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1 **IgG subclass-dependent pulmonary antigen retention during**
2 **acute IgG-dependent systemic anaphylaxis in mice**

3
4 **Running Title:** The lung retains antigen during anaphylaxis

5
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ABSTRACT

30
31 Mouse models of active systemic anaphylaxis rely predominantly on IgG
32 antibodies forming IgG-allergen immune complexes that induce IgG receptor-expressing
33 neutrophils and monocytes/macrophages to release potent mediators, leading to systemic
34 effects. Whether anaphylaxis initiates locally or systemically remains unknown. Here we
35 aimed at identifying the anatomical location of IgG-allergen immune complexes during
36 anaphylaxis. Active systemic anaphylaxis was induced following immunization with
37 BSA and intravenous challenge with fluorescently-labelled BSA. Antigen retention
38 across different organs was examined using whole body fluorescence imaging, comparing
39 immunized and naïve animals. Various mouse models and *in vivo* deletion strategies were
40 employed to determine the contribution of IgG receptors, complement component C1q,
41 myeloid cell types and anaphylaxis mediators. We found that following challenge,
42 antigen diffused systemically, but specifically accumulated in the lungs of mice sensitized
43 to that antigen, where it formed large antibody-dependent aggregates in the vasculature.
44 Antigen retention in the lungs did not rely on IgG receptors, C1q, neutrophils or
45 macrophages. IgG2a-mediated, but neither IgG1- nor IgG2b- mediated, passive systemic
46 anaphylaxis led to antigen retention in the lung. Neutrophils and monocytes significantly
47 accumulated in the lungs after challenge and captured high amounts of antigen, which
48 lead to downmodulation of surface IgG receptors and triggered their activation. Thus,
49 within minutes of systemic injection in sensitized mice, antigen formed aggregates in the
50 lung and liver vasculature, but accumulated specifically and dose-dependently in the lung.
51 Neutrophils and monocytes recruited to the lung captured antigen and became activated.
52 However, antigen aggregation in the lung vasculature was not necessary for anaphylaxis
53 induction.

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KEY POINTS

55 - Active systemic anaphylaxis in mice shows antigen retention specifically in the lung

56 - Antigen is predominantly captured by neutrophils and monocytes

57 - Neutrophils and monocytes activate locally in the lung during anaphylaxis

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KEY WORDS

65 Anaphylaxis; IgG; immune complexes; lungs; neutrophil; monocyte; macrophage; FcγR;

66 Platelet-activating Factor; Histamine.

ABBREVIATIONS USED

67

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69 Alum: Aluminum hydroxide

70 CFA: Complete Freund's adjuvant

71 IFA: Incomplete Freund's adjuvant

72 FcγR: IgG Fc receptor

73 PAF: Platelet-activating Factor

74 WT: Wild-Type

75 PSA: Passive Systemic Anaphylaxis

76 ASA: Active Systemic Anaphylaxis

77 BSA: Bovine Serum Albumin

78 OVA: Ovalbumin

79 mAb: Monoclonal Antibody

80 PBS: Phosphate Buffered Saline

81 SEM: Standard Error of the Mean

82 NETs: Neutrophil-extracellular traps

83 MPO: Myeloperoxidase

84 Gfi-1: Growth factor independence 1

85 C1q: Complement component 1q

86 TNP: Trinitrophenyl

INTRODUCTION

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Anaphylaxis is a severe, systemic allergic reaction with increasing worldwide incidence (1, 2). Reactions usually appear rapidly (within minutes to hours) and principally affect the vasculature and the lungs, i.e., intense vasodilation and bronchoconstriction, which can lead to hypotension, hypothermia, tachycardia, respiratory distress and eventually lung or heart failure. Anaphylaxis is classically attributed to an IgE-mediated reaction involving mast cell activation and release of histamine and tryptase (3), but increasing numbers of reports describe non-IgE-mediated anaphylaxis (reviewed in (4-6)). Our recent clinical study on human drug-induced anaphylaxis (mechanisms reviewed in (7)) demonstrated the existence of an IgG-mediated reaction driven by neutrophil activation via their IgG receptors (Fc γ R), which contributes to severe anaphylaxis in patients; including patients lacking biomarkers of IgE-dependent anaphylaxis (8). This IgG-mediated reaction was associated with the release of platelet-activating factor, elastase and neutrophil-extracellular traps (NETs). Serum levels of platelet-activating factor (PAF) have been found to directly correlate with anaphylaxis severity (9), whereas histamine and mast cell tryptase levels do not reflect the severity of reactions (10, 11).

Experimental models of anaphylaxis can be induced following systemic antigen/allergen challenge in mice previously immunized against that antigen/allergen (called active systemic anaphylaxis, ASA). These models largely entail IgG-driven reactions, triggered by IgG-immune complexes forming in circulation and subsequent activation of Fc γ R-expressing myeloid cells. Indeed, for the most part, mouse ASA models proceed independently of IgE antibodies and their receptors (Fc ϵ R) (12, 13). Depending on the experimental anaphylaxis model used, several Fc γ R-expressing cell

112 types have been proposed to drive the anaphylactic response, including neutrophils,
113 monocytes/macrophages, basophils and mast cells (reviewed in (3, 4, 14)). PAF release
114 by neutrophils and monocytes/macrophages, or histamine release by basophils and mast
115 cells, can cause anaphylactic symptoms. In these models, no overt symptoms of
116 anaphylaxis are observed when mice are treated with both PAF and histamine receptor
117 antagonists (13, 15, 16). We and others recently demonstrated that Fc γ R-expressing
118 platelets could also be major players in anaphylaxis induction and severity by releasing
119 serotonin (17, 18) (reviewed in (19)). Thus IgG-mediated anaphylaxis can rely on
120 neutrophils, monocytes/macrophages, basophils and mast cells in humans and wild-type
121 mice, but also on platelets in humans.

122 In mice, four IgG subclasses exist– IgG1, IgG2a/c, IgG2b, IgG3 – without
123 structural or functional equivalence to human IgG subclasses. We have shown that mouse
124 IgG1, IgG2a/c and IgG2b are all capable of triggering anaphylaxis in mice; almost
125 exclusively via mouse Fc γ RIII (20). This reaction can be inhibited by mouse Fc γ RIIB in
126 the case of IgG1 and IgG2b, but not IgG2a. Models of anaphylaxis relying on
127 immunization of mice result in polyclonal IgG responses comprised mostly of IgG1;
128 while IgG2a/c and IgG2b are provoked only by certain adjuvants and/or genetic
129 backgrounds e.g., alum immunization induces IgG1 but not IgG2 antibodies in C57BL/6
130 mice (13).

131 In both humans and mice, IgG-dependent anaphylaxis is considered to occur
132 chiefly when high amounts of antigen/allergen are present in circulation, thereby enabling
133 the formation of a critical amount of circulating IgG-immune complexes; as seen in drug-
134 induced anaphylaxis in humans (8) or in a mouse model of food-induced anaphylaxis, for
135 example (21). This notion has nevertheless been challenged by titrating down the
136 quantities of IgG antibodies and antigen: using cocktails of monoclonal IgG binding

137 different epitopes on a model allergen (OVA), merely tens of micrograms of IgG and
138 micrograms of antigen were sufficient to induce severe IgG-mediated anaphylaxis in mice
139 (22). This study also demonstrated that IgG-mediated anaphylaxis could potentiate IgE-
140 mediated anaphylaxis, in line with our recent results on human cases of severe drug-
141 induced anaphylaxis (8). Anaphylaxis can arise in humans after encounter with inhaled,
142 ingested or injected antigens/allergens (including intravenous, intradermal, subcutaneous,
143 intramuscular routes), and by virtually any route of challenge in sensitized mice, and
144 entail cutaneous, respiratory, oral, gastrointestinal and/or cardiac symptoms. Whether a
145 particular anatomical location favors the initiation of systemic anaphylaxis remains
146 unknown. In the case of IgG-dependent anaphylaxis, the circulatory system and highly
147 vascularized tissues are prime candidates for the site of anaphylaxis initiation because
148 IgG-antigen/allergen immune complexes must be formed prior to interacting with Fc γ R-
149 expressing cells.

150 Here, we sought to interrogate the anatomical location of IgG-dependent
151 anaphylaxis initiation. We postulated that this would correspond to a site with high
152 antigen density after injection and evidence of antigen capture by local Fc γ R-expressing
153 myeloid cells, and their activation. We characterized antigen retention sites *in vivo* in
154 mice and identified the myeloid cell populations responsible for immune complex
155 capture, requirements for Fc γ R expression and reliance on IgG subclasses.

MATERIAL & METHODS

156

157 **Mice**

158 C57BL/6J mice, myeloperoxidase-deficient (MPO^{KO}) mice, and CX3CR1^{GFP} knock-in
159 mice that express EGFP in monocytes, dendritic cells, NK cells, and brain microglia(23)
160 were purchased from Charles River, BALB/cJRj mice from Janvier, and used for
161 experiments after maintaining the mice for at least 1 week in SPF conditions after arrival
162 in Institut Pasteur's animal facility. FcγR^{null} mice (18), NOTAM mice (24), C1q^{KO} mice
163 on the C57BL/6 background (generated by Marina Botto, London, UK) (25) and Gfi-1^{KO}
164 mice on the C57BL/6 background (18, 24-26) were described previously (26). FcγR^{null}
165 C1q^{KO} mice were generated by intercrossing of FcγR^{null} mice and C1q^{KO} mice on a mixed
166 C57BL/6NTac x 129S6/SvEvTac (generated by Regeneron Pharmaceuticals; VG598
167 mice) (27). Mice were bred at Institut Pasteur and used for experiments at 7-11 weeks of
168 age. All animal care and experimentation were conducted in compliance with the
169 guidelines and specific approval of the Animal Ethics committee CETEA (Institut Pasteur,
170 Paris, France) registered under #170043, and by the French Ministry of Research.

171

172 **Reagents**

173 PBS-liposomes and Clodronate-liposomes were purchased from Liposoma (The
174 Netherlands). BSA was from Miltenyi Biotec. OVA, Freund's adjuvant, Alum, luminol
175 sodium salt, ABT-491 and cetirizine were from Sigma-Aldrich. Anti-Ly6G antigen-
176 specific antibody clone NIMP-R14, IgG1 anti-TNP (TIB-191) provided by D. Voehringer
177 (Universitätsklinikum, Erlangen, Germany), IgG2a anti-TNP (Hy1.2) provided by Shozo
178 Izui (University of Geneva, Geneva, Switzerland), and IgG2b anti-TNP (GORK)
179 provided by B. Heyman (Uppsala Universitet, Uppsala, Sweden) were reported
180 previously (20) and purified from hybridoma supernatant on Protein G affinity columns.
181 BSA or OVA were conjugated to VivoTag680 fluorochrome using the VivoTag 680XL
182 Protein Labeling Kit (Perkin Elmer). Antibodies against CD31 (MEC 13.3), Ly6G (1A8),
183 CD11b (M1/70), MHC class II (M5/114), Siglec F (REA798), CD45 (30F11), Ly6C
184 (AL21), CD16/32 (2.4G2) were purchased from eBioscience, Becton Dickinson, Miltenyi
185 Biotec or Biolegend, anti-myeloperoxidase (MPO; AF3667) from R&D Systems.

186

187 **Active Systemic Anaphylaxis**

188 Mice were injected intraperitoneally on day 0 with 200μg BSA in complete Freund's
189 adjuvant (CFA) or Alum, as indicated, and boosted intraperitoneally on day 14 and day

190 21 with 200µg BSA in incomplete Freund's adjuvant (IFA) or Alum, as indicated. Control
191 mice were immunized with OVA instead of BSA following the same protocol. BSA-
192 specific IgG1 and IgG2a/b/c antibodies in serum were titered by ELISA on day 30, as
193 described (13). Mice with comparable antibody titers were challenged i.v. with 500 µg
194 BSA (unless otherwise specified) or 500 µg OVA as control 10 days after the last
195 immunization. Central temperature was monitored using a digital thermometer with rectal
196 probe (YSI), and time of death was recorded. Absolute counts and proportions of
197 granulocytes in peripheral mouse blood were determined using an ABC Vet automatic
198 blood analyzer (Horiba ABX).

199 If indicated, 300µg/mouse anti-Ly6G (NIMP-R14) or corresponding rat IgG2b isotype
200 control mAbs, or 300µL /mouse PBS- or clodronate-liposomes were injected
201 intravenously 24 hours before challenge. In the case of double depletion of neutrophils
202 and monocytes/macrophages, 300µg/mouse anti-Ly6G (NIMP-R14) were injected
203 intravenously 29 hours before challenge, followed 5 hours later by 300µL/mouse
204 clodronate-liposomes intravenously. PAF-R antagonist ABT-491 (25µg/mouse) or H1-
205 receptor antagonist cetirizine DiHCl (300µg/mouse) diluted in 0.9% NaCl were injected
206 intravenously or intraperitoneally, respectively, 15min prior to challenge.

207

208 **Passive Systemic Anaphylaxis**

209 Mouse IgG1, IgG2a or IgG2b anti-TNP antibodies were administered intravenously at a
210 dose of 4 mg, if not otherwise indicated, in 200 µL 0.9% NaCl, followed by an
211 intravenous challenge with 200 µg of the antigen (TNP-BSA) in 0.9% NaCl 16 hours
212 later. Lung and liver tissues were collected 15 min after challenge for further analysis by
213 microscopy.

214

215 **Whole body and tissue fluorescence imaging**

216 Depilated and anesthetized mice were challenged i.v. with 500 µg BSA (composed of 50
217 µg BSA-VT680 + 450 µg unlabeled BSA to avoid instrument saturation) and its
218 anatomical distribution was analyzed on an IVIS 100 (Caliper Life Sciences) using 1 sec
219 exposition time, small binning and 675 – 720 nm filter band. Whole body imaging was
220 performed from 1 min to 30 min post-challenge, then mice were perfused with 10 ml of
221 PBS and tissues (lung, liver, salivary gland, thymus, spleen, kidney, heart, stomach) were
222 collected for ex vivo imaging using the same acquisition settings. Average radiant

223 efficiency (photons/seconds/cm²/surface radiance) of indicated regions of interest were
224 calculated using Living Image software (Perkin Elmer).

225

226 **Whole body bioluminescence imaging of myeloperoxidase activity**

227 Luminol has been proposed as a reporter for myeloperoxidase activity in vivo (28). Mice
228 were injected i.p. with 15 mg/mouse luminol before image acquisition on an IVIS 100
229 (Caliper Life Sciences) using 3 min exposure time and medium binning. For ex vivo
230 imaging on explanted tissue/organs, mice were perfused with 10 ml of PBS, tissues were
231 collected and imaged 20 min after luminol (Sigma Aldrich) injection and 30 min post
232 challenge. Total photon flux of each region of interest was calculated using Living Image
233 software.

234

235 **Immunofluorescence**

236 Fifteen minutes post challenge mice were perfused with 10 ml of PBS, lung and liver
237 tissues were collected and fixed in 1 % paraformaldehyde overnight at 4°C. Tissue
238 dehydration was performed in successive sucrose gradient baths (10%, 20% and 30% for
239 2h each) then tissues were embedded in OCT compound. Eight µm-thick sections were
240 permeabilized with PBS containing 0.1% Saponin and stained with DAPI, anti-CD31
241 antibody (1/500) and/or anti-MPO antibody (4 µg/mL) for 2h at room temperature and
242 revealed with FITC-coupled goat anti-rat Fab fragments (Jackson laboratories). Slides
243 were washed 3 times and mounted with coverslips using Prolong Gold antifade reagent
244 (Invitrogen) with DAPI. Samples were imaged by using a confocal microscope Leica SP5
245 and analyzed using ImageJ software.

246

247 **Flow cytometry**

248 BSA was fluorescently labeled with FITC. Lung cells were freshly isolated using Lung
249 Dissociation kit and gentleMACS dissociator (Miltenyi Biotec). Cells were stained with
250 the following antibodies for 30 min at 4°C: Ly6G (1A8), CD11b (M1/70), MHC class II
251 (M5/114), SiglecF (REA798), CD45 (30F11), Ly6C (AL21), CD16/32 (2.4G2). Data
252 were acquired using MACSQuant (Miltenyi Biotec) flow cytometer and analyzed by
253 FlowJo software. Dead cells, labeled with propidium iodide were excluded from the
254 analysis. Immune cell populations were identified as follows: neutrophils
255 (CD45⁺/CD11b⁺/Ly6G⁺), alveolar macrophages (CD45⁺/CD11b^{mid}/SiglecF^{high}),

256 eosinophils (CD45⁺/CD11b⁺/SiglecF⁺), Ly6C⁺ monocytes (CD45⁺/CD11b⁺/ SiglecF/
257 Ly6G⁻/ MHCII⁻/Ly6C⁺), Ly6C⁻ monocytes (CD45⁺/CD11b⁺/ SiglecF⁻/Ly6G⁻/MHCII⁻
258 /Ly6C⁻), CD11b⁺ dendritic cells (CD45⁺/CD11b⁺/SiglecF⁻/Ly6G⁻/MHCII^{mid}/Ly6C^{+/-}) and
259 interstitial macrophages (CD45⁺/CD11b⁺/SiglecF⁻/Ly6G⁻/MHCII^{high}/Ly6C⁻), B cells
260 (CD45⁺/CD11b⁻/MHCII^{high}).

261

262 **Fluorescence microscopy on fresh lung explant**

263 OVA-sensitized (CFA+IFA) CX3CR1^{GFP} mice were injected i.v. with 5 µg anti-Ly6G-
264 BV421 antibody. Challenge with 500 µg of OVA-VT680 was performed i.v. 5 min later.
265 Five minutes after challenge mice were euthanized and the lungs perfused with 10 ml
266 PBS, explanted and immediately sliced into 300 µm slices using a vibratome (Leica).
267 Samples were glued onto a petri dish filled with 37°C RPMI medium (minus phenol red)
268 using Vetbond (3M Co., Maplewood, MN). Two-photon excitation fluorescence imaging
269 was performed on a Zeiss LSM 710 NLO microscope equipped with a W Plan-
270 Apochromat 20× numerical aperture (NA) 1.0 differential interference contrast (DIC)
271 M27 75-mm water immersion objective. Excitation was produced at 940 nm by a tunable
272 pulsed laser (Chameleon; Coherent, Santa Clara, CA). SHG was epicollected by a
273 dedicated nondescanned detector (NDD) coupled with a 427- ± 20-nm bandpass filter,
274 green fluorescent protein (GFP) by a 500-to-550-nm NDD, rhodamine B or Alexa Fluor
275 568 by a 565-to-610-nm NDD, and Alexa Fluor 633 by a 660-to-730-nm NDD. Z-stacks
276 were rendered using a maximum-intensity projection.

277

278 **Statistical analyses**

279 Statistical analyses were performed with GraphPad Prism version 9.0 (GraphPad
280 Software Inc.). Fluorescence data between two groups were compared with a Mann-
281 Whitney two-tailed test (Fig.1B, 2A-B, 3A, 3D, Supp.1C, Supp.3B, Supp.4A-B).
282 Fluorescence data between more than two groups were compared with a two-way
283 ANOVA (Fig.1D, 3B, 3E, 4C-D, 5C, Supp.1D, Supp.3C (top), Supp.3D, Supp.4C).
284 Survival data between two groups were compared using the Log-rank (Mantel-Cox) test
285 (Supp.3C bottom). Time course responses were compared with an unpaired t-test, two-
286 tailed (Fig.5B).

288 We hypothesized that the anatomical location of IgG-mediated anaphylaxis
289 initiation would correspond to a site with high antigen density immediately after antigen
290 injection. Antigen accumulation would favor local generation of IgG antibody-antigen
291 immune complexes, and subsequent complement activation and/or myeloid cell
292 activation through their Fc γ Rs, leading to clinical signs of anaphylaxis. We therefore took
293 an approach to track the antigen during a mouse model of ASA: we labeled a model
294 antigen, BSA, with a near-infrared fluorochrome compatible with non-invasive whole-
295 body imaging (VivoTag680; VT680). C57BL/6J mice were immunized with BSA or
296 control antigen OVA in Freund's adjuvant (CFA/IFA). Five minutes after challenge with
297 BSA-VT680, we observed an accumulation of fluorescence signal in the liver area using
298 whole body imaging, and in the lung area specifically in BSA-sensitized mice (Fig.1A;
299 Supp.Fig.S1A). Quantification of antigen accumulation in explanted tissues 30 min after
300 challenge demonstrated a 120-fold increase of fluorescence in the lungs of BSA-
301 sensitized mice, but only a ~2-fold increase in the liver, spleen or thymus compared to
302 naive animals (Fig.1B; Supp.Fig.S1B). No increase in antigen-associated fluorescence
303 was detected in the salivary glands or in the stomach, whereas the kidneys and heart
304 showed an inverse ~2-fold reduction in fluorescence in sensitized compared to naive
305 animals (Fig.1B; Supp.Fig.S1C).

306 Immunofluorescence assessment of frozen sections of lung and liver from
307 sensitized mice collected 15 min post-challenge demonstrated the presence of large
308 extracellular aggregates of antigen in the lung alveolar capillaries and liver sinusoids.
309 These aggregates were multifocally distributed exclusively in vascular spaces. Such
310 aggregates were not seen in the lungs of naive mice injected with BSA-VT680, and barely
311 detectable in the liver (Fig.1C). In sensitized mice, the accumulation of antigen in the

312 lung was dose-dependent, whereas the degree to which antigen was detected in the liver
313 did not change with varying antigen dose (Fig.1D), nor in other tissues (Supp.Fig.1D).
314 Of note, challenge with a low dose (10 μ g) of antigen resulted in minimal antigen
315 accumulation in the lung 30 min after challenge (Fig.1D) and did not significantly reduce
316 central body temperature (Fig.1E), a hallmark of anaphylaxis in the mouse. Higher
317 antigen doses (i.e., 50 and 500 μ g) induced significant antigen accumulation in the lung
318 and >5°C drop of body temperature, with up to 50% mortality in mice challenged with
319 500 μ g antigen (Fig.1D-E). Altogether, these experiments indicate that antigen
320 accumulates specifically and dose-dependently in the lung during experimental active
321 systemic anaphylaxis, with massive antigen aggregates observed in the lung vasculature.

322 We next investigated if antigen was associating with specific immune cell
323 populations in the lung. We examined cell populations reported to be involved in
324 experimental anaphylaxis, i.e., neutrophils, macrophages (alveolar and interstitial),
325 Ly6C^{lo} and Ly6C^{hi} monocytes, but also Fc γ R-expressing eosinophils, CD11b⁺ dendritic
326 cells (DCs) and B cells. To ensure similar proportions of immune cells and total
327 circulating antibody between experimental and control groups, mice were either
328 immunized with BSA and challenged with FITC-labeled BSA (experimental group,
329 leading to BSA-immune complex formation and anaphylaxis) or immunized with OVA
330 and challenged with FITC-labeled BSA (control group, no immune complexes and no
331 anaphylaxis). Among all the cell types examined, the numbers of Ly6G⁺ neutrophils,
332 Ly6C^{hi} monocytes and Ly6C^{lo} monocytes significantly increased in the lung during
333 anaphylaxis (Fig.2A, gating information in Supp.Fig.2A). >95% of neutrophils and
334 Ly6C^{hi} monocytes were antigen-positive, along with the vast majority of interstitial
335 macrophages, eosinophils and alveolar macrophages, and 20% of B cells (Fig.2B).
336 Neutrophils, interstitial macrophages and Ly6C^{hi} monocytes exhibited the highest amount

337 of antigen-associated fluorescence on a per cell basis (Supp.Fig.2B-C) As a proportion of
338 the antigen-capturing cells, however, neutrophils and Ly6C^{hi} monocytes represented
339 >20% and >10%, respectively, whereas Ly6C^{lo} monocytes were barely detectable. ~40%
340 of all antigen-capturing cells were alveolar macrophages and interstitial macrophages
341 (Fig.2C).

342 To further investigate the anatomical distribution of antigen aggregates and
343 antigen-capturing cells, we performed 2-photon microscopy analysis of lung explants of
344 OVA-sensitized CX3CR1^{GFP} mice that express enhanced GFP (EGFP) in monocytes,
345 dendritic cells and a minor subset of NK cells, DCs or macrophages (29, 30) (but neither
346 neutrophils, eosinophils, B cells, T cells nor alveolar macrophages) (23), and which were
347 injected with BrilliantViolet421-labelled anti-Ly6G to visualize neutrophils in another
348 channel. As expected, labeled OVA antigen (OVA-VT680) could be readily imaged in
349 the lung explants of OVA-sensitized mice, but not in non-sensitized mice, taken 5 min
350 after OVA challenge. We observed accumulation of GFP⁺ cells and neutrophils in the
351 lungs of immunized and challenged mice, with evidence of antigen-neutrophil-GFP⁺ cell
352 foci and antigen capture by neutrophils (Fig.2D) and GFP⁺ cells (Supp.Fig.3A). The
353 velocity of neutrophils, but not of CX3CR1⁺ cells, was also significantly increased within
354 the lung tissue (Supp.Fig.3B) suggestive of persistent neutrophil recruitment and
355 activation. These experiments indicate that neutrophils and inflammatory monocytes
356 infiltrate the lung and take up antigen during anaphylaxis and represent, together with
357 tissue-resident monocytes/macrophages, grossly 80-90% of antigen-capturing cells.

358 We have previously found that FcγR-expression decreased on neutrophils and
359 monocytes during experimental anaphylaxis in mice expressing human FcγRs (31), and
360 in patients suffering from drug-induced anaphylaxis (8), due to receptor aggregation on
361 the cell surface and subsequent cell activation and endocytosis of antigen-IgG immune

362 complexes. As a simple readout of mouse FcγR engagement on lung resident or
363 infiltrating cells, we analyzed their surface expression of activating FcγRIII, the main IgG
364 receptor involved in IgG-anaphylaxis in mice (14), using an antibody recognizing both
365 FcγRIII and inhibitory FcγRIIB. FcγRIII/FcγRIIB expression decreased significantly
366 (1.5-2-fold) on neutrophils, Ly6C^{hi} and Ly6C^{lo} monocytes, eosinophils and B cells within
367 15 min of anaphylaxis induction, compared to cells from control animals immunized
368 against an alternate antigen (Fig.3A). Thus IgG-antigen immune complexes engage FcγR
369 on immune cells in the lung rapidly after challenge.

370 To understand if antigen accumulation in the lungs was dependent on immune
371 complex retention via FcγRs, we immunized and challenged FcγR^{null} mice, which are
372 knocked-out for FcγRI, FcγRIIB, FcγRIII and FcγRIV (18). Surprisingly, antigen
373 accumulation in the lung was unchanged in FcγR^{null} mice compared to wt mice (Fig.3B).
374 The same result was obtained in NOTAM mice (32) that express normal surface levels of
375 activating FcγRs, but lack functional signaling through the essential ITAM of the FcRγ
376 subunit associated with FcγRI, FcγRIII and FcγRIV. Complement component C1q-
377 deficient (C1q^{KO}) mice and double-deficient FcγR^{null}C1q^{KO} mice also demonstrated lung
378 antigen accumulation similar to wt mice after immunization and challenge (Fig.3B).
379 Neither FcγR^{null} mice nor NOTAM mice presented signs of anaphylaxis after antigen
380 challenge, whereas C1qKO mice developed temperature drop and mortality comparable
381 to wt mice (Fig.3C). Thus, antigen retention in the lung during anaphylaxis induction is
382 not contingent upon immune complex-mediated, FcγR-dependent cell activation, nor
383 complement component C1q (7, 33).

384 We previously reported that a concomitant depletion of neutrophils (using anti-
385 Ly6C/G antibodies) and monocytes/macrophages (using clodronate liposomes) in mice
386 protects from active systemic anaphylaxis (13). Here, we show that neutrophils and

387 monocytes/macrophages contribute to a large extent to antigen capture in the lung
388 (Fig.2B-D; Supp.Fig.2B-C,3A). Antigen accumulation in the lung was, however, not
389 impacted in the absence of mature neutrophils, using mice deficient for the transcriptional
390 repressor Gfi-1 (26) (Fig.3D), or following the depletion of neutrophils alone or of both
391 monocyte/macrophages and neutrophils before challenge (Fig.3E). Neutrophil-deficient
392 Gfi-1^{-/-} mice were nonetheless significantly protected from the loss of temperature and
393 mortality associated with anaphylaxis challenge, compared to Gfi-1^{+/-} littermate controls
394 (Supp.Fig.3C). Accumulation of antigen in the lungs was also unaffected by PAF or
395 histamine receptor blockade, although injection with PAF-receptor (ABT-491) or
396 histamine-receptor (cetirizine) antagonists inhibited anaphylaxis symptoms
397 (Supp.Fig.3D-E) (15, 33). Altogether, these results demonstrate that specific antigen
398 accumulation in the lung after challenge of immunized mice does not rely on FcγRs,
399 complement component C1q, neutrophils or monocyte/macrophages, nor on their
400 activation, or on the anaphylaxis mediators PAF and histamine.

401 The composition or size of the immune complexes formed after challenge in this
402 anaphylaxis model could determine antigen retention in the lung vasculature, conceivably
403 via physical restraints in the small capillaries. We therefore investigated antigen retention
404 after immunization with an alternative adjuvant, alum, that is expected to induce lower
405 levels and different subclasses of IgG, compared to Freund's adjuvant (13); and that will
406 likely result in the formation of immune complexes of a different composition or size
407 after challenge. Anaphylactic reactions in alum-immunized C57BL/6J mice after antigen
408 challenge were of similar intensity to that of CFA/IFA-immunized mice (Fig.4A) and
409 also relied on PAF and histamine (Supp.Fig.3E); yet did not lead to specific antigen
410 retention in the lung (Fig.4B-C). The difference in lung antigen retention between mice
411 challenged after CFA/IFA compared to alum immunization was also observed in FcγR^{null}

412 mice bred on a C57BL/6 dominant mixed background (C57BL/6N + C57BL/6J +
413 129S6/SvEvTac) and in C57BL/6 Gfi-1^{-/-} mice (Supp.Fig.4A-B). However, when we
414 performed alum immunization and challenge in BALB/c mice, we observed antigen
415 retention in the lungs with a strikingly similar pattern and intensity as that following
416 CFA/IFA immunization and challenge in C57BL/6J mice (Fig.4B-C). Alum
417 immunizations in C57BL/6J mice, compared to BALB/c mice, lead to significantly lower
418 antigen-specific total IgG responses, 4-fold lower IgG1 responses, undetectable IgG2a/c
419 and IgG2b responses (Fig.4D), as we reported earlier (13), and 2-fold lower IgG3
420 responses (Supp.Fig.4C).

421 Next, we tested if antigen-specific mouse IgG2 could be responsible for antigen
422 retention in the lung by employing a passive systemic anaphylaxis model based on
423 administration of anti-trinitrophenyl (TNP) IgG1, IgG2a and IgG2b mAbs, followed by
424 challenge with TNP-labeled BSA 18h later. Importantly, these antibodies all have similar
425 affinity for TNP (20). Whereas mouse IgG2a anti-TNP administered at doses of 1, 2 and
426 4 mg generated equivalent antigen aggregates in the liver, the 4 mg dose led to significant
427 antigen aggregates in the lung capillaries after antigen challenge (Supp.Fig.4D, Fig.4E).
428 Under the same conditions, neither anti-TNP IgG1 nor IgG2b lead to any detectable
429 antigen aggregates in the lung (Fig.4E). These results demonstrate that IgG2a antibodies
430 can mediate antigen retention in the lung; and suggest that IgG2a may also be responsible
431 for antigen retention in the lung in antigen-immunized and challenged mice.

432 We reported previously that active systemic anaphylaxis following CFA/IFA
433 immunization and challenge relied predominantly on neutrophils and PAF release, with
434 the release of the neutrophil enzyme myeloperoxidase (MPO) detected within minutes
435 after antigen challenge (13). MPO activity can be monitored *in vivo* via luminescence
436 generated by the oxidation of luminol (28) and can thus be considered a real-time

437 neutrophil activation marker. We found that the main anatomical sites of MPO activity
438 after challenge were the left and right lungs (Fig.5A): luminescence was readily
439 detectable at 2 min and remained significantly elevated for at least 20 min after challenge
440 (Fig.5B). Luminol oxidation was dependent on the presence of MPO and neutrophils: as
441 expected, immunized MPO^{KO} mice and neutrophil-deficient Gfi-1^{KO} mice did not display
442 elevated luminescence in the lungs over baseline unimmunized wt mice (Fig.5C),
443 although both of these mouse strains do undergo ASA after antigen challenge
444 (Supp.Fig.3C and Supp.Fig.4E). In wt mice undergoing ASA, MPO foci were detected
445 in the lungs by immunofluorescence, but not in the lungs of control mice (Fig.5D).
446 Altogether, these results suggest that antigen-specific antibodies within immune
447 complexes allow antigen retention in the lung capillaries, leading to local neutrophil
448 activation and degranulation.

449

DISCUSSION

450 We demonstrate here that the initiation of an IgG-dependent active systemic
451 anaphylactic reaction in mice coincides with antigen retention specifically in the lungs.
452 Lungs present with large antigen aggregates in the capillaries, myeloid cell recruitment
453 and antigen capture predominantly by neutrophils and monocyte/macrophage
454 populations. Antigen retention in the lung was observed independently of activating IgG
455 receptors (Fc γ R), complement component C1q, neutrophil and monocyte/macrophage
456 depletion. IgG2a-mediated passive systemic anaphylaxis, but neither IgG1- nor IgG2b-
457 driven reactions, led to antigen retention in the lung. Antigen accumulation in the lung
458 was associated with local neutrophil activation, which may contribute to pulmonary
459 damage and anaphylaxis severity.

460 Clinical signs of anaphylaxis are driven by the release of anaphylactogenic
461 mediators from myeloid cells (34, 35) downstream of specific antigen recognition by
462 antibodies and their corresponding receptors. Active mouse models of anaphylaxis entail
463 a polyclonal antibody response, the relative composition of which is determined, among
464 other factors, by the use of particular adjuvants (16), and genetic backgrounds that favor
465 certain antibody classes and subclasses. The large majority of these anaphylaxis models
466 rely on IgG- rather than IgE-mediated mechanisms, because mice lacking IgG receptors
467 are resistant to most anaphylaxis models, whereas mice lacking IgE receptors remain
468 susceptible (14). The contribution of any given cell population is therefore determined by
469 their expression of activating Fc γ Rs and/or Fc ϵ RI, the capacity of the cells to release
470 anaphylactogenic mediators, and a cells' potential for negative inhibition of FcR
471 signaling by expression of the inhibitory IgG receptor Fc γ RIIB (20, 36). In wild-type
472 mice, pathways of active systemic anaphylaxis and passive IgG anaphylaxis rely
473 predominantly on monocyte and/or neutrophil activation via Fc γ RIII, with a minor

474 contribution of FcγRIV, and subsequent PAF release (13, 15, 37). Herein, we employed
475 a model of IgG-dependent active anaphylaxis. Mice were immunized with relatively large
476 amounts of antigen (200 μg) in Freund's adjuvant or alum, leading to an antibody
477 response and anaphylaxis induction upon antigen re-exposure. Anaphylaxis
478 predominantly relied on IgG, because FcγR^{null} mice were resistant to anaphylaxis,
479 excluding a significant contribution of antigen specific IgE and FcεRI. Although
480 complement component C1q may contribute to myeloid cell activation through bridging
481 immune complexes and complement receptors (38), C1q-deficiency did not alter
482 anaphylaxis kinetics or severity in this model.

483 Immune complexes can form in both circulation and tissues after antigen
484 encounter with circulating specific IgG. IgG concentrations are significantly higher in the
485 blood than in tissues, however, which would favor IgG-antigen encounter in circulation
486 or in highly vascularized tissues. Furthermore, as antigen was injected intravenously in
487 this study, it is likely that IC formation starts in the vasculature. We indeed detected
488 higher antigen levels in highly vascularized organs such as the lung, liver and spleen, but
489 also in the thymus of sensitized compared to non-sensitized mice; together with lower
490 detectable antigen levels in the kidney and heart. Surprisingly, among all these tissues,
491 only in the lungs did antigen accumulation increase dose-dependently, and >100-fold
492 more in sensitized compared to non-sensitized mice. Overall, lung antigen retention
493 reached >10-fold higher than any other tissue. Supporting our findings in mice, antigen-
494 antibody aggregates have been reported in the lungs of immunized rabbits that were
495 intravenously challenged with antigen, principally in regions of peri-bronchial vascular
496 dilatation and edema (39, 40). Apart from the lung, antigen accumulation was highest in
497 the liver compared to other tissues after intravenous antigen injection; yet liver antigen
498 levels were equivalent between naive and immunized mice.

499 Lung antigen retention required the presence of antigen-specific antibodies.
500 Physical rather than biological constraints may provoke antigen retention in the lung, due
501 to the small size of some lung vessels (41) that could favor aggregates or clots of immune
502 complexes. In this study we used antigens of two different sizes, OVA (45 kD) and BSA
503 (66 kD), however no difference in antigen accumulation was observed between mice
504 challenged with the different antigens. Moreover, physical constraints alone could not
505 explain our findings using a passive systemic anaphylaxis model in which neither IgG1-
506 nor IgG2b-immune complexes, but only IgG2a-immune complexes, were retained in the
507 lungs. Coherent with this finding, C57BL/6 mice immunized with alum – eliciting IgG1
508 but not IgG2 antibodies to antigen – also lacked antigen retention in the lung. On the
509 contrary, BALB/c mice immunized with alum – generating IgG1, IgG2a and IgG2b
510 antibodies (as in C57BL/6 mice immunized with Freund’s adjuvant) – demonstrated
511 antigen retention in the lung after challenge. Lung antigen retention during these
512 anaphylaxis models might therefore rely on the quality of the antibody response, for
513 example the presence of IgG2a antibodies. Certainly, the degree of antigen retention
514 seems to depend on the amount of circulating antibody, because high levels of injected
515 IgG2a were necessary to see antigen accumulation in the lung in the passive anaphylaxis
516 model.

517 Reminiscent of our findings, Cloutier *et al* reported platelet sequestration and
518 thrombi formation in the lungs in a mouse model of systemic immune complex-induced
519 shock (17). Although this model required Fc γ R expression on platelets (via transgenic
520 expression of human Fc γ RIIA/CD32A), and platelet activation led to local serotonin
521 release and anaphylactic-like clinical symptoms, in parallel to our work these authors also
522 identified that immune complex-containing thrombi in the lung were not required for
523 anaphylaxis to occur. However, both in our study and in the Cloutier study (17), immune

524 complex accumulation in the lung did lead to local neutrophil activation and
525 degranulation that may contribute to pulmonary damage and the severity of immune
526 complex-induced anaphylaxis.

527 IgG-containing immune complexes are the primary ligands for FcγR. High-
528 affinity IgG receptors are able to bind monomeric IgG, like FcγRI for IgG2a and FcγRIV
529 for IgG2a and IgG2b, whereas low-affinity receptors like mouse FcγRIIB and FcγRIII
530 are unable to bind monomeric IgG (42). All these receptors bind immune complexes made
531 of mouse IgG2a and IgG2b, but only FcγRIIB and FcγRIII also bind immune complexes
532 made of mouse IgG1(43). In this study, we demonstrate significantly reduced
533 FcγRIIB/FcγRIII expression on neutrophils, Ly6C^{hi} and Ly6C^{lo} monocytes and
534 eosinophils during anaphylaxis, which is considered a marker of cell involvement in
535 immune complex-mediated reactions (20, 44). Among these cells, neutrophils and Ly6C^{hi}
536 monocytes were actively recruited to the lung, and bound and engulfed antigen, and
537 together constituted 40% of antigen-capturing cells. Supporting these findings, lung
538 neutrophilia was reported previously in a model of casein-induced anaphylaxis (45).

539 Both neutrophils and monocytes have been described, by us and others, to
540 contribute to IgG-dependent active anaphylaxis using depletion and reconstitution
541 experiments (13, 15). Yet here we demonstrate that neither cell type is required for
542 antigen retention in the lung. Neutrophils were necessary for local MPO release in the
543 lung during anaphylaxis that was detectable (and maximal) already 2 minutes post-
544 challenge. Nevertheless, MPO was not required for anaphylaxis to occur. MPO, together
545 with PAF release, by neutrophils in the lung and vasculature has been proposed to
546 contribute to platelet activation and favor neutrophil-platelet aggregates and clotting,
547 thereby participating in systemic mediator release (45).

548 Our data indicate that, within minutes of injection, circulating antigen formed
549 aggregates in the lung and liver vasculature of immunized mice, with a dose-dependent
550 accumulation exclusively in the lung. Neutrophils and monocytes recruited to the lung
551 captured antigen and became activated. Antigen aggregation in the lung vasculature was
552 not necessary for anaphylaxis to occur, but could be responsible for local damage. Results
553 from a passive systemic anaphylaxis model suggest that antigen retention may rely on the
554 presence of antibodies of a single IgG subclass, IgG2a, that enable Fc γ R- and C1q-
555 independent formation of large aggregates in the lung. The mechanism behind IgG2a-
556 mediated lung-specific antigen retention remains to be determined, and if a similar
557 mechanism exists in humans for one or several human IgG subclasses.

558

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563

564

AUTHORSHIP

565 BT performed most experiments, with contributions from OG, EC, CMG, DF, BI
566 and ORL; AJM, LEM and JL designed mouse targeting and generated mouse strains; BT,
567 LR, FJ and PB analyzed and discussed results; LR, FJ and PB supervised the research.
568 PB and BT prepared the figures. PB designed the research and secured funding. PB wrote
569 the manuscript with the help of CMG. All authors read and reviewed the manuscript
570 before submission.

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- 584 1. Turner, P. J., E. Jerschow, T. Umasunthar, R. Lin, D. E. Campbell, and R. J. Boyle.
585 2017. Fatal Anaphylaxis: Mortality Rate and Risk Factors. *J Allergy Clin Immunol*
586 *Pract* 5: 1169-1178.
- 587 2. Cardona, V., I. J. Ansotegui, M. Ebisawa, Y. El-Gamal, M. Fernandez Rivas, S.
588 Fineman, M. Geller, A. Gonzalez-Estrada, P. A. Greenberger, M. Sanchez Borges,
589 G. Senna, A. Sheikh, L. K. Tanno, B. Y. Thong, P. J. Turner, and M. Worm. 2020.
590 World allergy organization anaphylaxis guidance 2020. *World Allergy Organ J*
591 13: 100472.
- 592 3. Reber, L. L., J. D. Hernandez, and S. J. Galli. 2017. The pathophysiology of
593 anaphylaxis. *J Allergy Clin Immunol* 140: 335-348.
- 594 4. Finkelman, F. D., M. V. Khodoun, and R. Strait. 2016. Human IgE-independent
595 systemic anaphylaxis. *J Allergy Clin Immunol* 137: 1674-1680.
- 596 5. Munoz-Cano, R., C. Picado, A. Valero, and J. Bartra. 2016. Mechanisms of
597 Anaphylaxis Beyond IgE. *J Investig Allergol Clin Immunol* 26: 73-82; quiz 72p
598 following 83.
- 599 6. Cianferoni, A. 2021. Non-IgE-mediated anaphylaxis. *J Allergy Clin Immunol* 147:
600 1123-1131.
- 601 7. Bruhns, P., and S. Chollet-Martin. 2021. Mechanisms of human drug-induced
602 anaphylaxis. *J Allergy Clin Immunol* 147: 1133-1142.
- 603 8. Jonsson, F., L. de Chaisemartin, V. Granger, A. Gouel-Cheron, C. M. Gillis, Q.
604 Zhu, F. Dib, P. Nicaise-Roland, C. Ganneau, M. Hurtado-Nedelec, C. Paugam-
605 Burtz, S. Necib, H. Keita-Meyer, M. Le Dorze, B. Cholley, O. Langeron, L. Jacob,
606 B. Plaud, M. Fischler, C. Sauvan, M. T. Guinépain, P. Montravers, M. Aubier,
607 S. Bay, C. Neukirch, F. Tubach, D. Longrois, S. Chollet-Martin, and P. Bruhns.
608 2019. An IgG-induced neutrophil activation pathway contributes to human drug-
609 induced anaphylaxis. *Sci Transl Med* 11.
- 610 9. Vadas, P., B. Perelman, and G. Liss. 2013. Platelet-activating factor, histamine,
611 and tryptase levels in human anaphylaxis. *J Allergy Clin Immunol* 131: 144-149.
- 612 10. Dybendal, T., A. B. Guttormsen, S. Elsayed, B. Askeland, T. Harboe, and E.
613 Florvaag. 2003. Screening for mast cell tryptase and serum IgE antibodies in 18
614 patients with anaphylactic shock during general anaesthesia. *Acta Anaesthesiol*
615 *Scand* 47: 1211-1218.
- 616 11. Laroche, D., P. Gomis, E. Gallimidi, J. M. Malinovsky, and P. M. Mertes. 2014.
617 Diagnostic value of histamine and tryptase concentrations in severe anaphylaxis
618 with shock or cardiac arrest during anesthesia. *Anesthesiology* 121: 272-279.
- 619 12. Oettgen, H. C., T. R. Martin, A. Wynshaw-Boris, C. Deng, J. M. Drazen, and P.
620 Leder. 1994. Active anaphylaxis in IgE-deficient mice. *Nature* 370: 367-370.
- 621 13. Jönsson, F., D. A. Mancardi, Y. Kita, H. Karasuyama, B. Iannascoli, N. Van
622 Rooijen, T. Shimizu, M. Daëron, and P. Bruhns. 2011. Mouse and human
623 neutrophils induce anaphylaxis. *J Clin Invest* 121: 1484-1496.
- 624 14. Gillis, C. M., A. Gouel-Chéron, and P. Bruhns. 2015. Anaphylaxis (Immediate
625 Hypersensitivity): From Old to New Mechanisms. *Encyclopedia of Inflammatory*
626 *Diseases*.
- 627 15. Strait, R. T., S. C. Morris, M. Yang, X. W. Qu, and F. D. Finkelman. 2002.
628 Pathways of anaphylaxis in the mouse. *J Allergy Clin Immunol* 109: 658-668.
- 629 16. Balbino, B., R. Sibilano, P. Starkl, T. Marichal, N. Gaudenzio, H. Karasuyama, P.
630 Bruhns, M. Tsai, L. L. Reber, and S. J. Galli. 2017. Pathways of immediate

- 631 hypothermia and leukocyte infiltration in an adjuvant-free mouse model of
632 anaphylaxis. *J Allergy Clin Immunol* 139: 584-596 e510.
- 633 17. Cloutier, N., I. Allaeys, G. Marcoux, K. R. Machlus, B. Mailhot, A. Zufferey, T.
634 Levesque, Y. Becker, N. Tessandier, I. Melki, H. Zhi, G. Poirier, M. T. Rondina,
635 J. E. Italiano, L. Flamand, S. E. McKenzie, F. Cote, B. Nieswandt, W. I. Khan, M.
636 J. Flick, P. J. Newman, S. Lacroix, P. R. Fortin, and E. Boilard. 2018. Platelets
637 release pathogenic serotonin and return to circulation after immune complex-
638 mediated sequestration. *Proc Natl Acad Sci U S A* 115: E1550-E1559.
- 639 18. Beutier, H., B. Hechler, O. Godon, Y. Wang, C. M. Gillis, L. de Chaisemartin, A.
640 Gouel-Cheron, S. Magnenat, L. E. Macdonald, A. J. Murphy, N. s. group, S.
641 Chollet-Martin, D. Longrois, C. Gachet, P. Bruhns, and F. Jonsson. 2018. Platelets
642 expressing IgG receptor FcγRIIIA/CD32A determine the severity of
643 experimental anaphylaxis. *Sci Immunol* 3.
- 644 19. Godon, O., B. Hechler, and F. Jonsson. 2021. The role of IgG subclasses and
645 platelets in experimental anaphylaxis. *J Allergy Clin Immunol* 147: 1209-1211.
- 646 20. Beutier, H., C. M. Gillis, B. Iannascoli, O. Godon, P. England, R. Sibilano, L. L.
647 Reber, S. J. Galli, M. S. Cragg, N. Van Rooijen, D. A. Mancardi, P. Bruhns, and
648 F. Jonsson. 2017. IgG subclasses determine pathways of anaphylaxis in mice. *J*
649 *Allergy Clin Immunol* 139: 269-280 e267.
- 650 21. Strait, R. T., A. Mahler, S. Hogan, M. Khodoun, A. Shibuya, and F. D. Finkelman.
651 2011. Ingested allergens must be absorbed systemically to induce systemic
652 anaphylaxis. *J Allergy Clin Immunol* 127: 982-989 e981.
- 653 22. Ishikawa, R., Y. Tsujimura, K. Obata, Y. Kawano, Y. Minegishi, and H.
654 Karasuyama. 2010. IgG-mediated systemic anaphylaxis to protein antigen can be
655 induced even under conditions of limited amounts of antibody and antigen.
656 *Biochem Biophys Res Commun* 402: 742-746.
- 657 23. Jung, S., J. Aliberti, P. Graemmel, M. J. Sunshine, G. W. Kreutzberg, A. Sher,
658 and D. R. Littman. 2000. Analysis of fractalkine receptor CX(3)CR1 function by
659 targeted deletion and green fluorescent protein reporter gene insertion. *Mol Cell*
660 *Biol* 20: 4106-4114.
- 661 24. de Haij, S., J. H. Jansen, P. Boross, F. J. Beurskens, J. E. Bakema, D. L. Bos, A.
662 Martens, J. S. Verbeek, P. W. Parren, J. G. van de Winkel, and J. H. Leusen. 2010.
663 In vivo cytotoxicity of type I CD20 antibodies critically depends on Fc receptor
664 ITAM signaling. *Cancer Res* 70: 3209-3217.
- 665 25. Botto, M., C. Dell'Agnola, A. E. Bygrave, E. M. Thompson, H. T. Cook, F. Petry,
666 M. Loos, P. P. Pandolfi, and M. J. Walport. 1998. Homozygous C1q deficiency
667 causes glomerulonephritis associated with multiple apoptotic bodies. *Nat Genet*
668 19: 56-59.
- 669 26. Yucel, R., C. Kosan, F. Heyd, and T. Moroy. 2004. Gfi1:green fluorescent protein
670 knock-in mutant reveals differential expression and autoregulation of the growth
671 factor independence 1 (Gfi1) gene during lymphocyte development. *J Biol Chem*
672 279: 40906-40917.
- 673 27. Balbino, B., P. Herviou, O. Godon, J. Stackowicz, O. R. Goff, B. Iannascoli, D.
674 Sterlin, S. Brule, G. A. Millot, F. M. Harris, V. A. Voronina, K. C. Nadeau, L. E.
675 Macdonald, A. J. Murphy, P. Bruhns, and L. L. Reber. 2020. The anti-IgE mAb
676 omalizumab induces adverse reactions by engaging Fcγ receptors. *J Clin*
677 *Invest* 130: 1330-1335.
- 678 28. Gross, S., S. T. Gammon, B. L. Moss, D. Rauch, J. Harding, J. W. Heinecke, L.
679 Ratner, and D. Piwnicka-Worms. 2009. Bioluminescence imaging of
680 myeloperoxidase activity in vivo. *Nat Med* 15: 455-461.

- 681 29. Liu, Z., Y. Gu, S. Chakarov, C. Bleriot, I. Kwok, X. Chen, A. Shin, W. Huang, R.
682 J. Dress, C. A. Dutertre, A. Schlitzer, J. Chen, L. G. Ng, H. Wang, Z. Liu, B. Su,
683 and F. Ginhoux. 2019. Fate Mapping via Ms4a3-Expression History Traces
684 Monocyte-Derived Cells. *Cell* 178: 1509-1525 e1519.
- 685 30. Abram, C. L., G. L. Roberge, Y. Hu, and C. A. Lowell. 2014. Comparative
686 analysis of the efficiency and specificity of myeloid-Cre deleting strains using
687 ROSA-EYFP reporter mice. *J Immunol Methods* 408: 89-100.
- 688 31. Gillis, C. M., F. Jonsson, D. A. Mancardi, N. Tu, H. Beutier, N. Van Rooijen, L.
689 E. Macdonald, A. J. Murphy, and P. Bruhns. 2017. Mechanisms of anaphylaxis
690 in human low-affinity IgG receptor locus knock-in mice. *J Allergy Clin Immunol*
691 139: 1253-1265 e1214.
- 692 32. Boross, P., N. van Montfoort, D. A. Stapels, C. E. van der Poel, C. Bertens, J.
693 Meeldijk, J. H. Jansen, J. S. Verbeek, F. Ossendorp, R. Wubbolts, and J. H.
694 Leusen. 2014. FcRgamma-chain ITAM signaling is critically required for cross-
695 presentation of soluble antibody-antigen complexes by dendritic cells. *J Immunol*
696 193: 5506-5514.
- 697 33. Bruhns, P., and F. Jonsson. 2015. Mouse and human FcR effector functions.
698 *Immunol Rev* 268: 25-51.
- 699 34. Lee, J. K., and P. Vadas. 2011. Anaphylaxis: mechanisms and management. *Clin*
700 *Exp Allergy* 41: 923-938.
- 701 35. Brown, S. G., S. F. Stone, D. M. Fatovich, S. A. Burrows, A. Holdgate, A. Celenza,
702 A. Coulson, L. Hartnett, Y. Nagree, C. Cotterell, and G. K. Isbister. 2013.
703 Anaphylaxis: clinical patterns, mediator release, and severity. *J Allergy Clin*
704 *Immunol* 132: 1141-1149 e1145.
- 705 36. Smith, K. G., and M. R. Clatworthy. 2010. FcgammaRIIB in autoimmunity and
706 infection: evolutionary and therapeutic implications. *Nat Rev Immunol* 10: 328-
707 343.
- 708 37. Khodoun, M. V., Z. Y. Kucuk, R. T. Strait, D. Krishnamurthy, K. Janek, C. D.
709 Clay, S. C. Morris, and F. D. Finkelman. 2013. Rapid desensitization of mice with
710 anti-FcgammaRIIb/FcgammaRIII mAb safely prevents IgG-mediated
711 anaphylaxis. *J Allergy Clin Immunol* 132: 1375-1387.
- 712 38. Holers, V. M. 2014. Complement and Its Receptors: New Insights Into Human
713 Disease. *Annu Rev Immunol*.
- 714 39. Dixon, F. J. 1953. The use of I131 in immunologic investigation. *J Allergy* 24:
715 547-555.
- 716 40. Mc, K. G., E. C. Andrews, Jr., R. H. Heptinstall, and F. G. Germuth, Jr. 1957. An
717 immunohistologic study on the occurrence of intravascular antigen-antibody
718 precipitation and its role in anaphylaxis in the rabbit. *Bull Johns Hopkins Hosp*
719 101: 258-280.
- 720 41. Townsley, M. I. 2012. Structure and composition of pulmonary arteries,
721 capillaries, and veins. *Compr Physiol* 2: 675-709.
- 722 42. Bruhns, P. 2012. Properties of mouse and human IgG receptors and their
723 contribution to disease models. *Blood* 119: 5640-5649.
- 724 43. Wang, Y., V. Kremer, B. Iannascoli, O. R. Goff, D. A. Mancardi, L. Ramke, L.
725 de Chaisemartin, P. Bruhns, and F. Jonsson. 2022. Specificity of mouse and
726 human Fcgamma receptors and their polymorphic variants for IgG subclasses of
727 different species. *Eur J Immunol*.
- 728 44. Khodoun, M. V., R. Strait, L. Armstrong, N. Yanase, and F. D. Finkelman. 2011.
729 Identification of markers that distinguish IgE- from IgG-mediated anaphylaxis.
730 *Proc Natl Acad Sci U S A* 108: 12413-12418.

- 731 45. Krishnamurthy, D., P. Starkl, K. Szalai, F. Roth-Walter, S. C. Diesner, M.
732 Mittlboeck, C. Mannhalter, E. Untersmayr, and E. Jensen-Jarolim. 2012.
733 Monitoring neutrophils and platelets during casein-induced anaphylaxis in an
734 experimental BALB/c mouse model. *Clin Exp Allergy* 42: 1119-1128.
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FIGURE LEGENDS

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739 **FIG 1. Specific antigen accumulation in the lung during active systemic anaphylaxis.**

740 A, In vivo quantification of antigen in sensitized mice before (-) or 5 min after intravenous

741 BSA-VT680 (500 μ g) challenge. B, 30 min post-challenge (500 μ g BSA-VT680) ex vivo

742 quantification of BSA-VT680 in explanted organs. C, 15 min post-challenge (500 μ g

743 BSA-VT680) immunofluorescence on frozen sections. Scale bar = 20 μ m. Arrows

744 indicate antigen aggregates. D, 30 min post-challenge ex vivo quantification of BSA-

745 VT680 in explanted organs. E, Post-challenge (BSA) changes in body temperature. (B,D)

746 Data are pooled from 2 or 3 independent experiments (n = 5-14 per group). (B,D)

747 Individual measurements, (E) mean \pm SEM and p values are indicated: ns: non-significant,

748 *P < .05, ***P < .001 or ****P < .0001.

749

750 **FIG 2. Infiltrating myeloid cells capture antigen in the lung during active systemic**

751 **anaphylaxis.** A-C, Flow cytometry quantification of (A) cell numbers, (B) proportion of

752 antigen-positive cells of a given cell type (Ag⁺) and (C) proportion of different cell types

753 among Ag⁺ cells in the lungs 15 min post-challenge with FITC-labeled BSA in (B) OVA-

754 or BSA-sensitized, and (C) BSA-sensitized mice. D, Two-photon microscopy images of

755 fresh lung slices 5 min post-challenge with BSA-VT680 in CX3CR1-GFP mice injected

756 with anti-Ly6G-BV421 mAb to label neutrophils. Arrows indicate evidence of antigen

757 uptake by Ly6G⁺ cells (A-C) Data are pooled from 2 or 3 independent experiments (n =

758 6-11 per group). Individual measurements, mean \pm SEM and (A-B) p values are indicated:

759 ns: non-significant, *P < .05, **P < .01, ***P < .001 or ****P < .0001.

760

761 **FIG 3. FcγR-, C1q- and effector cell-independent antigen retention in the lung.** A,
762 FcγRIIB/III expression on lung immune cells 15 min post-challenge of wt C57BL/6J mice.
763 B, Post-challenge ex vivo quantification of BSA-VT680 in explanted lungs. C,
764 Temperature drop and survival during experimental anaphylaxis. D-E, Post-challenge ex
765 vivo quantification of BSA-VT680 in explanted lungs of (D) *gfi-1* mice and (E) C57BL/6
766 mice non-depleted (wt) or depleted of neutrophils (-neutro), or of neutrophils and
767 monocyte/macrophages (-neutro-Mφ). (A-E) Data are represented as mean ± SEM and
768 are representative of at least 2 independent experiments. (A,B,D,E) Individual
769 measurements, mean ± SEM and p values are indicated: ns: non-significant, *P < .05,
770 **P < .01, ***P < .001 or ****P < .0001.

771

772 **FIG 4. IgG2a antibody mediates antigen retention in the lung.** A-C, (A) Body
773 temperature drop (n≥7) in C57BL/6 mice, (B) in vivo quantification and (C) ex vivo
774 quantification post-challenge of BSA-VT680 in explanted organs of C57BL/6 and
775 BALB/c mice, during experimental anaphylaxis following BSA immunizations in
776 CFA/IFA or alum (n≥6). D, Serum anti-BSA IgG titers of indicated mice immunized with
777 BSA in CFA/IFA 10 days after the last immunization. E, 15 min post-challenge
778 immunofluorescence on frozen sections during passive systemic IgG-subclass dependent
779 anaphylaxis in C57BL/6 mice. Arrows indicate antigen aggregates. (C-D) Data are pooled
780 from 2 or 3 independent experiments. Individual measurements, mean ± SEM and p
781 values are indicated: ns: non-significant, *P < .05, **P < .01 or ****P < .0001.

782

783 **FIG 5. Myeloperoxidase release in the lung during active systemic anaphylaxis.** A,
784 Representative images show color-coded maps of photon flux superimposed on black and
785 white photographs of C57BL/6 mice 20 min post-challenge and injection of luminol

786 revealing myeloperoxidase activity. B, Time course of in vivo quantification of
787 myeloperoxidase activity after challenge or not (-) in C57BL/6 mice. C, In vivo
788 quantification on the lung area of myeloperoxidase activity 30 min after BSA challenge.
789 D, 15 min post-challenge immunofluorescence on lung slices from C57BL/6 wt mice;
790 arrows indicate MPO foci. (A-D) Data are representative of ≥ 2 independent experiments;
791 (B) mean \pm SEM and (C) individual measurements, mean \pm SEM, and p values are
792 indicated: ns: non-significant, ****P < .0001.