Of note, neutrophils, and to a much lower extent basophils,⁴⁴ express Fc γ RIIIB, the activating capacities of which are debated.^{40,45} As a consequence, Fc γ RIIA should be able to activate *in vivo* all of the cell types reported to be necessary for the induction of models of inflammatory and allergic disease in mice. Mast cells were reported to be required for mouse Fc γ RIIA-dependent and for mouse Fc ϵ RI-dependent PCA.^{3,6} We demonstrate here that mast cells are also required for human Fc γ RIIA-dependent PCA using novel mast cell-deficient Fc γ RIIA-transgenic mice. Noticeably, mast cells were also reported to be required for IgE-induced PSA (mouse Fc ϵ RI-dependent), but not

for IgG1-induced PSA (mouse Fc γ RIIA-dependent).^{5,6} Although basophils were proposed to be responsible for IgG1-induced PSA,⁷ basophil-deficient mice were, however, not protected.⁸ We reported recently that neutrophils are required for both IgG2-induced PSA and polyclonal IgG-induced PSA.¹ Depending on the PSA model used, either mast cells, basophils, or neutrophils have therefore been reported to be mandatory for mouse FcR-dependent anaphylaxis.

We demonstrate here that neutrophils and monocytes/macrophages both contribute to Fc γ RIIA-dependent polyclonal IgG-PSA. Indeed, the depletion of both cell populations, but not of either one, was necessary to abolish the shock. Supporting these results, monocytes/macrophages and neutrophils contributed to another Fc γ RIIA-dependent PSA model, ie, when induced by IV injections of a high dose anti-Fc γ RIIA mAb. These results provide the first evidence that monocytes/macrophages contribute to a model of PSA. In agreement with IgG-induced PSA models in WT mice,^{1,6} mast cells were not mandatory for Fc γ RIIA-dependent PSA. Basophils did not detectably contribute to Fc γ RIIA-dependent PSA. This latter result could be explained by the lower expression level of Fc γ RIIA on basophils than on neutrophils or monocytes/macrophages in transgenic mice, as it is in humans.

Active models of allergic diseases in mice have been reported to depend on specific cell types. Indeed, monocytes/macrophages, but not neutrophils, have been reported to be mandatory for a model of ASA performed in mice immunized with goat IgG anti-mouse IgD, and challenged with goat IgG.⁹ Inversely, in a different model of ASA, performed in BSA-immunized mice challenged with BSA, we reported that neutrophils, but not monocytes/macrophages, were mandatory.¹ In addition, we could show that the transfer of human neutrophils, which express Fc γ RIIA and Fc γ RIIIB, restored ASA in resistant mice. Because Fc γ RIIA, but not Fc γ RIIIB, binds mouse IgG-immune complexes, Fc γ RIIA is probably responsible for the activation of human neutrophils in this model. We could not address the contribution of neutrophils and basophils in Fc γ RIIA-dependent ASA because their depletion by specific mAbs is impaired on the FcR γ -deficient background. Indeed, whereas mouse Fc γ Rs enabled efficient cell depletion using anti-Gr1, anti-Ly-6G, or anti-CD200R3 mAbs, human Fc γ RIIA did not. All of these mAbs are of the rat IgG2b isotype that is poorly bound by human Fc γ RIIA (data not shown). Nevertheless, one may speculate that monocytes/macrophages and neutrophils also contribute to Fc γ RIIA-dependent ASA, as they contribute to Fc γ RIIA-dependent PSA. Whatever the responsible cell population for Fc γ RIIA-dependent ASA, Fc γ RIIA is sufficient to promote the release of mediators leading to anaphylactic reactions. Depending on the immunization protocol, PAF was reported to be responsible,^{5,9} or to contribute partially¹ to ASA. On one hand, PAF was found to be predominant in IgG1-induced PSA⁷ and we reported increased PAF levels in plasma during IgG2-induced PSA.¹ On the other hand, histamine was found mandatory for IgG- and IgE-induced PCA using histidine decarboxylase-deficient mice⁴⁶ as well as for IgE-induced PSA.^{7,47} It follows that Fc γ RIIA engagement should enable the release of PAF and/or of histamine by monocytes/macrophages, neutrophils, and mast cells. Supporting this line of reasoning, we report here that Fc γ RIIA-dependent cell activation *in vitro* induces human monocytes and neutrophils to produce PAF and human mast cells to release histamine. Whereas 3 to 5 times higher PAF amounts were produced by monocytes than by neutrophils, neutrophils are 10 times more numerous than monocytes in human blood, which may compensate their lower

production of PAF. Intriguingly, activated monocytes but not neutrophils produced leukotrienes (ie, LTB_4 and LTC_4) which may also relate to anaphylaxis induction/severity in humans.

$\text{Fc}\gamma\text{RIIA}$ is a nonconventional activating $\text{Fc}\gamma\text{R}$. Indeed, unlike all other activating $\text{Fc}\gamma\text{Rs}$ ($\text{Fc}\gamma\text{RI}$, $\text{Fc}\gamma\text{RIIA}$, and $\text{Fc}\gamma\text{RIV}$ in mice; $\text{Fc}\gamma\text{RI}$ and $\text{Fc}\gamma\text{RIIA}$ in humans), $\text{Fc}\gamma\text{RIIA}$ does not associate with the $\text{Fc}\gamma\text{R}$ -subunit, and contains an ITAM in its intracytoplasmic domain. The $\text{Fc}\gamma\text{RIIA}$ ITAM is, however, noncanonical, as it is several amino acids longer and kinked because of the presence of proline residues.⁴⁸ $\text{Fc}\gamma\text{RIIA}$ has been considered less potent to activate cells than $\text{Fc}\gamma\text{Rs}$ signaling through $\text{Fc}\gamma\text{R}$ -subunits as these subunits are expressed as dimers, thus providing 2 ITAMs per receptor. An elegant study using protein complementation⁴⁹ and crystallographic data⁵⁰ both suggest that $\text{Fc}\gamma\text{RIIA}$ are expressed as noncovalent dimers. Thus, $\text{Fc}\gamma\text{RIIA}$ and other activating $\text{Fc}\gamma\text{Rs}$ have the same number of ITAMs when expressed at the cell membrane. Whereas both types of receptors have the ability to induce intracellular calcium concentration increase,⁵¹ tyrosine phosphorylation events,⁵² and mediator release from granules,^{23,27} only the $\text{Fc}\gamma\text{RI}$ ITAM was reported to enable antigen presentation and cytokine secretion^{13,14} upon receptor crosslinking. The inability of $\text{Fc}\gamma\text{RIIA}$ to induce cytokine secretion after engagement has been contradicted by reports on human skin-derived mast cells²⁷ or on human macrophages.⁵³ Here, we detected chemokine (KC and $\text{MIP-1}\alpha$) secretion by ex vivo alveolar macrophages, but failed to detect cytokine secretion by in vitro-derived murine mast cells (data not shown), after $\text{Fc}\gamma\text{RIIA}$ engagement. Nevertheless, this latter stimulation led to the release of lipid mediators (LTC_4 , PGD_2), granular mediators (histamine, β -hexosaminidase), calcium signaling, and to tyrosine phosphorylation of intracellular proteins. Among them, Syk, $\text{PLC}\gamma 1$, Akt, Erk1/2 , and SHIP1 were readily phosphorylated upon $\text{Fc}\gamma\text{RIIA}$ engagement. These results correlate with previous reports showing that Syk and SHIP associate to $\text{Fc}\gamma\text{RIIA}$, are phosphorylated upon receptor aggregation,^{29,54} and that $\text{Fc}\gamma\text{RIIA}$ requires $\text{PLC}\gamma 1$ for calcium signaling.¹⁴ Phosphorylation of most intracellular signaling proteins was, however, less prominent after $\text{Fc}\gamma\text{RIIA}$ engagement than after $\text{Fc}\gamma\text{RI}$ engagement. Similar results were reported when comparing $\text{Fc}\gamma\text{RIIA}$ with $\text{Fc}\gamma\text{RI}$ downstream signaling.¹⁴ Altogether, these data suggest that $\text{Fc}\gamma\text{RIIA}$ enables cell activation through similar, although not identical, signaling pathways compared with those activated by $\text{Fc}\gamma\text{R}$ -associated $\text{Fc}\gamma\text{Rs}$. This may lead to differences in biologic outcome, in particular when considering cytokine production.

Finally, when considering its pattern of expression and its ability to activate myeloid cells, human $\text{Fc}\gamma\text{RIIA}$ appears as a functional homolog of mouse $\text{Fc}\gamma\text{RIIA}$. Noticeably, both receptors are the only activating $\text{Fc}\gamma\text{Rs}$ expressed on mast cells, basophils, and eosinophils in humans and mice, respectively. $\text{Fc}\gamma\text{RIIA}$ is, however, coexpressed in humans with inhibitory $\text{Fc}\gamma\text{RIIB}$ on basophils (L. Cassard and F.J., unpublished data, 2011), and on some monocytes and rare neutrophils.⁵⁵ Because mouse and human $\text{Fc}\gamma\text{RIIB}$ both inhibit cell activation by the same mechanism,⁵⁶ and because human $\text{Fc}\gamma\text{RIIA}$ is coexpressed with mouse $\text{Fc}\gamma\text{RIIB}$ in $\text{Fc}\gamma\text{R}^{-/-}\text{IIA}$ mice on basophils, monocytes, and neutrophils, we wondered if mouse $\text{Fc}\gamma\text{RIIB}$ could negatively regulate human $\text{Fc}\gamma\text{RIIA}$ -triggered PSA. We found that PSA was less profound in $\text{Fc}\gamma\text{R}^{-/-}\text{IIA}$ mice (expressing m $\text{Fc}\gamma\text{RIIB}$) than in 3KOIIA (lacking m $\text{Fc}\gamma\text{RIIB}$; supplemental Figure 3A). Similar to mouse $\text{Fc}\gamma\text{RIIB}$, human $\text{Fc}\gamma\text{RIIB}$ may therefore also negatively regulate $\text{Fc}\gamma\text{RIIA}$ -triggered anaphylaxis in humans. In vivo, $\text{Fc}\gamma\text{RIIA}$ demonstrated strong potential to activate myeloid cells and induce inflammatory and allergic pathologies in transgenic mice. Indeed, $\text{Fc}\gamma\text{RIIA}$ engagement can activate neutrophils leading to nephritis⁴⁰ or anaphylactic shock (this paper and Jönsson et al¹). $\text{Fc}\gamma\text{RIIA}$

engagement can also activate macrophages leading to rheumatoid arthritis,³⁹ thrombocytopenia,¹⁷ lung inflammation, or anaphylactic shock and activate mast cells leading to PCA (this paper). Therefore, even though $\text{Fc}\gamma\text{RIIA}$ may appear less efficient in vitro than other $\text{Fc}\gamma\text{R}$ -associated $\text{Fc}\gamma\text{Rs}$, its properties are sufficient for the induction of severe allergic, autoimmune and inflammatory pathologies in vivo. Targeting $\text{Fc}\gamma\text{RIIA}$ with specific blocking molecules in inflammation and autoimmune/allergic reactions in humans might lead to similar inhibition as we reported recently for mouse $\text{Fc}\gamma\text{RIIA}$ in a murine model of rheumatoid arthritis,⁵⁷ in PSA and in ASA.¹ Supporting this assumption, we report here that blocking $\text{Fc}\gamma\text{RIIA}$ protected transgenic mice from local and systemic anaphylaxis. Blocking $\text{Fc}\gamma\text{RIIA}$ using divalent ligands (eg, mAb IV.3) to prevent allergic and autoimmune disease in humans, however, should not be envisioned, as we report here that high-doses of mAb IV.3 induced rather than prevented anaphylaxis. Small chemical entities, which prevent immune-complex binding to $\text{Fc}\gamma\text{RIIA}$, have proven efficient in a murine model of arthritis in $\text{Fc}\gamma\text{RIIA}$ -transgenic mice,²⁰ and may not induce these adverse effects. In conclusion, blocking $\text{Fc}\gamma\text{RIIA}$ might be a potential approach for various allergic diseases, including non-IgE-mediated anaphylactic shocks that may be induced after $\text{Fc}\gamma\text{RIIA}$ engagement on monocyte/macrophages and neutrophils.

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Authorship

Contribution: F.J. performed experiments and designed part of the research; D.A.M. contributed to anaphylaxis experiments; W.Z. and L.B.S. performed analysis of human mast cells; Y.K. and T.S. performed analysis of lipid mediators; B.I. genotyped mice and produced essential reagents; H.K. performed histology; N.v.R. provided reagents; P.B., M.D., and F.J. analyzed results; P.B. designed and supervised the research; and P.B., with help from F.J. and M.D., wrote the paper.

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References

- Jönsson F, Mancardi DA, Kita Y, et al. Mouse and human neutrophils induce anaphylaxis. *J Clin Invest*. 2011;121(4):1484-1496.
- Daéron M. Fc receptor biology. *Annu Rev Immunol*. 1997;15:203-234.
- Wershil BK, Mekori YA, Murakami T, Galli SJ. 125I-fibrin deposition in IgE-dependent immediate hypersensitivity reactions in mouse skin. Demonstration of the role of mast cells using genetically mast cell-deficient mice locally reconstituted with cultured mast cells. *J Immunol*. 1987;139(8):2605-2614.
- Arimura A, Nagata M, Takeuchi M, Watanabe A, Nakamura K, Harada M. Active and passive cutaneous anaphylaxis in WBB6F1 mouse, a mast cell-deficient strain. *Immunol Invest*. 1990;19(3):227-233.
- Arimura A, Nagata M, Watanabe A, Nakamura K, Takeuchi M, Harada M. Production of active and passive anaphylactic shock in the WBB6F1 mouse, a mast cell-deficient strain. *Experientia*. 1990;46(7):739-742.
- Miyajima I, Dombrowicz D, Martin TR, Ravetch JV, Kinet JP, Galli SJ. Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and Fc gammaRIII. Assessment of the cardiopulmonary changes, mast cell degranulation, and death associated with active or IgE- or IgG1-dependent passive anaphylaxis. *J Clin Invest*. 1997;99(5):901-914.
- Tsujimura Y, Obata K, Mukai K, et al. Basophils play a pivotal role in immunoglobulin-G-mediated but not immunoglobulin-E-mediated systemic anaphylaxis. *Immunity*. 2008;28(4):581-589.
- Ohnmacht C, Schwartz C, Panzer M, Schiedewitz I, Naumann R, Voehringer D. Basophils orchestrate chronic allergic dermatitis and protective immunity against helminths. *Immunity*. 2010;33(3):364-374.
- Strait RT, Morris SC, Yang M, Qu XW, Finkelman FD. Pathways of anaphylaxis in the mouse. *J Allergy Clin Immunol*. 2002;109(4):658-668.
- Nimmerjahn F, Bruhns P, Horiuchi K, Ravetch JV. Fc gamma RIV: a novel FcR with distinct IgG subclass specificity. *Immunity*. 2005;23(1):41-51.
- Dombrowicz D, Flamand V, Brigman KK, Koller BH, Kinet JP. Abolition of anaphylaxis by targeted disruption of the high affinity immunoglobulin E receptor alpha chain gene. *Cell*. 1993;75(5):969-976.
- Hazenbos WL, Gessner JE, Hofhuis FM, et al. Impaired IgG-dependent anaphylaxis and Arthus reaction in Fc gamma RIII (CD16) deficient mice. *Immunity*. 1996;5(2):181-188.
- Van den Herik-Oudijk IE, Ter Bekke MW, Tempelman MJ, Capel PJ, Van de Winkel JG. Functional differences between two Fc receptor ITAM signaling motifs. *Blood*. 1995;86(9):3302-3307.
- Dai X, Jayapal M, Tay HK, et al. Differential signal transduction, membrane trafficking, and immune effector functions mediated by Fc gammaRI versus Fc gammaRIIa. *Blood*. 2009;114(2):318-327.
- Bruhns P, Iannascoli B, England P, et al. Specificity and affinity of human Fc(gamma) receptors and their polymorphic variants for human IgG subclasses. *Blood*. 2009;113(16):3716-3725.
- Gulen F, Tanac R, Altinoz S, et al. The Fc gamma-RIIa polymorphism in Turkish children with asthma bronchial and allergic rhinitis. *Clin Biochem*. 2007;40(5-6):392-396.
- McKenzie SE, Taylor SM, Malladi P, et al. The role of the human Fc receptor Fc gamma RIIa in the immune clearance of platelets: a transgenic mouse model. *J Immunol*. 1999;162(7):4311-4318.
- Tan Sardjono C, Mottram PL, van de Velde NC, et al. Development of spontaneous multisystem autoimmune disease and hypersensitivity to antibody-induced inflammation in Fc gamma receptor IIa-transgenic mice. *Arthritis Rheum*. 2005;52(10):3220-3229.
- Reilly AF, Norris CF, Surrey S, et al. Genetic diversity in human Fc receptor II for immunoglobulin G: Fc gamma receptor IIa ligand-binding polymorphism. *Clin Diagn Lab Immunol*. 1994;1(6):640-644.
- Pietersz GA, Mottram PL, van de Velde NC, et al. Inhibition of destructive autoimmune arthritis in Fc gammaRIIa transgenic mice by small chemical entities. *Immunol Cell Biol*. 2009;87(1):3-12.
- Mancardi DA, Iannascoli B, Hoos S, England P, Daeron M, Bruhns P. Fc gammaRIIa is a mouse IgE receptor that resembles macrophage Fc epsilonRI in humans and promotes IgE-induced lung inflammation. *J Clin Invest*. 2008;118(11):3738-3750.
- Ujike A, Ishikawa Y, Ono M, et al. Modulation of immunoglobulin (Ig)E-mediated systemic anaphylaxis by low-affinity Fc receptors for IgG. *J Exp Med*. 1999;189(10):1573-1579.
- Daéron M, Latour S, Malbec O, et al. The same tyrosine-based inhibition motif, in the intracytoplasmic domain of Fc gamma RIIB, regulates negatively BCR-, TCR-, and FcR-dependent cell activation. *Immunity*. 1995;3(5):635-646.
- Kita Y, Takahashi T, Uozumi N, Shimizu T. A multiplex quantitation method for eicosanoids and platelet-activating factor using column-switching reversed-phase liquid chromatography-tandem mass spectrometry. *Anal Biochem*. 2005;342(1):134-143.
- Zhou JS, Xing W, Friend DS, Austen KF, Katz HR. Mast cell deficiency in Kit(W-sh) mice does not impair antibody-mediated arthritis. *J Exp Med*. 2007;204(12):2797-2802.
- Tsai M, Grimbaldston MA, Yu M, Tam SY, Galli SJ. Using mast cell knock-in mice to analyze the roles of mast cells in allergic responses in vivo. *Chem Immunol Allergy*. 2005;87:179-197.
- Zhao W, Kepley CL, Morel PA, Okumoto LM, Fukuoka Y, Schwartz LB. Fc gamma RIIa, not Fc gamma RIIB, is constitutively and functionally expressed on skin-derived human mast cells. *J Immunol*. 2006;177(1):694-701.
- Malbec O, Roget K, Schiffer C, et al. Peritoneal cell-derived mast cells: an in vitro model of mature serosal-type mouse mast cells. *J Immunol*. 2007;178(10):6465-6475.
- Maresco DL, Osborne JM, Cooney D, Coggeshall KM, Anderson CL. The SH2-containing 5'-inositol phosphatase (SHIP) is tyrosine phosphorylated after Fc gamma receptor clustering in monocytes. *J Immunol*. 1999;162(11):6458-6465.
- Ono M, Bolland S, Tempst P, Ravetch JV. Role of the inositol phosphatase SHIP in negative regulation of the immune system by the receptor Fc(gamma)RIIB. *Nature*. 1996;383(6597):263-266.
- Malbec O, Schmitt C, Bruhns P, Krystal G, Fridman WH, Daeron M. Src homology 2 domain-containing inositol 5-phosphatase 1 mediates cell cycle arrest by Fc gammaRIIB. *J Biol Chem*. 2001;276(32):30381-30391.
- Korsgren M, Erjefält JS, Korsgren O, Sundler F, Persson CG. Allergic eosinophil-rich inflammation develops in lungs and airways of B cell-deficient mice. *J Exp Med*. 1997;185(5):885-892.
- Mehlhop PD, van de Rijn M, Goldberg AB, et al. Allergen-induced bronchial hyperreactivity and eosinophilic inflammation occur in the absence of IgE in a mouse model of asthma. *Proc Natl Acad Sci U S A*. 1997;94(4):1344-1349.
- Syed SN, Konrad S, Wiege K, et al. Both Fc gammaRIIa and Fc gammaRIII are essential receptors mediating type II and type III autoimmune responses via Fc gamma-LAT-dependent generation of C5a. *Eur J Immunol*. 2009;39(12):3343-3356.
- Lotner GZ, Lynch JM, Betz SJ, Henson PM. Human neutrophil-derived platelet activating factor. *J Immunol*. 1980;124(2):676-684.
- Klebanoff SJ. Myeloperoxidase: friend and foe. *J Leukoc Biol*. 2005;77(5):598-625.
- Skokowa J, Ali SR, Felda O, et al. Macrophages induce the inflammatory response in the pulmonary Arthus reaction through G alpha i2 activation that controls C5aR and Fc receptor cooperation. *J Immunol*. 2005;174(5):3041-3050.
- van Royen-Kerkhof A, Sanders EA, Walraven V, et al. A novel human CD32 mAb blocks experimental immune haemolytic anaemia in Fc gammaRIIa transgenic mice. *Br J Haematol*. 2005;130(1):130-137.
- Tsuboi N, Hernandez T, Li X, et al. Regulation of human neutrophil Fc gamma receptor IIa by C5a receptor promotes inflammatory arthritis in mice. *Arthritis Rheum*. 2011;63(2):467-478.
- Tsuboi N, Asano K, Lauterbach M, Mayadas TN. Human neutrophil Fc gamma receptors initiate and play specialized nonredundant roles in antibody-mediated inflammatory diseases. *Immunity*. 2008;28(6):833-846.
- Alves-Rosa F, Stanganelli C, Cabrera J, van Rooijen N, Palermo MS, Isturiz MA. Treatment with liposome-encapsulated clodronate as a new strategic approach in the management of immune thrombocytopenic purpura in a mouse model. *Blood*. 2000;96(8):2834-2840.
- Solomon S, Rajasekaran N, Jeisy-Walder E, Snapper SB, Illes H. A crucial role for macrophages in the pathology of K/B x N serum-induced arthritis. *Eur J Immunol*. 2005;35(10):3064-3073.
- Wipke BT, Allen PM. Essential role of neutrophils in the initiation and progression of a murine model of rheumatoid arthritis. *J Immunol*. 2001;167(3):1601-1608.
- Meknache N, Jönsson F, Laurent J, Guinneeain MT, Daéron M. Human basophils express the glycosylphosphatidylinositol-anchored low-affinity IgG receptor Fc gammaRIIB (CD16B). *J Immunol*. 2009;182(4):2542-2550.
- Ravetch JV, Bolland S. IgG Fc receptors. *Annu Rev Immunol*. 2001;19:275-290.
- Makabe-Kobayashi Y, Hori Y, Adachi T, et al. The control effect of histamine on body temperature and respiratory function in IgE-dependent systemic anaphylaxis. *J Allergy Clin Immunol*. 2002;110(2):298-303.
- Winbery SL, Lieberman PL. Histamine and antihistamines in anaphylaxis. *Clin Allergy Immunol*. 2002;17:287-317.
- Brooks DG, Qiu WQ, Luster AD, Ravetch JV. Structure and expression of human IgG FcRII(CD32). Functional heterogeneity is encoded by the alternatively spliced products of multiple genes. *J Exp Med*. 1989;170(4):1369-1385.
- Powell MS, Barnes NC, Bradford TM, et al. Alteration of the Fc gamma RIIa dimer interface affects receptor signaling but not ligand binding. *J Immunol*. 2006;176(12):7489-7494.
- Maxwell KF, Powell MS, Hulett MD, et al. Crystal

- structure of the human leukocyte Fc receptor, Fc gammaRIIa. *Nat Struct Biol.* 1999;6(5):437-442.
51. Van Den Herik-Oudijk IE, Westerdaal NA, Henriquez NV, Capel PJ, Van De Winkel JG. Functional analysis of human Fc gamma RII (CD32) isoforms expressed in B lymphocytes. *J Immunol.* 1994;152(2):574-585.
52. Van den Herik-Oudijk IE, Capel PJ, van der Bruggen T, Van de Winkel JG. Identification of signaling motifs within human Fc gamma RIIa and Fc gamma RIIb isoforms. *Blood.* 1995;85(8):2202-2211.
53. Clavel C, Nogueira L, Laurent L, et al. Induction of macrophage secretion of tumor necrosis factor alpha through Fc gamma receptor IIa engagement by rheumatoid arthritis-specific autoantibodies to citrullinated proteins complexed with fibrinogen. *Arthritis Rheum.* 2008;58(3):678-688.
54. Chacko GW, Brandt JT, Coggeshall KM, Anderson CL. Phosphoinositide 3-kinase and p72syk noncovalently associate with the low affinity Fc gamma receptor on human platelets through an immunoreceptor tyrosine-based activation motif. Reconstitution with synthetic phosphopeptides. *J Biol Chem.* 1996;271(18):10775-10781.
55. Veri MC, Gorlatov S, Li H, et al. Monoclonal antibodies capable of discriminating the human inhibitory Fc gamma-receptor IIB (CD32B) from the activating Fc gamma-receptor IIA (CD32A): biochemical, biological and functional characterization. *Immunology.* 2007;121(3):392-404.
56. Isnardi I, Bruhns P, Bismuth G, Fridman WH, Daeron M. The SH2 domain-containing inositol 5-phosphatase SHIP1 is recruited to the intracytoplasmic domain of human Fc gammaRIIB and is mandatory for negative regulation of B cell activation. *Immunol Lett.* 2006;104(1-2):156-165.
57. Mancardi DA, Jonsson F, Iannascoli B, et al. The murine high-affinity IgG receptor Fc(gamma)RIV is sufficient for autoantibody-induced arthritis. *J Immunol.* 2011;186(4):1899-1903.

Antibodies, reagents, and cells

PBS-liposomes and Clodronate-liposomes were prepared as published.²⁶ Alum hydroxide gel, bovine serum albumin (BSA), albumin from chicken egg grade V (ova), DNP₃₀-coupled human serum albumin (DNP₃₀-HSA), rabbit Glucose-6-phosphate Isomerase (GPI), gadolinium(III)-chloride (GdCl₃), Pluronic F-127, complete and incomplete Freund's adjuvant (CFA, IFA), β -hexosaminidase substrate, anti-FLAG mAb, anti-ovalbumin mIgG1 (OVA-14), anti-ova rabbit serum and purified human serum IgG were obtained from Sigma-Aldrich. Anti-mouse CD11b, CD11c, CD3, CD19, Gr1, SiglecF, NK1.1, c-kit, anti-IgE and anti-human Fc γ RI, Fc γ RII, Fc γ RIII, CD3, CD19, CD56, CD24, CD14, CDw125 mAbs were purchased from BD Biosciences (San Diego, CA); isotype controls from BD and Clinisciences (Montrouge, France); anti-mouse DX5, anti-mouse Fc ϵ RI and anti-human Fc ϵ RI mAbs from eBioscience (San Diego, CA); anti-mouse Fc γ RI (290322), anti-mouse Fc γ RIIIA (275003) and anti-human CCR3 from R&D systems; anti-human Fc γ RIIA mAb (IV.3) from StemCell Technologies. The hybridoma producing mAbs anti-mFc γ RIV (9E9) was provided by J.V. Ravetch (Rockefeller University, NY, USA), anti-Gr1 (RB6-8C5) by R. Coffman (DNAX, Palo Alto, CA), anti-human Fc γ RIIA (IV.3) by C.L. Anderson (Heart & Lung Research Institute, Columbus, OH, USA), anti-Ly-6G (NIMP-R14) by C. Leclerc (Institut Pasteur, Paris, France), IgE anti-DNP (2682I) by C.A. Molinaro (Scripps Clinic and Research Foundation, La Jolla, CA, USA). Purified mAbs mouse anti-hFc γ RIIB/C (GB3) were provided by U. Jacob (SuppreMol, Martinsried/München, Germany), anti-CD200R3 (Ba103) by H. Karasuyama (JST, CREST, Tokyo Medical and Dental University Graduate School, Tokyo, Japan), anti-CCR3 by J.J. Lee (Mayo Clinic, AZ, USA).

Rabbit antiserum against the intracellular portion of human Fc γ RIIA was described before.²⁷ ELISA kit for MPO was obtained from HyCult Biotech, for MIP2 from R&D Systems, for LTC₄ and histamine from Neogen and for PDG₂ from Cayman Chemical. CHO-K1 cells stably transfected with FLAG-tagged mouse Fc γ Rs²⁵ or human FLAG-tagged Fc γ Rs¹⁸ were cultured as described.

Flow cytometry analysis

Human blood from healthy donors or murine blood from Fc γ RIIA transgenic mice was incubated with IV.3-FITC or isotype control (mIgG2b-FITC). Human blood cell populations were discriminated based on FSC/SSC properties and staining of CD3 (T cells), CD19 (B cells), CD14 (monocytes), CD56 (NK cells), Fc ϵ RI/CD203c (basophils), CD24 (neutrophils) and CCR3/CDw125 (eosinophils). Mouse blood cell populations were discriminated based on FSC/SSC properties and staining of CD3 (T cells), B220 (B cells), CD11b/CD115 (monocytes), NK1.1/DX5 (NK cells), IgE/DX5 (basophils), CD11b/Gr1^{high} (neutrophils) and Gr1^{int}/SiglecF (eosinophils). Macrophages in the peritoneum were identified as CD11b⁺/Gr1^{neg} cells, mast cells as IgE⁺/c-kit⁺ expressing cells. Cells in BAL were stained with CD11c/Gr1 and enumerated using Counting beads (Caltag). Expression of different Flag-tagged FcRs in CHO-K1 cells was compared using anti-FLAG antibody.

Immune complex binding: CHO-K1 cells were incubated with preformed ICs made of 0.5 μ g/mL GPI and 1 μ g/mL anti-GPI polyclonal antibodies purified from KRN serum, or of 1 μ g/mL ova and rabbit anti-ova antiserum (1/500 dilution), for 1 hour at 4°C. PCMC were incubated with preformed ICs made of 20 μ g/mL GPI and KRN serum (1/100 dilution) or of 100 μ g/mL ovalbumin and 100 μ g/mL mAb OVA-14, for 30 min at 4°C. Bound ICs were detected using F(ab')₂ GAM-F(ab')₂-PE or DAM-FITC.

Flow cytometric analysis of Ca²⁺ mobilization: 1×10^6 PCMC/ml were preloaded with 50 μ g Fluo-3 AM (Molecular Probes, Eugene, OR) in 0.2% Pluronic F-127 for 30 min and [Ca²⁺]_i was monitored by flow cytometry.

***In vitro* cell activation**

PCMC degranulation and mediator production

PCMC were generated as described²² and challenged with preformed ICs made of 50 μ g/ml GPI either with 1/100 dilution of anti-GPI antiserum or with Protein G-purified IgG anti-GPI (100 μ g/ml), or ICs made of 100 μ g/ml mAb OVA-14 and 100 μ g/ml ova, at 37°C. As a positive control, mast cells were sensitized with IgE anti-DNP 2682I and challenged with 0,01 μ g/ml DNP-HSA. β -hexosaminidase was assayed after 15 min stimulation, and histamine, LTC₄ and PDG₂ after 30 min stimulation.

Human skin-derived mast cells

Mast cells were obtained from human skin as described²³ and resuspended in serum-free X-VIVO 15 medium containing 100 ng/ml of rhSCF (gift of Amgen Inc.). 10^6 /ml 6-16 week-old mature mast cells were incubated at 37°C for 30 min with anti-hFc ϵ RI mAb 22E7 (1 μ g/ml) or with a mix of IV.3 mAb (1 μ g/ml) and F(ab')₂ goat-anti-mouse IgG Fc γ -specific (2 μ g/ml) to cross-link Fc ϵ RI and Fc γ RIIA, respectively. β -hexosaminidase release was determined as described.²⁴

Activation of primary mouse peritoneal mast cells

Mouse peritoneal cells were stimulated *ex vivo* with preformed IC or 10 μ g/ml anti-IgE (R35-72, BD Pharmingen) for 15 min at 37°C, followed by incubation with toluidine blue at 0°C. Degranulation was assessed morphologically under the microscope on at least 200 cells per experimental point.

Western blot analysis

Immediately after stimulation with indicated reagents, PCMC were lysed in 10 mM Tris (pH 7.2) 1% SDS lysis buffer at 95°C. 20 μ g of proteins (quantified by D_c protein assay, Bio-Rad) were separated by electrophoresis, transferred on PDVF membranes. Membranes were incubated with anti-pERK1/2 (Thr202/Tyr204), anti-pSyk (Tyr525/526), anti-pAkt (S473), anti-pI κ B (Ser32), anti-actin (I-19) mAbs (Cell Signaling), anti-pSHIP (Stemcell Technologies), anti-pLat (Tyr191) (Millipore), anti-pPLC γ 1 (Tyr783) (Santa Cruz) or rabbit serum anti-hFc γ RIIA intracellular domain, followed by incubations with HRP-labeled secondary antibodies (Santa Cruz), detected using enhanced chemoluminescence (Amersham Biosciences).

Human monocyte and neutrophil activation

Blood samples from normal human donors were fractionated by density centrifugation on Lympholyte-poly (Cedarlane Labs). Monocytes were purified from PBMCs, and neutrophils from granulocytes, using anti-CD14 or anti-CD15 magnetic beads (Miltenyi), respectively. $0.5-1 \times 10^6$ purified cells were incubated or not with HA-human IgG or -IV.3 mAb (100 μ g/ml) for 20 min at 37°C in HBSS buffer supplemented with 1.26mM CaCl₂ and 0.9mM MgCl₂. A small fraction of the cells was used to assess cell activation using CD62L expression by flow cytometry; the major fraction of the cells was centrifuged and used for lipid mediator measurement.

Lipid mediator measurement: Culture supernatants were mixed 1:10 v/v in methanol, or cell pellets resuspended in methanol, and centrifuged at 15,000rpm for 10min. Resulting supernatants were frozen at -20°C. Lipid mediators were quantified as described. After adding deuterized PAF and LTB₄ (PAF-d4 and LTB₄-d4, Cayman chemical, Ann Arbor, MI) as internal standards, lipid mediators were extracted with Oasis HLB solid phase extraction cartridge (1cc/10mg, Waters, Milford, MA). PAF, LTB₄ and LTC₄ were quantified using liquid chromatography-tandem mass spectrometry on a TSQ Quantum Ultra mass spectrometer (Thermo Fischer Scientific, Waltham, MA) that was operated in negative electrospray ionization. Selected reaction monitoring (SRM) mode was used to monitor 568.5 to 59 *m/z* transitions for PAF, 572.5 to 59 *m/z* transitions for PAF-d4, 335 to 195 *m/z* transitions for LTB₄, and 624 to 272 *m/z* transitions for LTC₄. Commercial PAF, LTB₄ and LTC₄ (Cayman chemical) mixed with PAF-d4 and LTB₄-d4 were used to draw calibration curves, and data were processed using the Xcalibur 2.0 software (Thermo Fischer Scientific). PAF, LTB₄ and LTC₄ values less than 4pg should be considered under the lower quantification limit (represented by a dashed line in Fig.4).

Lung histology

3µm sections of paraffin-embedded mouse lungs or human lung biopsies were stained for FcγRIIA using rabbit antiserum against the intracellular portion of human FcγRIIA and Histofine (simple stain mouse MAX PO, Nichirei Bioscience Inc.) followed by revelation with AEC (Sigma), and counterstained with Hematoxylin (Merck).

Statistical analyses

Data was analyzed using one-way ANOVA with Bonferroni post-test (Fig. 2A,C-D, and 2E-F lower panels, Fig. 3, 4, 6H, 7H-I), two-way ANOVA with Bonferroni post-test (Fig. 1G, 2E and F upper panels, Fig. 5A-C, 6E-F, 7K-L, S3A-B), Mantel Cox test for all Survival curves, or Student's t-test (all other data).

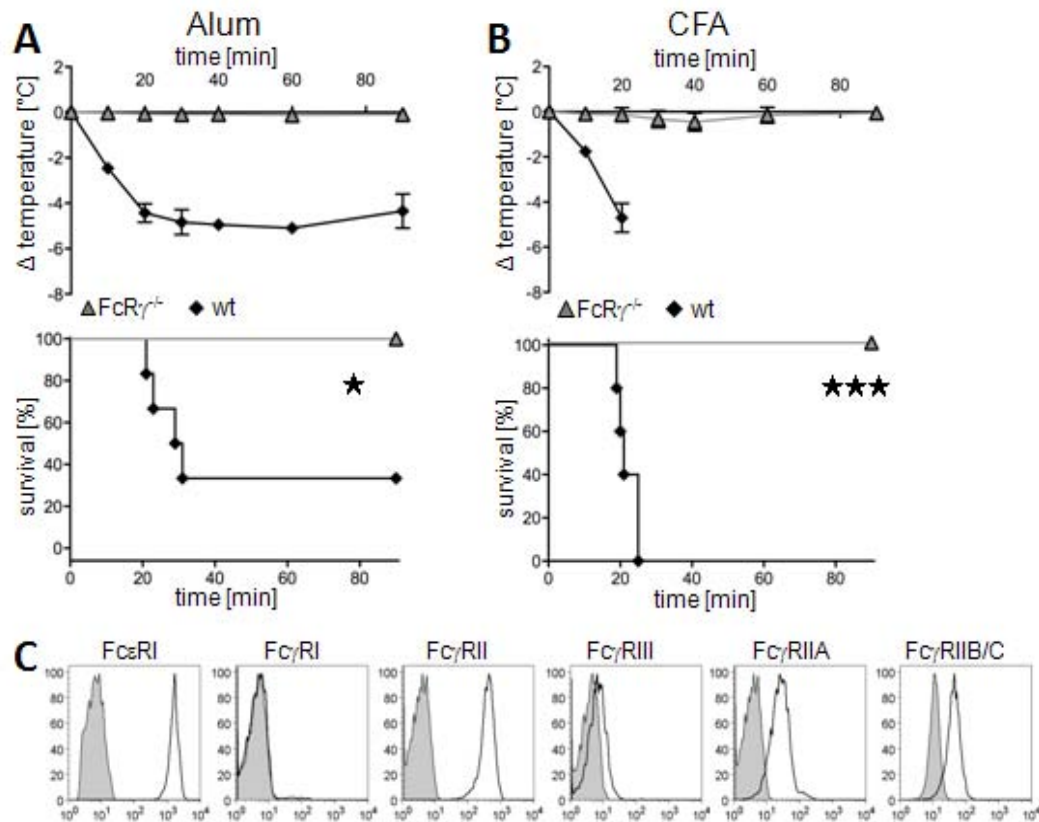


Figure S1

(A–B) Indicated mice were immunized with BSA in alum (A) or in Freund's adjuvant (B) and challenged with BSA. Central body temperatures and survival rates were monitored. ASA in wt (A, n=6; B, n=5) and FcR $\gamma^{-/-}$ mice (A, n=7; B, n=7). (C) Representative histogram plots of human FcR expression on human blood basophils (gated on Fc ϵ RI⁺/CD203c⁺ cells).

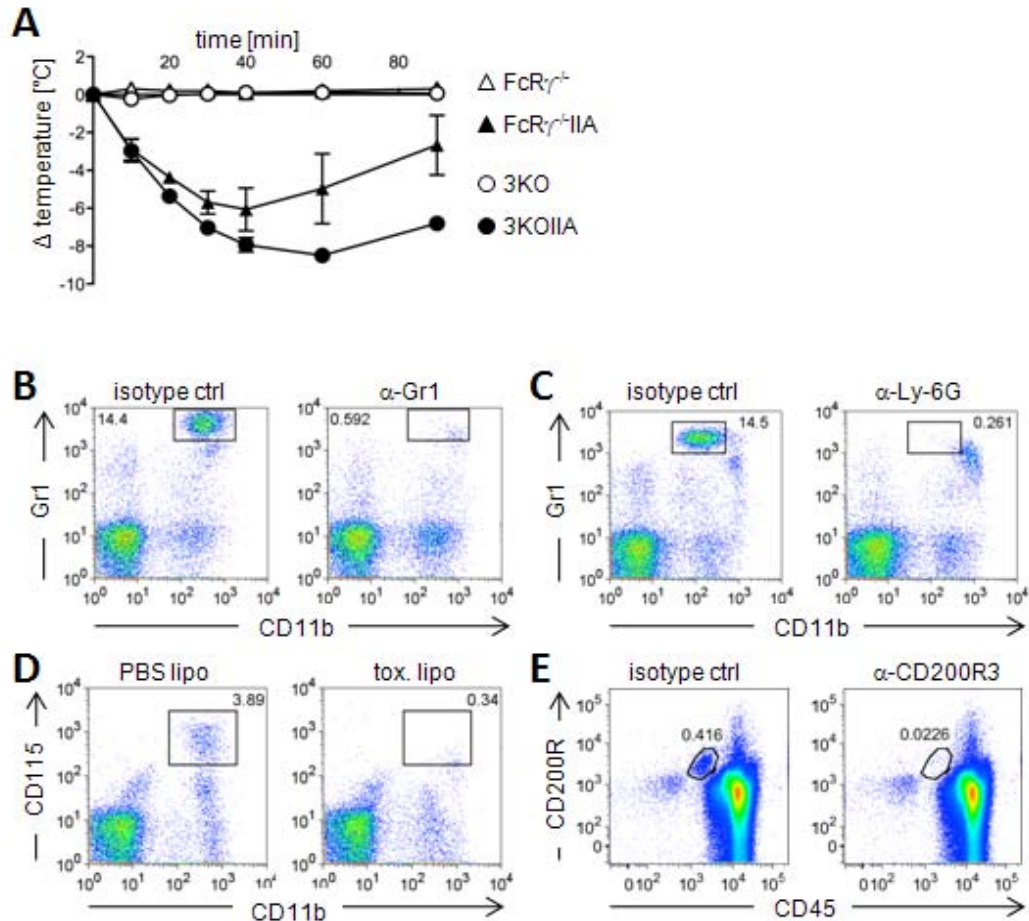


Figure S2

(A) Indicated mice were injected with preformed polyclonal IgG-IC (mouse anti-GPI antiserum plus GPI), and central temperatures were monitored (n=3). (B–E) Representative density plots of mouse blood stained with indicated antibodies upon cell depletion as used in experiments represented in Fig. 3. (B,C) Depletion of mouse neutrophils following injection of anti-Gr1 (B) or anti-Ly6G (C) mAbs. (D) Depletion of monocytes by injection of toxic liposomes. PBS liposomes were used as controls. (E) Depletion of basophils following anti-CD200R3 injection. (B,C,E; left panels) Matched isotype controls are shown for each specific mAb injection. (A) Data are represented as mean $\pm$ SEM. (A–E) Data are representative from at least two independent experiments.

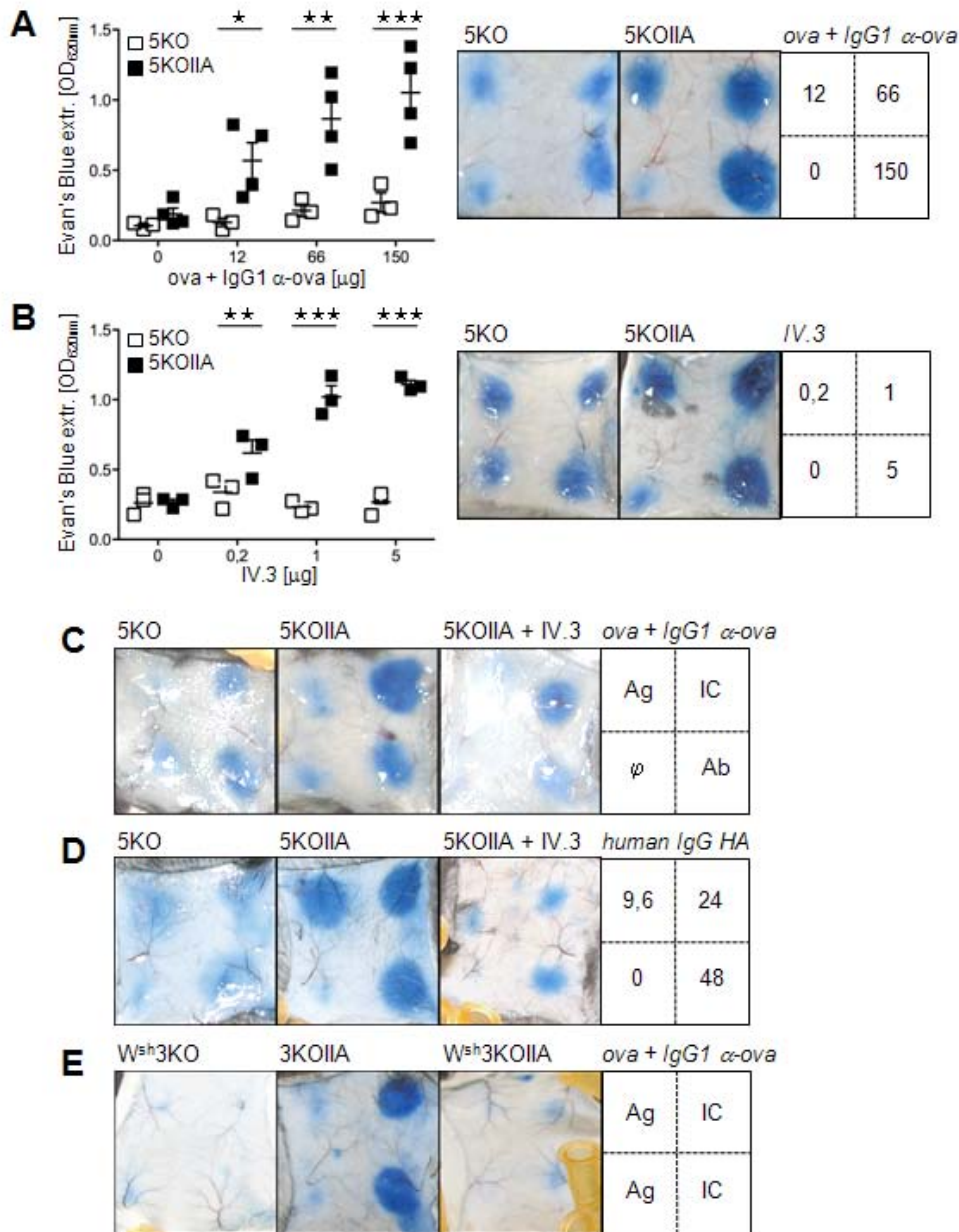


Figure S3

(A–E) Mice were injected intradermally with indicated reagents and i.v. with Evan's blue. (A,B) PCA in 5KO (open symbols: A, n=3; B, n=3), 5KOIIA (black symbols: A, n=4, B n=3). Quantification of Evan's blue extracted from skin tissue are represented (left panel). Data are represented as single measured points, and mean $\pm$ SEM and are representative of at least 2 independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001. Representative photographs of the four injections sites on the skin (middle panel) and the corresponding doses of reagents (right panel) are indicated. (C–E) Representative photographs of the four injections sites on the skin (left panel) and the corresponding doses of reagents (right panel) corresponding to the PCA reactions from Fig. 5A (C), Fig. 5B (D) and Fig. 5C (E).