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## Conditional neutrophil depletion challenges their contribution to mouse models of anaphylaxis

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1 **Conditional neutrophil depletion challenges their contribution to mouse**  
2 **models of anaphylaxis**

3

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28 To the Editor,

29

30 Anaphylaxis is an acute and potentially lethal systemic allergic reaction. In humans, it is  
31 largely accepted that anaphylaxis relies predominately on IgE antibodies<sup>1</sup>. However, IgG  
32 might also contribute to anaphylaxis induced by infused drugs<sup>1</sup>. Several mouse models have  
33 been developed to identify key effector cells and mediators of anaphylaxis. Consequently, two  
34 main pathways have been identified in mice: a “classical” pathway consisting of IgE, FcεRI,  
35 histamine and mast cells, and an “alternative” pathway involving IgG, FcγRIII, platelet-  
36 activating factor (PAF) and - depending on the anaphylaxis model studied - macrophages,  
37 basophils and/or neutrophils<sup>1-3</sup>.

38

39 We and others have reported that neutrophils are potentially important drivers of IgG  
40 anaphylaxis in mice, based on experiments using neutrophil-depleting mAbs<sup>1,4</sup>. ~~However, the~~  
41 ~~use of such mAbs has caveats, considering potential off target effects.~~ In contrast, Strait et al.<sup>3</sup>  
42 showed that injection of anti-Gr-1 mAb 2 days before antigen challenge failed to suppress  
43 IgG-mediated anaphylaxis, indicating that the effect of these mAbs likely depends on the  
44 dose, timing of injection and specific model used. We recently described an inducible,  
45 antibody-independent, neutrophil depletion mouse model (*PMN<sup>DTR</sup>* mice), relying on the  
46 selective expression of the diphtheria toxin (DT) receptor on neutrophils<sup>5</sup>. Injection of DT in  
47 *PMN<sup>DTR</sup>* mice leads to a marked depletion of blood, spleen and bone marrow neutrophils (**Fig.**  
48 **S1A-D**)<sup>5</sup>. We therefore used *PMN<sup>DTR</sup>* mice to re-evaluate the contribution of neutrophils to  
49 anaphylaxis models using *PMN<sup>DTR</sup>* mice.

50

51 To elicit active systemic anaphylaxis (ASA), mice were immunized with BSA emulsified in  
52 Freund’s adjuvant, and challenged i.v. with BSA (**Fig. 1A**), a model that mostly relies on

53 IgG<sup>4</sup>. As expected, pre-treatment of mice with anti-Ly6G or anti-Gr-1 neutrophil-depleting  
54 mAbs markedly reduced hypothermia and mortality in this model (**Fig. 1B-C**). Strikingly,  
55 however, DT-treated neutrophil-deficient *PMN<sup>DTR</sup>* mice exhibited similar anaphylaxis  
56 severity to neutrophil-sufficient controls (**Fig. 1D-E**). We obtained similar results in models  
57 of IgG2a- or IgG2b-induced passive systemic anaphylaxis (PSA) (**Fig. 1F-J**).

58  
59 Interestingly, while performing the initial treatment to deplete neutrophils, we observed that  
60 injection of either anti-Ly6G or anti-Gr-1 mAbs induced a transient hypothermia in WT mice,  
61 whereas injection of DT in *PMN<sup>DTR</sup>* mice did not induce any apparent side effects (**Fig. 2A-**  
62 **B**). We therefore questioned whether engagement of FcγRs and/or complement by the  
63 depleting mAbs might induce an initial anaphylactic-like event that could desensitize mice to  
64 subsequent IgG-mediated anaphylaxis. To test this, we administered anti-Ly6G mAb to  
65 FcγR<sup>Null</sup> mice that do not express FcγRs, and to FcγR<sup>Null</sup>/C1q<sup>KO</sup> mice that lack both FcγRs and  
66 complement component C1q. As expected, neutrophil depletion by mAbs was impaired (**Fig.**  
67 **S1E-G**), which suggests that this depletion occurs at least partly through antibody-dependent  
68 cellular cytotoxicity or phagocytosis mediated by FcγRs, and potentially via complement-  
69 dependent cytotoxicity. Moreover, transient hypothermia after mAb injection was abolished  
70 in FcγR and complement deficient animals (**Fig. 2C**).

71  
72 PAF is a key driver of anaphylaxis<sup>1,2</sup>. We therefore assessed the role of PAF in anti-Ly6G  
73 mAb-induced hypothermia using PAF receptor-deficient mice (PtafR<sup>KO</sup>) which are resistant  
74 to PAF-induced shock (**Fig. S2A**). Neutrophil depletion efficacy was unaltered in PtafR<sup>KO</sup>  
75 mice (**Fig. S2B-D**), however these mice exhibited reduced hypothermia upon injection of  
76 anti-Ly6G mAbs (**Fig. 2D**). In agreement with these results, pre-treatment of mice with a PAF  
77 receptor antagonist (ABT-491) also largely prevented hypothermia upon injection of anti-

78 Ly6G mAbs (**Fig. 2E-F**), without affecting neutrophil depletion efficacy (**Fig. S2G-I**). ABT-  
79 491 has a short half-life *in vivo* (**Fig. S2E-F**). We could then demonstrate that when the PAF-  
80 induced hypothermia after anti-Ly6G mAb injection is blocked, then the protective effect of  
81 this mAb on IgG anaphylaxis is reduced (**Fig 2G**).

82

83 Our data demonstrate that IgG-anaphylaxis can still occur in neutropenic mice and that  
84 neutrophil-targeting mAbs likely protect from anaphylaxis through their capacity to  
85 desensitize effector cells by engaging Fc $\gamma$ Rs and inducing the release of PAF, rather than via  
86 neutrophil depletion *per se*. Neutrophils are therefore dispensable for IgG-anaphylaxis in  
87 mice. They may nevertheless be activated during IgG-anaphylaxis, since their Fc $\gamma$ Rs are  
88 engaged<sup>6,7</sup>, as in other IgG-mediated inflammatory processes.

89

90 In addition, we used *PMN<sup>DTR</sup>* mice to confirm that neutrophils do play a pivotal role in two  
91 other models of IgG-mediated inflammation: immune complex-induced airway inflammation  
92 (**Fig. S3A-E**) and autoantibody-induced arthritis (**Fig. S3F-H**). This extends the validation of  
93 *PMN<sup>DTR</sup>* mice as a meaningful model in neutrophil research.

94

95 This study highlights that that the use of depleting mAbs *in vivo*, and especially in antibody-  
96 driven reactions such as anaphylaxis, should be carefully evaluated to avoid overinterpretation  
97 of the actual contribution of neutrophils to disease models. Not only should we consider the  
98 potential off-target effects of mAbs, but also the ramifications of on-target depletion  
99 strategies which engage Fc receptors and consequently affect other immune activation  
100 pathways.

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112

## 113 **Authorship Contributions**

114 JS together with CMG, OG, FJ, EL, WW and EC performed all experiments, BI prepared  
115 essential reagents, JS, CMG, PB, LR and FJ analyzed and discussed results, and SJG, PB, LR  
116 and FJ supervised the research. JS, CMG, SJG, PB, LR, and FJ prepared the figures and wrote  
117 the manuscript. All authors were given the opportunity to read and make amendments to the  
118 manuscript.

119

## 120 **Conflicts of interest**

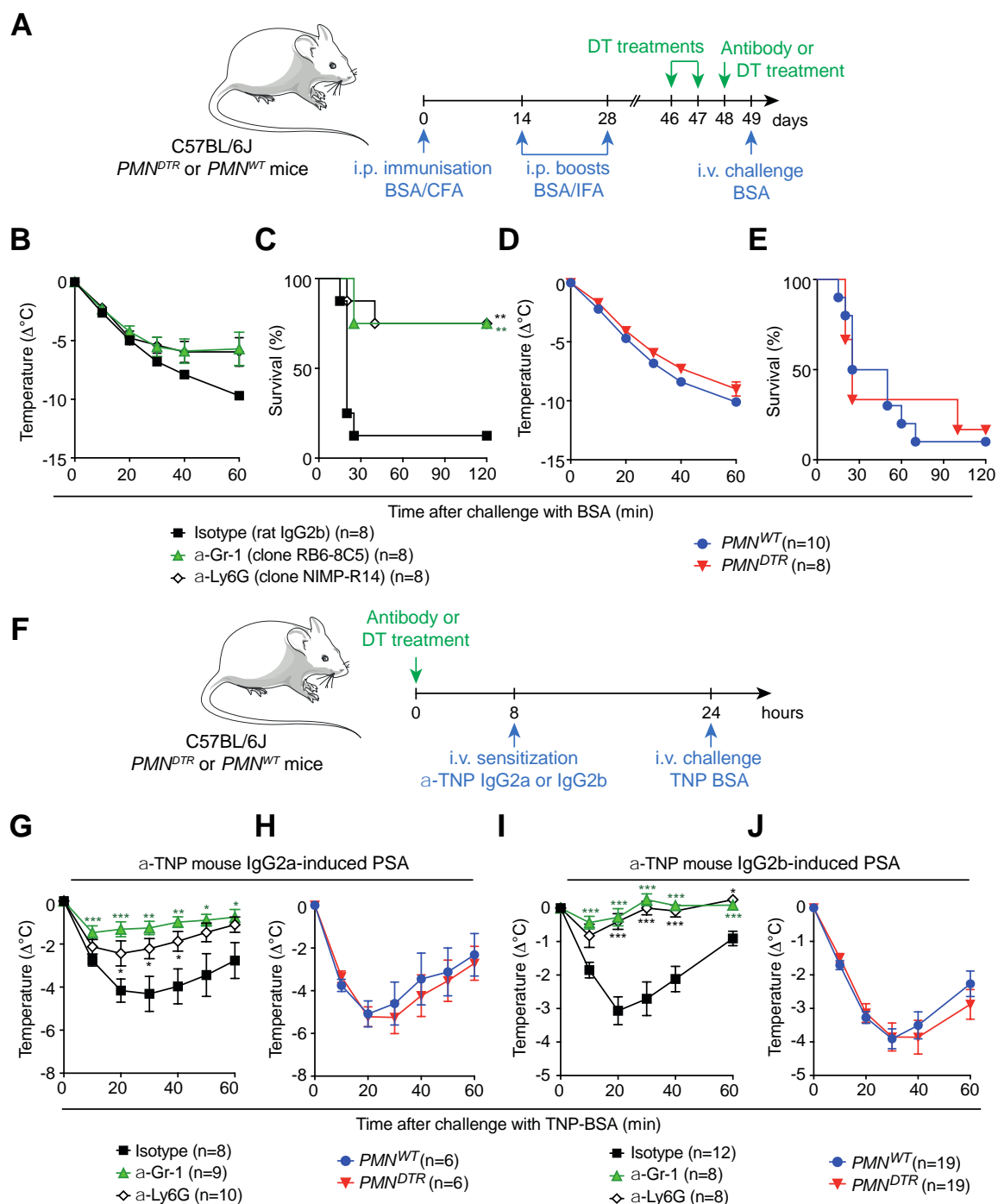
121 PB is a paid consultant to Regeneron Pharmaceuticals. The rest of the authors declare that  
122 they have no relevant conflicts of interest.

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## 126 **References**

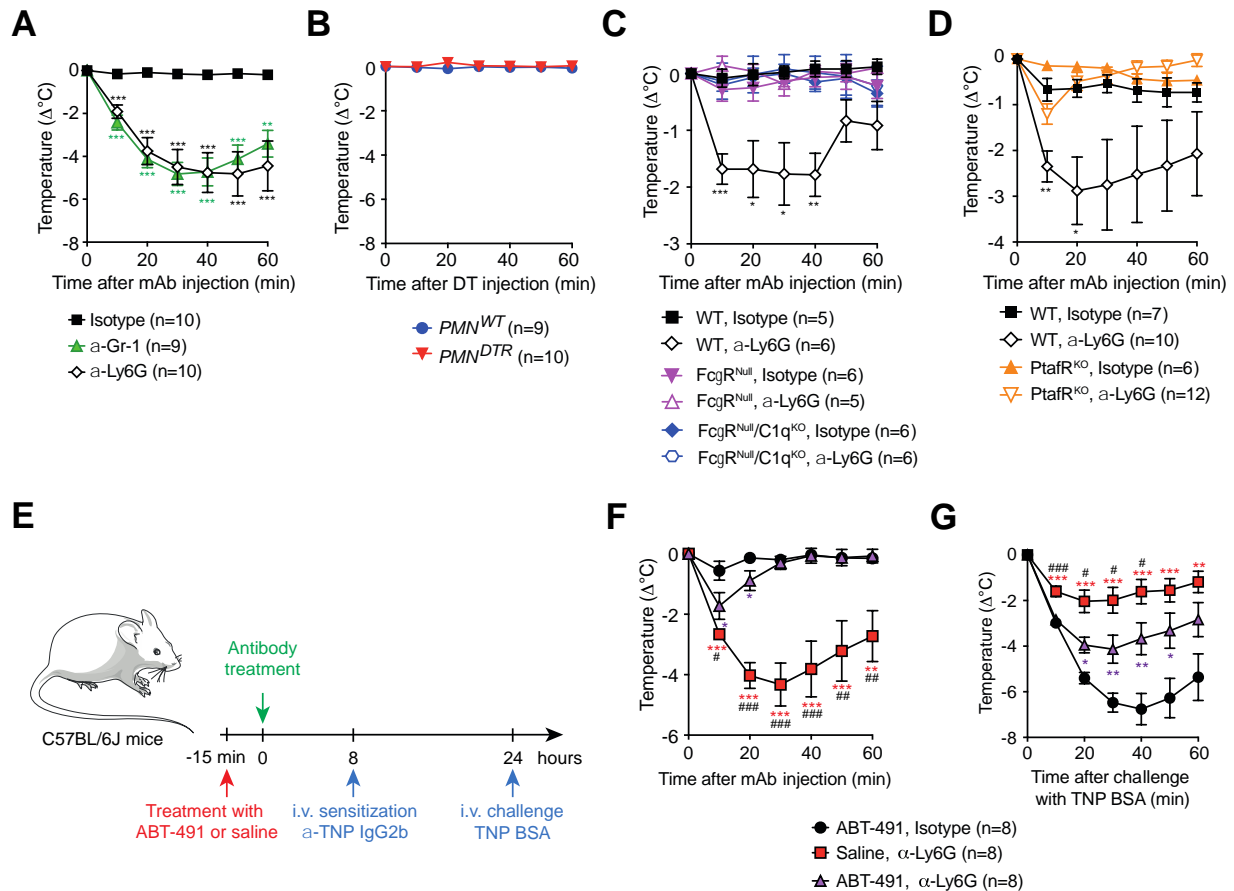
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145

146 **Figure 1. Neutrophil depletion using antibodies, but not the  $PMN^{DTR}$  model, protects**  
 147 **mice from active or passive anaphylaxis. (A-E)** Active systemic anaphylaxis (ASA) in WT  
 148 mice pre-treated with neutrophil-depleting  $\alpha$ -Gr-1 or  $\alpha$ -Ly6G mAbs (or an isotype control),  
 149 and in  $PMN^{DTR}$  and  $PMN^{WT}$  mice pre-treated with DT. (A) Protocol outline. (B, D) Changes  
 150 in body temperature ( $\Delta^{\circ}\text{C}$ ) and (C, E) survival after challenge with BSA. (F) IgG2a- or  
 151 IgG2b-mediated passive systemic anaphylaxis (PSA) protocol outline. (G-J) Changes in body  
 152 temperature ( $\Delta^{\circ}\text{C}$ ) after challenge with TNP-BSA. Data are pooled from at least two



153 independent experiments, means  $\pm$  s.e.m. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.005$ , using Mann-  
 154 Whitney test (two-tailed) or Mantel-Cox log-rank test.



155

156 **Figure 2.  $\alpha$ -Ly6G mAbs induce  $Fc\gamma R$ - and PAF-dependent hypothermia which protects**  
 157 **mice from subsequent IgG anaphylaxis. (A-B)** Changes in body temperature ( $\Delta^{\circ}\text{C}$ ) after  
 158 injection of (A) neutrophil-depleting  $\alpha$ -Ly6G or  $\alpha$ -Gr-1 mAbs, or a rat IgG2b isotype control  
 159 (Isotype) in C57BL/6J mice, or (B) DT into  $PMN^{WT}$  or  $PMN^{DTR}$  mice. (C-D) Changes in body  
 160 temperature following injection of  $\alpha$ -Ly6G mAbs or a rat IgG2b isotype control (Isotype) in  
 161 (C) WT,  $Fc\gamma R^{Null}$  and  $Fc\gamma R^{Null}/C1q^{KO}$ , or (D) controls vs.  $PtafR^{KO}$  mice. (E-G) Mice were  
 162 pre-treated with ABT-491 or saline 15 minutes prior to injection of  $\alpha$ -Ly6G mAbs or isotype  
 163 control. Mice were then sensitized with  $\alpha$ -TNP IgG2a and challenged 16 hours later with  
 164 TNP-BSA (E). Changes in body temperature were monitored after injection of  $\alpha$ -Ly6G mAbs  
 165 (F) and after challenge with TNP-BSA (G). Data are pooled from at least two different  
 166 experiments, means  $\pm$  s.e.m. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.005$  vs. respective isotype  
 167 control group; #  $P < 0.05$ , ##  $P < 0.01$ , ###  $P < 0.005$  between saline,  $\alpha$ -Ly6G and ABT-491,  $\alpha$ -  
 168 Ly6G groups (in F and G) using Mann-Whitney test (two-tailed).