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1 **Basophils are inept at promoting human Th17 responses**

2
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25 **Abbreviated Title:** basophils and human Th17 response
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28

29 **Abstract**

30 Basophils are the rare granulocytes and play an important role in the polarization of
31 Th2 responses and protection against helminth parasites. In addition, basophils
32 contribute to the pathogenesis of several diseases such as asthma, chronic allergy and
33 lupus. Notably, Th17 cells are also implicated in the pathogenesis of these diseases
34 suggesting that basophils support the activation and expansion of this subset of CD4⁺
35 T cells. Therefore, we explored whether basophils promote the expansion of human
36 Th17 cells. We show that basophils lack the capacity to expand Th17 cells and to
37 induce the secretion of Th17 cytokines either directly or indirectly via antigen
38 presenting cells such as monocytes. As human basophils lack HLA-DR and co-
39 stimulatory molecules, their inability to confer T cell receptor- and co-stimulatory
40 molecule-mediated signals to CD4⁺ T cells might explain the lack of Th17 responses
41 when memory CD4⁺ T cells were co-cultured with basophils.

42

43

44 **Key words:** basophils; IL-17; Th17; IL-22; monocytes

45

46

47 **1. Introduction**

48 Basophils are the rare granulocytes and represent less than 1% of circulating
49 leukocytes. They play an important role in the polarization of Th2 responses and in
50 the protection against helminth parasites [1-5]. Recent studies have identified several
51 surface markers of human and mouse basophils that could be used for the
52 identification and isolation of these cells. These markers include CD49b (DX5),
53 CD123 (IL-3 receptor α chain), CD200R3 (a disulfide-linked dimeric CD200R-like
54 receptor belonging to the immunoglobulin superfamily), CD203c, 2B4 (or CD244, a
55 66-kDa protein from the CD2 family), CCR2, CCR3, CD45R (intermediate level of
56 expression) and Fc ϵ RI. Further, in contrary to mast cells, basophils are c-Kit⁻
57 (CD117⁻) and this marker could be used to discriminate basophils from mast cells in
58 the tissues [2].

59

60 Since long time, basophils have been neglected in immunology due to their low
61 number in the circulation and their shared features with tissue-resident mast cells.
62 However, recent studies indicate that basophils have a major impact on the immune
63 responses and diverse roles of these cells in autoimmune and inflammatory diseases
64 are emerging. Because basophils express several sensing molecules including Fc ϵ RI,
65 toll-like receptors (TLRs such as TLR2 and TLR4) and receptors for various
66 cytokines including IL-3, IL-33 and IL-25, basophils can readily respond to various
67 stimuli and release immune modulators such as cytokines, chemokines, histamine and
68 lipid mediators [2]. Therefore, a higher number of activated basophils could tilt the
69 homeostatic balance of the immune system leading to inflammatory conditions.

70

71 Activated basophils act as accessory cells to provide Th2 environment and enhance
72 dendritic cell-mediated Th2 responses. In fact, recent reports indicate that the function
73 of basophils in the polarization of Th2 responses is not only important for the
74 protection against helminth parasites but it can also contribute to the pathogenesis of
75 asthma, allergy and autoimmune diseases such as systemic lupus erythematosus [1, 2,
76 6-8].

77

78 A newly identified subset of CD4⁺ T cells namely Th17 cells are also implicated in
79 the pathogenesis of asthma, chronic allergy and lupus suggesting that basophils might
80 support the activation and expansion of this subset of CD4⁺ T cells [9, 10]. Th17 cells
81 express lineage specific transcription factor RORC and IL-17A is the prototype
82 cytokine of these cells. In addition, Th17 cells secrete other inflammatory mediators
83 such as IL-17F and IL-22 [9]. As basophils have an important role in the regulation of
84 immune responses such as T and B cell responses, we explored whether basophils
85 promote the expansion of human Th17 cells.

86

87 **2. Materials and Methods**

88 *2.1. Isolation of circulating human basophils and monocytes*

89 Buffy coats of healthy donors were purchased from Centre Necker-Cabanel,
90 Etablissement Français du Sang, Paris, France upon ethical committee permission
91 (N°12/EFS/079). Basophils from the buffy coats were isolated by two-step process.
92 By percoll density gradient centrifugation, we first obtained peripheral blood
93 mononuclear cells (PBMCs). These PBMCs were subjected to MicroBead-based
94 negative isolation of basophils by using basophil isolation kit II (Miltenyi Biotec,
95 Paris, France) [11]. Monocytes from PBMCs were purified by using CD14

96 MicroBeads (Miltenyi Biotec). The purity of basophils as well as that of monocytes
97 was in the range of 94±5% as analyzed by flow cytometry (BD LSR II, BD
98 Biosciences, Le Pont de Claix, France). Basophils were analyzed by using
99 fluorochrome-conjugated mAbs to CD203c (eBioscience, Paris, France) FcεRI and
100 CD123 (both from Miltenyi Biotec) [12] while monocytes were monitored by using
101 fluorochrome -conjugated mAb to CD14 (BD Biosciences).

102

103 *2.2. Isolation of memory CD4⁺ T cells*

104 To isolate memory CD4⁺ T cells, untouched total CD4⁺ T cells were first purified
105 from PBMCs by using CD4⁺ T-cell isolation kit II (Miltenyi Biotec). Further, by
106 using CD45RA MicroBeads (Miltenyi Biotec), naïve CD4⁺CD45RA⁺ T cells were
107 depleted from total CD4⁺ T cells. Finally, CD4⁺CD45RO⁺CD25⁻ memory T cells
108 were obtained by depleting CD25⁺ cells with CD25 MicroBeads (Miltenyi Biotec).
109 The purity of isolated cells was in the range of 95±4%.

110

111

112 *2.3. Co-culture of basophils and monocytes with CD4⁺CD45RO⁺CD25⁻ memory T* 113 *cells*

114 Allogeneic memory CD4⁺ T cells were cultured in U-bottomed 96 wells plate
115 (0.1x10⁶ cells/200 µl/well) in X-vivo-10% human AB serum and IL-2 (100
116 IU/0.5x10⁶ cells, ImmunoTools, Friesoythe, Germany) either alone; or with basophils
117 in the presence of IL-3 (100 ng/1x10⁶ cells, Miltenyi Biotec) or IL-3 and monoclonal
118 anti-human IgE (10 ng/0.1x10⁶ cells, clone GE1, Sigma-Aldrich, Saint Quentin
119 Fallavier, France); or with peptidoglycan-stimulated monocytes (5 µg/0.5 x10⁶ cells,
120 Invivogen, Toulouse, France); or with peptidoglycan-stimulated monocytes and IL-3-
121 primed basophils; or with peptidoglycan-stimulated monocytes and IL-3-anti-IgE-

122 treated basophils. The activation of basophils was analyzed by the expression of
123 CD63 by using fluorescence-conjugated mAb (BD Bioscience). Monocytes and
124 basophils were stimulated in the co-culture and were not pre-activated. The ratio of
125 memory CD4⁺ T cells and monocytes and/or basophils was maintained at 5:1. After
126 five days of culture, the cells were harvested and cell-free culture supernatants were
127 collected for the analysis of IL-17A and IL-17F. The cells were processed for staining
128 and flow cytometry as described below.

129

130 *2.4. Intracellular staining and flow cytometry*

131 The harvested cells were re-stimulated with phorbol 12-myristate 13-
132 acetate/ionomycin (Sigma-Aldrich) for 6 hours, with GolgiStop (BD Biosciences)
133 during last 3 hours. Surface staining was done with fluorescence-conjugated CD4
134 mAb (BD Biosciences) and fixable viability dye (eBioscience), in order to gate and
135 analyze viable CD4⁺ T cells. Further, cells were fixed, permeabilized (Fix/Perm;
136 eBioscience), and incubated at 4°C with fluorochrome-conjugated mAbs to IFN- γ , IL-
137 4 (BD Biosciences) and IL-17A (eBioscience). The stained cells were subjected to
138 flow cytometry (BD LSR II). Ten thousand cells were acquired for each sample and
139 data were processed by using FACS DIVA software (BD Biosciences).

140

141 *2.5. Cytokines analysis*

142 Levels of IL-17A (DuoSet ELISA kits, R&D Systems), IL-17F and IL-6 (ELISA
143 Ready-SET-Go, eBioscience) in cell-free culture supernatants were quantified by
144 ELISA. The detection limits were 15 pg/mL for IL-17A, 16 pg/mL for IL-17F and 2
145 pg/mL for IL-6.

146

147 *2.6. Measurement of plasma IgE*

148 The IgE in the plasma of healthy donors was measured by an automated classical
149 sandwich immunoassay by ImmunoCap technology (Thermo Fischer, Phadia SAS, St
150 Quentin Yvelines, France). Results are expressed in kU/L and the admitted
151 correspondence is 2.4 ng/ml per kU/L

152

153 *2.7. Statistical analysis*

154 Statistical analysis was done by one-Way ANOVA (Friedman test) or two-tailed
155 Student's-t-test using Prism 5 software (GraphPad softwares). Values of $P < 0.05$
156 were considered as statistically correlated.

157

158 **3. Results**

159

160 *3.1. Activated human basophils lack the capacity to promote Th17 expansion*

161 We investigated the direct effect of basophils on the expansion of Th17 cells. As
162 stimulated basophils are known to secrete variety of cytokines and other chemical
163 mediators, we also examined if enhanced degranulation of basophils through FcεRI
164 cross-linking would augment Th17 responses. IL-3-primed basophils were co-
165 cultured with CD4⁺CD45RO⁺ memory T cells either in the presence or absence of
166 FcεRI cross-linking. To avoid nonspecific stimulatory effects of xeno-proteins in the
167 fetal calf serum, we utilized X-vivo medium-containing 10% human AB serum for the
168 experiments. Also, survival of basophils in the co-cultures was ensured by the
169 addition of IL-3 at the time of co-culture of cells. As activated CD4⁺ T cells produce
170 IL-3, this will further ensure the survival of basophils [13, 14].

171

172 FcεRI cross-linking led to activation of basophils as analyzed by the expression of
173 CD63 (Fig. 1A). We observed that neither IL-3-primed nor FcεRI-activated basophils
174 could amplify IL-17A⁺ Th17 cells from memory CD4⁺ T cells (Fig. 1B and 1C). The
175 percentage of IL-17A⁺/IFN-γ⁻ and IL-17A⁺/IFN-γ⁺ T cells remained unaltered in the
176 presence of either IL-3-primed or FcεRI-activated basophils. In addition, basophils
177 did not activate Th17 cells to secrete Th-17-derived cytokines. Only marginal changes
178 in the secretion pattern of IL-17A and IL-17F were observed (Fig. 2A and 2B). Thus,
179 our results imply that basophils alone are poor inducers of Th17 cell expansion and
180 hence ruled out the possibility of the direct association of basophils in the
181 development of Th17 responses. We also analyzed the proportion of IFNγ⁺CD4⁺ T
182 cells and IL-4⁺CD4⁺ T cells among CD4⁺ T cells that were co-cultured with
183 basophils. We observed an increased tendency of Th2 response and decreased Th1
184 response. However, results were statistically non-significant due to variations among
185 the individual donors (data not shown).

186

187 *3.2. Activation of basophils is not influenced by the donor-dependent variations in the* 188 *level of plasma IgE and the expression of FcεRI on the basophils*

189 We examined whether the concentration of IgE in the plasma of healthy donors and
190 the expression of FcεRI on the basophils influence the activation of basophils. We
191 found that donors had uniform level of plasma IgE (28.25±5.1 kU/L, n=7) (Fig. 3A)
192 and the expression of FcεRI on the basophils (mean fluorescence intensity:
193 6367±1045, n=8) (Fig. 3B). These data thus ruled out the possibility of significant
194 donor-dependent variations in basophil stimulation due to plasma IgE and FcεRI
195 expression on the basophils.

196

197 3.3. *Human basophils are inapt at promoting antigen presenting cell-mediated Th17*
198 *expansion*

199 It is known that basophils secrete various inflammatory mediators and hence could
200 influence the activation of other immune cells [2, 15]. Therefore, by mimicking
201 closely the tissue microenvironment i.e. in the presence of activated antigen
202 presenting cells (APCs, TLR2-activated monocytes in our experiments) that would
203 provide all different signals required for CD4⁺ T cell activation, we investigated the
204 effect of activated basophils on APC-mediated Th17 responses.

205

206 In line with previous reports, we found that IL-17A⁺ Th17 cells were significantly
207 enhanced when memory CD4⁺ T cells were co-cultured with monocytes, thus
208 confirming the ability of activated APCs to expand Th17 cells [9, 10, 16]. Whereas,
209 IL-3 treated basophils did not further amplify monocyte-mediated Th17 responses
210 (Fig. 1B and 1C). The proportion of IL-17A⁺/IFN- γ ⁻ and IL-17A⁺/IFN- γ ⁺ T cells was
211 not significantly altered in the presence of IL-3-primed basophils with monocytes
212 (Fig. 1B and 1C). Interestingly, similar results were also obtained in the presence of
213 Fc ϵ RI-activated-basophils. These flow-cytometry results were further confirmed by
214 the analysis of secretion of Th-17-derived cytokines. Monocytes significantly
215 enhanced the production of IL-17A and IL-17F by ten to fifteen times (Fig. 2A and
216 2B). Although, there was a slight increase in the production of these cytokines in the
217 presence of basophils, the values were not statistically significant (Fig. 2A and 2B).
218 We have recently demonstrated that basophils also lack the capacity to modulate
219 another Th17 cytokine IL-22 from CD4⁺ T cells [17]. Together, these results thus
220 provide a pointer that circulating human basophils lack the capacity to enhance APC-
221 mediated Th17 responses.

222 *3.4. Human basophils produce minute amounts of IL-6 following activation*

223 A slender increase in the production of monocyte-mediated Th17 cytokines in the
224 presence of activated basophils suggest that basophils secrete cytokines or soluble
225 factors that stimulate Th17 cytokines. However, human basophils produce
226 undetectable levels of other Th17 propagating cytokines such as IL-23 and PGE₂ [18].
227 On the other hand, basophils have been shown to secrete small amounts of IL-6 that
228 could explain marginal increase in the level of Th17 cytokines. In fact, IL-3 and
229 FcεRI-activated-basophils (0.2×10^5 cells) produced 57.4 ± 52.8 pg (n=4) of IL-6.
230 However, equivalent number of TLR2-activated monocytes produced 4829.5 ± 1426.3
231 pg (n=4) of IL-6 (Fig. 4). As activated innate cells such as monocyte, macrophages
232 and dendritic cells (DCs) secrete massive quantities of Th17-amplifying cytokines
233 [19, 20], the basophil-secreted IL-6 effect would be nullified.

234

235 **4. Discussion**

236 Various receptor-ligand interactions between APCs and responder CD4⁺ T cells, and
237 cytokine milieu in the microenvironment determine the activation, polarization and
238 expansion of CD4⁺ T cells. Previous reports have shown that murine basophils at
239 secondary lymphoid organs display the features of professional APCs and polarize
240 Th2 responses [21-24]. However, these reports are contradictory due to the basophil
241 depletion method employed [25, 26] and also DCs could mediate Th2 polarization
242 independent of IL-4 via Notch ligand Jagged and OX-40 ligand [27, 28]. In contrast to
243 murine basophils, several reports including ours demonstrated that circulating human
244 basophils lack HLA-DR and co-stimulatory molecules CD80 and CD86 and were
245 unable to function as APCs to promote T cell polarization [11, 29-32]. Although,
246 stimulation of basophils with GM-CSF and IFN-γ was shown to induce HLA-DR

247 expression to a smaller extent in some donors, these cells did not express co-
248 stimulatory molecules [33]. Thus, the inability of human basophils to confer T-cell
249 receptor- and co-stimulatory molecule-mediated signals to CD4⁺ T cells might explain
250 the lack of Th17 responses when CD4⁺ T cells were co-cultured with basophils.

251

252 Recently Wakahara et al., demonstrated that human basophils enhance Th17
253 responses upon interaction with memory CD4⁺ T cells [34]. The reasons for the
254 discrepancies in the results are not clear. Differences in the type of serum used and
255 stimulatory conditions could be the possible reasons. Based on their results and the
256 presence of basophils in the inflamed mucosal tissues, Wakahara et al., also suggested
257 a role for basophils in the pathogenesis of inflammatory bowel disease [34]. However,
258 on the contrary, a recent report demonstrates that basophils limit the disease severity
259 in experimental murine colitis model [35]. Also, a recent randomized, double-blind
260 placebo-controlled clinical trial failed to demonstrate effectiveness of a human anti-
261 IL-17A monoclonal antibody Secukinumab for moderate to severe Crohn's disease
262 [36]. Therefore, the pathogenic role of Th17 cells in inflammatory bowel disease
263 remains controversial.

264

265 To conclude, our results indicate that basophils lack the ability to augment Th17 cell
266 responses either directly or via APCs. Therefore, we suggest that increased activation
267 and accumulation of Th17 cells in various inflammatory diseases such as asthma,
268 chronic allergy and lupus are under the control of innate cells such as monocytes,
269 macrophages or DCs but not basophils.

270

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272

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278

279 **Conflict of interests:** The authors declare no competing financial interests.

280

281 **Author contributions**

282 M.S. performed the experiments, analyzed the data, drawn the figures and wrote the
283 paper.

284 E.S-V. performed the experiments and analyzed the data.

285 P.P. performed the experiments and analyzed the data.

286 S.V.K. analyzed the data.

287 J.B. analyzed the data, drawn the figures and wrote the paper.

288 All authors reviewed the manuscript and approved the final version.

289

290

291 **References**

- 292 [1] Karasuyama H, Yamanishi Y. Basophils have emerged as a key player in
293 immunity. *Curr Opin Immunol* 2014;31:1-7
- 294 [2] Voehringer D. Protective and pathological roles of mast cells and basophils.
295 *Nat Rev Immunol* 2013;13:362-75.
- 296 [3] Min B, Prout M, Hu-Li J, Zhu J, Jankovic D, Morgan ES, et al. Basophils
297 produce IL-4 and accumulate in tissues after infection with a Th2-inducing
298 parasite. *J Exp Med.* 2004; 200:507-17.
- 299 [4] Otsuka A, Nakajima S, Kubo M, Egawa G, Honda T, Kitoh A, et al. Basophils
300 are required for the induction of Th2 immunity to haptens and peptide
301 antigens. *Nat Commun.* 2013;4:1739. doi:10.1038/ncomms2740
- 302 [5] Suurmond J, Stoop JN, Rivellese F, Bakker AM, Huizinga TW, Toes RE.
303 Activation of human basophils by combined toll-like receptor-and FcεRI-
304 triggering can promote Th2 skewing of naive T helper cells. *Eur J Immunol.*
305 2014;44:386-96
- 306 [6] Deckers J, Branco Madeira F, Hammad H. Innate immune cells in asthma.
307 *Trends Immunol* 2013;34:540-7.
- 308 [7] Charles N, Hardwick D, Daugas E, Illei GG, Rivera J. Basophils and the T
309 helper 2 environment can promote the development of lupus nephritis. *Nat*
310 *Med* 2010;16:701-7.
- 311 [8] Kaveri SV, Mouthon L, Bayry J. Basophils and nephritis in lupus. *N Engl J*
312 *Med* 2010;363:1080-2.
- 313 [9] Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. *Annu Rev*
314 *Immunol* 2009;27:485-517.

315

- 316 [10] Maddur MS, Miossec P, Kaveri SV, Bayry J. Th17 cells: biology,
317 pathogenesis of autoimmune and inflammatory diseases, and therapeutic
318 strategies. *Am J Pathol* 2012;181:8-18.
- 319 [11] Sharma M, Hegde P, Aimaniananda V, Beau R, Maddur MS, Senechal H, et al.
320 Circulating human basophils lack the features of professional antigen
321 presenting cells. *Sci Rep* 2013;3:1188. doi: 10.1038/srep01188
- 322 [12]. Sharma M, Schoindre Y, Hegde P, Saha C, Maddur MS, Stephen-Victor E, et
323 al. Intravenous immunoglobulin-induced IL-33 is insufficient to mediate
324 basophil expansion in autoimmune patients. *Sci Rep* 2014; 4: 5672. doi:
325 10.1038/srep05672.
- 326 [13] Shen T, Kim S, Do JS, Wang L, Lantz C, Urban JF, et al. T cell-derived IL-3
327 plays key role in parasite infection-induced basophil production but is
328 dispensable for in vivo basophil survival. *Int Immunol*. 2008;20:1201-9.
- 329 [14] Sullivan BM, Liang HE, Bando JK, Wu D, Cheng LE, McKerrow JK, et al.
330 Genetic analysis of basophil function in vivo. *Nat Immunol*. 2011;12:527-35.
- 331 [15] Egawa M, Mukai K, Yoshikawa S, Iki M, Mukaida N, Kawano Y, et al.
332 Inflammatory monocytes recruited to allergic skin acquire an anti-
333 inflammatory M2 phenotype via basophil-derived interleukin-4. *Immunity*
334 2013;38:570-80.
- 335 [16] Holzer U, Reinhardt K, Lang P, Handgretinger R, Fischer N. Influence of a
336 mutation in IFN- γ receptor 2 (IFNGR2) in human cells on the generation of
337 Th17 cells in memory T cells. *Hum Immunol*. 2013;74:693-700.
- 338 [17] Sharma M, Kaveri SV, Bayry J. Human basophils lack the capacity to drive
339 memory CD4⁺ T cells toward the IL-22 response. *J Allergy Clin Immunol*.
340 2013;132:1457-8.

- 341 [18] Ugajin T, Satoh T, Kanamori T, Aritake K, Urade Y, Yokozeki H. FcεRI, but
342 not FcγR, signals induce prostaglandin D2 and E2 production from basophils.
343 *Am J Pathol.* 2011;179:775-82
- 344 [19] Holla S, Sharma M, Vani J, Kaveri SV, Balaji KN, Bayry J. GM-CSF along
345 with IL-4 but not alone is indispensable for the differentiation of human
346 dendritic cells from monocytes. *J Allergy Clin Immunol* 2014; 133:1500-
347 1502.e1.
- 348 [20] Chávez-Sánchez L, Garza-Reyes MG, Espinosa-Luna JE, Chávez-Rueda K,
349 Legorreta-Haquet MV, Blanco-Favela F. The role of TLR2, TLR4 and CD36
350 in macrophage activation and foam cell formation in response to oxLDL in
351 humans. *Hum Immunol* 2014;75:322-9.
- 352 [21] Sokol CL, Chu NQ, Yu S, Nish SA, Laufer TM, Medzhitov R. Basophils
353 function as antigen-presenting cells for an allergen-induced T helper type 2
354 response. *Nat Immunol* 2009;10:713-20.
- 355 [22] Yoshimoto T, Yasuda K, Tanaka H, Nakahira M, Imai Y, Fujimori Y, et al.
356 Basophils contribute to T_H2-IgE responses in vivo via IL-4 production and
357 presentation of peptide-MHC class II complexes to CD4⁺ T cells. *Nat*
358 *Immunol* 2009;10:706-12.
- 359 [23] Perrigoue JG, Saenz SA, Siracusa MC, Allenspach EJ, Taylor BC, Giacomini
360 PR, et al. MHC class II-dependent basophil-CD4⁺ T cell interactions promote
361 T_H2 cytokine-dependent immunity. *Nat Immunol* 2009;10:697-705.
- 362 [24] Maddur MS, Kaveri SV, Bayry J. Basophils as antigen presenting cells.
363 *Trends Immunol.* 2010; 31:45-48

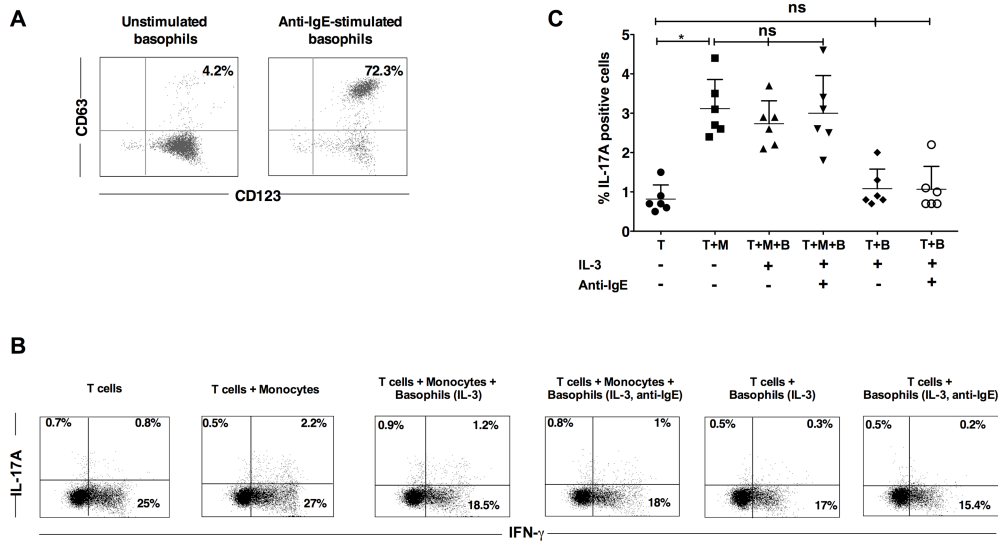
- 364 [25] Phythian-Adams AT, Cook PC, Lundie RJ, Jones LH, Smith KA, Barr TA, et
365 al. CD11c depletion severely disrupts Th2 induction and development in vivo.
366 J Exp Med 2010;207:2089-96.
- 367 [26] Hammad H, Plantinga M, Deswarte K, Pouliot P, Willart MA, Kool M, et al.
368 Inflammatory dendritic cells--not basophils--are necessary and sufficient for
369 induction of Th2 immunity to inhaled house dust mite allergen. J Exp Med
370 2010;207:2097-111.
- 371 [27] Amsen D, Blander JM, Lee GR, Tanigaki K, Honjo T, Flavell RA. Instruction
372 of distinct CD4 T helper cell fates by different notch ligands on antigen-
373 presenting cells. Cell. 2004;117:515-26.
- 374 [28] Maddur MS, Sharma M, Hegde P, Stephen-Victor E, Pulendran B, Kaveri SV,
375 et al. Human B cells induce dendritic cell maturation and favour Th2
376 polarization by inducing OX-40 ligand. Nat Commun. 2014; 5:4092. doi:
377 10.1038/ncomms5092
- 378 [29] Kitzmuller C, Nagl B, Deifl S, Walterskirchen C, Jahn-Schmid B, Zlabinger
379 GJ, et al. Human blood basophils do not act as antigen-presenting cells for the
380 major birch pollen allergen Bet v 1. Allergy 2012;67:593-600.
- 381 [30] Dijkstra D, Hennig C, Witte T, Hansen G. Basophils from humans with
382 systemic lupus erythematosus do not express MHC-II. Nat Med 2012;18:
383 488-9.
- 384 [31] Eckl-Dorna J, Ellinger A, Blatt K, Ghanim V, Steiner I, Pavelka M, et al.
385 Basophils are not the key antigen-presenting cells in allergic patients. Allergy
386 2012;67:601-8.

- 387 [32] Sharma M, Lecerf M, Friboulet A, Kaveri SV, Dissous C, Bayry J. Mediation
388 of T-helper 17 responses to schistosomes by dendritic cells but not basophils. *J*
389 *Infect Dis.* 2014; 209:2019-21.
- 390 [33] Voskamp AL, Prickett SR, Mackay F, Rolland JM, O'Hehir RE. MHC Class II
391 Expression in Human Basophils: Induction and Lack of Functional
392 Significance. *PLoS One.* 2013;8:e81777.
- 393 [34] Wakahara K, Baba N, Van VQ, Begin P, Rubio M, Ferraro P, et al. Human
394 basophils interact with memory T cells to augment Th17 responses. *Blood*
395 2012;120:4761-71.
- 396 [35] Gomez MR, Talke Y, Hofmann C, Ketelsen I, Hermann F, Reich B, et al.
397 Basophils control T-cell responses and limit disease activity in experimental
398 murine colitis. *Mucosal Immunol* 2014;7:188-99.
- 399 [36] Hueber W, Sands BE, Lewitzky S, Vandemeulebroecke M, Reinisch W,
400 Higgins PD, et al. Secukinumab, a human anti-IL-17A monoclonal antibody,
401 for moderate to severe Crohn's disease: unexpected results of a randomised,
402 double-blind placebo-controlled trial. *Gut* 2012;61:1693-700.
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408 **Figure Legends**

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411 **Fig 1.** Human basophils are mute-spectators in Th17 expansion. (A) The expression
 412 of CD63 on the surface of unstimulated and anti-IgE stimulated basophils. (B and C)
 413 Memory CD4⁺ T cells were cultured alone with IL-2 (T) or with basophils (T+B) or
 414 peptidoglycan-stimulated monocytes (T+M) or peptidoglycan-stimulated monocytes
 415 and basophils (T+M+B). Basophils were stimulated either with IL-3 or combination
 416 of IL-3 and anti-IgE. (B) A representative flow-cytometry analysis of intracellular IL-
 417 17A and IFN- γ , and (C) percentage (mean \pm SD) of CD4⁺CD45RO⁺ memory T cells
 418 positive for IL-17A⁺ (n=6) were shown. *, P<0.05; ns, not-significant as analyzed by
 419 one-way ANOVA test.

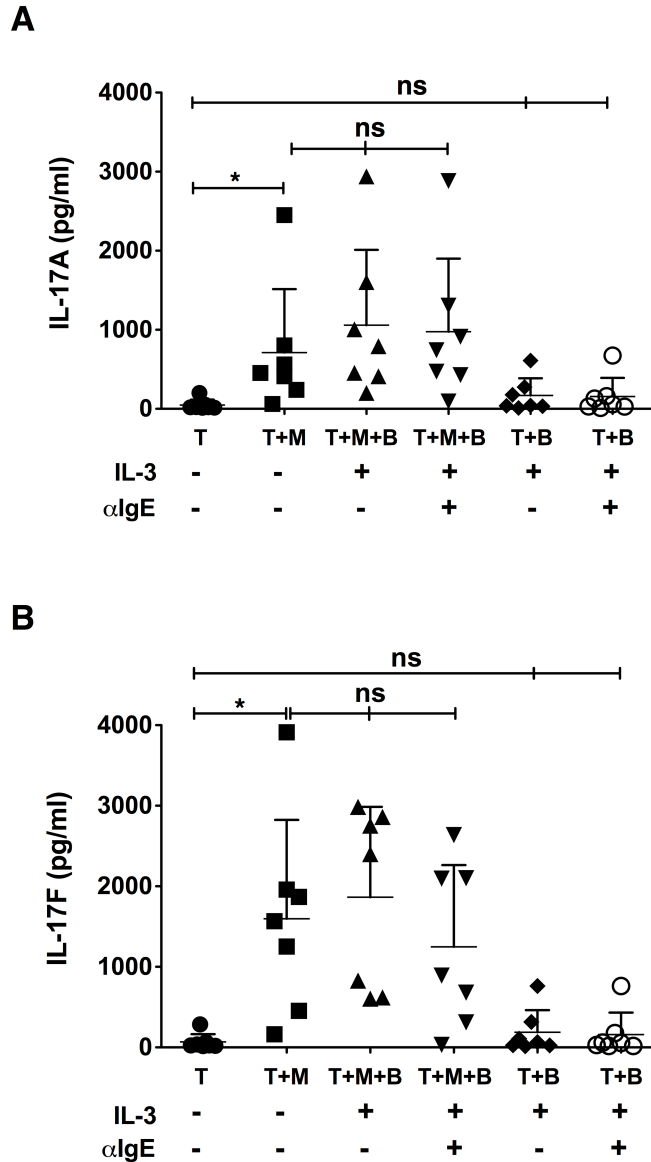
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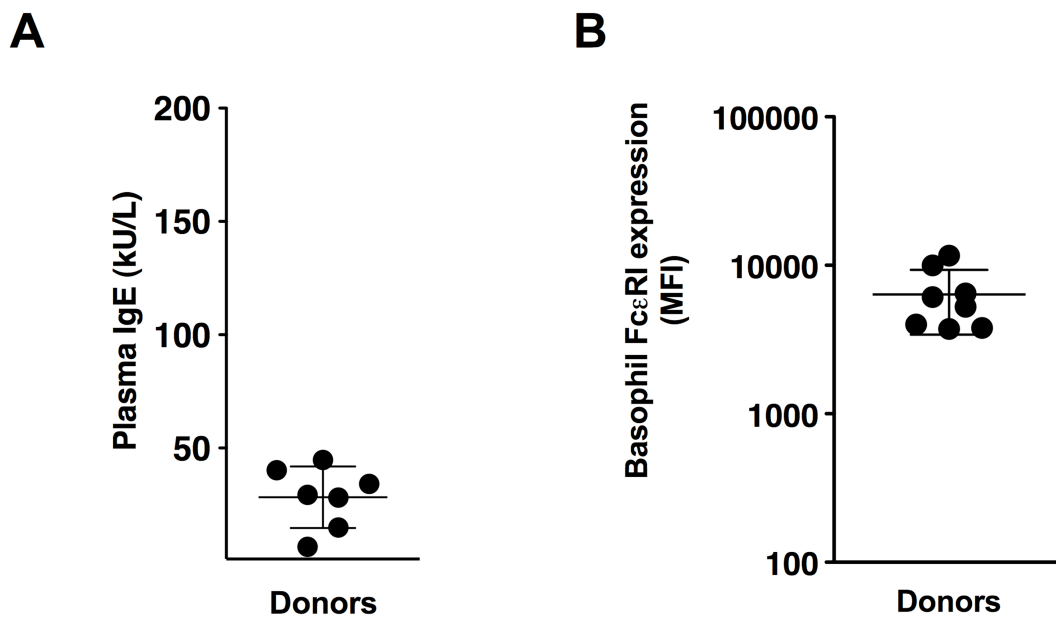


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426 **Fig 2.** Human basophils do not promote Th17 cytokine secretion. (A-B) The amount
 427 of secretion (pg/ml) of (A) IL-17A and (B) IL-17F in the culture supernatants of
 428 memory CD4⁺ T cells that were cultured alone with IL-2 (T) or with basophils (T+B)
 429 or peptidoglycan-stimulated monocytes (T+M) or peptidoglycan-stimulated
 430 monocytes and basophils (T+M+B). Basophils were stimulated either with IL-3 or
 431 combination of IL-3 and anti-IgE. The cytokines were measured by ELISA. The data
 432 represent mean \pm SD from six independent experiments using cells from different
 433 donors. *, P<0.05; ns, not-significant as analyzed by one-way ANOVA test.

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437 **Fig 3.** FcεRI-mediated activation of basophils is not influenced by the level of plasma
438 IgE and the expression of FcεRI on the basophils. (A) The level of IgE (kU/L) in the
439 plasma of healthy donors (n=7). (B) The expression (MFI) of FcεRI on the basophils
440 of healthy donors (n=8). The lines represent mean and SD values.

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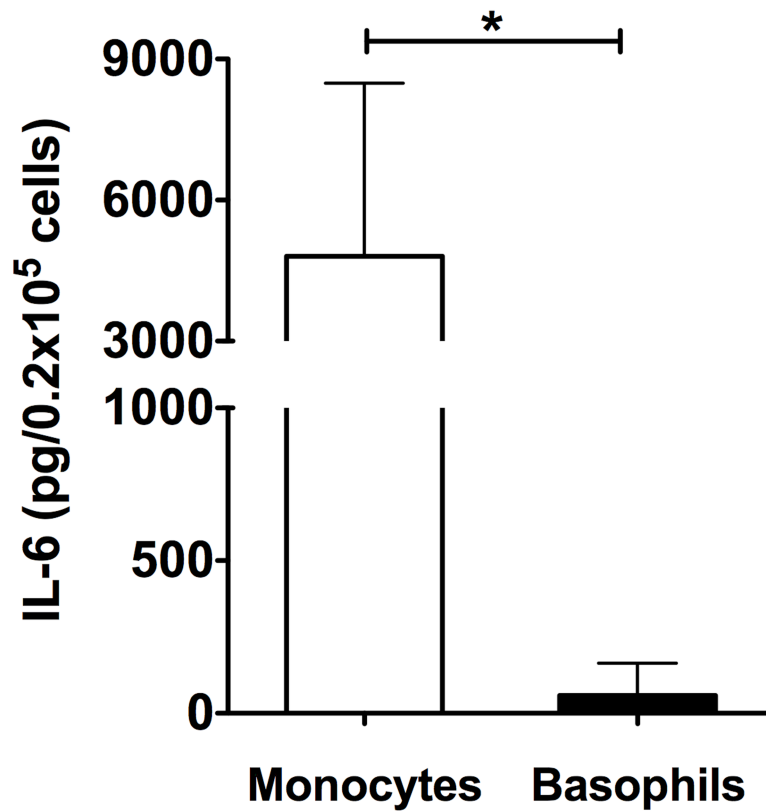
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452 **Fig 4.** Human basophils produce minute amounts of IL-6. Basophils were stimulated
453 with a combination IL-3 and anti-IgE for 24 hours. Monocytes were activated with
454 peptidoglycan. IL-6 in the culture supernatants was quantified (pg/0.2x10⁵ cells) by
455 ELISA. The results are mean±SD from four donors. *, P<0.05 as analyzed by two-
456 tailed Student's -t- test.