



**HAL**  
open science

## The anti-IgE mAb Omalizumab induces adverse reactions by engaging Fc $\gamma$ receptors

Bianca Balbino, Pauline Herviou, Ophélie Godon, Julien Stackowicz, Odile Richard-Le Goff, Bruno Iannascoli, Delphine Sterlin, Sébastien Brûlé, Gaël A Millot, Faith Harris, et al.

► **To cite this version:**

Bianca Balbino, Pauline Herviou, Ophélie Godon, Julien Stackowicz, Odile Richard-Le Goff, et al.. The anti-IgE mAb Omalizumab induces adverse reactions by engaging Fc $\gamma$  receptors. *Journal of Clinical Investigation*, 2019, 10.1172/JCI129697 . pasteur-02451133

**HAL Id: pasteur-02451133**

**<https://pasteur.hal.science/pasteur-02451133>**

Submitted on 23 Jan 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

3 **Authors**

4 Bianca Balbino<sup>1,2</sup>, Pauline Herviou<sup>1</sup>, Ophélie Godon<sup>1</sup>, Julien Stackowicz<sup>1,2</sup>, Odile Richard-  
5 Le Goff<sup>1</sup>, Bruno Iannascoli<sup>1</sup>, Delphine Sterlin<sup>1,3</sup>, Sébastien Brûlé<sup>4</sup>, Gael A. Millot<sup>5</sup>, Faith  
6 M. Harris<sup>6</sup>, Vera A. Voronina<sup>6</sup>, Kari C. Nadeau<sup>7,8</sup>, Lynn E. Macdonald<sup>6</sup>, Andrew J.  
7 Murphy<sup>6</sup>, Pierre Bruhns<sup>1,\*</sup> and Laurent L. Reber<sup>1,9,\*</sup>

8  
9 **Affiliations**

10 <sup>1</sup>Unit of Antibodies in Therapy and Pathology, Institut Pasteur, UMR1222 INSERM,  
11 75015 Paris, France. <sup>2</sup>Sorbonne Université, Paris, France. <sup>3</sup>Assistance Publique–Hôpitaux  
12 de Paris, La Pitié-Salpêtrière, Département d’Immunologie, France, Paris. <sup>4</sup>Plateforme de  
13 biophysique moléculaire, Institut Pasteur, UMR 3528 CNRS, Paris 75015, France. <sup>5</sup>Hub  
14 de Bioinformatique et Biostatistique – Département Biologie Computationnelle, Institut  
15 Pasteur, USR 3756 CNRS, Paris, France. <sup>6</sup>Regeneron Pharmaceuticals Inc., Tarrytown,  
16 NY 10591, USA. <sup>7</sup>Sean N. Parker Center for Allergy and Asthma Research, Stanford  
17 University, Stanford, California, USA. <sup>8</sup>Division of Pulmonary and Critical Care,  
18 Department of Medicine, Stanford University, California, USA. <sup>9</sup>Center for  
19 Physiopathology of Toulouse-Purpan (CPTP), UMR 1043, University of Toulouse,  
20 INSERM, CNRS, Toulouse, France. \*These authors contributed equally to this work.

21

22

23 **Corresponding authors**

24

25 Laurent L. Reber, Ph.D

26 ATIP-Avenir team “Asthma, Allergy & Immunotherapy”

27 Center for Physiopathology Toulouse-Purpan

28 CHU Purpan – BP 3028

29 31024 Toulouse Cedex 3, France

30 Phone: +33-5-6274-4529

31 e-mail: laurent.reber@inserm.fr

32

33 Pierre Bruhns, Ph.D

34 Unit of Antibodies in Therapy and Pathology

35 Department of Immunology

36 Institut Pasteur

37 25 rue du Docteur Roux

38 Paris, 75015, France

39 Phone: +33-1-4568-8629

40 e-mail: bruhns@pasteur.fr

41

42 **Running title**

43 *Xolair induces Fc $\gamma$ 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 Fc $\epsilon$ RI 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<sup>®</sup>) is a recombinant humanized IgG1 mAb directed against IgE  
67 (4). Omalizumab binds to the C $\epsilon$ 3 domain of free IgE, and thereby impairs binding of IgE  
68 to both Fc $\epsilon$ RI and the low-affinity IgE receptor CD23 (Fc $\epsilon$ RII) (5-7). Omalizumab does  
69 not recognize IgE already bound to Fc $\epsilon$ RI 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<sup>KI</sup> mice (in which all mouse FcγRs have  
112 been replaced with human FcγRs) (20, 21) or FcγR<sup>null</sup> mice (deficient for all FcγRs) (**Figure**  
113 **1F**). Omalizumab/IgE ICs induced a downregulation of CD62L in neutrophils from  
114 hFcγR<sup>KI</sup> mice, but not in neutrophils from FcγR<sup>null</sup> 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<sup>KI</sup> mice and nude FcγR<sup>null</sup> 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<sup>KI</sup> 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<sup>null</sup> 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<sup>KI</sup> mice.  
130 Intravenous injection of ICs induced significant hypothermia (the main readout of  
131 anaphylaxis in mice (23)) in hFcγR<sup>KI</sup> mice (**Figure 2C**). Importantly, hypothermia was not  
132 observed upon ICs injection in hFcγR<sup>null</sup> mice (**Figure 2C**), or injection of IgE or



133 Omalizumab alone in hFcγR<sup>KI</sup> 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<sup>null</sup>hFcγRIIA<sup>Tg</sup> 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<sup>KI</sup> 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<sup>KI</sup>C1q<sup>-/-</sup> 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<sub>H</sub> and V<sub>L</sub> sequences (4)) lacking the N-linked glycan  
154 attached to asparagine 297 in the Fc portion (N<sub>297</sub>A 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 N<sub>297</sub>A 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<sup>KI</sup>hFcRn<sup>KI</sup>hβ2m<sup>KI</sup> 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<sup>KI</sup>hFcRn<sup>KI</sup>hβ2m<sup>KI</sup> 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 N<sub>297</sub>A mutation  
177 does not affect the half-life of the anti-IgE mAb in vivo.

178

179 We also verified that the N<sub>297A</sub> 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 $\epsilon$ RI<sup>Tg</sup> mice (which express the human IgE receptor hFc $\epsilon$ 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 $\gamma$ R<sup>KI</sup> 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 $\gamma$ R<sup>KI</sup> 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 $\gamma$ R<sup>KI</sup> 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 $\gamma$ 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 $\gamma$ 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

232 **Acknowledgments**

233

234 This work was supported by the European Commission (Marie Skłodowska-Curie  
235 Individual Fellowship H2020-MSCA-IF-2014 656086 to L.L.R.) and the European  
236 Research Council (ERC)–Seventh Framework Program (ERC-2013-CoG 616050 to P.B.),  
237 the Institut Pasteur initiative for valorizing the applications of research (ValoExpress

238 2017), the Institut National de la Santé et de la Recherche Médicale (INSERM) and the  
239 ATIP-Avenir program (to L.L.R.). Part of this work was performed on a platform member  
240 of France Life Imaging network partly funded by the French program “Investissement  
241 d’Avenir” (grant ANR-11-INBS-0006). B.B. was supported partly by a stipend from the  
242 Pasteur - Paris University (PPU) International Ph.D program and a fellowship from the  
243 French “Fondation pour la Recherche Médicale FRM”. D.S. benefited from a stipend  
244 (“Poste d’accueil”) provided by AP-HP, Paris, France and by the Institut Pasteur, Paris,  
245 France.

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.

254

255

256

257 **References**

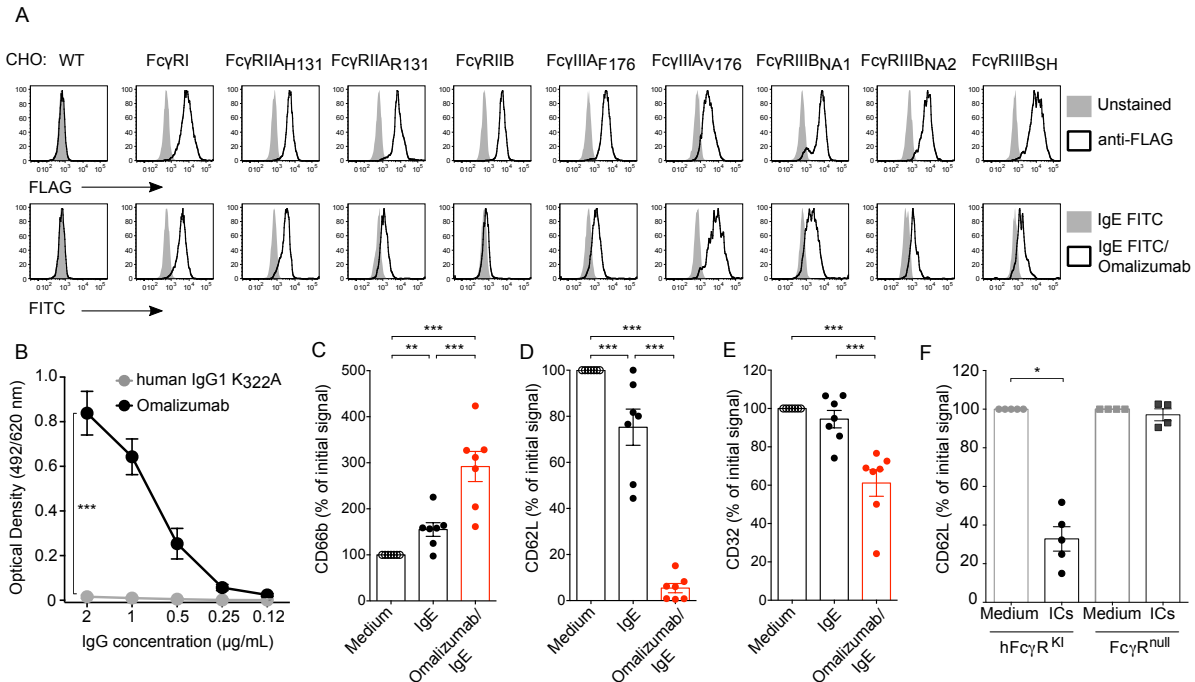
258

- 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.

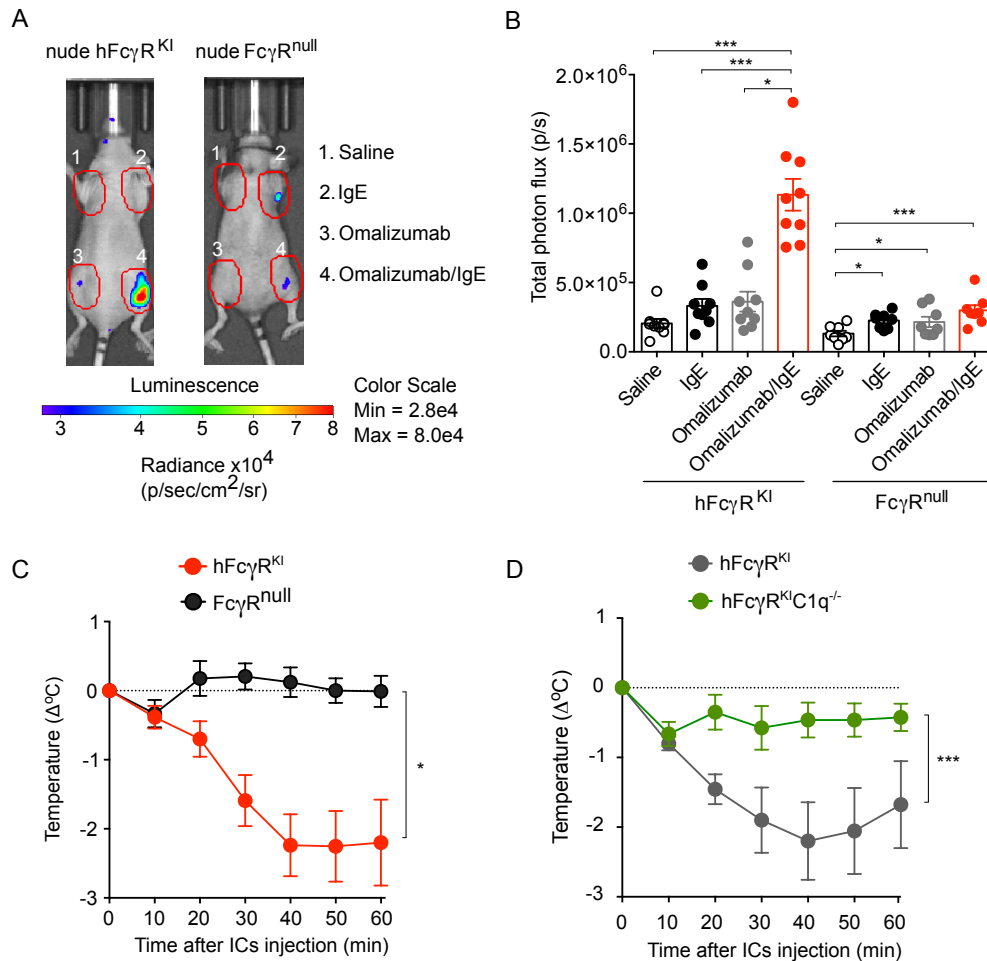


- 346 31. Jonsson F, de Chaisemartin L, Granger V, Gouel-Cheron A, Gillis CM, Zhu Q, et  
347 al. An IgG-induced neutrophil activation pathway contributes to human drug-  
348 induced anaphylaxis. *Sci Transl Med.* 2019;11(500).  
349



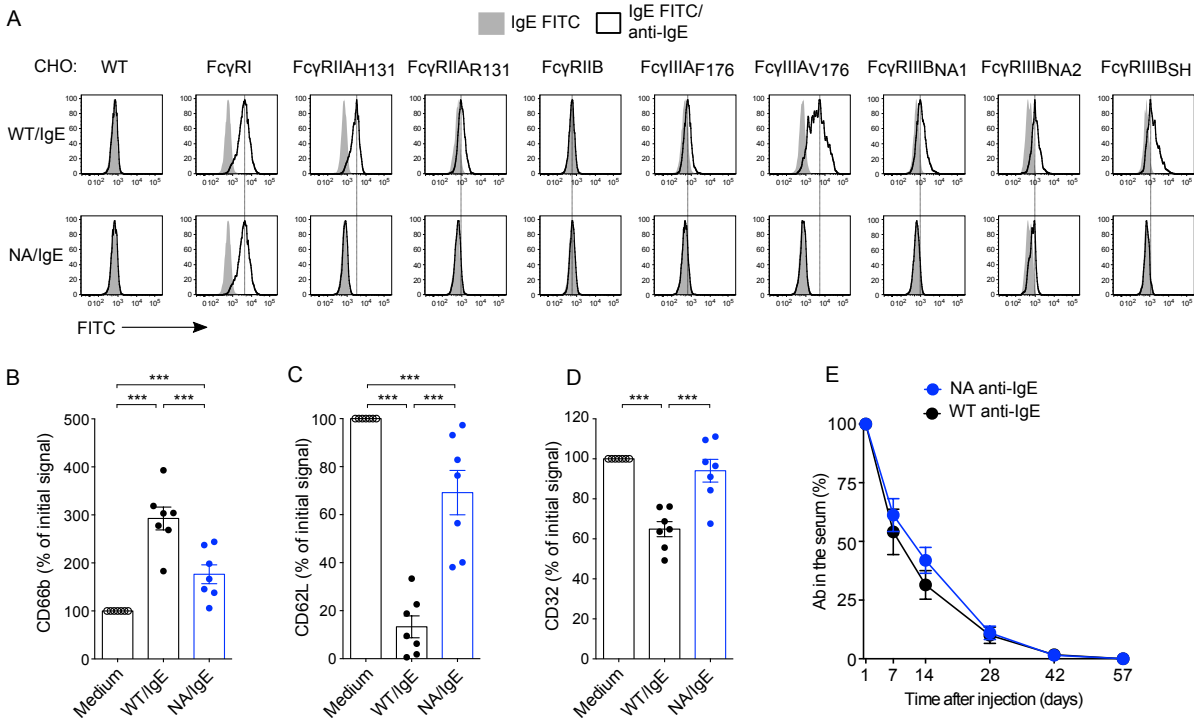
350

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 (K<sub>322</sub>A) 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<sup>+</sup>CD15<sup>+</sup> 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<sup>+</sup>Ly6G<sup>+</sup> neutrophils purified from hFcγR<sup>KI</sup> or FcγR<sup>null</sup> 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<sup>null</sup>; total *n*=4/group) or three (hFcγR<sup>KI</sup>;  
 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.  
 370



371

372 **Figure 2. Omalizumab/IgE ICs induce skin inflammation and anaphylaxis through**  
 373 **engagement of Fc $\gamma$ Rs in Fc $\gamma$ 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 $\gamma$ R<sup>KI</sup> mice ( $n=9$ ) or nude Fc $\gamma$ R<sup>null</sup> 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 $\gamma$ R<sup>KI</sup> mice ( $n=13$ ) or  
 379 Fc $\gamma$ R<sup>null</sup> mice ( $n=9$ ) (C), or hFc $\gamma$ R<sup>KI</sup> mice ( $n=9$ ) or hFc $\gamma$ R<sup>KI</sup> C1q<sup>-/-</sup> 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.

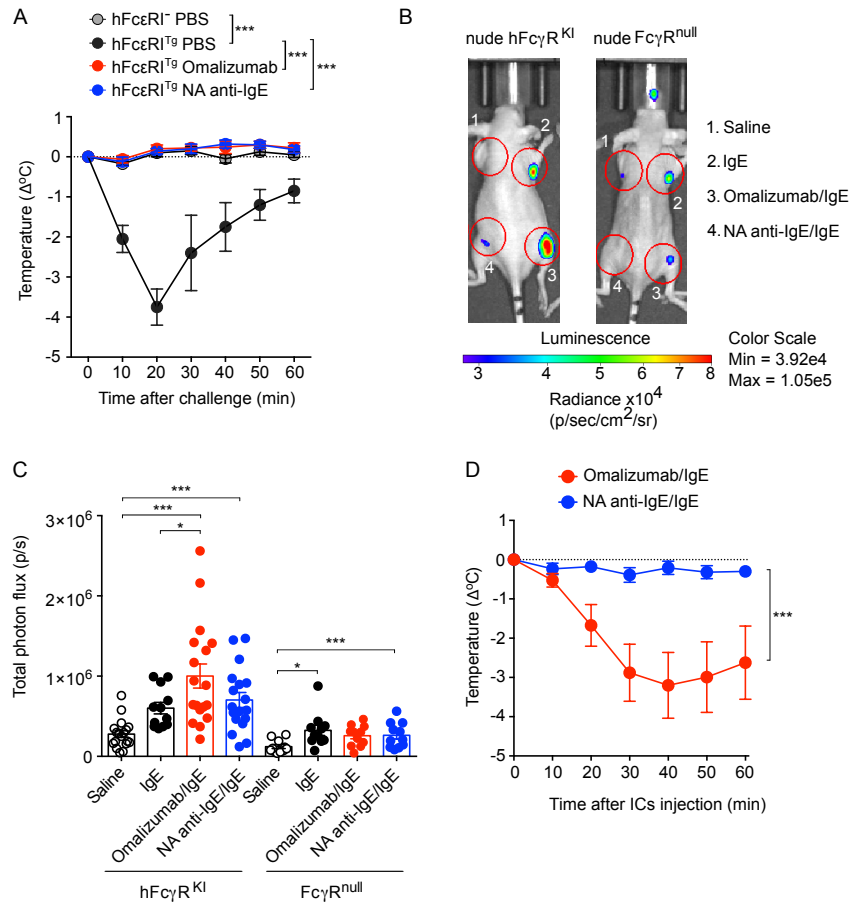


383

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<sup>+</sup>CD15<sup>+</sup> 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<sup>K1</sup>hFcRn<sup>K1</sup>hβ2m<sup>K1</sup>  
 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.

397

398



399

**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  $\mu\text{g}$  of nitrophenyl-coupled BSA (NP-BSA) in hFcεRI<sup>+/+</sup> mice pre-treated i.v. with 700  $\mu\text{g}$  Omalizumab, NA anti-IgE or PBS 30 min before sensitization with anti-NP IgE (10  $\mu\text{g}$ ). Data in A are pooled from two independent experiments (total  $n=4-6/\text{group}$ ). hFcεRI<sup>-/-</sup> 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<sup>KI</sup> or nude FcγR<sup>null</sup> mice. Regions of interest outlined in red surround the site of injection. Bars in C indicate means  $\pm$  SEM pooled from five (nude hFcγR<sup>KI</sup>, total  $n=18$ ) or four (nude FcγR<sup>null</sup>, total  $n=11$ ) independent experiments. The color bar represents bioluminescent signal in radiance (p/sec/cm<sup>2</sup>/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<sup>KI</sup> 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.