IgG subclasses determine pathways of anaphylaxis in mice

Héloïse Beutier, Caitlin M. Gillis, Bruno Iannascoli, Ophélie Godon, Patrick England, Riccardo Sibilano, Laurent L. Reber, Stephen J. Galli, Mark S. Cragg, Nico van Rooijen, et al.

IgG subclasses determine pathways of anaphylaxis in mice

Héloïse Beutier, PharmD[1,2,3], Caitlin M. Gillis, B.Sci. [1,2,3], Bruno Iannascoli, [no degree][1,2], Ophélie Godon, M.Sc.[1,2], Patrick England, PhD[4], Riccardo Sibilano, PhD[5,6], Laurent L. Reber, PhD[1,2], Stephen J. Galli, MD[5,6], Mark S. Cragg, PhD[7], Nico Van Rooijen, PhD[8], David A. Mancardi, PhD[1,2], Pierre Bruhns, PhD[1,2,*] and Friederike Jönsson, PhD[1,2,*]

Authors' affiliations

[1]Institut Pasteur, Department of Immunology, Unit of Antibodies in Therapy and Pathology, Paris, France;

[2]INSERM, U1222, Paris, France;

[3]Université Pierre et Marie Curie, Paris, France;

[4]Institut Pasteur, Plate-Forme de Biophysique Moléculaire, Centre d'Innovation et Recherche Technologique (CiTech), CNRS-UMR3528, Paris, France;

[5]Department of Pathology, Stanford University School of Medicine, Stanford, CA, USA.

[6]Sean N. Parker Center for Allergy Research, Stanford University School of Medicine, Stanford, CA, USA.

[7]Antibody and Vaccine Group, Cancer Sciences Unit, University of Southampton Faculty of Medicine, Southampton General Hospital, Southampton, UK

[8]Department of Molecular Cell Biology, VU Medical Center, Amsterdam, The Netherlands;

[*]co-senior authorship.

Sources of funding: none of the sources of funding have an interest in the subject matter or materials discussed in the submitted manuscript

Correspondence to: Friederike Jönsson and Pierre Bruhns, Unit of Antibodies in Therapy and Pathology, Department of Immunology, Institut Pasteur, 25 rue du Docteur Roux, 75015 Paris, France. Phone: +33144389144 or +33145688629. E-mail: joensson@pasteur.fr; bruhns@pasteur.fr

ABSTRACT

Background: Animal models have demonstrated that allergen-specific IgG confers sensitivity to systemic anaphylaxis that relies on IgG receptors (FcγRs). Mouse IgG2a and IgG2b bind activating FcγRI, FcγRIII and FcγRIV, and inhibitory FcγRIIB; mouse IgG1 binds only FcγRIII and FcγRIIB. Although these interactions are of strikingly different affinities, these three IgG subclasses have been shown to enable induction of systemic anaphylaxis.

Objective: Determine which pathways control the induction of IgG1-, IgG2a- and IgG2b-passive systemic anaphylaxis.

Methods: Mice were sensitized with IgG1, IgG2a or IgG2b anti-TNP mAbs and challenged with TNP-BSA intravenously to induce systemic anaphylaxis that was monitored using rectal temperature. Anaphylaxis was evaluated in mice deficient for FcγRs, injected with mediator antagonists or in which basophils, monocyte/macrophages or neutrophils had been depleted. The expression of FcγRs was evaluated on these cells before and after anaphylaxis.

Results: Activating FcγRIII is the receptor primarily responsible for all three models of anaphylaxis, and subsequent down regulation of this receptor was observed. These models differentially relied on histamine release and on the contribution of mast cells, basophils, macrophages and neutrophils. Strikingly, basophil contribution and histamine predominance in IgG1- and IgG2b-mediated anaphylaxis correlated with the ability of inhibitory FcγRIIB to negatively regulate these models of anaphylaxis.

Conclusion: We propose that the differential expression of inhibitory FcγRIIB on myeloid cells and its differential binding of IgG subclasses controls the contributions of mast cells, basophils, neutrophils and macrophages to IgG subclass-dependent anaphylaxis. Collectively, our results unravel novel complexities in the involvement and regulation of cell populations in IgG-mediated reactions *in vivo*.

CLINICAL IMPLICATIONS

Anaphylactic pathways induced by different IgG subclasses in mice vary in terms of contributions by different cell types, mediators and antibody receptors. These results may help in the design of efforts to understand and treat IgG-mediated anaphylaxis in humans, e.g., as seen following intravenous IgG or administration of therapeutic IgG antibodies.

CAPSULE SUMMARY

Antibodies of the IgG class can contribute to anaphylaxis. This report reveals pathways induced by each IgG subclass in experimental anaphylaxis, demonstrating varying contributions of cells, mediators and antibody receptors.

KEY WORDS

Anaphylaxis; IgG; mouse model; basophil; neutrophil; monocyte; macrophage; FcγR; Platelet-activating Factor; Histamine.

ABBREVIATIONS USED

FcγR: IgG Fc receptor

PAF: Platelet-activating factor

K_A: Affinity constant

WT: C57Bl/6 Wild-type

PSA: Passive systemic anaphylaxis

TNP: Trinitrophenyl

BSA: Bovine serum albumin

mAb: Monoclonal antibody

PBS: Phosphate Buffered Saline

Gfi1: Growth Factor Independence-1

GeoMean: Geometric Mean

SEM: Standard error of the mean

INTRODUCTION

Anaphylaxis is a hyperacute allergic reaction that occurs with increasing incidence in the population and can be of fatal consequence. Symptoms include skin rashes, hypotension, hypothermia, abdominal pain, bronchospasm and heart and lung failure that may lead to asphyxia and sometimes death[1]. The main treatment remains epinephrine (adrenaline) injection to restore heart and lung function. Since anaphylaxis represents an emergency situation, few clinical studies have been possible to address the mechanisms leading to anaphylaxis in patients. Experimental models of anaphylaxis identified mechanisms involving allergen-specific antibodies that trigger activating antibody receptors on myeloid cells, leading to the release of mediators. These mediators can, by themselves, recapitulate the symptoms of anaphylaxis as observed in humans[2, 3].

The "classical" mechanism of anaphylaxis states that allergen-specific IgE binds the activating IgE receptor FcεRI on mast cells, which upon allergen encounter become activated and release histamine, among other mediators. Notably, histamine injection suffices to induce the signs of anaphylaxis in animal models[4]. In many cases, detectable allergen-specific IgE and elevated histamine levels do not accompany anaphylaxis in humans (discussed in [5]), leading to the notion that "atypical" or "alternate" mechanisms of induction could explain these cases. One of these atypical/alternate models proposes a similar cascade of events, but instead based on allergen-specific IgG binding to allergen, forming IgG-allergen immune complexes that trigger activating IgG receptors (FcγRs) expressed on myeloid cells (*i.e.* macrophages, basophils and/or neutrophils), which in turn release Platelet-Activating Factor (PAF)[2,3]. Importantly, PAF injection suffices to induce the signs of anaphylaxis in animal models [6]. IgG-induced anaphylaxis

120 can be elicited by intravenous injection of allergen-specific IgG followed by allergen
121 administration, and is termed IgG-induced passive systemic anaphylaxis (PSA).

122 IgG receptors in the mouse comprise four "classical" IgG receptors termed FcγRs, but
123 also the neonatal IgG receptor (FcRn) and the intracellular FcR tripartite motif-containing protein
124 21 (TRIM21)[7, 8]. Whereas FcRn and TRIM21 both participate in the intracellular routing of IgG,
125 and FcRn in protection from catabolism and distribution to tissues[9], FcγRs control cell activation
126 in the presence of immune complexes. FcγRs in mice are subdivided into i) activating FcγRs, *i.e.*
127 FcγRI, FcγRIII and FcγRIV, that lead to cell activation upon immune complex binding, and ii) an
128 inhibitory FcγR, *i.e.* FcγRIIB, that inhibits cell activation when co-engaged by an immune
129 complex with an activating FcγR co-expressed on the same cell[10]. Inhibition of cell activation by
130 FcγRIIB thus requires that the immune complex contains IgG that are bound both by the
131 activating and by the inhibitory FcγR.

132 Four IgG subclasses exist in mice, IgG1, IgG2a, IgG2b and IgG3. Among those, only
133 IgG2a and IgG2b bind to all FcγRs, whereas IgG1 binds only to FcγRIIB and FcγRIII. It remains
134 under debate whether IgG3 binds to FcγRs, particularly FcγRI[11, 12]. The affinities of these FcγRs
135 towards IgG subclasses are strikingly different (Table 1) leading to the notion of "high-affinity"
136 receptors that retain monomeric IgG and "low-affinity" receptors that do not[8]. The avidity of
137 IgG-immune complexes, however, enables both types of receptors to retain IgG-immune
138 complexes, leading to receptor clustering, intracellular signaling events and, eventually, to cell
139 activation. FcγRI is a high-affinity receptor for IgG2a[13], and FcγRIV is a high-affinity receptor
140 for IgG2a and IgG2b[14]. All other FcγR-IgG interactions are of low affinity (reviewed in [7]).

141 Three out of the four IgG subclasses in the mouse, *i.e.* IgG1, IgG2a and IgG2b, have been
142 reported to enable the induction of systemic anaphylaxis, inducing mild to severe hypothermia[5,
143 15, 16]. This is rather surprising for IgG1, considering that inhibitory FcγRIIB binds IgG1 with a

144 10-fold higher affinity (K_A=3.3x10^6 M^{-1}) than activating FcγRIII (K_A=3.1x10^5 M^{-1})[17] (Table 1),
145 implying that inhibition should dominate over activation. WT mice, indeed, develop a very mild
146 anaphylactic reaction during IgG1-PSA compared to FcγRIIB$^{-/-}$ mice[18], indicating that inhibition
147 by FcγRIIB occurs in WT mice during IgG1-PSA, reducing, but not protecting from,
148 anaphylaxis. IgG1-PSA has been reported to rely on basophils[19] that co-express FcγRIIB and
149 FcγRIII[20]. In this apparently simple situation, only one activating receptor and one inhibitory
150 receptor are engaged on a single cell type that, once activated, produces an anaphylactogenic
151 mediator, like PAF[19].

152 IgG2a and IgG2b, however, bind three activating FcγRs and inhibitory FcγRIIB with
153 different affinities ranging over 2 logs. In particular, the affinity of FcγRIIB for IgG2a is
154 significantly lower than for IgG2b, whereas activating IgG receptors FcγRIII and FcγRIV bind
155 IgG2a and IgG2b with similar affinities, respectively (Table 1). Notably, FcγRIV is not expressed
156 on basophils, but on monocytes/macrophages and neutrophils[21] that have both been reported to
157 contribute to experimental anaphylaxis[16, 22-24]. In addition, mice expressing only FcγRIV can
158 develop IgG-PSA[16]. Together with expression and binding data, one would therefore hypothesize
159 that FcγRIV contributes predominantly to IgG2a- and IgG2b-PSA. In this work, we present
160 evidence contrary to this hypothesis, and reveal which activating FcγR on which cell type(s)
161 releasing which mediator(s) are responsible for IgG2a-PSA and IgG2b-PSA, and the differential
162 regulation of these models of anaphylaxis by FcγRIIB. Our results unravel a complex balance
163 determined by FcγR expression patterns, inhibition potential by FcγRIIB and respective affinities
164 of activating and inhibitory FcγRs for IgG subclasses that, altogether, regulate the contribution of
165 cells and anaphylactogenic mediators to a given model of IgG-induced anaphylaxis.
166

METHODS

Mice. Female C57Bl/6J mice (herein referred to as "WT") were purchased from Charles River, female Balb/cJRj mice from Janvier Labs, FcγRIIB$^{-/-}$ (MGI:1857166), FcγRIII$^{-/-}$ mice (MGI: 3620982) and Rosa26-YFP mice from Jackson Laboratories. FcγRI$^{-/-}$ mice (MGI: 3664782) were provided by J. Leusen (University Medical Center, Utrecht, The Netherlands), FcγRIV$^{-/-}$ mice (MGI: 5428684) by J.V. Ravetch (The Rockefeller University, New York, NY, USA), Gfi1$^{-/-}$ mice by T. Moroy (Montreal University, Montreal, QC, Canada) and MRP8-cre mice by Clifford Lowell (University of California at San Francisco, CA, USA). MRP8-cre and Rosa26-YFP mice were intercrossed to generate MRP8-cre; Rosa26-YFP mice. Cpa3-Cre; Mcl-1$^{fl/fl}$ mice[25] (backcrossed for at least 9 generations on a C57Bl/6J background) were kept in the Stanford University animal facility. All mouse protocols were approved by the Animal Ethics committee CETEA (Institut Pasteur, Paris, France) registered under #C2EA-89, and the Institutional Animal Care and Use Committee of Stanford University.

Antibodies and reagents. PBS- and clodronate-liposomes were prepared as previously described[26]. $TNP_{(21-31)}$-BSA was obtained from Santa Cruz, ABT-491 from Sigma-Aldrich; cetirizine DiHCl from Selleck Chemicals; anti-mouse FcγRIII (275003) from R&D Systems; rat IgG2b isotype control (LTF-2) from Bio X Cell. Purified anti-CD200R3 (Ba103) was provided by H. Karasuyama (Tokyo Medical and Dental University Graduate School, Tokyo, Japan). The hybridoma producing mAbs anti-mouse FcγRIV (9E9) was provided by J.V. Ravetch (Rockefeller University, New York, New York, USA), anti-Ly6G (NIMP-R14) by C. Leclerc (Institut Pasteur, Paris, France), IgG1 anti-TNP (TIB-191) by D. Voehringer (Universitätsklinikum, Erlangen, Germany), IgG2a anti-TNP (Hy1.2) by Shozo Izui (University of Geneva, Geneva, Switzerland) and IgG2b anti-TNP (GORK) by B. Heyman (Uppsala

191 Universitet, Uppsala, Sweden): corresponding antibodies were purified as described[16]. Purified
192 mouse IgE anti-TNP was purchased from BD Pharmingen. MAb 9E9 was coupled to FITC using
193 the Pierce™ FITC Antibody labeling kit (Life Technologies). The antibodies used for flow
194 cytometry staining of c-Kit (clone 2B8), CD49b (clone DX5), IgE (clone R35-72), CD11b (clone
195 M1/70), F4/80 (clone 6F12), CD115 (clone T38-320), Ly6G (clone 1A8) and Ly6C (clone AL-
196 21) were purchased from BD Pharmingen; CD45 (clone 30F11) and Gr1 (clone RB6-8C5) were
197 purchased from Miltenyi Biotec. FcγRIIB was detected using FITC-coupled mAb AT130-2
198 mIgG1 N297A[27].
199
200 ***Passive Systemic Anaphylaxis***. *IgG-induced PSA:* IgG1, IgG2a or IgG2b anti-TNP antibodies
201 were administered intravenously at a dose of 500 μg, if not otherwise indicated, in 200 μL
202 physiological saline, followed by an intravenous challenge with 200 μg of the antigen (TNP-
203 BSA) in physiological saline 16 hours later. *IgE-induced PSA*: IgE anti-TNP antibodies were
204 administered intravenously at a dose of 50 μg in 200 μL physiological saline followed by an
205 intravenous challenge with 500 μg of TNP-BSA in physiological saline 24 hours later. The body
206 temperature of mice was monitored using a digital thermometer with rectal probe (YSI).
207
208 ***In vivo blocking and cellular depletion***. 300 μg/mouse of PBS- or clodronate-liposomes, 300
209 μg/mouse of rat IgG2b isotype control or anti-Ly6G, and 30 μg/mouse of anti-CD200R3 mAbs
210 were injected i.v. 24 hours before challenge. Specificity of cell depletion was evaluated using
211 flow cytometry on blood, bone marrow, spleen and peritoneum taken from naïve WT mice 24
212 hours after injection of the depleting antibody or clodronate-liposomes (Examples are shown in
213 Supplemental Figures 1 & 2). 25 μg/mouse of ABT-491 or 300 μg/mouse of cetirizine were

injected intravenously 20 minutes or intraperitoneally 30 minutes before challenge, respectively. 200 µg/mouse of anti-FcγRIV mAb were injected intravenously 30 minutes before challenge.

Flow cytometry analysis. Freshly isolated cells were stained with indicated fluorescently labeled mAbs for 30 minutes at 4°C. Cell populations were defined as follows: neutrophils ($CD45^+/CD11b^+/Ly6G^{hi}/Ly6C^{int}$), monocytes ($CD45^+/CD11b^+/Ly6G^{lo}/Ly6C^{lo\ or\ hi}$), basophils ($CD45^{int}/DX5^+/IgE^+$); spleen macrophages ($CD45^+/CD11b^+/Gr\text{-}1^{lo}/CD115^+/F4/80^{hi}$); peritoneal macrophages ($CD45^+/CD11b^+/F4/80^+$); peritoneal mast cells ($CD45^+/c\text{-}Kit^+/IgE^+$). Expression of FcγR on indicated cell population is represented as Δ Geomean between specific and isotype control staining. *NB*: In Figure 5: 1 or 0.5 mg IgG2b was injected to assess expression on neutrophils/monocytes or basophils, respectively.

Surface plasmon resonance analysis. Experiments were performed at 25°C using a ProteOn XPR36 real-time SPR biosensor (BioRad). Anti-TNP antibodies were immobilized covalently through amine coupling on the surface of a GLC chip. TNP-BSA was then injected on the chip at a flow rate of 25 µl.min-1, with contact and dissociation time of 8 minutes each. Binding responses were recorded in real time as resonance units (RU; 1 RU ≈ 1 pg/mm^2). Background signals were subtracted, and binding rates (k_{on} and k_{off}) and equilibrium constants (*Kd)* were determined using the Biaevaluation software (GE Healthcare).

ELISAs. After the induction of IgG1-, IgG2a-, IgG2b- or IgE-induced PSA, plasma and serum were collected at 5 minutes and 3 hours later to determine the histamine and mMCP-1 content, respectively. Histamine and mMCP-1 concentration were determined using commercially available ELISA kits (Beckman Coulter; eBioscience) following the manufacturer's instructions.

Relative binding affinity of IgG1, IgG2a and IgG2b anti-TNP antibodies to TNP-BSA was determined by ELISA. Briefly, TNP-BSA-coated plates were incubated with dilutions of IgG1, IgG2a or IgG2b anti-TNP antibodies. After washing, bound anti-TNP IgG were revealed using the same HRP-coupled anti-mouse IgG and SIGMAFAST OPD solution.

Mast cell histology. Mouse back skin biopsies were collected 24 hours after the induction of specific cell depletion and mouse ear skin biopsies were collected 30 minutes after IgE, IgG1, IgG2a or IgG2b-induced PSA, and embedded in paraffin prior to sectioning. Mast cells in toluidine blue-stained biopsies were counted visually in at least 15 FOV/mouse and > 6 mice per treatment (Supplemental Figure 1I).

Statistics. Data were analyzed using one-way or two-way ANOVA with Tukey's post-test. A *p*-value less than .05 was considered significant: (*$p < .05$; **p < .01; ***$p < .001$; ****$p < .0001$). If not stated otherwise, data are represented as mean +/- SEM.

RESULTS

FcγRIII dominates anaphylaxis induced by IgG subclasses

Passive systemic anaphylaxis was induced by an intravenous injection of one of the different anti-TNP IgG isotypes (IgG1, IgG2a, IgG2b) followed by an intravenous challenge with TNP-BSA 16 h later. This protocol induces a transient decrease in body temperature that is most pronounced between 30 and 40 minutes. As reported previously[3, 16, 19, 22, 28], all three IgG isotypes were capable of inducing anaphylaxis in WT mice (Figure 1A-C). In these experimental conditions IgG1-PSA triggered a maximum temperature loss of ≈2°C, IgG2a-PSA of ≈4°C and IgG2b-PSA of ≈3°C in WT mice. Using single FcγR-knockout mice we evaluated the contribution of each of the four mouse FcγRs to these anaphylaxis models. The absence of either FcγRIV (with the exception of a single time point in IgG2b-PSA) or FcγRI had no significant impact on IgG-PSA-induced hypothermia, regardless of the subclass of IgG antibodies used to induce anaphylaxis (Figure 1A-C). The lack of FcγRIII, however, protected mice from anaphylaxis in all models. Mice lacking the inhibitory receptor FcγRIIB had a significantly more severe temperature drop than WT mice in both IgG1- and IgG2b-PSA, but showed no significant difference in the severity of IgG2a-PSA (Figure 1A-C). Even though the three anti-TNP IgG mAbs used are not switch variants of a unique anti-TNP antibody, they show comparable binding to TNP-BSA by ELISA, similar affinity (nanomolar range) and dissociation rates (k_{off}) by surface plasmon resonance analysis, particularly the IgG2a and IgG2b anti-TNP antibodies (Supplemental Figures 3A, B & C). Of note, untreated FcγR-deficient mice presented modest variations in FcγR expression levels (Supplemental Figure 5) and leukocyte representation among blood cells compared to WT mice (Supplemental Figure 6). In particular, a mild lymphopenia in FcγRIV$^{-/-}$ mice and in FcγRIIB$^{-/-}$ mice (the latter also have a tendency to express higher levels of

278 FcγRIII and FcγRIV); and a mild eosinophilia in FcγRIII$^{-/-}$ mice, that also express significantly
279 more FcγRIIB on neutrophils and Ly6C^{hi} monocytes. Together, we think that these variations do
280 not explain the drastic phenotypes observed for PSA in FcγRIIB$^{-/-}$ and FcγRIII$^{-/-}$ mice compared
281 to WT mice. Thus, these data demonstrate that FcγRIII predominates in the induction of IgG1-,
282 IgG2a- and IgG2b-PSA, and that FcγRIIB specifically dampens anaphylaxis severity in IgG1-
283 and IgG2b-PSA.
284
285 **Basophils, mast cells, monocytes/macrophages and neutrophils contribute differentially to**
286 **IgG isotype-dependent anaphylaxis models**
287 FcγRIII is expressed by all myeloid cells[7, 20] and to a lesser extent by NK cells[29]. One may
288 therefore anticipate that IgG immune complexes formed *in vivo* as a consequence of TNP-BSA
289 injection in anti-TNP sensitized mice would therefore engage FcγRIII on these cells, leading to
290 cell activation and possibly contributing to anaphylaxis. Basophils, mast cells, neutrophils and
291 monocyte/macrophages have indeed been reported to contribute to IgG-PSA[16, 19, 22, 15], however
292 the respective contribution of each of these different cell types remains debated[2, 28]. To
293 investigate which cell types contribute to PSA induced by different IgG subclasses, we depleted
294 basophils (anti-CD200R3 mAb), monocytes/macrophages (clodronate-filled liposomes) or
295 neutrophils (anti-Ly6G) prior to anaphylaxis induction or evaluated anaphylaxis induction in
296 transgenic mice deficient in certain cell populations.
297 Of note, the relatively mild temperature loss in IgG1-PSA in WT mice (Supplemental
298 Figure 4A), did not allow us to address reliably the contribution of either basophils or neutrophils
299 to this model of anaphylaxis. We therefore restricted our analysis of the contribution of myeloid
300 cell populations to IgG2a-PSA and IgG2b-PSA. Antibody-induced basophil depletion or
301 genetically-induced mast cell and basophil deficiency (Supplemental Figure 2H, Cpa3-Cre; Mcl-

302 $1^{fl/fl}$ mice[25]), did not affect IgG2a–PSA (Figure 2A&B), but significantly inhibited IgG2b-PSA
303 (Figure 2F&G). Monocyte/macrophage depletion (Figure 2C&H) significantly inhibited both
304 IgG2a- and IgG2b-PSA. The absence of neutrophils, either following antibody-mediated
305 depletion (Figure 2D&I) or using neutropenic $Gfi1^{-/-}$ mice[30] (Figure 2E&J), significantly
306 inhibited both IgG2a- and IgG2b-PSA. Whereas monocytes/macrophages and neutrophils appear
307 to contribute to both models of anaphylaxis, basophils and possibly mast cells therefore
308 contribute specifically to IgG2b-PSA, but not to IgG2a-PSA.
309
310 **FcγRIII is down-regulated specifically on neutrophils following IgG2a PSA**
311 Khodoun *et al* proposed to use the reduced expression level of FcγRIII on mouse
312 neutrophils as a marker to distinguish IgE- from IgG1-induced PSA, both of which required
313 priming with an antigen-specific IgG1 and challenge with that antigen[31]. We therefore wondered
314 if FcγRIII expression on neutrophils might also be a marker for IgG2a- and IgG2b-PSA. In
315 addition, reduced expression of FcγR(s) following IgG-PSA may document that a particular cell
316 population is activated following engagement of its FcγR(s) by IgG-immune complexes during
317 anaphylaxis. This parameter may thus be used to discriminate cell populations contributing to
318 anaphylaxis following direct activation by IgG-immune complexes from those contributing
319 following activation by mediators liberated by IgG-immune complex-activated cells (*e.g.*
320 histamine, PAF, leukotrienes and prostaglandins).
321 Among mouse IgG receptors, only FcγRIIB, FcγRIII and FcγRIV are significantly
322 expressed on circulating myeloid cells, but not FcγRI[7, 32, 33]. Of circulating monocyte populations,
323 "classical" $Ly6C^{hi}$ monocytes are $Fc\gamma RIIB^{med}$, $Fc\gamma RIII^{med}$ $Fc\gamma RIV^{-}$, whereas "non-classical"
324 $Ly6C^{lo}$ monocytes are $Fc\gamma RIIB^{lo}$, $Fc\gamma RIII^{lo}$ $Fc\gamma RIV^{hi}$ [34]. We therefore determined the expression
325 of FcγRIIB, FcγRIII and FcγRIV before and after IgG2a-PSA induction on neutrophils and

monocyte subsets. The expression of FcγRIII was down regulated on neutrophils, but not on Ly6C^{hi} monocytes, during IgG2a-PSA (Figure 3A&D). The expression of FcγRIV was also down regulated on neutrophils, but not on Ly6C^{lo} monocytes, during IgG2a-PSA (Figure 3B&D). This was unexpected considering that FcγRIV does not significantly contribute to this PSA model (Figure 1B). The expression of FcγRIIB, however, remained unchanged on Ly6C^{hi} and Ly6C^{lo} monocytes and neutrophils (Figure 3C&D), in agreement with the lack of contribution of this receptor to IgG2a-PSA (Figure 1B). Together these data suggest that neutrophils may directly be activated through FcγRIII by immune complexes formed during IgG2a-PSA. They also suggest that neutrophils, but not Ly6C^{lo} monocytes, may be similarly activated through FcγRIV, even if no contribution of this receptor was identified in this model using FcγRIV$^{-/-}$ mice (Figure 1B).

Elevated IgG2 antibody doses reveal FcγRIV contribution to IgG2a-PSA and IgG2b-PSA

In mice, FcγRIV binds monomeric IgG2a and IgG2b. At physiological concentrations of IgG2a (≈ 2.5 mg/mL) and IgG2b (≈ 1.5 mg/mL) in the serum, FcγRIV may therefore be occupied *in vivo*, particularly on circulating neutrophils and monocytes. Nevertheless, the short binding half-lives of monomeric IgG2a ($t_{1/2}$ ≈ 3 min) and monomeric IgG2b ($t_{1/2}$ ≈ 10 min) by FcγRIV, and their ability to be displaced from this receptor by immune complexes,[14] may enable IgG2-immune complexes to interact with FcγRIV during anaphylaxis and therefore contribute to its induction and/or severity.

To explore this possibility, we primed FcγRIII$^{-/-}$ mice with various doses of anti-TNP IgG2a before challenge with TNP-BSA, in order to induce a range of *in vivo* concentrations of immune complexes. As expected, the low doses did not trigger FcγRIII$^{-/-}$ mice to develop anaphylaxis after challenge. Elevated doses (1 or 2 mg), however, enabled significant temperature drops in FcγRIII$^{-/-}$ mice, comparable to those observed in WT mice primed with 500

μg IgG2, particularly at the highest dose of IgG2a (2 mg) (Figure 4A). Already at a dose of 1mg of IgG2, FcγRIII$^{-/-}$ mice developed mild hypothermia in IgG2a-PSA but not in IgG2b-PSA (Figure 4B&C). Unexpectedly in the same conditions, FcγRIV contributed to IgG2b-PSA that was not anymore dampened by inhibitory FcγRIIB (Figure 4C). At a dose of 2 mg of IgG, FcγRIII$^{-/-}$ mice developed hypothermia in both IgG2a-PSA and IgG2b-PSA that was abolished when FcγRIII$^{-/-}$ mice were pre-treated with a blocking antibody against FcγRIV (Figure 4D&E). FcγRI did not contribute to either model of IgG2-PSA at an elevated dose (Figure 4B&C). Furthermore, the expression of FcγRIII was down regulated on neutrophils and basophils, but not on Ly6C^{hi} monocytes, following IgG2b-PSA (Figure 5A&D). The expression of FcγRIV was also down regulated on neutrophils, but not on Ly6C^{lo} monocytes (Figure 5B&D). The expression of FcγRIIB, however, did not change on either neutrophils or Ly6C^{hi} and Ly6C^{lo} monocytes even though this inhibitory receptor regulates IgG2b-PSA (Figures 1C and 5C&D). This observation is in agreement with the report by Khodoun et al, reporting that FcγRIIB expression did not change on neutrophils following IgG1-PSA[31]. Altogether high doses of antigen-specific IgG2 reveal the contribution of FcγRIV to IgG2a-PSA and to IgG2b-PSA, and suggest the direct activation of neutrophils and basophils by IgG2b-immune complexes.

IgG1 PSA in the absence of inhibitory FcγRIIB

The unexpected differences observed between IgG2a- and IgG2b-PSA induction pathways prompted us to find a mouse model more sensitive to IgG1-PSA than WT mice, to be able to evaluate the contribution of cell types and mediators also in this model. Indeed, as mentioned earlier, WT mice respond poorly to IgG1-PSA (Figure 1A; Supplemental Figure 4A)[18]. FcγRIIB$^{-/-}$ mice, however, develop a temperature drop of ≈4°C during IgG1-PSA, comparable to temperature losses observed in WT mice during IgG2a- or IgG2b-PSA (Figure

374 1B&C). We therefore analyzed the contribution of cell types to IgG1-PSA in FcγRIIB$^{-/-}$ mice.
375 Basophil depletion mildly - but significantly - inhibited IgG1-PSA (Figure 6A), in agreement
376 with previous data[19]. The depletion of neutrophils had the same effect, although not consistently
377 as strongly as basophil depletion (Figure 6B and data not shown). Monocyte/macrophage
378 depletion had only a tendency to ameliorate anaphylaxis that was reproducible but not significant
379 (Figure 6C). These results suggest that IgG1-PSA relies on basophils and neutrophils, and
380 possibly also on monocytes.
381
382 **PAF and histamine contribute differentially to IgG2a- and IgG2b-PSA**
383 Because cell types contribute differently to IgG2-PSA models (*i.e.* IgG2a-PSA,
384 neutrophils and monocytes; IgG2b-PSA, basophils, neutrophils and monocytes), one can expect
385 that the mediators responsible for clinical signs also may differ between them. Platelet activating
386 factor (PAF) has been shown to be responsible for anaphylactic reactions that required basophil[19],
387 neutrophil[16, 24] and/or monocyte/macrophage[22] activation, whereas histamine has been shown to
388 be responsible for mast cell- and basophil-dependent anaphylaxis[35, 36]. Neutrophils are the main
389 producers of PAF[37], whereas mast cells and basophils are the main producers of histamine[38, 39].
390 We therefore analyzed the relative contribution of these two mediators to the three models of
391 PSA using the histamine-receptor 1 antagonist cetirizine and the PAF-R antagonist ABT-491.
392 Surprisingly, histamine-receptor 1 antagonist cetirizine significantly inhibited IgG1-PSA whereas
393 PAF-R antagonist ABT-491 had no significant effect, in opposition with previous data[19]. The
394 combination of both antagonists had an additive effect, and almost abolished IgG1-PSA (Figure
395 7A). These results obtained in FcγRIIB$^{-/-}$ mice were confirmed in WT mice (Figure 7A). Whereas
396 cetirizine mildly reduced hypothermia in IgG2a-PSA, it significantly inhibited IgG2b-PSA. ABT-
397 491 mildly reduced hypothermia in IgG2a-PSA, but had no significant effect on IgG2b-PSA

(Figure 7B&C). The combination of cetirizine and ABT-491, however, almost abolished both IgG2a- and IgG2b-PSA. Elevated plasma histamine levels were detected 5 minutes post challenge in all three IgG-PSA models, and particularly high levels were observed in mice undergoing IgE-PSA (as a positive control) or undergoing IgG2a-PSA (Figures 7D&E). This latter finding is surprising as IgG2a-PSA is unaffected by the absence of both mast cells and basophils that are considered major sources of histamine. Mast cell protease-1 (mMCP-1), which is released upon activation of mucosal mast cells, could be detected in the serum of mice undergoing IgE-PSA, but not in those undergoing any one of the three models of IgG-PSA, 3 hours post-PSA induction (Figure 7F). Collectively these results suggest that histamine predominantly contributes to IgG1- and IgG2b-PSA, whereas histamine and PAF, together, are necessary for IgG2a-PSA.

DISCUSSION

Our work suggests that the activating IgG receptor FcγRIII predominantly contributes to IgG-dependent passive systemic anaphylaxis, whether induced by IgG1, IgG2a or IgG2b antibodies. A contribution of the activating IgG receptor FcγRIV was only identified when using very high amounts of IgG2 antibodies, whereas the activating IgG receptor FcγRI played no detectable role. Remarkably, the inhibitory IgG receptor FcγRIIB controlled the severity of IgG1- and IgG2b-, but not IgG2a-induced anaphylaxis. The ability of FcγRIIB to inhibit a given model of IgG-induced anaphylaxis correlated with the contribution of basophils and histamine to that model. Indeed, basophils, and possibly mast cells, contributed with neutrophils to IgG1-PSA, and with neutrophils and monocytes to IgG2b-PSA, but not to IgG2a-PSA that appeared to depend entirely on neutrophils and monocytes/macrophages. Altogether our data propose that the three IgG subclasses IgG1, IgG2a and IgG2b induce three qualitatively different pathways of anaphylaxis that are nevertheless triggered primarily by a single IgG receptor, FcγRIII.

FcγRIII is a low-affinity receptor for IgG1, IgG2a and IgG2b, whereas FcγRI is a high-affinity receptor for IgG2a, and FcγRIV is a high affinity receptor for IgG2a and IgG2b. One would therefore assume that FcγRIII predominates in IgG1-PSA, FcγRI and FcγRIV in IgG2a-PSA, and FcγRIV in IgG2b-PSA. However, our data from FcγRIII$^{-/-}$ mice indicate that this receptor predominates in all three models. Notably, we found an increased expression of FcγRIIB on neutrophils and Ly6C^{hi} monocytes in FcγRIII$^{-/-}$ mice, which could mask a potential contribution of FcγRIV in these conditions. In support of the notion that FcγRIII predominates IgG-PSA induction, an alternative model of PSA induced by sensitization and challenge with goat antibodies was found to be driven by FcγRIII[22] and blocking antibodies against FcγRIII were protective in a model of PSA induced by IgG immune complexes[16]. In addition, IgG2a-PSA

437 in FcγRIIB$^{-/-}$ mice was abolished following injection of anti-FcγRIIB/III blocking mAbs[5].
438 FcγRIII is the only activating IgG receptor in the mouse that does not bind an IgG subclass with
439 high affinity, thus it remains unoccupied by monomeric IgG and accessible for binding of
440 immune complexes. This is theoretically not the case for FcγRI and FcγRIV, which at
441 physiological serum concentrations of IgG2a (≈ 2.5 mg/mL) and IgG2b (≈ 1.5 mg/mL), are likely
442 occupied *in vivo*, particularly on circulating cells. Of note, C57Bl/6 mice produce IgG2c, but not
443 IgG2a antibodies, whose amino acid sequence varies by about 15%. Experiments performed in
444 Balb/c mice that express endogenous IgG2a (but no IgG2c) gave similar results regarding the
445 contribution of basophils, neutrophils and monocytes to IgG2a (Supplemental Figure 4B),
446 indicating that IgG2a and IgG2c sequence variations probably do not affect the mechanisms of
447 anaphylaxis induction that we describe herein.

448 Adult female mice of 20 g, as used in this study, possess a circulating blood volume of
449 1.4-1.5 mL. Injection of 500 µg antibody thus corresponds to ≈330 µg/mL of circulating
450 antibody, injection of 1 mg to ≈660 µg/mL, and injection of 2 mg to ≈1,3 mg/mL. In cases of
451 anaphylaxis the circulating concentration of allergen-specific IgG has not been evaluated due to
452 lack of testing and appropriate controls (*i.e.* monoclonal anti-allergen antibodies); although we
453 have reported high circulating antigen-specific IgG levels in an autoimmune model of arthritis[33].
454 It seems rather unlikely that patients suffering from anaphylaxis possess such elevated circulating
455 levels of IgG anti-allergen as in the mice receiving the high doses we used in this study.
456 Nevertheless, our results in high-dose IgG2a- and IgG2b-PSA demonstrate that FcγRIV can by
457 itself (*i.e.* in the absence of FcγRIII) trigger anaphylaxis. Similar results have been obtained in
458 mice expressing only FcγRIV: "FcγRIV-only" mice developed IgG2b-PSA after injection of pre-
459 formed IgG2b immune complexes and also upon injection of polyclonal anti-sera followed by a
460 challenge with the antigen[16]. We reported previously that IgG2b-PSA triggered by the injection

461 of preformed IgG2b-immune complexes in WT mice was abolished following injection of anti-
462 FcγRIV blocking mAb 9E9. This contrasts with the findings of the current study, in which we
463 show that FcγRIII is the major activating receptor in all models of IgG-PSA, and FcγRIV
464 contributes only at high antibody concentrations. Two hypotheses may explain these discrepant
465 results: i) the injection of preformed IgG2b-immune complexes leads to an immediate circulating
466 bolus of immune complexes, which are similarly formed only after injection of high amounts of
467 IgG2b and antigen, thus triggering FcγRIV; 2) as recently reported[40] mAb 9E9 may not only
468 block FcγRIV through its Fab portions, but also FcγRIII via its Fc portion once 9E9 is bound to
469 FcγRIV. In our view, it is likely that a combination of these mechanisms reconcile our previous
470 and herein described results, and suggest that IgG2b-PSA induced following injection of
471 preformed IgG2b-immune complexes relies rather on both FcγRIII and FcγRIV than on FcγRIV
472 alone as we reported previously[16]. Together this body of evidence supports the notion that
473 FcγRIV is capable of triggering cell activation leading to anaphylaxis, yet in restricted conditions,
474 *i.e.* in the absence/blockade of FcγRIII or in presence of large amounts of IgG2a and/or IgG2b
475 antibodies.
476
477 The differential contribution of FcγRs to IgG-PSA may rely on their respective expression
478 patterns on myeloid cells. Indeed, FcγRI is not[32, 33] or only barely[34] expressed on circulating
479 monocytes, and its expression is largely restricted to tissue-resident macrophages. The level of its
480 expression on cells reported to contribute to anaphylaxis (*i.e.* monocytes in this case) may
481 therefore not suffice to induce their activation. This notion is supported by the absence of any
482 detectable effect of FcγRI deficiency in IgG2-PSA that we report in this study, even at high doses
483 of IgG2 antibodies. FcγRIII, however, is expressed on all myeloid cells[7] and moreover at
484 comparably high levels on all those cell types that have been reported to contribute to

anaphylaxis; basophils, monocytes and neutrophils[20]. This pattern of cellular expression may explain its predominant contribution to all models of IgG-induced anaphylaxis. FcγRIV is expressed on neutrophils and Ly6C^{lo} monocytes. It remains unclear, however, if Ly6C^{lo}, Ly6C^{hi} or both monocyte subsets contribute to anaphylaxis. FcγRIV could contribute to PSA induction in exceptional conditions (FcγRIII deficiency or high IgG2 antibody doses). The lack of FcγRIV contribution in classical conditions of PSA may suggest that its expression level is not sufficient in WT mice. Notably, it has been reported previously that particular FcγR deficiencies modify the expression levels of other FcγRs. In particular FcγRIII$^{-/-}$ mice, but not FcγRI$^{-/-}$ mice, presented a significant increase in FcγRIV expression levels on neutrophils[16, 41, 42] and a tendency for increased expression on Ly6C^{lo} monocytes (Supplemental Figure 5B). This could explain why the contribution of FcγRIV to IgG2-PSA becomes apparent in FcγRIII$^{-/-}$ mice. FcγRIV$^{-/-}$ mice did not, conversely, present alterations of FcγRIII expression on neutrophils or Ly6C^{hi} monocytes compared to WT littermates (Supplemental Figure 5A). FcγRIIB$^{-/-}$ mice expressed significantly higher levels of FcγRIII and FcγRIV on neutrophils and increased FcγRIII on Ly6C^{hi} monocytes that may, altogether, contribute to their higher susceptibility to anaphylaxis induction (Supplemental Figure 5A&B).

The contribution of a rather restricted subset of myeloid cells to these (and other) models of anaphylaxis[2, 3] appears to be determined by at least two factors: their capacity to release anaphylactogenic mediators (*e.g.* histamine or PAF) and their expression of sufficient levels of activating IgG receptors. Mast cells and basophils release histamine, and neutrophils, monocytes/ macrophages and basophils release PAF, upon FcγR-triggering. Other mediators may induce anaphylaxis or contribute to its severity, among them lipid mediators like prostaglandins, thromboxanes and leukotrienes. Some of these have indeed been reported to trigger

509 bronchoconstriction and an increase in vascular permeability[43]. The release of such mediators is
510 sufficiently rapid to coincide with the celerity of hypothermia, which is detectable within minutes
511 after allergen challenge. It is therefore surprising that eosinophils do not contribute to IgG-PSA,
512 as they express high levels of activating FcγRIII and FcγRIIB[20] (but no FcγRI or FcγRIV), and
513 are capable of releasing Leukotriene C4, Prostaglandin E2, thromboxane and PAF upon
514 activation[43]. Though eosinophils represent relatively low numbers among blood cells
515 ($\approx 2x10^5$/mL), this is an unlikely explanation because basophils are significantly less numerous
516 ($\approx 5x10^4$/mL) but do contribute to anaphylaxis models. Most revealingly, it has been reported that
517 eosinophils do not release PAF following IgG-dependent activation[44]. Whether eosinophils
518 produce other potentially anaphylactogenic mediators following IgG-immune complex activation
519 has not been investigated, but the lack of such an effect appears the most reasonable hypothesis
520 to explain why eosinophils have not been found to contribute to IgG-induced anaphylaxis.

521 We investigated the contribution of neutrophils and monocytes to IgG-PSA models using
522 depletion approaches. $Ly6G^+$ cell depletion using NIMP-R14 resulted in an efficient depletion of
523 neutrophils in the blood and the spleen (Supplemental Figures 1B&2B). The same treatment
524 resulted only in a partial depletion in the bone marrow, in which a proportion of $Ly6G^+$ cells are
525 masked from fluorescent anti-Ly6G staining, but not depleted by NIMP-R14 treatment (refer to
526 bone marrow panels in Supplemental Figures 1C,D & 2C,D,I). Importantly, we found that NIMP-
527 R14 depletion has a significant impact on monocyte populations in the blood and to some extent
528 in the spleen. This should be taken into consideration when interpreting the results of NIMP-R14
529 depletion experiments. All IgG-PSA models were ameliorated following NIMP-R14 depletion,
530 but also when monocytes/macrophages were targeted using clodronate liposomes. Intravenous
531 injection of clodronate liposomes resulted in a significant depletion of monocytes from the blood
532 and monocytes/macrophages from the spleen and BM, but not from the skin (data not shown) and

peritoneum (Supplemental Figures 1&2, as reported[26]), and to a significant increase in blood leukocyte counts and particularly of neutrophils (Supplemental Figures 1&2). Thus the anti-Ly6G and the clodronate liposome treatments alter also the monocytes and neutrophil compartment, respectively, but reduced hypothermia in the three models of IgG-PSA studied. Constitutive deficiency in neutrophils, studied using $Gfi1^{-/-}$ mice, confirmed the role of neutrophils in IgG2a- and IgG2b-PSA models. Both neutrophils and monocytes can therefore be considered to contribute to IgG-induced anaphylaxis in mice, whether dependent on IgG1, IgG2a or IgG2b. The role of macrophages in the different IgG-PSA models remains to be investigated more deeply, as clodronate liposomes injected intravenously efficiently targeted macrophages in the spleen, but not in other tissues like peritoneum or skin, and thus do not allow conclusions on their contribution.

The contribution of basophils to models of anaphylaxis has been a recent matter of debate. Tsujimura *et al* reported that depletion of basophils using anti-CD200R3 (clone Ba103) monoclonal antibodies strongly inhibited IgG1-PSA and rescued mast cell-deficient mice from active anaphylaxis[19]. Ohnmacht *et al*, however, found that basophil-deficient $Mcpt8^{cre}$ mice demonstrated slightly decreased but significant hypothermia in response to IgG1-PSA (induced with the same antibody clone) when compared to WT mice[45]. More recently, Reber *et al.* reported that peanut-induced anaphylaxis was reduced following Diphtheria toxin injection in $Mcpt8^{DTR}$ mice that selectively depletes basophils, and confirmed that basophil depletion using anti-CD200R3 mAbs inhibited anaphylaxis[36]. Moreover, Khodoun *et al* found a contribution of basophils to anaphylaxis mortality, but not to hypothermia, in a model of IgG2a-PSA following anti-CD200R3 mAb injection[5]. It therefore appears that differences between inducible basophil depletion using specific antibodies or toxin administration and a constitutive lack of basophils,

557 possibly leading to compensatory mechanisms during development of these mice, may account
558 for the divergent results observed. Intriguingly however, basophils have been reported to be
559 resistant to IgG-immune complex triggering *ex vivo* due to dominant inhibition by FcγRIIB over
560 activation by FcγRIII[20]. In this study, we report that both basophil depletion following anti-
561 CD200R3 mAb (Ba103) injection or constitutive deficiency of basophils and mast cells in Cpa3-
562 Cre; Mcl-1$^{fl/fl}$ mice inhibits IgG2b-PSA but not IgG2a-PSA, confirming a role for basophils (and
563 potentially mast cells) to specific IgG-PSA models. Of note, Ba103 efficiently depleted basophils
564 from the blood and partially from the spleen and the bone marrow, but had no significant effect
565 on mast cells in the peritoneum or skin (Supplemental Figures 1A&1E and 2A&2E). The
566 difference in the ability of basophils to respond to IgG-immune complex triggering *in vitro* and
567 the various *in vivo models* may be explained by functional alterations during basophil purification
568 or a requirement for co-stimulation by other cells or their products that are present *in vivo*, but not
569 *ex vivo*, for basophils to respond to IgG-immune complexes. Our results using Cpa3-Cre; Mcl-
570 1$^{fl/fl}$ mice indicate that mast cells were not necessary for IgG2a-PSA. We could not formally
571 define their role in IgG2b-PSA as basophil depletion and deficiency in basophils and mast cells
572 lead to similar reduction in IgG2b-PSA. Notably, increased plasma histamine levels, but no
573 increase in mMCP-1 levels could be detected, suggesting that mucosal mast cells were not
574 activated during IgG-PSA. Intriguingly, however, dermal mast cells displayed a degranulated
575 morphology 30 minutes after challenge in all IgG PSA models tested (Supplemental Figure 7).
576 Whether their degranulation is a cause or a consequence of anaphylaxis remains however elusive.
577
578 The ability of cells expressing activating FcγRs to respond to IgG-immune complexes has
579 been proposed to be regulated by co-expression of FcγRIIB[46]. FcγRIIB$^{-/-}$ mice develop increased
580 hypersensitivity and anaphylactic reactions to IgG1-PSA (this report and[16, 18]). Our results further

demonstrate that FcγRIIB inhibits IgG2b-, but not IgG2a-PSA. This latter finding is supported by results from Khodoun *et al*[5]: these authors proposed that the lack of this inhibitory receptor may lead to increased spontaneous formation of immune complexes in FcγRIIB$^{-/-}$ mice, that could compete with IgG2a-immune complexes. In light of our results comparing IgG1-, IgG2a- and IgG2b-PSA, we rather propose that the significantly lower affinity of inhibitory FcγRIIB for IgG2a (K_A = 4.2 10^5 M^{-1}) than for IgG1 (K_A = 3.3 10^6 M^{-1}) and IgG2b (K_A = 2.2 10^6 M^{-1}) is the determining factor (Table 1). Another factor may be the variance in expression of FcγRIIB on circulating myeloid cells: basophils > monocytes > eosinophils >> neutrophils[20]. Whereas the exact numbers of expressed activating FcγRIII and inhibitory FcγRIIB per cell remain unknown, flow cytometric analysis allowed the estimation of their relative expression: indeed, the ratio FcγRIII/FcγRIIB is higher on neutrophils than on monocytes and basophils. These differential expression levels may thus explain why neutrophils contribute to anaphylaxis, as the receptor balance is in favor of the activating receptor. Strikingly, FcγRIIB is co-expressed only with FcγRIII on basophils and Ly6C^{hi} monocytes, whereas it is co-expressed with FcγRIII and FcγRIV on neutrophils and Ly6C^{lo} monocytes[34]. Contribution of a given cell type to anaphylaxis may therefore be favored when inhibitory FcγRIIB is required to dampen the stimulatory potential of two activating IgG receptors instead of one. This concept extends to IgG1-immune complexes that only engage one activating receptor, FcγRIII.

Our results on the contribution of mouse IgG receptors, cells and mediators in IgG-induced anaphylaxis can potentially be translated to human IgG-mediated anaphylaxis, *e.g.* following intravenous IgG or therapeutic IgG antibody administration. Indeed, even though IgG receptors are different in the two species, we have already reported that human FcγRI (hFcγRI) and human FcγRIIA (hFcγRIIA) can induce anaphylaxis when expressed under the control of

their own promoter in transgenic mice[23, 24]. hFcγRI (CD64) is the equivalent of mouse FcγRI whereas hFcγRIIA (CD32A) can be regarded as the equivalent of mouse FcγRIII, and hFcγRIIIA (CD16A) the equivalent of mouse FcγRIV[7]. hFcγRIIA, like mouse FcγRIII, is expressed on all myeloid cells and could therefore act as the principal IgG receptor responsible for anaphylaxis in humans. hFcγRIIB, the equivalent of mouse FcγRIIB, is scarcely expressed on most circulating myeloid cells[47] except for its high expression on basophils[20], suggesting that among myeloid cells only human basophils are highly sensitive to hFcγRIIB-mediated inhibition. In contrast to mouse FcγRI, hFcγRI is constitutively expressed on circulating monocytes and inducibly on neutrophils, allowing this receptor to induce anaphylaxis[24]. The binding of human IgG subclasses to hFcγRs differs strikingly from the binding of mouse IgG subclasses to mouse FcγRs. Noticeably, the affinity of hFcγRIIB for any human IgG subclass is the lowest among human IgG-hFcγR interactions. For example, human IgG1, the equivalent of mouse IgG2a, is bound by all activating hFcγRs ($K_A > 10^6$ M^{-1}) with at least a ten-fold higher affinity than by inhibitory hFcγRIIB ($K_A \approx 10^5$ M^{-1})[48]. If we consider the translation of our results obtained in the mouse to human IgG-induced anaphylaxis, one could anticipate that hFcγRIIB-mediated inhibition of IgG-induced anaphylaxis is inefficient in human neutrophils and monocytes, and efficient only in human basophils for which the elevated hFcγRIIB expression may compensate for the low-affinity of this receptor for human IgG subclasses. Certainly, FcγR-engagement by IgG immune complexes on human basophils could not trigger any detectable basophil activation *in vitro*[20], similar to the results we reported for mouse basophil activation. Our data altogether propose that the differential expression of inhibitory FcγRIIB on myeloid cells and its differential binding of IgG subclasses control the contribution of basophils, neutrophils and monocytes to IgG-dependent anaphylaxis, thus revealing novel complexities in the mechanism of regulation of cell populations, and therefore their contribution to IgG-mediated reactions *in vivo*.

REFERENCES

1. Brown SG, Stone SF, Fatovich DM, Burrows SA, Holdgate A, Celenza A, et al. Anaphylaxis: Clinical patterns, mediator release, and severity. J Allergy Clin Immunol 2013.
2. Finkelman FD, Rothenberg ME, Brandt EB, Morris SC, Strait RT. Molecular mechanisms of anaphylaxis: lessons from studies with murine models. J Allergy Clin Immunol 2005; 115:449-57; quiz 58.
3. Jonsson F, Mancardi DA, Albanesi M, Bruhns P. Neutrophils in local and systemic antibody-dependent inflammatory and anaphylactic reactions. J Leukoc Biol 2013; 94:643-56.
4. Iff ET, Vaz NM. Mechanisms of anaphylaxis in the mouse. Similarity of shock induced by anaphylaxis and by mixtures of histamine and serotonin. Int Arch Allergy Appl Immunol 1966; 30:313-22.
5. Khodoun MV, Kucuk ZY, Strait RT, Krishnamurthy D, Janek K, Clay CD, et al. Rapid desensitization of mice with anti-FcgammaRIIb/FcgammaRIII mAb safely prevents IgG-mediated anaphylaxis. J Allergy Clin Immunol 2013; 132:1375-87.
6. Million M, Fioramonti J, Zajac JM, Bueno L. Effects of neuropeptide FF on intestinal motility and temperature changes induced by endotoxin and platelet-activating factor. Eur J Pharmacol 1997; 334:67-73.
7. Bruhns P. Properties of mouse and human IgG receptors and their contribution to disease models. Blood 2012; 119:5640-9.
8. Guilliams M, Bruhns P, Saeys Y, Hammad H, Lambrecht BN. The function of Fcgamma receptors in dendritic cells and macrophages. Nat Rev Immunol 2014; 14:94-108.
9. Roopenian DC, Akilesh S. FcRn: the neonatal Fc receptor comes of age. Nat Rev Immunol 2007; 7:715-25.
10. Bruhns P, Fremont S, Daëron M. Regulation of allergy by Fc receptors. Curr Opin Immunol 2005; 17:662-9.
11. Gavin AL, Barnes N, Dijstelbloem HM, Hogarth PM. Identification of the mouse IgG3 receptor: implications for antibody effector function at the interface between innate and adaptive immunity. J Immunol 1998; 160:20-3.
12. Saylor CA, Dadachova E, Casadevall A. Murine IgG1 and IgG3 isotype switch variants promote phagocytosis of Cryptococcus neoformans through different receptors. J Immunol 2010; 184:336-43.
13. Unkeless JC, Eisen HN. Binding of monomeric immunoglobulins to Fc receptors of mouse macrophages. J Exp Med 1975; 142:1520-33.
14. Mancardi DA, Iannascoli B, Hoos S, England P, Daeron M, Bruhns P. FcgammaRIV is a mouse IgE receptor that resembles macrophage FcepsilonRI in humans and promotes IgE-induced lung inflammation. J Clin Invest 2008; 118:3738-50.
15. 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; 99:901-14.

673 16. Jönsson F, Mancardi DA, Kita Y, Karasuyama H, Iannascoli B, Van Rooijen N, et al.
674 Mouse and human neutrophils induce anaphylaxis. J Clin Invest 2011; 121:1484-96.
675 17. Nimmerjahn F, Ravetch JV. Divergent immunoglobulin g subclass activity through
676 selective Fc receptor binding. Science 2005; 310:1510-2.
677 18. Ujike A, Ishikawa Y, Ono M, Yuasa T, Yoshino T, Fukumoto M, et al. Modulation of
678 immunoglobulin (Ig)E-mediated systemic anaphylaxis by low-affinity Fc receptors for
679 IgG. J Exp Med 1999; 189:1573-9.
680 19. Tsujimura Y, Obata K, Mukai K, Shindou H, Yoshida M, Nishikado H, et al. Basophils
681 play a pivotal role in immunoglobulin-G-mediated but not immunoglobulin-E-mediated
682 systemic anaphylaxis. Immunity 2008; 28:581-9.
683 20. Cassard L, Jonsson F, Arnaud S, Daeron M. Fcgamma receptors inhibit mouse and human
684 basophil activation. J Immunol 2012; 189:2995-3006.
685 21. Nimmerjahn F, Bruhns P, Horiuchi K, Ravetch JV. Fc gamma RIV: a novel FcR with
686 distinct IgG subclass specificity. Immunity 2005; 23:41-51.
687 22. Strait RT, Morris SC, Yang M, Qu XW, Finkelman FD. Pathways of anaphylaxis in the
688 mouse. J Allergy Clin Immunol 2002; 109:658-68.
689 23. Jonsson F, Mancardi DA, Zhao W, Kita Y, Iannascoli B, Khun H, et al. Human
690 FcgammaRIIA induces anaphylactic and allergic reactions. Blood 2012; 119:2533-44.
691 24. Mancardi DA, Albanesi M, Jonsson F, Iannascoli B, Van Rooijen N, Kang X, et al. The
692 high-affinity human IgG receptor FcgammaRI (CD64) promotes IgG-mediated
693 inflammation, anaphylaxis, and antitumor immunotherapy. Blood 2013; 121:1563-73.
694 25. Lilla JN, Chen CC, Mukai K, BenBarak MJ, Franco CB, Kalesnikoff J, et al. Reduced
695 mast cell and basophil numbers and function in Cpa3-Cre; Mcl-1fl/fl mice. Blood 2011;
696 118:6930-8.
697 26. Van Rooijen N, Sanders A. Liposome mediated depletion of macrophages: mechanism of
698 action, preparation of liposomes and applications. J Immunol Methods 1994; 174:83-93.
699 27. Williams EL, Tutt AL, French RR, Chan HT, Lau B, Penfold CA, et al. Development and
700 characterisation of monoclonal antibodies specific for the murine inhibitory
701 FcgammaRIIB (CD32B). Eur J Immunol 2012; 42:2109-20.
702 28. Jiao D, Liu Y, Lu X, Liu B, Pan Q, Liu Y, et al. Macrophages are the dominant effector
703 cells responsible for IgG-mediated passive systemic anaphylaxis challenged by natural
704 protein antigen in BALB/c and C57BL/6 mice. Cell Immunol 2014; 289:97-105.
705 29. Biburger M, Nimmerjahn F. Low level of FcgammaRIII expression on murine natural
706 killer cells. Immunol Lett 2012; 143:53-9.
707 30. Yucel R, Kosan C, Heyd F, Moroy T. Gfi1:green fluorescent protein knock-in mutant
708 reveals differential expression and autoregulation of the growth factor independence 1
709 (Gfi1) gene during lymphocyte development. J Biol Chem 2004; 279:40906-17.
710 31. Khodoun MV, Strait R, Armstrong L, Yanase N, Finkelman FD. Identification of markers
711 that distinguish IgE- from IgG-mediated anaphylaxis. Proc Natl Acad Sci U S A 2011;
712 108:12413-8.
713 32. Tan PS, Gavin AL, Barnes N, Sears DW, Vremec D, Shortman K, et al. Unique
714 monoclonal antibodies define expression of Fc gamma RI on macrophages and mast cell
715 lines and demonstrate heterogeneity among subcutaneous and other dendritic cells. J
716 Immunol 2003; 170:2549-56.
717 33. Mancardi DA, Jonsson F, Iannascoli B, Khun H, Van Rooijen N, Huerre M, et al. The
718 murine high-affinity IgG receptor Fc(gamma)RIV is sufficient for autoantibody-induced
719 arthritis. J Immunol 2011; 186:1899-903.

34. Biburger M, Aschermann S, Schwab I, Lux A, Albert H, Danzer H, et al. Monocyte subsets responsible for immunoglobulin G-dependent effector functions in vivo. Immunity 2011; 35:932-44.
35. Makabe-Kobayashi Y, Hori Y, Adachi T, Ishigaki-Suzuki S, Kikuchi Y, Kagaya Y, et al. The control effect of histamine on body temperature and respiratory function in IgE-dependent systemic anaphylaxis. J Allergy Clin Immunol 2002; 110:298-303.
36. Reber LL, Marichal T, Mukai K, Kita Y, Tokuoka SM, Roers A, et al. Selective ablation of mast cells or basophils reduces peanut-induced anaphylaxis in mice. J Allergy Clin Immunol 2013; 132:881-8 e11.
37. Camussi G, Aglietta M, Coda R, Bussolino F, Piacibello W, Tetta C. Release of platelet-activating factor (PAF) and histamine. II. The cellular origin of human PAF: monocytes, polymorphonuclear neutrophils and basophils. Immunology 1981; 42:191-9.
38. Wedemeyer J, Tsai M, Galli SJ. Roles of mast cells and basophils in innate and acquired immunity. Curr Opin Immunol 2000; 12:624-31.
39. Jonsson F, Daeron M. Mast cells and company. Front Immunol 2012; 3:16.
40. Tipton TR, Mockridge CI, French RR, Tutt AL, Cragg MS, Beers SA. Anti-mouse FcgammaRIV antibody 9E9 also blocks FcgammaRIII in vivo. Blood 2015; 126:2643-5.
41. Syed SN, Konrad S, Wiege K, Nieswandt B, Nimmerjahn F, Schmidt RE, et al. Both FcgammaRIV and FcgammaRIII are essential receptors mediating type II and type III autoimmune responses via FcRgamma-LAT-dependent generation of C5a. Eur J Immunol 2009; 39:3343-56.
42. Nimmerjahn F, Lux A, Albert H, Woigk M, Lehmann C, Dudziak D, et al. FcgammaRIV deletion reveals its central role for IgG2a and IgG2b activity in vivo. Proc Natl Acad Sci U S A 2010; 107:19396-401.
43. Stone KD, Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. J Allergy Clin Immunol 2010; 125:S73-80.
44. Capron M. Eosinophils: receptors and mediators in hypersensitivity. Clin Exp Allergy 1989; 19 Suppl 1:3-8.
45. Ohnmacht C, Schwartz C, Panzer M, Schiedewitz I, Naumann R, Voehringer D. Basophils orchestrate chronic allergic dermatitis and protective immunity against helminths. Immunity 2010; 33:364-74.
46. Smith KG, Clatworthy MR. FcgammaRIIB in autoimmunity and infection: evolutionary and therapeutic implications. Nat Rev Immunol 2010; 10:328-43.
47. Veri MC, Gorlatov S, Li H, Burke S, Johnson S, Stavenhagen J, et al. Monoclonal antibodies capable of discriminating the human inhibitory Fcgamma-receptor IIB (CD32B) from the activating Fcgamma-receptor IIA (CD32A): biochemical, biological and functional characterization. Immunology 2007; 121:392-404.
48. Bruhns P, Iannascoli B, England P, Mancardi DA, Fernandez N, Jorieux S, et al. Specificity and affinity of human Fc{gamma} receptors and their polymorphic variants for human IgG subclasses. Blood 2009; 113:3716-25.

ACKNOWLEDGMENTS

We are thankful to our colleagues at Institut Pasteur, Paris: D. Sinnaya for administrative help, Stéphane Petres for help with antibody purifications and Laurence Fiette for help with histological analyses. We are thankful to our colleagues for their generous gifts: T. Moroy (Montreal University, Montreal, QC, Canada), Clifford Lowell (University of California at San Francisco, CA, USA), J.V. Ravetch (Rockefeller University, New York, NY, USA) and J. Leusen (University Medical Center, Utrecht, The Netherlands) for mice; R. Coffman (DNAX, Palo Alto, CA, USA), R. Good (USFCM, Tampa, FL, USA), B. Heyman (Uppsala Universitet, Uppsala, Sweden), H. Karasuyama (Tokyo Medical and Dental University Graduate School, Tokyo, Japan) and D. Voehringer (Universitätsklinikum, Erlangen, Germany) for antibodies. Cl_2MDP was a gift of Roche Diagnostics GmbH. This work was supported by the Institut Pasteur, the Institut National de la Santé et de la Recherche Médicale (INSERM), the European Research Council (ERC)–Seventh Frame-work Program (ERC-2013-CoG 616050), the Société Française d'Allergologie (SFA; *Soutien de la Recherche en Allergologie*) and the Balsan company. H.B. is supported by a fellowship from the *University Pierre et Marie Curie*. C.M.G. is a scholar of the Pasteur Paris University International Doctoral Program and supported by a stipend from the Institut Carnot Pasteur *Maladies Infectieuses*. F.J. is an employee of the *Centre National de La Recherche Scientifique* (CNRS). R.S. and S.J.G. are supported by NIH/NIAMS grant R01 AR067145 and the Department of Pathology at Stanford University.

AUTHORSHIP AND CONFLICT OF INTEREST STATEMENTS

H.B. performed all experiments at the Institut Pasteur with contributions from P.E, C.M.G, F.J.; R.S. and L.L.R contributed experiments using Cpa3-Cre; Mcl-$1^{fl/fl}$ mice; B.I. and

O.G. genotyped mice and produced reagents; M.S.C., S.J.G. and N.v.R. provided reagents; H.B., P.B., P.E., C.M.G., S.J.G, F.J., D.A.M., L.L.R. and R.S. analyzed and discussed results; F.J., P.B. and D.A.M. supervised and designed the research; P.B. and F.J. wrote the manuscript. All authors read and had an opportunity to contribute to the editing of the manuscript, and declare no competing financial interests.

TABLES

Table 1: Affinities of mouse FcγR-IgG subclass interactions (K_A values in M^{-1})

	IgG1	**IgG2a**	**IgG2b**	**IgG3**
FcγRI	-	$1x10^8$	$1x10^5$	(+)
FcγRIIB	$3.3x10^6$	$4.2x10^5$	$2.2x10^6$	-
FcγRIII	$3.1x10^5$	$6.8x10^5$	$6.4x10^5$	-
FcγRIV	-	$2.9x10^7$	$1.7x10^7$	-

"-", no detectable affinity.

"(+)", under debate[11, 12].

Data compiled from [17, 21]

FIGURE LEGENDS

Figure 1. FcγRIII dominates in IgG-PSA models. Mice injected with anti-TNP mAbs were challenged with TNP-BSA and body temperatures monitored. **(A)** IgG1-, **(B)** IgG2a- or **(C)** IgG2b-induced PSA in indicated mice (n≥3/group). Data are representative of at least two independent experiments (A: n=2; B: n=3; C: n=2). Significant differences compared to the WT group are indicated.

Figure 2. Basophils, mast cells, monocytes/macrophages and neutrophils contribute differentially to IgG-PSA models. Indicated mice (n≥8/group) were injected with IgG2a **(A-E)** or IgG2b **(F-J)** anti-TNP mAbs, challenged with TNP-BSA and body temperatures were monitored. WT mice (n=8/group) were pretreated as indicated (A, C-D, F, H-I). Lipo-PBS: PBS liposomes; Lipo-Cd: clodronate liposomes. Data are pooled from at least two independent experiments.

Figure 3. Reduced expression of FcγRIII and FcγRIV, but not FcγRIIB, on neutrophils following IgG2a-PSA. (A) FcγRIII, **(B)** FcγRIV and **(C)** FcγRIIB expression on blood cells from WT mice (A&B: n=11/group; C: n≥6/group) left untreated, injected with IgG2a anti-TNP mAbs, or injected with IgG2a anti-TNP mAbs and challenged with TNP-BSA. **(D)** Compilation of Δ Geomean +/- SEM data from A-C.

Figure 4. High doses of IgG2 antibodies reveal FcγRIV contribution to IgG2-PSA. (A) PSA in indicated mice injected with various doses of IgG2a anti-TNP mAbs (n=2/group). **(B-E)** PSA

822 in indicated mice (B&C: n=8/group; D&E: n≥3/group) injected with indicated doses of anti-TNP
823 mAbs. Data are pooled from two independent experiments. Significant differences compared to
824 the untreated WT group are indicated.
825
826 **Figure 5. Expression of FcγRs on myeloid cells following IgG2b-PSA. (A)** FcγRIII (left:
827 n=8/group, right: n=3/group), **(B)** FcγRIV (n=8/group) and **(C)** FcγRIIB expression (n≥6/group)
828 on cells from WT mice (n=8/group) left untreated, injected with IgG2b anti-TNP mAbs, or
829 injected with IgG2b anti-TNP mAbs and challenged with TNP-BSA. **(D)** Compilation of Δ
830 Geomean +/- SEM data from A-C.
831
832 **Figure 6. Cell contributions to IgG1-PSA in the absence of inhibitory FcγRIIB.** FcγRIIB$^{-/-}$
833 mice were pretreated as indicated, then injected with IgG1 anti-TNP mAbs, challenged with
834 TNP-BSA and central temperatures were monitored (A: n=8/group; B: n=7/group; C:
835 n=10/group). Data are represented as mean +/- SEM. Data are pooled from two independent
836 experiments.
837
838 **Figure 7. Contributions of histamine and PAF to IgG-PSA.** Body temperatures of pretreated
839 mice during **(A)** IgG1-PSA in FcγRIIB$^{-/-}$ (n=6/group) or WT mice (n=4/group), **(B)** IgG2a-PSA,
840 **(C)** IgG2b-PSA or (**D**) IgE-PSA in WT mice (n≥7/group). **(E)** Histamine and **(F)** mMCP-1
841 concentrations post-PSA (n=3/group). Data are representative of at least two independent
842 experiments, except for A&C (pooled from two independent experiments).
843
844

845

Figure 1

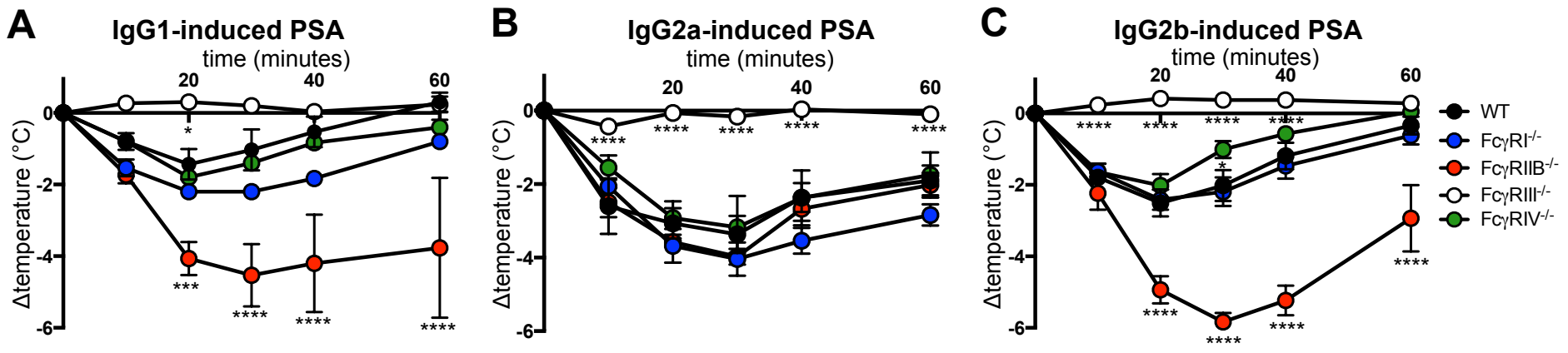

A
IgG1-induced PSA
time (minutes)
20
40
60
0
-2
-4
-6
Δtemperature (°C)
*
***
****
****
****
B
IgG2a-induced PSA
time (minutes)
20
40
60
0
-2
-4
-6
Δtemperature (°C)
****
****
****
****
****
C
IgG2b-induced PSA
time (minutes)
20
40
60
0
-2
-4
-6
Δtemperature (°C)
****
****
****
***
*
****
****
****
WT
FcγRI-/-
FcγRIIB-/-
FcγRIII-/-
FcγRIV-/-

Figure 2

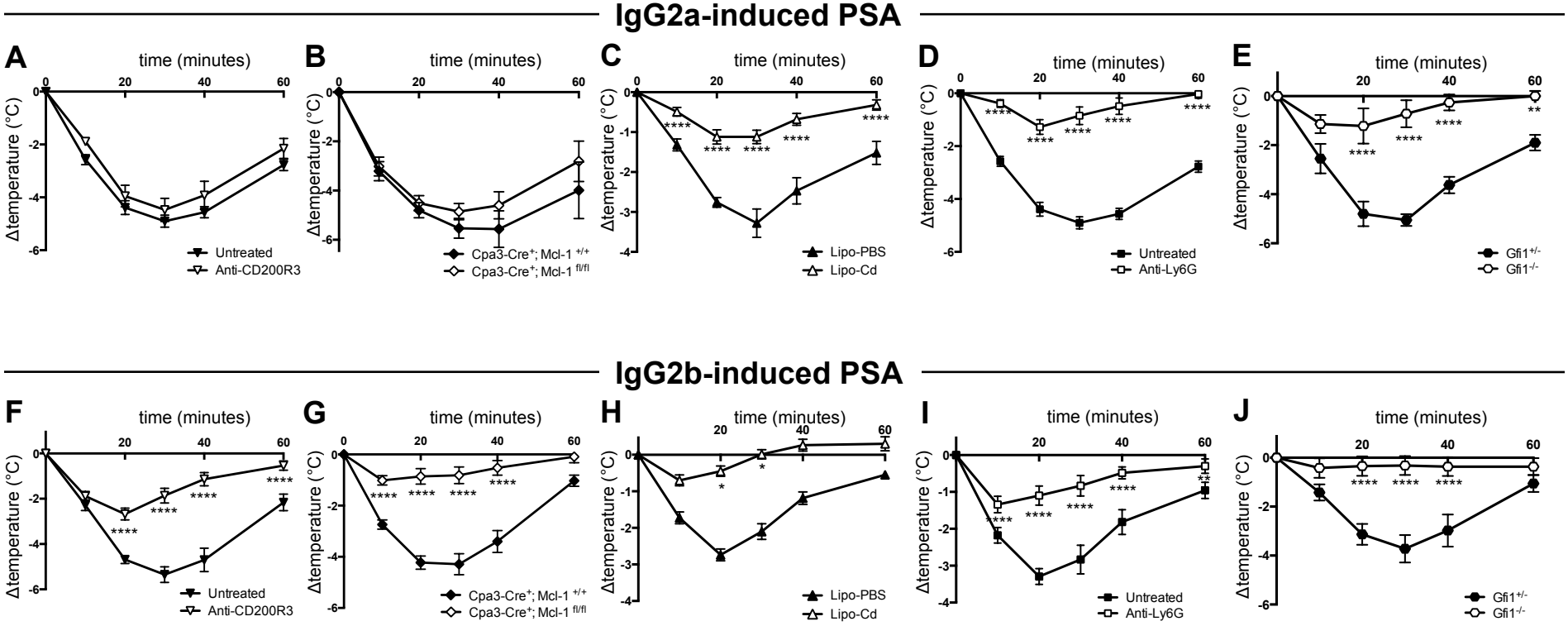
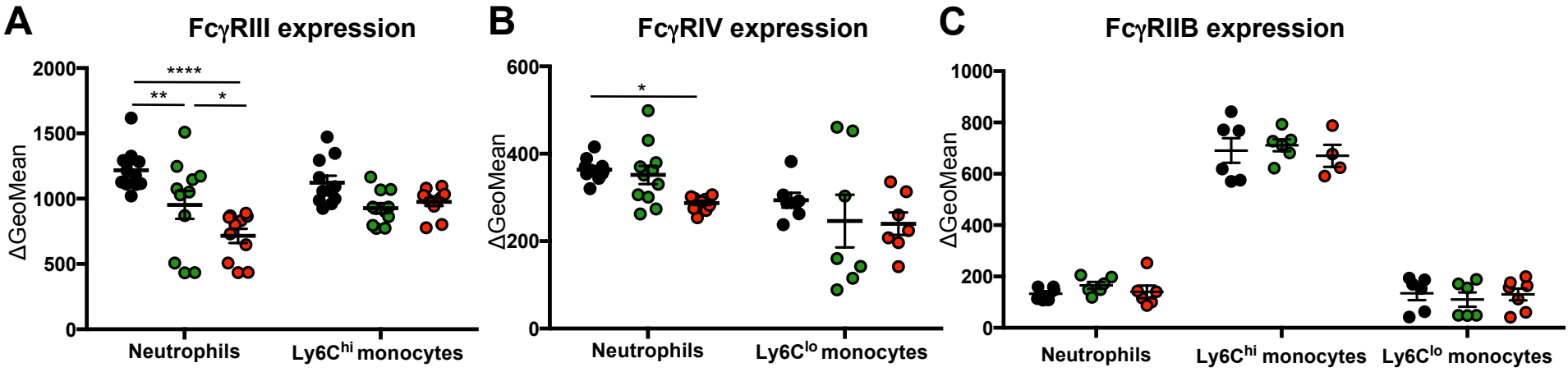

IgG2a-induced PSA
A
B
C
D
E
time (minutes)
Δtemperature (°C)
Untreated
Anti-CD200R3
Cpa3-Cre+; Mcl-1 +/+
Cpa3-Cre+; Mcl-1 fl/fl
Lipo-PBS
Lipo-Cd
Untreated
Anti-Ly6G
Gfi1+/-
Gfi1-/-
IgG2b-induced PSA
F
G
H
I
J
time (minutes)
Δtemperature (°C)
Untreated
Anti-CD200R3
Cpa3-Cre+; Mcl-1 +/+
Cpa3-Cre+; Mcl-1 fl/fl
Lipo-PBS
Lipo-Cd
Untreated
Anti-Ly6G
Gfi1+/-
Gfi1-/-

Figure 3



D

FcγRIII expression	Untreated	19h post-IgG2a	19h post-IgG2a, 3h post-Ag
Neutrophils	1217 ± 49	952 ± 106	715 ± 54
Ly6C^{hi} monocytes	1123 ± 53	927 ± 39	975 ± 31

FcγRIV expression	Untreated	19h post-IgG2a	19h post-IgG2a, 3h post-Ag
Neutrophils	363 ± 7.3	352 ± 21	287 ± 5
Ly6C^{lo} monocytes	294 ± 17	246 ± 60	240 ± 26

FcγRIIB expression	Untreated	19h post-IgG2a	19h post-IgG2a, 3h post Ag
Neutrophils	133 ± 10	165 ± 13	140 ± 24
Ly6C^{hi} monocytes	691 ± 48	711 ± 23	670 ± 43
Ly6C^{lo} monocytes	134 ± 27	110 ± 28	130 ± 23

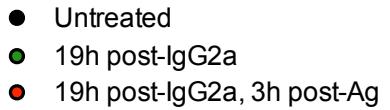


Figure 4

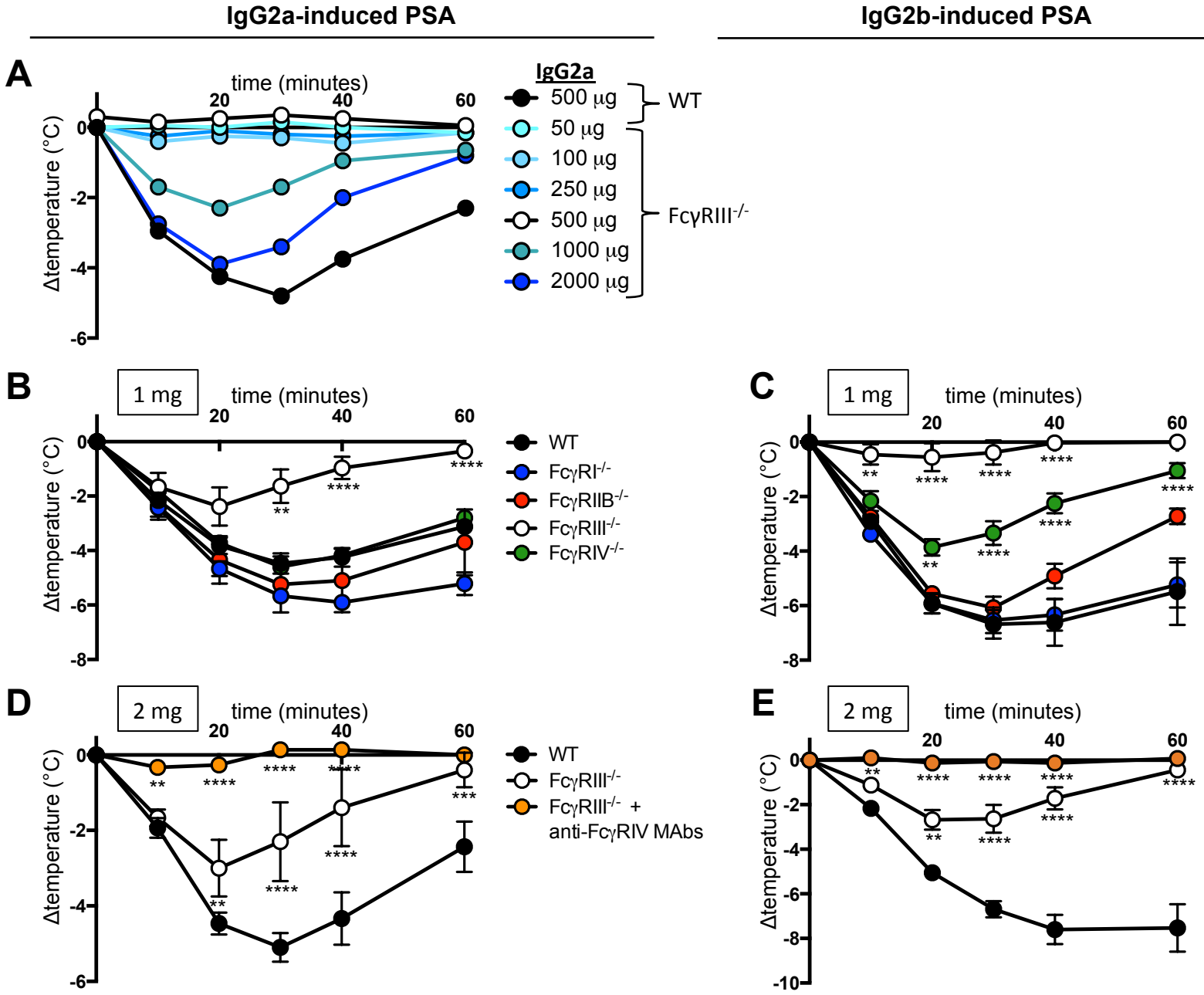
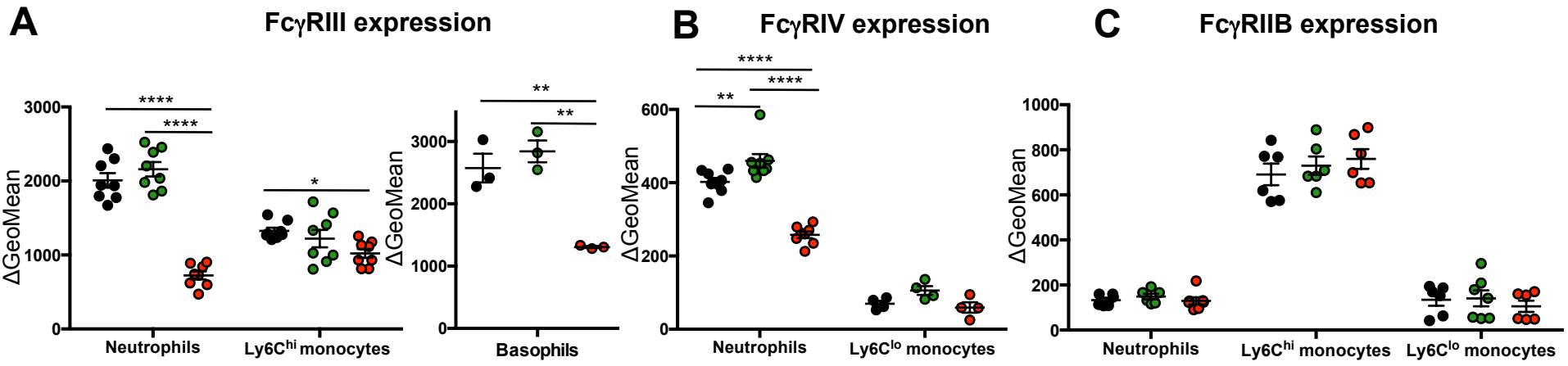

IgG2a-induced PSA
IgG2b-induced PSA
A
time (minutes)
Δtemperature (°C)
IgG2a
500 μg
50 μg
100 μg
250 μg
500 μg
1000 μg
2000 μg
WT
FcγRIII-/-
B
1 mg
WT
FcγRI-/-
FcγRIIB-/-
FcγRIII-/-
FcγRIV-/-
C
1 mg
D
2 mg
WT
FcγRIII-/-
FcγRIII-/- + anti-FcγRIV MAbs
E
2 mg

Figure 5



D

FcγRIII expression	Untreated	19h post-IgG2b	19h post-IgG2b, 3h post Ag
Neutrophils	2008 ± 97	2158 ± 98	724 ± 54
Ly6C^{hi} monocytes	1326 ± 42	1222 ± 117	1021 ± 60
BM basophils	2574 ± 231	2842 ± 176	1307 ± 15

FcγRIV expression	Untreated	19h post-IgG2b	19h post-IgG2b, 3h post Ag
Neutrophils	402 ± 11	459 ± 19	258 ± 9
Ly6C^{lo} monocytes	70 ± 8	106 ± 12	59 ± 14

FcγRIIB expression	Untreated	19h post-IgG2b	19h post-IgG2b, 3h post Ag
Neutrophils	133 ± 10	149 ± 12	130 ±16
Ly6C^{hi} monocytes	691 ± 48	730 ± 41	759 ± 44
Ly6C^{lo} monocytes	135 ± 27	141 ± 35	105 ± 25

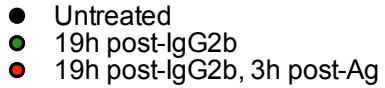


Figure 6

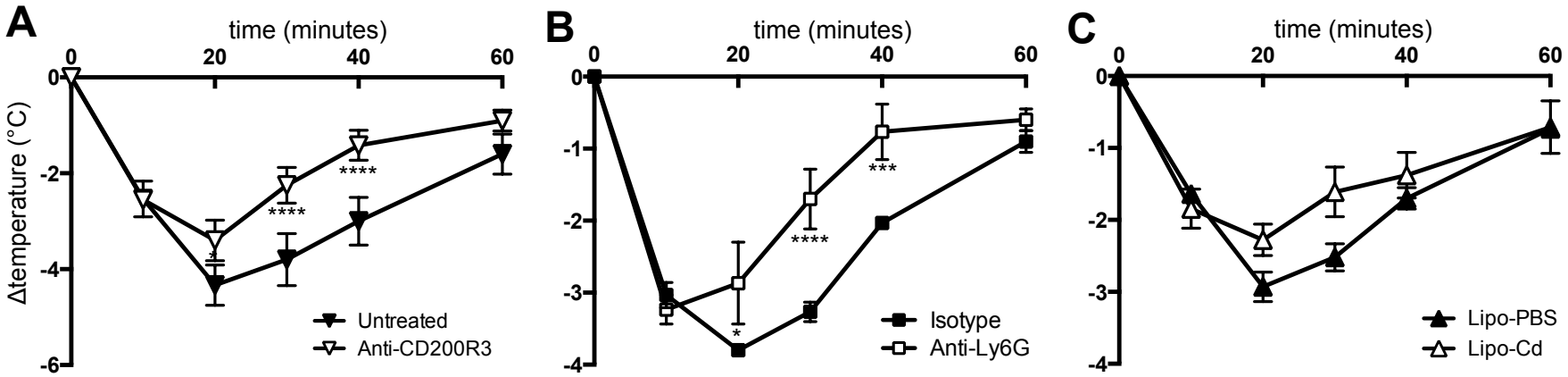
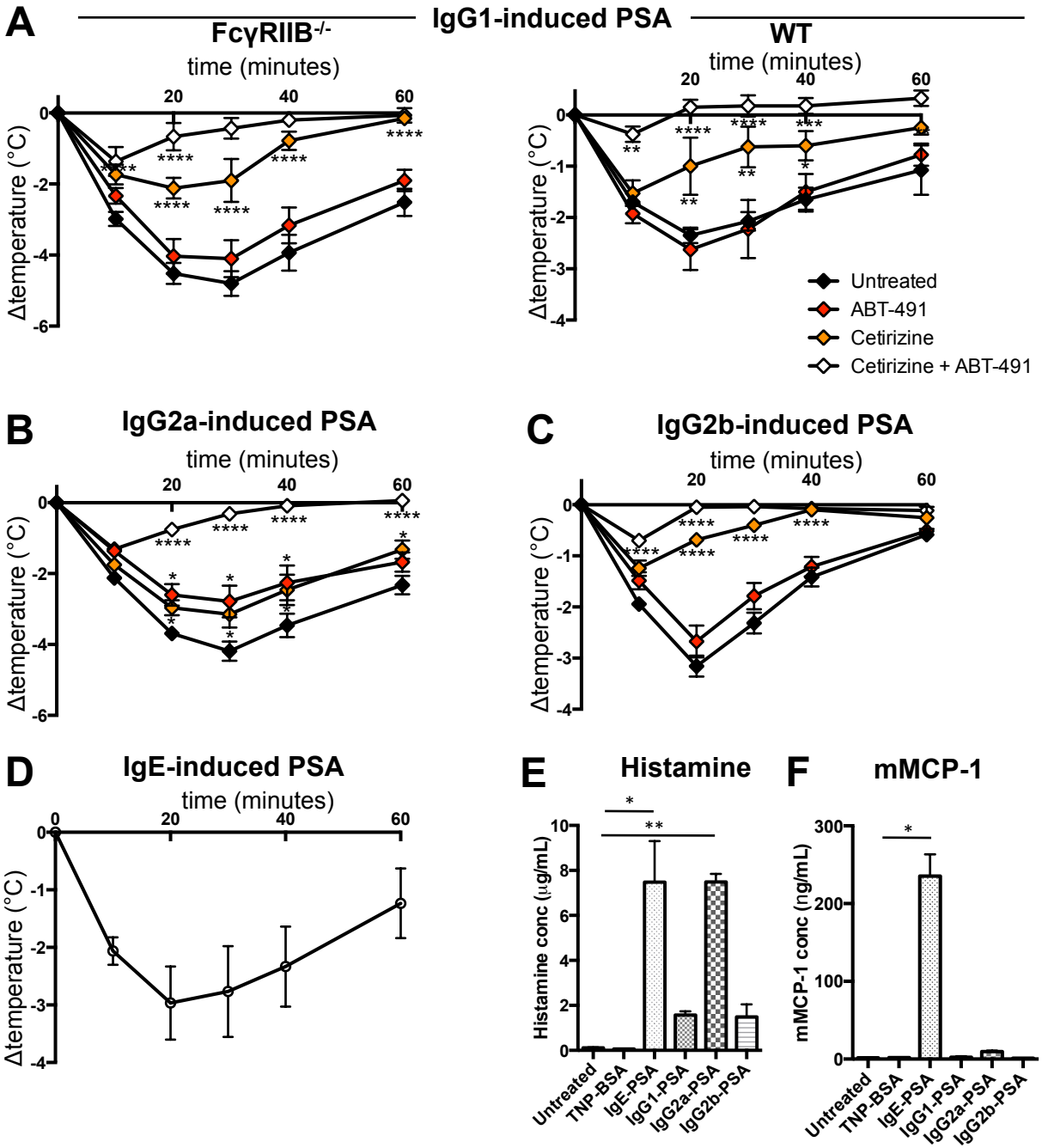

A
time (minutes)
0
20
40
60
0
-2
-4
-6
Δtemperature (°C)
****
****
Untreated
Anti-CD200R3
B
time (minutes)
0
20
40
60
0
-1
-2
-3
-4
*
****
***
Isotype
Anti-Ly6G
C
time (minutes)
0
20
40
60
0
-1
-2
-3
-4
Lipo-PBS
Lipo-Cd

Figure 7


A
IgG1-induced PSA
FcγRIIB-/-
WT
time (minutes)
Δtemperature (°C)
Untreated
ABT-491
Cetirizine
Cetirizine + ABT-491
B
IgG2a-induced PSA
C
IgG2b-induced PSA
D
IgE-induced PSA
E
Histamine
Histamine conc (µg/mL)
F
mMCP-1
mMCP-1 conc (ng/mL)
Untreated
TNP-BSA
IgE-PSA
IgG1-PSA
IgG2a-PSA
IgG2b-PSA

Supplemental Figure 1. Effects of depletion strategies on myeloid cell populations – Cell counts. WT mice were treated with indicated reagents. 24 hours after injection, counts of specific cell populations were determined by flow cytometry (A-G) or histology (I&J); leukocyte counts in total blood were measured with an automatic blood analyzer (H). Counts of **(A)** basophils, **(B)** neutrophils, **(C)** Ly6C^{hi} monocytes and **(D)** Ly6C^{lo} monocytes in blood, spleen and bone marrow, **(E)** peritoneal mast cells **(F)** peritoneal macrophages and **(G)** splenic macrophages. **(I)** Representation of a toluidine blue-stained back skin section with two mast cells (arrows). **(J)** Counts of mast cells/mm^2 in the dermis of WT mice. (A-H) Figures show one of three independent experiments. Individual measurements and mean +/- SEM are represented**.** Iso = isotype rat IgG2b, Ba103 = anti-CD200R3 mAb, NIMP = anti-Ly6G mAb, PBS = PBS liposomes, CS= clodronate liposomes.

Supplemental Figure 2. Effects of depletion strategies on myeloid cell populations – Frequencies. WT mice were treated with indicated reagents. 24 hours after injection, percentages of specific cell populations among CD45$^+$ cells were determined by flow cytometry (A-H): **(A)** basophils, **(B)** neutrophils, **(C)** Ly6C^{hi} monocytes and **(D)** Ly6C^{lo} monocytes in blood, spleen and bone marrow, **(E)** peritoneal mast cells **(F)** peritoneal macrophages and **(G)** splenic macrophages. **(H)** Percentages of peritoneal mast cells (pMC FcεRI$^+$/cKit$^+$) and blood basophils (FcεRI$^+$/DX5$^+$) in Cpa3-Cre; Mcl-1$^{fl/fl}$ mice and in Cpa3-Cre; Mcl-1$^{+/+}$ mice. (**I**) Left: Percentages of YFP-positive cells in MRP8-Cre; Rosa26-YFP mice. Right: Effect of NIMP-R14 injection on neutrophils (percentages and counts CD45$^+$/YFP$^+$/Ly6C^{neg}/CD115^{neg} cells) in blood, spleen and bone marrow of MRP8-Cre; Rosa26-YFP mice. (A-H) Figures show corresponding percentages to cell counts shown in Supplemental Figure 1 and display values for individually measured mice

and the mean and SEM**.** Iso = isotype rat IgG2b, PBS = PBS liposomes, CS= clodronate liposomes.

Supplemental Figure 3. Relative affinity of IgG1 (TIB191), IgG2a (Hy1.2) and IgG2b (GORK) anti-TNP to TNP-BSA. (A) ELISA anti-TNP. Comparison of binding capacity of TIB 191, Hy1.2 or GORK to immobilized TNP-BSA. Data are represented as mean +/- SEM and representative of results from five independent experiments. **(B) Surface plasmon resonance analysis.** Comparison of binding affinity TNP-BSA to immobilized TIB 191, Hy1.2 or GORK clones. (**C**) The table recapitulates the k_{on}, k_{off} and Kd for each condition.

Supplemental Figure 4. IgG1-PSA induces mild hypothermia in WT mice and monocytes/macrophages and neutrophils contribute to IgG2a-PSA in Balb/c mice. (A) WT mice were injected with IgG1 anti-TNP mAbs, challenged with TNP-BSA and body temperatures were monitored. PSA in mice left untreated, injected with anti-Ly6G or anti-CD200R3 (n=4/group). **(B)** Balb/c mice were left untreated, injected with anti-Ly6G, anti-CD200R3 (n=6/group), lipo-PBS (n=6/group) or lipo-Cd (n=6/group) prior to IgG2a-PSA induction. Body temperatures were monitored. Data are represented as mean +/- SEM. Data are pooled from two independent experiments. Significant differences compared to the untreated group are indicated.

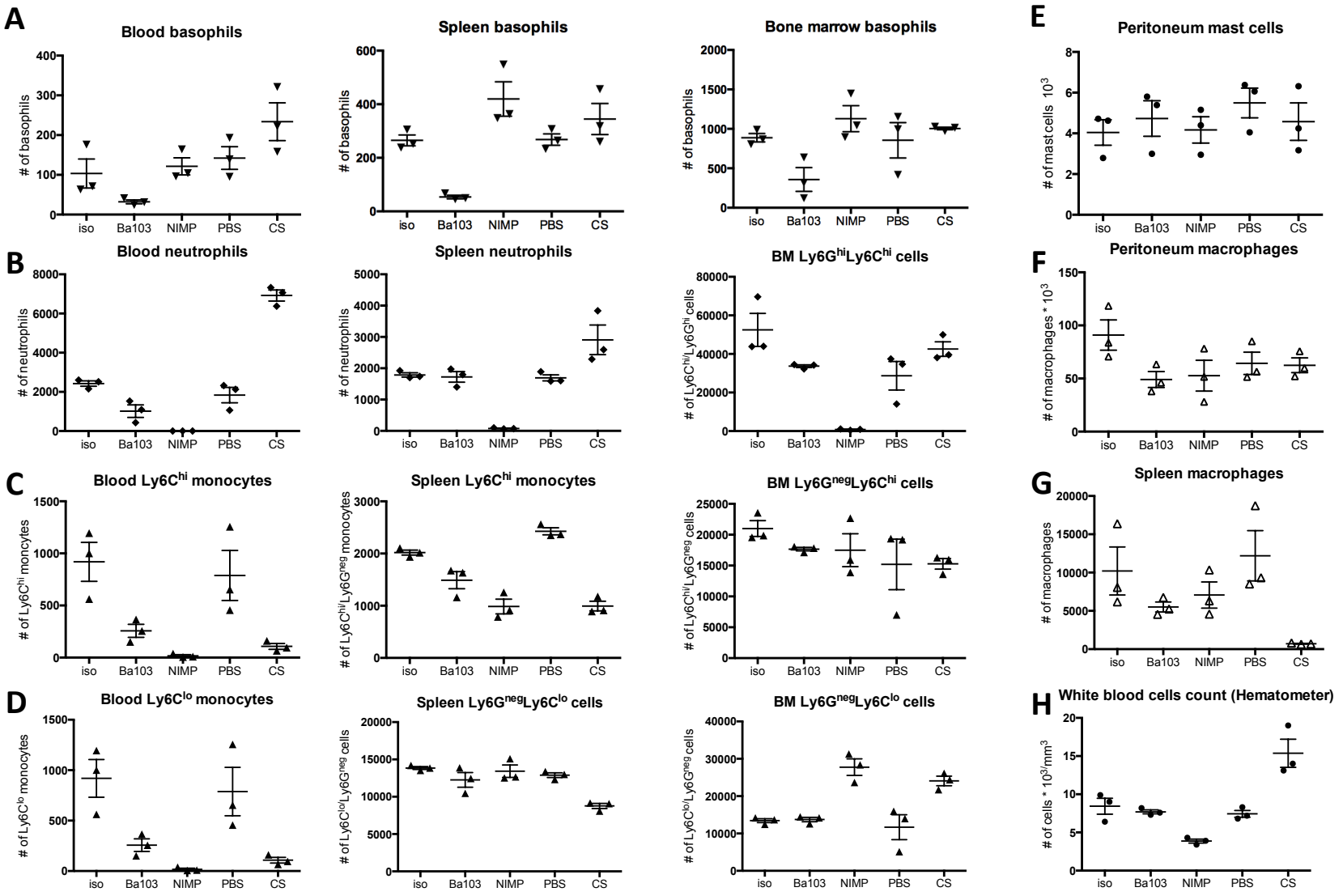
Supplemental Figure 5. FcγR expression in FcγR-deficient mice. Expression of **(A)** FcγRIII, **(B)** FcγRIV and **(C)** FcγRIIB is represented as the ΔGeomean of FcγR-specific staining compared to isotype control staining from blood leukocytes collected from untreated WT, $Fc\gamma RI^{-/-}$, $Fc\gamma RIIB^{-/-}$, $Fc\gamma RIII^{-/-}$ and $Fc\gamma RIV^{-/-}$ mice (n=4/group). Data are represented as mean +/- SEM.

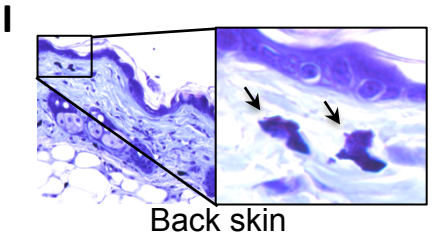
Supplemental Figure 6. Blood leukocyte numbers in FcγR-deficient mice. Leukocyte populations were assessed using an ABC Vet automatic blood analyzer (Horiba ABX) from blood collected from untreated WT, FcγRI$^{-/-}$, FcγRIIB$^{-/-}$, FcγRIII$^{-/-}$ and FcγRIV$^{-/-}$ mice (n=4/group). "Granulocytes" represent mainly neutrophils (as judged by their size and granularity). Data are represented as mean +/- SEM; each point represents one mouse.

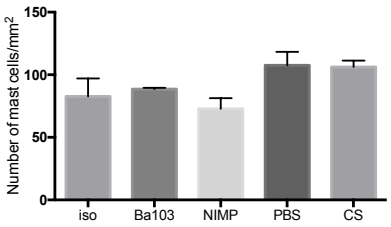
Supplemental Figure 7. Mast cell degranulation after IgG1, IgG2a and IgG2b-induced PSA. WT mice were injected with IgE, IgG1, IgG2a, IgG2b anti-TNP mAbs or left untreated (n=3 for all groups) and challenged with TNP-BSA. Mouse ear skin biopsies were collected 30 minutes after TNP-BSA injection. Representation of a toluidine blue-stained ear skin sections with one mast cell (indicated by an arrow) for one mouse of each group of mice.

Supplemental Figure 1

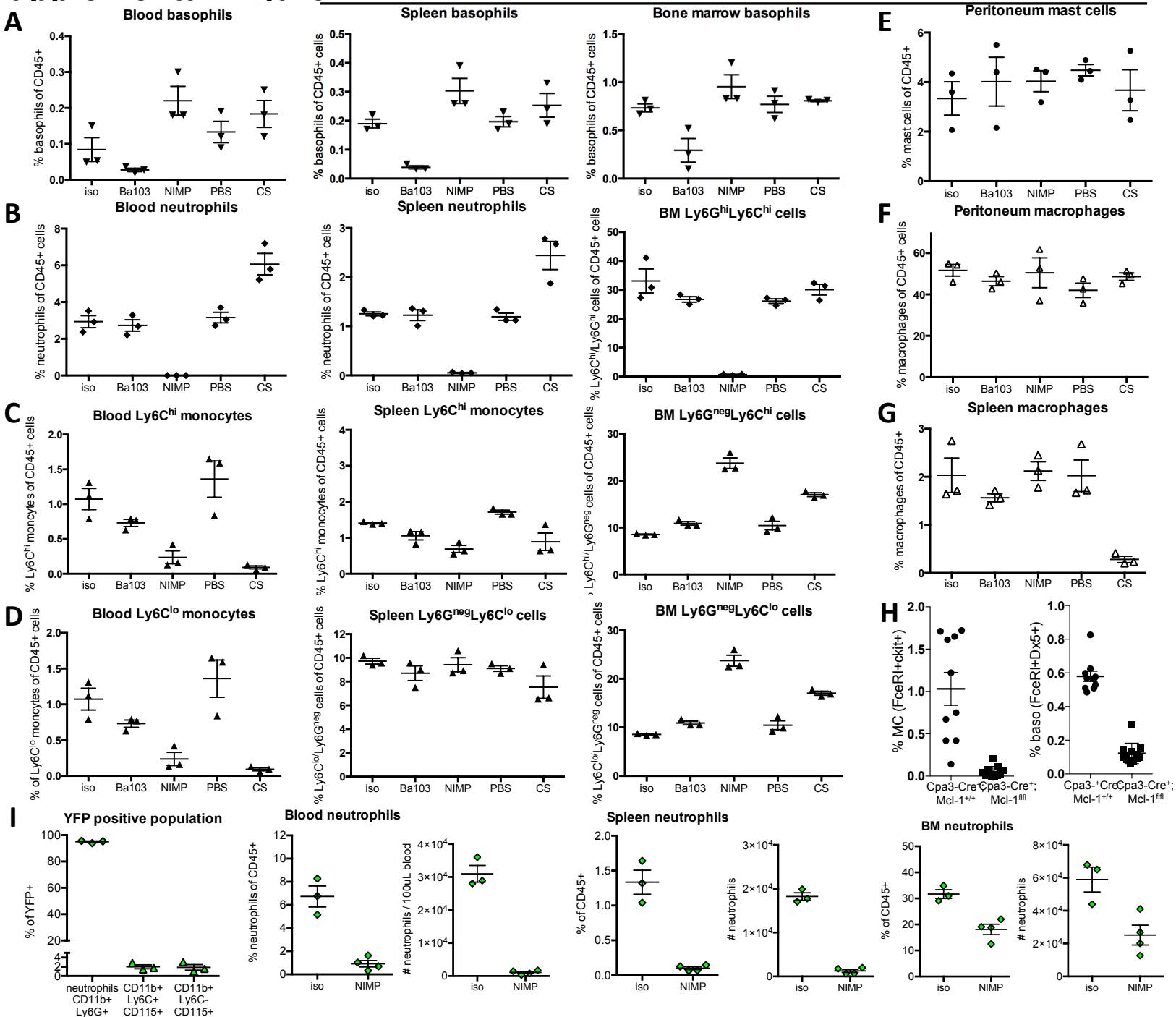
Tissues



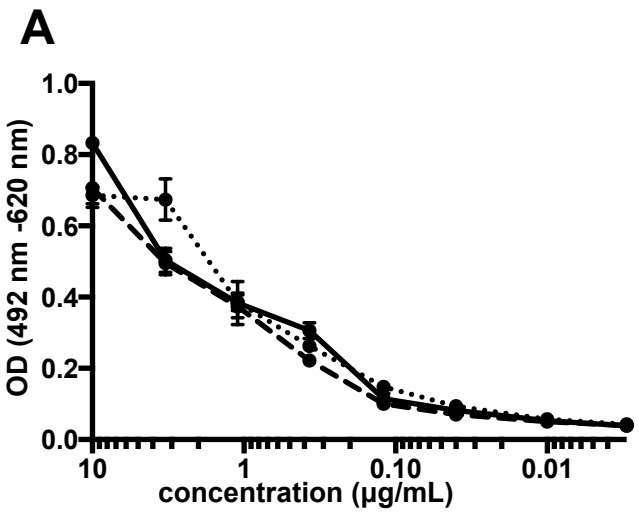


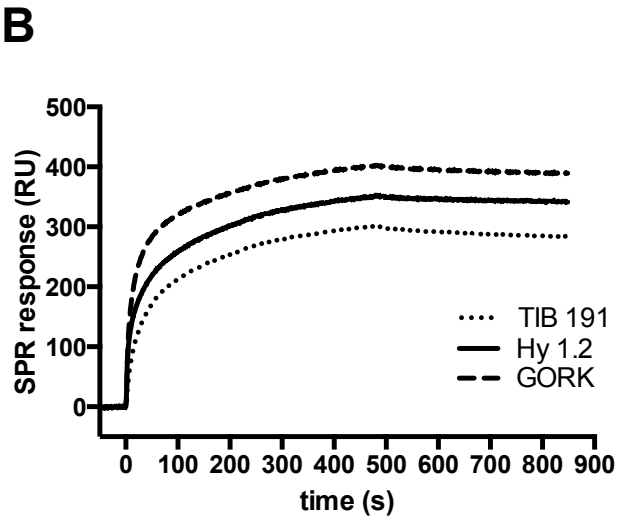


Supplemental Figure 2



Supplemental Figure 3

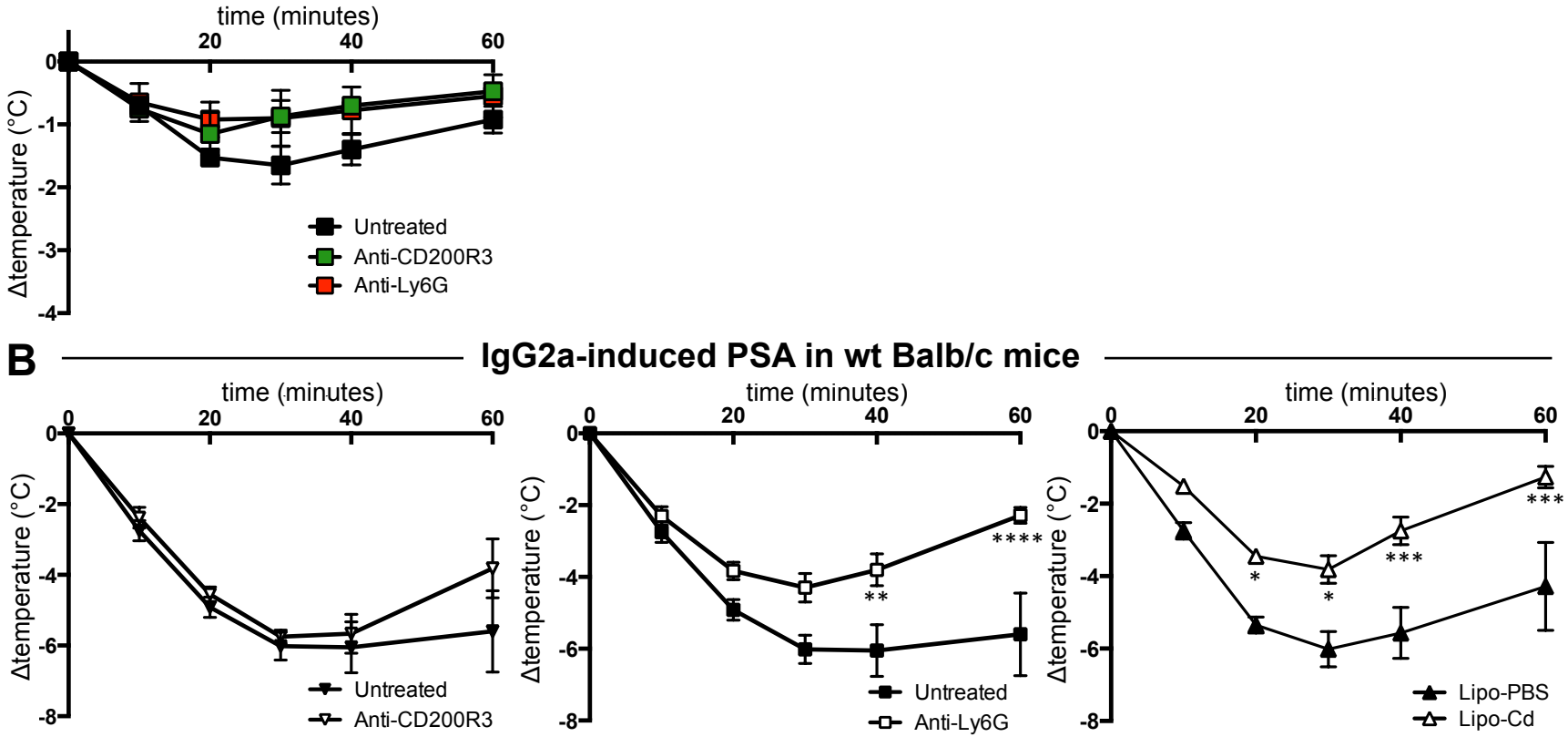




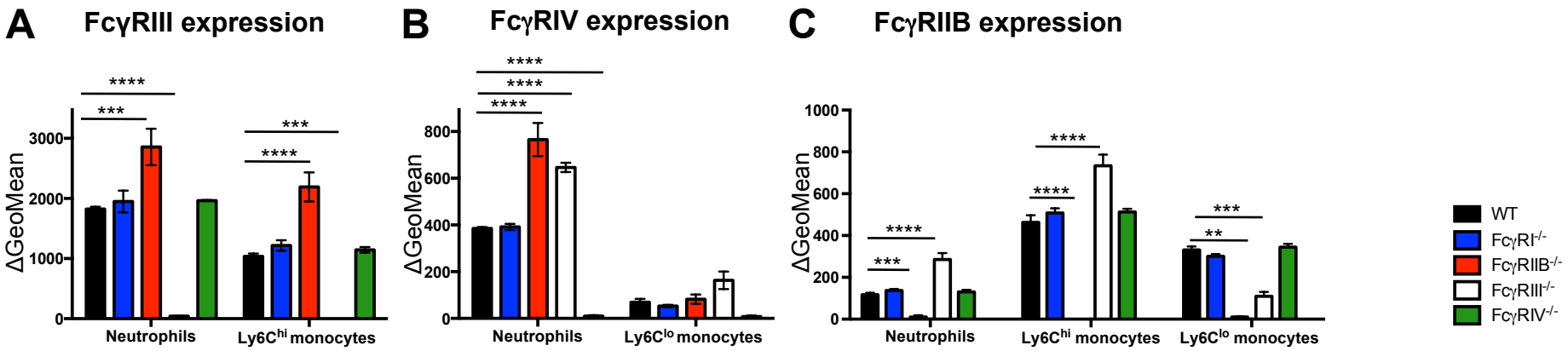
C

	k_{on} ($10^5 M^{-1}s^{-1}$)	k_{off} (10^{-4} s^{-1})	Kd (nM)
TIB 191 (IgG1)	0.97 (± 0.29)	2.27 (± 0.32)	2.34 (± 0.33)
Hy1.2 (IgG2a)	1.43 (± 0.43)	1.08 (± 0.30)	0.76 (± 0.31)
GORK (IgG2b)	2.15 (± 0.65)	1.19 (± 0.24)	0.55 (± 0.20)

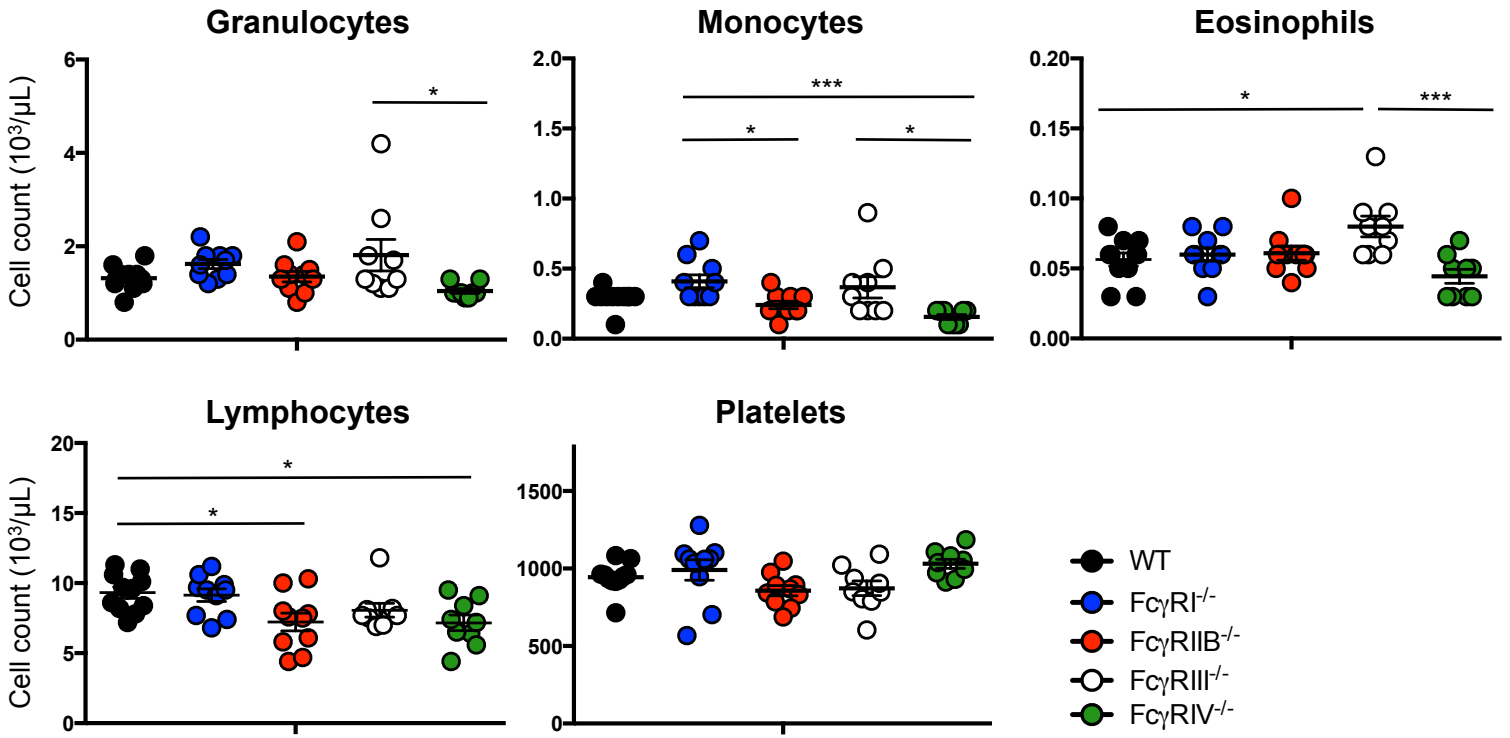
Supplemental Figure 6



Supplemental Figure 5



Supplemental Figure 6



Supplemental Figure 7