



HAL
open science

Anti-IgE IgG autoantibodies isolated from therapeutic normal IgG intravenous immunoglobulin induce basophil activation

Caroline Galeotti, Anupama Karnam, Jordan Dimitrov, Alain Chevailler, Sridhar V. Kaveri, Jagadeesh Bayry

► To cite this version:

Caroline Galeotti, Anupama Karnam, Jordan Dimitrov, Alain Chevailler, Sridhar V. Kaveri, et al.. Anti-IgE IgG autoantibodies isolated from therapeutic normal IgG intravenous immunoglobulin induce basophil activation. Cellular and molecular immunology, 2019, 17 (4), pp.426-429. 10.1038/s41423-019-0334-x . inserm-02564293

HAL Id: inserm-02564293

<https://inserm.hal.science/inserm-02564293v1>

Submitted on 27 Jun 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 *Correspondence*

2
3 **Anti-IgE IgG autoantibodies isolated from therapeutic normal IgG intravenous**
4 **immunoglobulin induce basophil activation**
5

6 **Caroline Galeotti^{1,2,*}, Anupama Karnam^{1,*}, Jordan D Dimitrov^{1,3}, Alain Chevailler⁴,**
7 **Srini V Kaveri^{1,3} and Jagadeesh Bayry^{1,3}**
8

9 ¹Institut National de la Santé et de la Recherche Médicale; Centre de Recherche des
10 Cordeliers, Equipe-Immunopathologie et Immunointervention Thérapeutique; Sorbonne
11 Université, Paris, F-75006, France

12 ²Service de Rhumatologie Pédiatrique, Centre de Référence des Maladies Auto-
13 Inflammatoires rares et des Amyloses, CHU de Bicêtre, le Kremlin Bicêtre, F-94270,
14 France

15 ³Université Paris Descartes, Sorbonne Paris Cité, Paris, F-75006, France

16 ⁴Laboratoire d'Immunologie et d'Allergologie; CHU d'Angers; Université d'Angers;
17 INSERM Unité 1232; LabEx IGO "Immuno-Graft-Onco", Angers, F-49933, France
18

19 * These authors contributed equally to this work.
20

21 **Corresponding author:** Jagadeesh Bayry (jagadeesh.bayry@crc.jussieu.fr)
22
23

24 **Keywords:** Basophils; anti-IgE IgG; IVIG; IL-3 ; CD69 ; IL-4 ; IL-8
25
26
27

28 Basophils are rare granulocytes. Despite represent only about 0.5% of the leukocytes,
29 basophils have several important functions in physiology^{1,2}. Although basophils lack the
30 classical features of professional antigen presenting cells³⁻⁷, through the secretion of
31 cytokines they orient the immune response by polarizing Th2 differentiation, and
32 supporting B cell differentiation and class-switch. Basophils are also critical for mediating
33 the protection against helminth infection^{1,2,8,9}.

34

35 Basophils receive activation signals from diverse sources. It is well recognized that
36 cytokines like IL-3, granulocyte-macrophage colony-stimulating factor (GM-CSF), thymic
37 stromal lymphopoietin (TSLP) and IL-33; various toll-like receptor ligands; and allergen-
38 bound IgE provide activation signals for the basophils and induce the release of
39 inflammatory mediators¹⁰⁻¹⁵. In addition, several reports also demonstrate the presence of
40 anti-IgE autoantibodies that possess the capacity to induce basophil activation in patients
41 with chronic spontaneous urticaria (CSU), atopic and non-atopic asthma and autoimmune
42 diseases¹⁶⁻²¹. However, isolation and functional exploration of such anti-IgE IgG
43 autoantibodies either from the healthy donors or from the patients are not attempted yet.
44 By using pooled normal IgG preparation from the healthy donors, intravenous
45 immunoglobulin G (IVIG)²² that represents a complete repertoire of IgG of a normal
46 individual, we attempted to address this outstanding question in the field.

47

48 Recently, we reported that at a concentration (25 mg/0.5 million cells/mL) corresponding
49 to the level of IgG reached in the patients immediately following high-dose IVIG therapy,
50 both IVIG and its F(ab')₂ fragments induce basophil activation²³. Mechanistically, IVIG
51 induces basophil activation by signaling through basophil surface-bound IgE in an IL-3
52 and Syk-dependent mechanism²³. We first performed dose-response experiments with

53 various concentrations of IVIG to comprehend if lower concentration of IVIG also induces
54 basophil activation.

55

56 As a source of IVIG, Sandoglobulin® (CSL Behring, Switzerland) was used in the
57 experiments. It was dialyzed several times against phosphate buffered saline followed by
58 final dialysis in RPMI-1640 at 4°C for the removal of stabilizing agents. Peripheral blood
59 basophils were isolated from the buffy bags of healthy donors obtained from Centre
60 Necker-Cabanel, EFS, Paris (INSERM-EFS ethical permission N°18/EFS/033). The
61 cellular fractions that contain peripheral blood mononuclear cells and basophils were
62 collected by Ficoll density gradient centrifugation. From these fractions, basophils were
63 isolated by basophil isolation kit II (Miltenyi Biotec) and autoMACS® (Miltenyi Biotec).
64 The purity of basophils was 96-97%.

65

66 Basophils (0.1×10^6 cells/well/200 μ L) were distributed in 96 well U-bottomed plates. For
67 the dose-response experiments with IVIG, basophils were cultured either alone in serum-
68 free X-VIVO 15 medium; or with IL-3 (100 ng/mL, ImmunoTools); or with IL-3 plus
69 three different doses of IVIG (25, 15 or 10 mg/mL) for 24 hours. IVIG was added
70 following two hours of stimulation of basophils with IL-3. Basophils were analyzed for the
71 expression of CD69 by flow cytometry (LSR II, BD Biosciences) using CD69-APC/Cy7
72 MAb from BD Biosciences. Flow cytometry data were analyzed by BD FACS DIVA (BD
73 Biosciences) and Flowjo (FlowJo LLC). Cell-free culture supernatants were used for the
74 analyses of cytokines by ELISA (ELISA Ready-SET-Go, eBioscience Affymetrix).

75

76 As IVIG did not modify the activation status of either resting basophils or basophils
77 primed with IL-33, TSLP, IL-25 or GM-CSF cytokines²³, we used IL-3 priming

78 throughout the experiments. The dose-response experiments revealed that even at 10
79 mg/mL concentration corresponding to the circulating IgG levels of healthy donors, IVIG
80 could induce basophil activation as shown by the enhanced expression of CD69 and IL-4
81 secretion (Figure 1a and b). These results suggest that occurrence of anti-IgE IgG
82 autoantibodies is a common feature in the healthy population.

83

84 We then investigated if the induction of basophil activation by IVIG was due to non-
85 specific cell activation caused by cell death. Therefore, we measured the viability of cells
86 by Annexin V staining. Basophils were cultured in the medium alone or with IL-3 or IL-3
87 plus IVIG (25 mg/mL) or IL-3 plus human serum albumin (HSA, 10 mg/mL, Laboratoire
88 Français du fractionnement et des Biotechnologies, France) for 24 hours. HSA was used an
89 irrelevant protein control for IVIG. The cells were stained with Annexin V (APC-Annexin
90 V, BD Biosciences) and propidium iodide (PI, Invitrogen™). We indeed confirm that
91 basophil activation induced by IVIG was not because of non-specific cell activation caused
92 by cell death. In fact, viability of cells as analyzed by Annexin V-PI staining was similar
93 between IL-3-treated, IVIG-treated and HSA-treated cells (Figure 1c and d).

94

95 These data prompted us to investigate anti-IgE IgG autoantibodies in IVIG. The ELISA
96 performed with recombinant human IgE-kappa (AbD Serotec) revealed that both IVIG and
97 its F(ab')₂ fragments recognize IgE in a dose-dependent manner (Figure 1e). Therefore, we
98 next aimed at isolating the anti-IgE IgG autoantibodies from IVIG by affinity
99 chromatography. IgE myeloma protein was coupled to a cyanogen bromide-activated
100 sepharose 4B (Sigma-Aldrich). IVIG at a concentration of 60 mg/mL was added to the
101 column and incubated at ambient temperature for 4 hours on a rotator. After draining the
102 unbound IgG, column was washed several times with phosphate buffered saline. The anti-

103 IgE IgG autoantibodies were eluted by using 3M potassium thiocyanate, dialyzed against
104 phosphate buffered saline and concentrated. IgG concentration was measured by
105 spectrophotometer (NanoDrop Technologies).

106

107 Based on the amount of eluted IgG, we estimate that anti-IgE IgG represent nearly 0.3-
108 0.5% of total IgG (IVIG). We then investigated if these isolated anti-IgE IgG are
109 functional. IL-3-primed basophils from healthy individuals were stimulated with either
110 IVIG (25 mg/0.5x10⁶ cells/mL) or isolated anti-IgE IgG (1.5 mg/0.5x10⁶/mL) for 24 hours.
111 This concentration of anti-IgE IgG was selected considering the levels of IgG and the
112 number of basophils in the circulation of healthy individuals. We found that isolated anti-
113 IgE IgG were able to induce basophil activation similar to that of IVIG even at 16-times
114 lesser concentrations: both induced similar level of CD69 expression (Figure 1f and g), and
115 secretion of IL-4 and IL-8 (Figure 1h).

116

117 The site of recognition of anti-IgE IgG autoantibodies from IVIG is not known. As we
118 used IgE myeloma protein for the isolation of anti-IgE IgG, and yet these isolated IgG
119 induced activation of basophils from various donors carrying distinct IgE molecules imply
120 that anti-IgE IgG do not signal basophils by interacting with Fab-region of basophil-bound
121 IgE. It is likely that these antibodies might recognize either Cε2 or Cε4 domains of IgE but
122 not Cε3 domain (which is binding site of FcεRI and hence inaccessible for the recognition
123 by anti-IgE IgG) similar to the previous report²⁴; although we do not rule out the
124 recognition of Cε1 domain as well.

125

126 The precise function of anti-IgE IgG autoantibodies in healthy individuals is not clear at
127 this stage. Isolation and characterization of such anti-IgE IgG monoclonal antibodies by

128 using techniques like phage display technology should provide further insight on them²⁵.
129 Since anti-IgE autoantibodies induce IL-4 secretion in basophils, this might support Th2
130 and B cell differentiation. In the context of IVIG immunotherapy however, we propose that
131 IL-4 induction in basophils might help in the suppression of effector Th1 and Th17 cells²²,
132 and in the upregulation of inhibitory Fc γ RIIB on phagocytic cells to reduce inflammatory
133 response to IgG-autoantigen immune complexes²⁶. Thus, distinct natural autoantibodies
134 diversify the action of IVIG on granulocytes²⁷.
135

136

137 **CONFLICT OF INTEREST**

138 The work is supported in part by research grant from CSL Behring, Switzerland.

139

140

141 **ACKNOWLEDGMENTS**

142

143 Supported by Institut National de la Santé et de la Recherche Médicale (INSERM),

144 Université Pierre et Marie Curie, Université Paris Descartes, ANR-AAPG2019-CE17-

145 BASIN, and CSL Behring, Switzerland. CG and AK were the recipient of fellowships from

146 La Fondation pour la Recherche Médicale (FDM20150633674 and FDT201805005552

147 respectively) France. We thank the staff of Centre d'Histologie, d'Imagerie et de

148 Cytométrie, Centre de Recherche des Cordeliers for the help; and F. Carrère at Royan

149 Hospital for sending the IgE myeloma serum.

150

151

152 **AUTHOR CONTRIBUTIONS**

153 JB designed the study. CG and AK performed the experiments. CG, AK, JD, SVK and JB

154 analyzed the data. AC provided the essential tools. JB drafted the manuscript, and all the

155 authors edited and approved the final version of the manuscript.

156

157

158

159

160

161

162

163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212

REFERENCES

- 1 Karasuyama, H., Miyake, K., Yoshikawa, S. & Yamanishi, Y. Multifaceted roles of basophils in health and disease. *J. Allergy Clin. Immunol.* **142**, 370-380, doi:10.1016/j.jaci.2017.10.042 (2018).
- 2 Voehringer, D. Protective and pathological roles of mast cells and basophils. *Nat. Rev. Immunol.* **13**, 362-375 (2013).
- 3 Eckl-Dorna, J. *et al.* Basophils are not the key antigen-presenting cells in allergic patients. *Allergy* **67**, 601-608 (2012).
- 4 Kitzmuller, C. *et al.* Human blood basophils do not act as antigen-presenting cells for the major birch pollen allergen Bet v 1. *Allergy* **67**, 593-600 (2012).
- 5 Sharma, M. *et al.* Circulating human basophils lack the features of professional antigen presenting cells. *Sci. Rep.* **3**, 1188 (2013).
- 6 Miyake, K. *et al.* Trogocytosis of peptide-MHC class II complexes from dendritic cells confers antigen-presenting ability on basophils. *Proc. Natl. Acad. Sci. U S A.* **114**, 1111-1116 (2017).
- 7 Stephen-Victor, E. *et al.* Demystification of enigma on antigen-presenting cell features of human basophils: data from secondary lymphoid organs. *Haematologica* **102**, e233-e237 (2017).
- 8 Gurram, R. K. & Zhu, J. Orchestration between ILC2s and Th2 cells in shaping type 2 immune responses. *Cell. Mol. Immunol.* **16**, 225-235 (2019).
- 9 Sharma, M. & Bayry, J. Autoimmunity: Basophils in autoimmune and inflammatory diseases. *Nat. Rev. Rheumatol.* **11**, 129-131 (2015).
- 10 Voehringer, D. Basophil modulation by cytokine instruction. *Eur. J. Immunol.* **42**, 2544-2550 (2012).
- 11 Siracusa, M. C. *et al.* TSLP promotes interleukin-3-independent basophil haematopoiesis and type 2 inflammation. *Nature* **477**, 229-233 (2011).
- 12 Leyva-Castillo, J. M. *et al.* Skin thymic stromal lymphopoietin initiates Th2 responses through an orchestrated immune cascade. *Nat. Commun.* **4**, 2847 (2013).
- 13 Sharma, M. *et al.* Regulatory T cells induce activation rather than suppression of human basophils. *Sci Immunol* **3**, aan0829 (2018).
- 14 Jiao, D. *et al.* NOD2 and TLR2 ligands trigger the activation of basophils and eosinophils by interacting with dermal fibroblasts in atopic dermatitis-like skin inflammation. *Cell. Mol. Immunol.* **13**, 535-550 (2016).
- 15 Suurmond, J. *et al.* Activation of human basophils by combined toll-like receptor- and FcepsilonRI-triggering can promote Th2 skewing of naive T helper cells. *Eur. J. Immunol.* **44**, 386-396 (2014).
- 16 MacGlashan, D. Autoantibodies to IgE and FcepsilonRI and the natural variability of spleen tyrosine kinase expression in basophils. *J. Allergy Clin. Immunol.* **143**, 1100-1107 e1111 (2019).
- 17 Ritter, C., Battig, M., Kraemer, R. & Stadler, B. M. IgE hidden in immune complexes with anti-IgE autoantibodies in children with asthma. *J. Allergy Clin. Immunol.* **88**, 793-801 (1991).
- 18 Chan, Y. C. *et al.* "Auto-anti-IgE": naturally occurring IgG anti-IgE antibodies may inhibit allergen-induced basophil activation. *J. Allergy Clin. Immunol.* **134**, 1394-1401 e1394 (2014).
- 19 Shakib, F. & Smith, S. J. In vitro basophil histamine-releasing activity of circulating IgG1 and IgG4 autoanti-IgE antibodies from asthma patients and the demonstration that anti-IgE modulates allergen-induced basophil activation. *Clin. Exp. Allergy* **24**, 270-275 (1994).

- 213 20 Iwamoto, I. *et al.* Relationship between anti-IgE autoantibody and severity of
214 bronchial asthma. *Int. Arch. Allergy Appl. Immunol.* **90**, 414-416 (1989).
- 215 21 Gruber, B. L., Kaufman, L. D., Marchese, M. J., Roth, W. & Kaplan, A. P. Anti-
216 IgE autoantibodies in systemic lupus erythematosus. Prevalence and biologic
217 activity. *Arthritis Rheum.* **31**, 1000-1006 (1988).
- 218 22 Galeotti, C., Kaveri, S. V. & Bayry, J. IVIG-mediated effector functions in
219 autoimmune and inflammatory diseases. *Int. Immunol.* **29**, 491-498 (2017).
- 220 23 Galeotti, C. *et al.* Intravenous immunoglobulin induces IL-4 in human basophils by
221 signaling through surface-bound IgE. *J. Allergy Clin. Immunol.* **144**, 524-535 e528
222 (2019).
- 223 24 Shakib, F. & Powell-Richards, A. Elucidation of the epitope locations of human
224 autoanti-IgE: recognition of two epitopes located within the C epsilon 2 and the C
225 epsilon 4 domains. *Int. Arch. Allergy Appl. Immunol.* **95**, 102-108 (1991).
- 226 25 Vogel, M. & Horn, M. P. Isolation of Natural Anti-FcepsilonRIalpha
227 Autoantibodies from Healthy Donors. *Methods Mol. Biol.* **1643**, 5-22 (2017).
- 228 26 Anthony, R. M., Kobayashi, T., Wermeling, F. & Ravetch, J. V. Intravenous
229 gammaglobulin suppresses inflammation through a novel T(H)2 pathway. *Nature*
230 **475**, 110-113 (2011).
- 231 27 von Gunten, S. & Simon, H. U. Granulocyte death regulation by naturally
232 occurring autoantibodies. *Adv. Exp. Med. Biol.* **750**, 157-172 (2012).
- 233

234

235
236
237

FIGURE LEGEND

238 **Figure 1. Anti-IgE IgG autoantibodies isolated from IVIG induce basophil activation.**

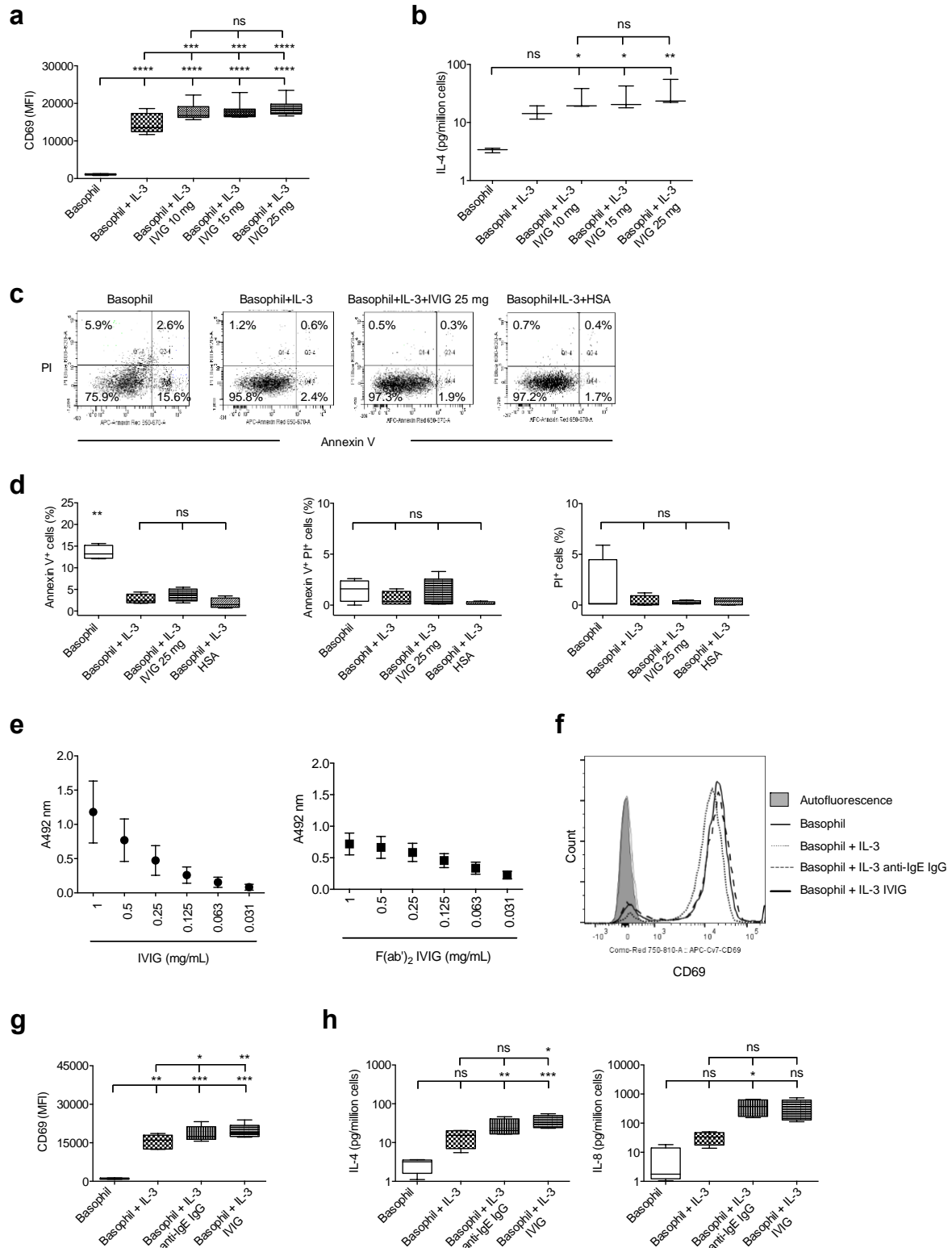
239 **(a, b)** Basophils isolated from the healthy donors were cultured either alone (0.1×10^6
240 cells/well/200 μ L) or with IL-3 (100 ng/mL). Three different concentrations of IVIG (25,
241 15 or 10 mg/ml) were added to the cells following 2 hours stimulation with IL-3. After 24
242 hours, the expression of CD69 (MFI, median fluorescence intensity, n=7 donors) and the
243 amounts of IL-4 (n=3 donors) in the culture supernatants were analyzed. **(c, d)** The
244 viability of basophils as analyzed by Annexin V and PI staining. Representative dot plots
245 (c) and data from four experiments (d) using different donors are presented. HSA, human
246 serum albumin used at 10 mg/mL concentration. **(e)** Recognition of IgE by IVIG (left
247 panel) or (Fab')₂ fragments (right panel). Data (mean \pm SEM) are from the two
248 experiments. **(f-g)** Basophils were cultured either alone or with IL-3 (100 ng/mL). IVIG
249 (25 mg/mL) or anti-IgE IgG (1.5 mg/mL) were added to the cells following 2 hours
250 stimulation with IL-3. After 24 hours, the expression (MFI, median fluorescence intensity)
251 of CD69 was analyzed. Representative histogram overlays (e) and data from 5 donors (f)
252 are presented. **(h)** The amounts (pg) of IL-4 and IL-8 in the culture supernatants of above
253 experiments. Data from 4 donors are presented. All data are plotted using box & whiskers
254 plot, wherein whiskers denote minimum and maximum values and the dividing line in the
255 box symbolises the median. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; ns, not
256 significant, one-way ANOVA with Tukey's multiple comparison tests.

257

258

259

260



261

262

263