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1 **The anti-IgE mAb Omalizumab induces adverse reactions by**
2 **engaging Fcγ receptors**

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41

42 **Running title**

43 *Xolair induces Fc γ R-dependent inflammation*

44 **Abstract**

45

46 Omalizumab is an anti-IgE monoclonal antibody (mAb) approved for the treatment of
47 severe asthma and chronic spontaneous urticaria. Use of Omalizumab is associated with
48 reported side effects, ranging from local skin inflammation at the injection site to systemic
49 anaphylaxis. To date, the mechanisms through which Omalizumab induces adverse
50 reactions are still unknown. Here, we demonstrated that immune complexes formed
51 between Omalizumab and IgE can induce both skin inflammation and anaphylaxis through
52 engagement of IgG receptors (FcγRs) in FcγR-humanized mice. We further developed an
53 Fc-engineered mutant version of Omalizumab, and demonstrated that this mAb is equally
54 potent as Omalizumab at blocking IgE-mediated allergic reactions, but does not induce
55 FcγR-dependent adverse reactions. Overall, our data indicate that Omalizumab can induce
56 skin inflammation and anaphylaxis by engaging FcγRs, and demonstrate that Fc-
57 engineered versions of the mAb could be used to reduce such adverse reactions.

58 Introduction

59

60 IgE antibodies (Abs) are key mediators of allergic diseases (1-3). Upon exposure to
61 an allergen in allergic patients, such allergen is recognized by IgE bound to their high-
62 affinity receptor FcεRI on the surface of mast cells and basophils, which promotes the
63 immediate activation of these cells, and the release of inflammatory mediators such as
64 histamine, responsible for allergic symptoms (3).

65

66 Omalizumab (Xolair®) is a recombinant humanized IgG1 mAb directed against IgE
67 (4). Omalizumab binds to the Cε3 domain of free IgE, and thereby impairs binding of IgE
68 to both FcεRI and the low-affinity IgE receptor CD23 (FcεRII) (5-7). Omalizumab does
69 not recognize IgE already bound to FcεRI or CD23, and therefore cannot induce cell
70 activation by crosslinking of IgE receptors (5, 7). Omalizumab is approved for the
71 treatment of severe asthma (8) and chronic spontaneous urticaria (9). It also shows
72 promises for the treatment of other allergic diseases, including food allergy (10).

73

74 However, treatment with Omalizumab is associated with adverse reactions,
75 ranging from skin inflammation at the injection site to anaphylaxis in ~0.1-0.2% of patients
76 (11-13). The mechanism of these side effects is still unknown. Notably, Omalizumab does
77 not induce the formation of anti-drug Abs, and most cases of anaphylaxis occur within the
78 first three injections of the drug (11-13).

79

80 We hypothesized that the formation of immune complexes (ICs) between
81 Omalizumab and IgE could be responsible for some of the adverse reactions observed with
82 this therapeutic mAb. Using mice humanized for all IgG receptors (FcγRs), we demonstrate
83 here that Omalizumab/IgE ICs can induce both skin inflammation at the site of injection of
84 the drug, as well as systemic anaphylaxis through engagement of FcγRs. Finally, we
85 developed an Fc-engineered version of Omalizumab which blocks IgE-mediated allergic
86 reactions without inducing FcγR-dependent adverse reactions.

87

88 **Results and Discussion**

89

90 We first co-incubated Omalizumab and human IgE (termed IgE herein) in vitro to
91 form ICs, and assessed the molecular mass of these ICs by size exclusion chromatography
92 coupled to static light scattering (SEC-SLS). As reported previously (14, 15), these ICs
93 were of limited size, mainly consisting of trimeric structures (**Supplemental Figure 1**). It
94 was initially suggested that such small ICs have a low potential to engage FcγRs (15).
95 However, we found that these ICs potently bind all activating human FcγRs (FcγRI, IIA,
96 IIIA and IIIB), but not the inhibitory FcγRIIB that has the lowest affinity for human IgG1
97 among FcγRs (16) (**Figure 1A**). As expected, we also observed that Omalizumab binds
98 human complement component C1q in a dose-dependent manner (**Figure 1B**).

99

100 As neutrophils were reported to contribute to IgG-mediated inflammation and
101 anaphylaxis (17), we next evaluated whether Omalizumab/IgE ICs can activate neutrophils
102 through engagement of FcγRs. We purified neutrophils from healthy donors and incubated
103 these cells with Omalizumab/IgE ICs. We found that such ICs induce marked upregulation
104 of CD66b and downregulation of CD62L on the surface of neutrophils, which are
105 considered hallmarks of neutrophil activation (18, 19) (**Figure 1, C and D**). The ICs also
106 induced downregulation of FcγRII (CD32) (**Figure 1E**). As human neutrophils express
107 FcγRIIA and not FcγRIIB (20), and Omalizumab/IgE ICs do not bind FcγRIIB (**Figure**
108 **1A**), our results indicate that the ICs induce active engagement of FcγRIIA in neutrophils.

109

110 To further confirm the role of FcγRs in neutrophil activation, we performed similar
111 experiments with neutrophils purified from hFcγR^{KI} mice (in which all mouse FcγRs have
112 been replaced with human FcγRs) (20, 21) or FcγR^{null} mice (deficient for all FcγRs) (**Figure**
113 **1F**). Omalizumab/IgE ICs induced a downregulation of CD62L in neutrophils from
114 hFcγR^{KI} mice, but not in neutrophils from FcγR^{null} mice (**Figure 1F**), demonstrating that
115 Omalizumab/IgE can activate neutrophils through engagement of human FcγRs.

116

117 The most frequent side effect observed with Omalizumab is skin inflammation (13).
118 We hypothesized that such local inflammation could be a consequence of FcγRs
119 engagement. To assess this, we injected Omalizumab/IgE ICs subcutaneously into hairless
120 (to avoid shaving-induced skin inflammation) nude hFcγR^{KI} mice and nude FcγR^{null} mice,
121 and assessed skin inflammation after 2 h by bioluminescence imaging of myeloperoxidase
122 (MPO) activity (20, 22). We observed a strong MPO activity at the site of ICs injection in
123 hFcγR^{KI} mice (**Figure 2, A and B**). By contrast, MPO activity was markedly reduced upon
124 injection of IgE alone or Omalizumab alone, or injection of ICs in FcγR^{null} mice. Thus, our
125 results indicate that Omalizumab/IgE ICs can induce skin inflammation through
126 engagement of hFcγRs.

127

128 The most dramatic side effect reported for Omalizumab is anaphylaxis (12, 13). We
129 thus assessed whether Omalizumab/IgE ICs can induce anaphylaxis in hFcγR^{KI} mice.
130 Intravenous injection of ICs induced significant hypothermia (the main readout of
131 anaphylaxis in mice (23)) in hFcγR^{KI} mice (**Figure 2C**). Importantly, hypothermia was not
132 observed upon ICs injection in hFcγR^{null} mice (**Figure 2C**), or injection of IgE or

133 Omalizumab alone in hFcγR^{KI} mice (**Supplemental Figure 2**), demonstrating that the ICs
134 induce systemic anaphylaxis through engagement of human FcγRs. Previous work
135 indicates that hFcγRIIA contributes to experimental anaphylaxis in humanized mice (21,
136 24, 25). We did not observe anaphylaxis in mFcγR^{null}hFcγRIIA^{Tg} mice (which express only
137 hFcγRIIA (21)), indicating that hFcγRIIA is not sufficient to trigger Omalizumab/IgE-
138 mediated anaphylaxis (**Supplemental Figure 3A**). By contrast, anaphylaxis was markedly
139 reduced in hFcγR^{KI} mice pre-treated with a blocking mAb against hFcγRIII (**Supplemental**
140 **Figure 3B**). This suggests that hFcγRIII plays an important role in Omalizumab/IgE-
141 mediated anaphylaxis. However, these results have to be interpreted carefully as we cannot
142 exclude that pre-treatment with the anti-hFcγRIII mAb induces engagement of the receptor
143 to some extent, thereby “desensitizing” cells expressing hFcγRIII.

144

145 Since Omalizumab also binds complement component C1q (**Figure 1B**), we assessed
146 the potential contribution of C1q to ICs-induced anaphylaxis. We found that anaphylaxis
147 is markedly reduced in hFcγR^{KI}C1q^{-/-} mice, which express all hFcγRs but lack mouse C1q
148 (**Figure 2D**). Although further work is required to confirm the implication of human C1q,
149 our data strongly suggest that the complement pathway plays an important role, through
150 C1q engagement, in Omalizumab/IgE-induced anaphylaxis.

151

152 Based on these results, we decided to produce an Fc-engineered form of Omalizumab
153 (using available Omalizumab V_H and V_L sequences (4)) lacking the N-linked glycan
154 attached to asparagine 297 in the Fc portion (N₂₉₇A mutation) to reduce binding to FcγRs
155 and complement (26, 27). We refer to this mAb as ‘NA anti-IgE’. As a control, we also

156 produced a non-mutated version of this mAb ('WT anti-IgE'). Both the WT and NA anti-
157 IgE mainly formed trimers when incubated with IgE in vitro (**Supplemental Figure 4, A-**
158 **D**), which is consistent with the data we obtained using commercial Omalizumab
159 (**Supplemental Figure 1**). As expected, ICs made of IgE and the WT anti-IgE could bind
160 all activating FcγRs, while binding to FcγRs was markedly reduced with ICs made of IgE
161 and the NA anti-IgE (**Figure 3A**). Indeed, IgE/NA anti-IgE ICs could only bind to FcγRI,
162 which is consistent with a previous report showing that the N₂₉₇A mutation does not
163 abrogate binding to this high-affinity FcγR (28). In addition, WT anti-IgE could bind
164 human C1q (**Supplemental Figure 4E**), but we detected no binding to C1q with the NA
165 anti-IgE (**Supplemental Figure 4E**). Finally, we observed activation of human neutrophils
166 with ICs made of IgE and the WT anti-IgE, but markedly reduced activation with ICs made
167 of IgE and the NA anti-IgE (**Figure 3, B-D**).

168

169 FcRn/β2m heterodimers extend the half-life of IgG by reducing lysosomal
170 degradation in endothelial cells (29). To assess the half-life of our anti-IgE mAbs in vivo,
171 we generated hFcγR^{KI}hFcRn^{KI}hβ2m^{KI} mice which recapitulate binding of IgG to all human
172 FcγRs and to the human FcRn-β2m complex (**Supplemental Figure 5**). We injected WT
173 or NA anti-IgE into hFcγR^{KI}hFcRn^{KI}hβ2m^{KI} mice, and observed similar mAbs levels in
174 sera collected at different time-points (**Figure 3E**). We obtained similar results when
175 comparing the half-life of commercial Omalizumab and the Fc-engineered NA anti-IgE
176 (**Supplemental Figure 6**). Altogether, these results demonstrate that the N₂₉₇A mutation
177 does not affect the half-life of the anti-IgE mAb in vivo.

178

179 We also verified that the N_{297A} mutation does not affect the ability of the anti-IgE
180 mAb to block IgE. Both the WT and NA anti-IgE recognized IgE with the same affinity
181 (**Supplemental Figure 7A**), and were equally potent at blocking binding of IgE to human
182 mast cells (**Supplemental Figure 7B**). Moreover, we showed that pre-treatment of
183 hFc ϵ RI^{Tg} mice (which express the human IgE receptor hFc ϵ RI (30)) with either
184 Omalizumab or the NA anti-IgE can block IgE-mediated anaphylaxis (**Figure 4A**).
185 Altogether, our results demonstrate that the Fc-engineered NA anti-IgE is equally potent
186 as Omalizumab at blocking IgE-mediated allergic reactions.

187

188 We then compared skin inflammation induced by IgE/Omalizumab or IgE/NA anti-
189 IgE ICs in hFc γ R^{KI} mice. Injection of IgE/Omalizumab ICs induced marked MPO activity
190 in the skin (**Figure 4, B and C**). This was reduced to levels observed with injection of IgE
191 alone in hFc γ R^{KI} mice injected with IgE/NA anti-IgE ICs (**Figure 4, B and C**). Finally, we
192 compared the ability of ICs made of IgE and Omalizumab or the NA anti-IgE to induce
193 anaphylaxis in hFc γ R^{KI} mice. We observed anaphylaxis in mice injected with
194 IgE/Omalizumab ICs but not in mice injected with IgE/NA anti-IgE ICs (**Figure 4D**).

195

196 In summary, our findings demonstrate that Omalizumab forms ICs with IgE which
197 can activate neutrophils, and induce skin inflammation and systemic anaphylaxis through
198 human Fc γ Rs in FcR-humanized mice. Such findings could explain some of the side effects
199 which have been described in patients treated with Omalizumab (12, 13). One must be
200 careful when extrapolating these findings obtained in humanized mice to humans, as very
201 few data have been reported supporting the existence of Fc γ R-mediated anaphylaxis in

202 humans. However, one recent report provides evidence of an IgG-induced, FcγR-
203 dependent neutrophil activation pathway in anaphylaxis to neuromuscular-blocking agents
204 (NMBAs) in humans (31), which reinforces the potential clinical relevance of our findings.
205 The Fc-engineered anti-IgE mAb we developed is equally potent as Omalizumab at
206 blocking IgE-mediated allergic reactions but does not induce FcγR-mediated
207 inflammation. It could thus potentially be used in patients with very high levels of IgE,
208 and/or in patients with a history of anaphylaxis or other adverse reactions to Omalizumab.
209 Finally, we envision that IC-mediated engagement of FcγRs could be a more general
210 mechanism of therapeutic mAbs-mediated adverse reactions.

211

212

213

214

215 **Materials and Methods**

216 See the supplemental Methods for the description of all experimental procedures and
217 statistical analyses.

218

219 **Study approval.** All animal care and experimentation were conducted in compliance with
220 the guidelines and specific approval of the Animal Ethics committee CETEA (Institut
221 Pasteur, Paris, France) registered under #2013-0103, and by the French Ministry of
222 Research under agreement #00513.02.

223

224 **Author contributions**

225

226 Experimental design, B.B, P.B and L.L.R; Conducting experiments, B.B, P.H, O.G, J.S
227 and L.L.R; Acquiring data B.B, P.H, O.G, J.S, O.R.G, B.I, D.S, S.B and L.L.R; Providing
228 mice: F.M.H, V.A.V, L.E.M and A.J.M; Providing reagents: K.C.N; Statistical analysis:
229 G.A.M; Formal analysis, B.B and L.L.R; Writing (original draft), B.B and L.L.R; Writing
230 (review and editing), all authors.

231

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233

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246

247 **Competing interests**

248

249 B.B, P.B and L.L.R are inventors on a patent related to this work (PCT/EP2019/059414).
250 L.E.M and A.M. are employees of Regeneron Pharmaceuticals, Inc. hold stock in the
251 company, and are inventors on patents and patent applications related to the mice used for
252 this work. P.B. is a paid consultant for Regeneron Pharmaceuticals. The authors declare no
253 additional competing financial interests.

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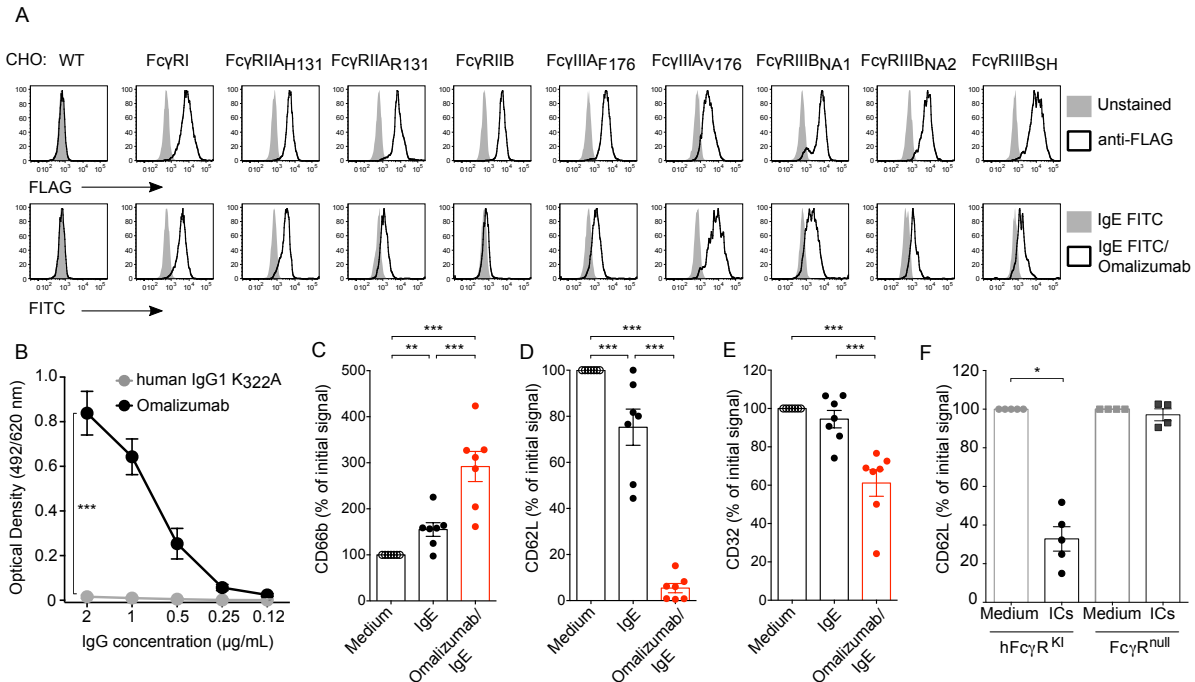
257 **References**

258

- 259 1. Gould HJ, and Sutton BJ. IgE in allergy and asthma today. *Nat Rev Immunol.* 2008;8(3):205-17.
260
- 261 2. Balbino B, Conde E, Marichal T, Starkl P, and Reber LL. Approaches to target IgE
262 antibodies in allergic diseases. *Pharmacol Ther.* 2018;191:50-64.
- 263 3. Galli SJ, and Tsai M. IgE and mast cells in allergic disease. *Nat Med.*
264 2012;18(5):693-704.
- 265 4. Presta LG, Lahr SJ, Shields RL, Porter JP, Gorman CM, Fendly BM, et al.
266 Humanization of an antibody directed against IgE. *J Immunol.* 1993;151(5):2623-
267 32.
- 268 5. Chang TW, Davis FM, Sun NC, Sun CR, MacGlashan DW, Jr., and Hamilton RG.
269 Monoclonal antibodies specific for human IgE-producing B cells: a potential
270 therapeutic for IgE-mediated allergic diseases. *Biotechnology (N Y).*
271 1990;8(2):122-6.
- 272 6. Selb R, Eckl-Dorna J, Twaroch TE, Lupinek C, Teufelberger A, Hofer G, et al.
273 Critical and direct involvement of the CD23 stalk region in IgE binding. *J Allergy*
274 *Clin Immunol.* 2016.
- 275 7. Davies AM, Allan EG, Keeble AH, Delgado J, Cossins BP, Mitropoulou AN, et al.
276 Allosteric mechanism of action of the therapeutic anti-IgE antibody omalizumab. *J*
277 *Biol Chem.* 2017;292(24):9975-87.
- 278 8. Busse W, Corren J, Lanier BQ, McAlary M, Fowler-Taylor A, Cioppa GD, et al.
279 Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the
280 treatment of severe allergic asthma. *J Allergy Clin Immunol.* 2001;108(2):184-90.
- 281 9. Maurer M, Rosen K, Hsieh HJ, Saini S, Grattan C, Gimenez-Arnau A, et al.
282 Omalizumab for the treatment of chronic idiopathic or spontaneous urticaria. *N*
283 *Engl J Med.* 2013;368(10):924-35.
- 284 10. MacGinnitie AJ, Rachid R, Gragg H, Little SV, Lakin P, Cianferoni A, et al.
285 Omalizumab facilitates rapid oral desensitization for peanut allergy. *J Allergy Clin*
286 *Immunol.* 2017;139(3):873-81 e8.
- 287 11. Limb SL, Starke PR, Lee CE, and Chowdhury BA. Delayed onset and protracted
288 progression of anaphylaxis after omalizumab administration in patients with
289 asthma. *J Allergy Clin Immunol.* 2007;120(6):1378-81.
- 290 12. Lieberman PL, Umetsu DT, Carrigan GJ, and Rahmaoui A. Anaphylactic reactions
291 associated with omalizumab administration: Analysis of a case-control study. *J*
292 *Allergy Clin Immunol.* 2016;138(3):913-5 e2.
- 293 13. Lieberman PL, Jones I, Rajwanshi R, Rosen K, and Umetsu DT. Anaphylaxis
294 associated with omalizumab administration: Risk factors and patient
295 characteristics. *J Allergy Clin Immunol.* 2017;140(6):1734-6 e4.
- 296 14. Liu J, Lester P, Builder S, and Shire SJ. Characterization of complex formation by
297 humanized anti-IgE monoclonal antibody and monoclonal human IgE.
298 *Biochemistry.* 1995;34(33):10474-82.
- 299 15. Fox JA, Hotaling TE, Struble C, Ruppel J, Bates DJ, and Schoenhoff MB. Tissue
300 distribution and complex formation with IgE of an anti-IgE antibody after

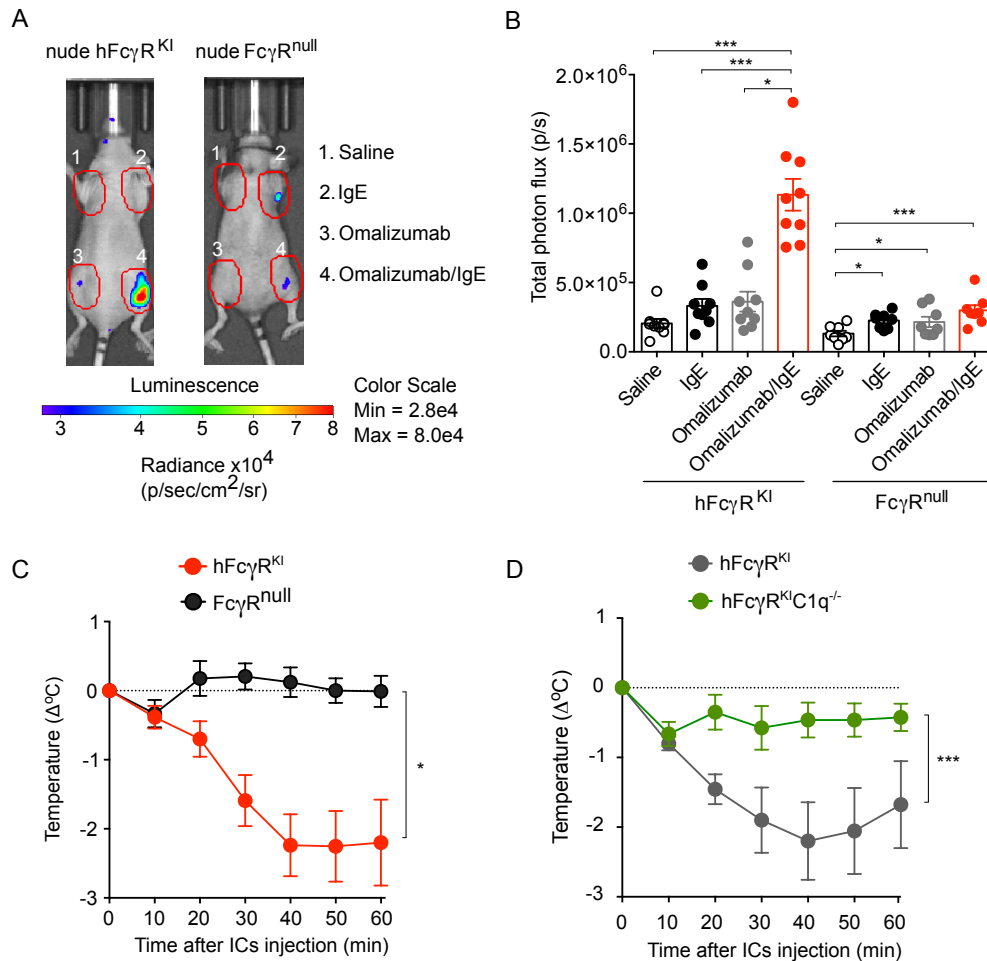
- 301 intravenous administration in cynomolgus monkeys. *J Pharmacol Exp Ther.*
302 1996;279(2):1000-8.
- 303 16. Bruhns P, Iannascoli B, England P, Mancardi DA, Fernandez N, Jorieux S, et al.
304 Specificity and affinity of human Fcγ receptors and their polymorphic
305 variants for human IgG subclasses. *Blood.* 2009;113(16):3716-25.
- 306 17. Jonsson F, Mancardi DA, Albanesi M, and Bruhns P. Neutrophils in local and
307 systemic antibody-dependent inflammatory and anaphylactic reactions. *J Leukoc*
308 *Biol.* 2013;94(4):643-56.
- 309 18. Zhao L, Xu S, Fjaertoft G, Pauksen K, Hakansson L, and Venge P. An enzyme-
310 linked immunosorbent assay for human carcinoembryonic antigen-related cell
311 adhesion molecule 8, a biological marker of granulocyte activities in vivo. *J*
312 *Immunol Methods.* 2004;293(1-2):207-14.
- 313 19. Pillay J, Kamp VM, van Hoffen E, Visser T, Tak T, Lammers JW, et al. A subset
314 of neutrophils in human systemic inflammation inhibits T cell responses through
315 Mac-1. *J Clin Invest.* 2012;122(1):327-36.
- 316 20. Reber LL, Gillis CM, Starkl P, Jonsson F, Sibilano R, Marichal T, et al. Neutrophil
317 myeloperoxidase diminishes the toxic effects and mortality induced by
318 lipopolysaccharide. *J Exp Med.* 2017;214(5):1249-58.
- 319 21. Beutier H, Hechler B, Godon O, Wang Y, Gillis CM, de Chaisemartin L, et al.
320 Platelets expressing IgG receptor FcγRIIA/CD32A determine the severity of
321 experimental anaphylaxis. *Sci Immunol.* 2018;3(22).
- 322 22. Gross S, Gammon ST, Moss BL, Rauch D, Harding J, Heinecke JW, et al.
323 Bioluminescence imaging of myeloperoxidase activity in vivo. *Nat Med.*
324 2009;15(4):455-61.
- 325 23. Strait RT, Morris SC, Yang M, Qu XW, and Finkelman FD. Pathways of
326 anaphylaxis in the mouse. *J Allergy Clin Immunol.* 2002;109(4):658-68.
- 327 24. Gillis CM, Jonsson F, Mancardi DA, Tu N, Beutier H, Van Rooijen N, et al.
328 Mechanisms of anaphylaxis in human low-affinity IgG receptor locus knock-in
329 mice. *J Allergy Clin Immunol.* 2017;139(4):1253-65 e14.
- 330 25. Jonsson F, Mancardi DA, Zhao W, Kita Y, Iannascoli B, Khun H, et al. Human
331 FcγRIIA induces anaphylactic and allergic reactions. *Blood.*
332 2012;119(11):2533-44.
- 333 26. Bruhns P, and Jonsson F. Mouse and human FcR effector functions. *Immunol Rev.*
334 2015;268(1):25-51.
- 335 27. Smith P, DiLillo DJ, Bournazos S, Li F, and Ravetch JV. Mouse model
336 recapitulating human Fcγ receptor structural and functional diversity. *Proc*
337 *Natl Acad Sci U S A.* 2012;109(16):6181-6.
- 338 28. Nesspor TC, Raju TS, Chin CN, Vafa O, and Brezski RJ. Avidity confers
339 FcγRIIb binding and immune effector function to aglycosylated
340 immunoglobulin G1. *J Mol Recognit.* 2012;25(3):147-54.
- 341 29. Stapleton NM, Einarsdottir HK, Stemerding AM, and Vidarsson G. The multiple
342 facets of FcRn in immunity. *Immunol Rev.* 2015;268(1):253-68.
- 343 30. Dombrowicz D, Brini AT, Flamand V, Hicks E, Snouwaert JN, Kinet JP, et al.
344 Anaphylaxis mediated through a humanized high affinity IgE receptor. *J Immunol.*
345 1996;157(4):1645-51.

- 346 31. Jonsson F, de Chaisemartin L, Granger V, Gouel-Cheron A, Gillis CM, Zhu Q, et
347 al. An IgG-induced neutrophil activation pathway contributes to human drug-
348 induced anaphylaxis. *Sci Transl Med.* 2019;11(500).
349



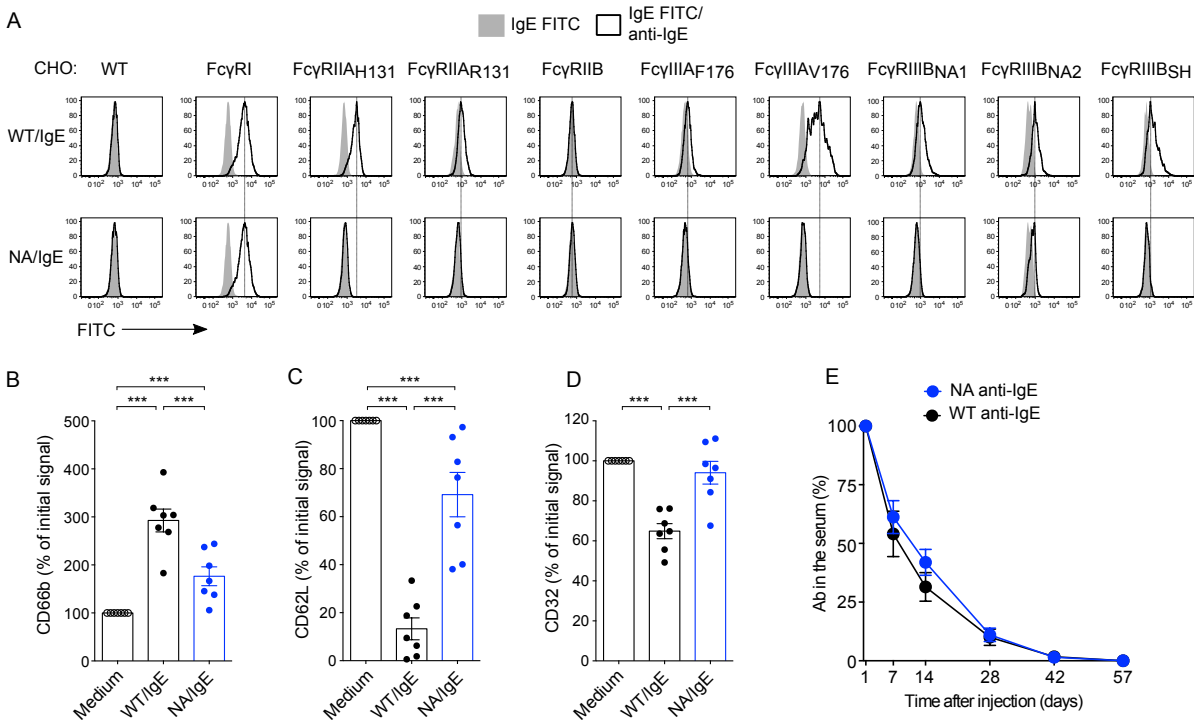
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351 **Figure 1. Omalizumab/IgE immune complexes (ICs) bind FcγRs and activate**
 352 **neutrophils.** (A) Binding of pre-formed IgE/Omalizumab ICs to FcγRs in CHO cells stably
 353 transfected with each one of the human FcγRs (16). Upper histograms show binding of an
 354 anti-FLAG mAb as a control for FcγR expression. Lower histograms show binding of ICs
 355 or IgE FITC alone. Data are representative of three independent experiments. (B) Binding
 356 of Omalizumab to human C1q assessed by ELISA. An irrelevant IgG1 mutated in its Fc
 357 portion at position 322 (K₃₂₂A) to preclude binding to C1q was used as a negative control.
 358 Results in B show means ± SD from data pooled from two independent experiments (total
 359 of *n*=4 replicates). (C-E) Expression of CD66b (C), CD62L (D) and CD32 (E) on purified
 360 CD45⁺CD15⁺ human neutrophils after 1 h incubation with Omalizumab/IgE immobilized
 361 ICs, IgE or medium alone. Results in C-E show values from neutrophils from individual
 362 donors normalized against cells stimulated with medium alone; bars indicate means ± SEM
 363 of *n*=7 total values per group pooled from three independent experiments. (F) CD62L
 364 expression on CD11b⁺Ly6G⁺ neutrophils purified from hFcγR^{KI} or FcγR^{null} mice after 1 h
 365 incubation with ICs or medium. Results in F show values from individual mice with bars
 366 indicating means ± SEM pooled from two (FcγR^{null}; total *n*=4/group) or three (hFcγR^{KI};
 367 total *n*=5/group) independent experiments. *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001 using
 368 one-way analysis of variance (ANOVA) in B, contrast linear model in C, D and E and
 369 Welch test in F. For further details on the statistical analysis, please refer to Table S1.
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372 **Figure 2. Omalizumab/IgE ICs induce skin inflammation and anaphylaxis through**
 373 **engagement of Fc γ R in Fc γ R-humanized mice.** Representative bioluminescent images
 374 (A) and quantification (B) of MPO activity 2 h after subcutaneous injection of
 375 IgE/Omalizumab ICs in nude hFc γ R^{KI} mice ($n=9$) or nude Fc γ R^{null} mice ($n=8$). Regions of
 376 interest outlined in red in A surround sites of injection. Data in B are means \pm SEM pooled
 377 from two independent experiments. (C-D) Changes in body temperature ($\Delta^\circ\text{C}$ [mean \pm
 378 SEM]) after intravenous injection of IgE/Omalizumab ICs into hFc γ R^{KI} mice ($n=13$) or
 379 Fc γ R^{null} mice ($n=9$) (C), or hFc γ R^{KI} mice ($n=9$) or hFc γ R^{KI} C1q^{-/-} mice ($n=8$). Data are
 380 pooled from three (C) or two (D) independent experiments. *, $P < 0.05$; ***, $P < 0.001$ by
 381 Contrast test in linear model (B and C) or ANOVA (D). For further details on the statistical
 382 analysis, please refer to Table S1.

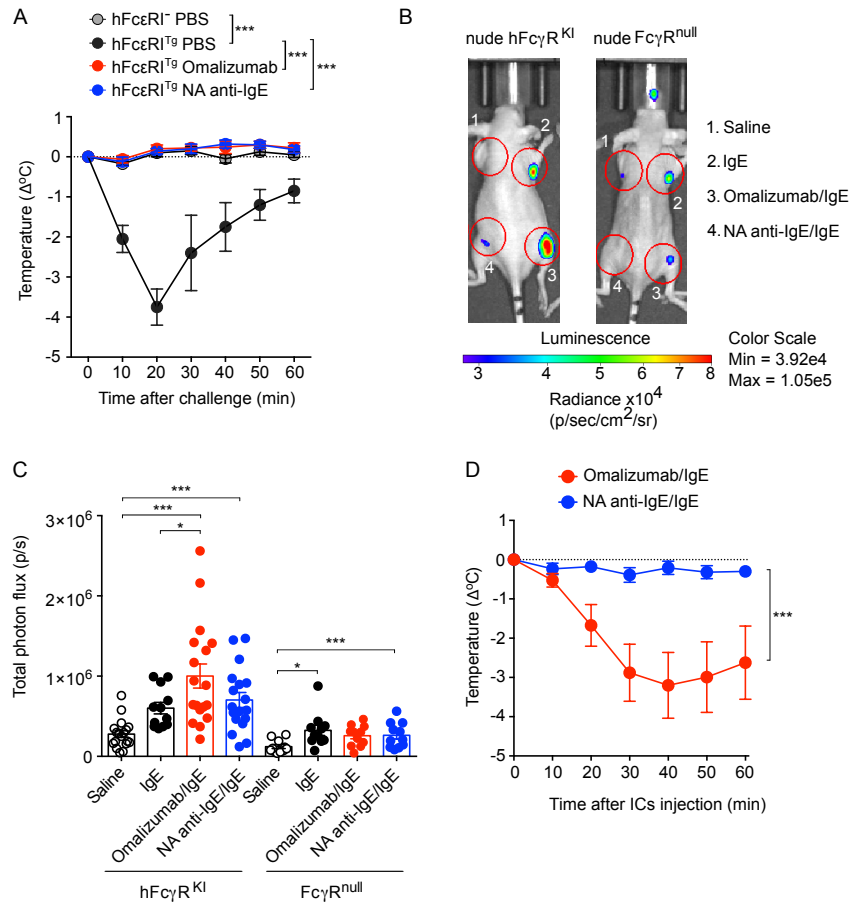


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384 **Figure 3. Fc-engineered anti-IgE antibodies display markedly reduced FcγR-binding**
 385 **and neutrophil activation.** (A) Binding of ICs made of FITC-IgE and WT anti-IgE or Fc-
 386 engineered N297A ('NA') anti-IgE. Data are representative of three independent
 387 experiments. (B-C) Expression of CD66b (B), CD62L (C) and CD32 (D) on purified
 388 CD45⁺CD15⁺ human neutrophils after 1 h incubation with IgE/WT anti-IgE or IgE/NA
 389 anti-IgE ICs or medium alone. Results in B-D show values from neutrophils from
 390 individual donors normalized against cells stimulated with medium alone. Bars indicate
 391 means ± SEM pooled from three independent experiments (total n=7/group). (E) 100 μg
 392 of WT or NA anti-IgE was injected intraperitoneally (i.p.) into hFcγR^{K1}hFcRn^{K1}hβ2m^{K1}
 393 mice, and serum was collected at different time-points. Levels of anti-IgE mAbs were
 394 measured by ELISA. Data are indicated as means ± SEM pooled from two independent
 395 experiments (n=13/group). ***, P < 0.001 by contrast linear model in B, C and D and
 396 ANOVA in E. For further details on the statistical analysis, please refer to Table S1.

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Figure 4. Fc-engineered anti-IgE antibodies block IgE-mediated anaphylaxis but do not induce FcγR-dependent inflammation. (A) Changes in body temperature ($\Delta^{\circ}\text{C}$ [mean \pm SEM]) after intravenous (i.v.) challenge with 500 μg of nitrophenyl-coupled BSA (NP-BSA) in hFcεRI⁺ mice pre-treated i.v. with 700 μg Omalizumab, NA anti-IgE or PBS 30 min before sensitization with anti-NP IgE (10 μg). Data in A are pooled from two independent experiments (total $n=4-6/\text{group}$). hFcεRI⁻ mice were used as a control. (B and C) Representative bioluminescent images (B) and quantification (C) of MPO activity 2 h after subcutaneous injection of IgE/Omalizumab or IgE/NA anti-IgE ICs in nude hFcγR^{KI} or nude FcγR^{null} mice. Regions of interest outlined in red surround the site of injection. Bars in C indicate means \pm SEM pooled from five (nude hFcγR^{KI}, total $n=18$) or four (nude FcγR^{null}, total $n=11$) independent experiments. The color bar represents bioluminescent signal in radiance (p/sec/cm²/sr). (D) Changes in body temperature ($\Delta^{\circ}\text{C}$ [mean \pm SEM]) after i.v. injection of IgE/Omalizumab ($n=10$) or IgE/NA anti-IgE ($n=11$) into hFcγR^{KI} mice. Data in D are pooled from two independent experiments. *, $P < 0.05$; ***, $P < 0.001$ by Contrast test linear model (A and C) or 2-way repeated-measures (ANOVA) (D). For further details on the statistical analysis, please refer to Table S1.