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## LETTER

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

To the Editor,

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

We and others have reported that neutrophils are potentially important drivers of IgG anaphylaxis in mice, based on experiments using neutrophil-depleting mAbs.<sup>1,4</sup> In contrast, Strait et al.<sup>3</sup> showed that injection of anti-Gr-1 mAb 2 days before antigen challenge failed to suppress IgG-mediated anaphylaxis, indicating that the effect of these mAbs likely depends on the dose, timing of injection, and specific model used. We recently described an inducible, antibody-independent, neutrophil depletion mouse model (*PMN<sup>DTR</sup>* mice), relying on the selective expression of the diphtheria toxin (DT) receptor on neutrophils.<sup>5</sup> Injection of DT in *PMN<sup>DTR</sup>* mice leads to a marked depletion of blood, spleen, and bone marrow neutrophils (Figure S1A–D).<sup>5</sup> We therefore used *PMN<sup>DTR</sup>* mice to reevaluate the contribution of neutrophils to anaphylaxis models.

To elicit active systemic anaphylaxis (ASA), mice were immunized with BSA emulsified in Freund's adjuvant, and challenged i.v. with BSA (Figure 1A), a model that mostly relies on IgG.<sup>4</sup> As expected, pretreatment of mice with anti-Ly6G or anti-Gr-1 neutrophil-depleting mAbs markedly reduced hypothermia and mortality in this model (Figure 1B,C). Strikingly, however, DT-treated neutrophil-deficient *PMN<sup>DTR</sup>* mice exhibited similar anaphylaxis severity to neutrophil-sufficient controls (Figure 1D,E). We obtained similar results in models of IgG2a- or IgG2b-induced passive systemic anaphylaxis (PSA) (Figure 1F–J).

Interestingly, while performing the initial treatment to deplete neutrophils, we observed that injection of either anti-Ly6G or anti-Gr-1 mAbs induced a transient hypothermia in WT mice, whereas injection of DT in *PMN<sup>DTR</sup>* mice did not induce any apparent side

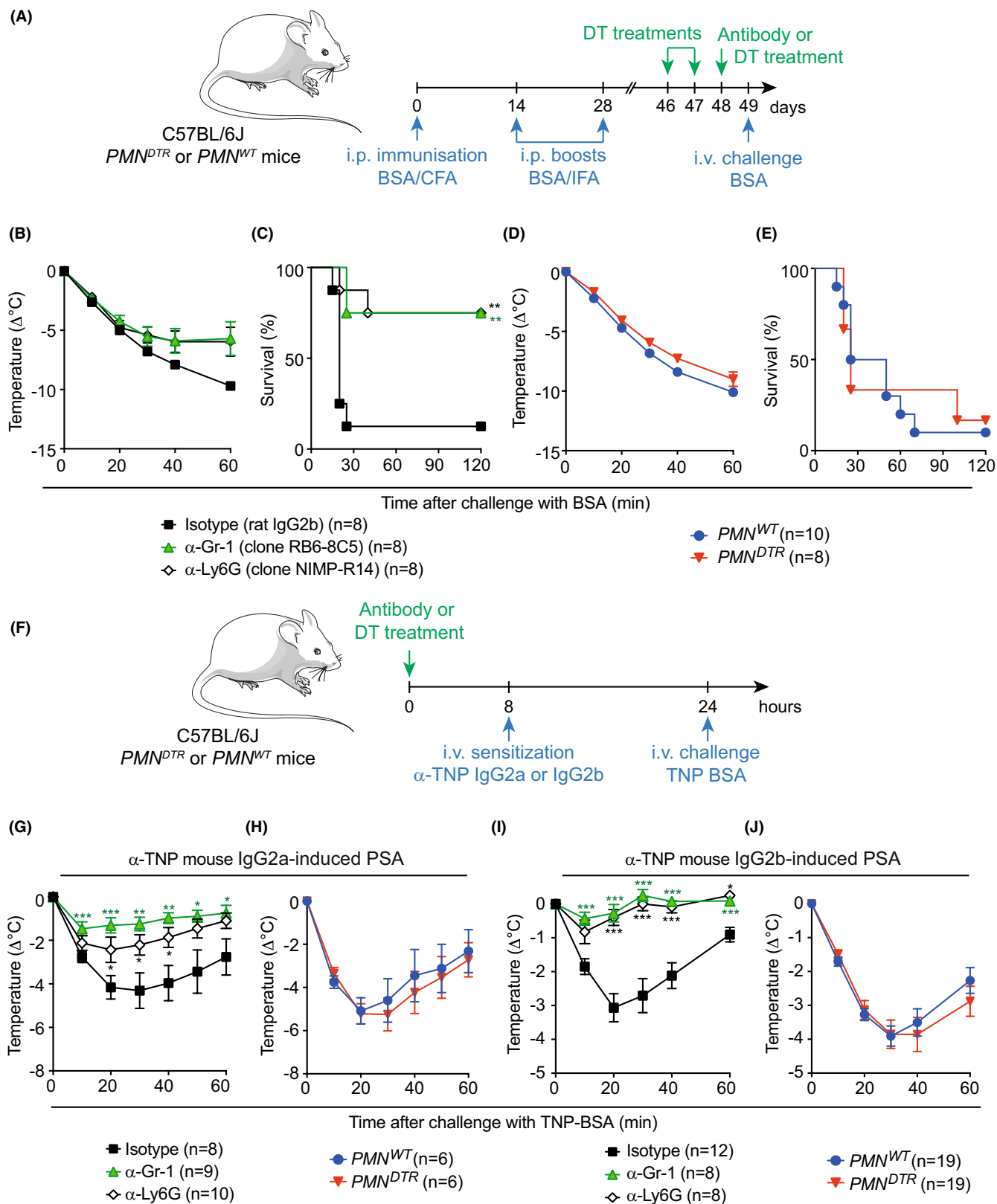
effects (Figure 2A,B). We therefore questioned whether engagement of FcγRs and/or complement by the depleting mAbs might induce an initial anaphylactic-like event that could desensitize mice to subsequent IgG-mediated anaphylaxis. To test this, we administered anti-Ly6G mAb to FcγR<sup>Null</sup> mice that do not express FcγRs, and to FcγR<sup>Null</sup>/C1q<sup>KO</sup> mice that lack both FcγRs and complement component C1q. As expected, neutrophil depletion by mAbs was impaired (Figure S1E–G), which suggests that this depletion occurs at least partly through antibody-dependent cellular cytotoxicity or phagocytosis mediated by FcγRs, and potentially via complement-dependent cytotoxicity. Moreover, transient hypothermia after mAb injection was abolished in FcγR and complement deficient animals (Figure 2C).

PAF is a key driver of anaphylaxis.<sup>1,2</sup> We therefore assessed the role of PAF in anti-Ly6G mAb-induced hypothermia using PAF receptor-deficient mice (PtafR<sup>KO</sup>) which are resistant to PAF-induced shock (Figure S2A). Neutrophil depletion efficacy was unaltered in PtafR<sup>KO</sup> mice (Figure S2B–D); however, these mice exhibited reduced hypothermia upon injection of anti-Ly6G mAbs (Figure 2D). In agreement with these results, pretreatment of mice with a PAF receptor antagonist (ABT-491) also largely prevented hypothermia upon injection of anti-Ly6G mAbs (Figure 2E,F), without affecting neutrophil depletion efficacy (Figure S2G–I). ABT-491 has a short half-life *in vivo* (Figure S2E,F). We could then demonstrate that when the PAF-induced hypothermia after anti-Ly6G mAb injection is blocked, then the protective effect of this mAb on IgG anaphylaxis is reduced (Figure 2G).

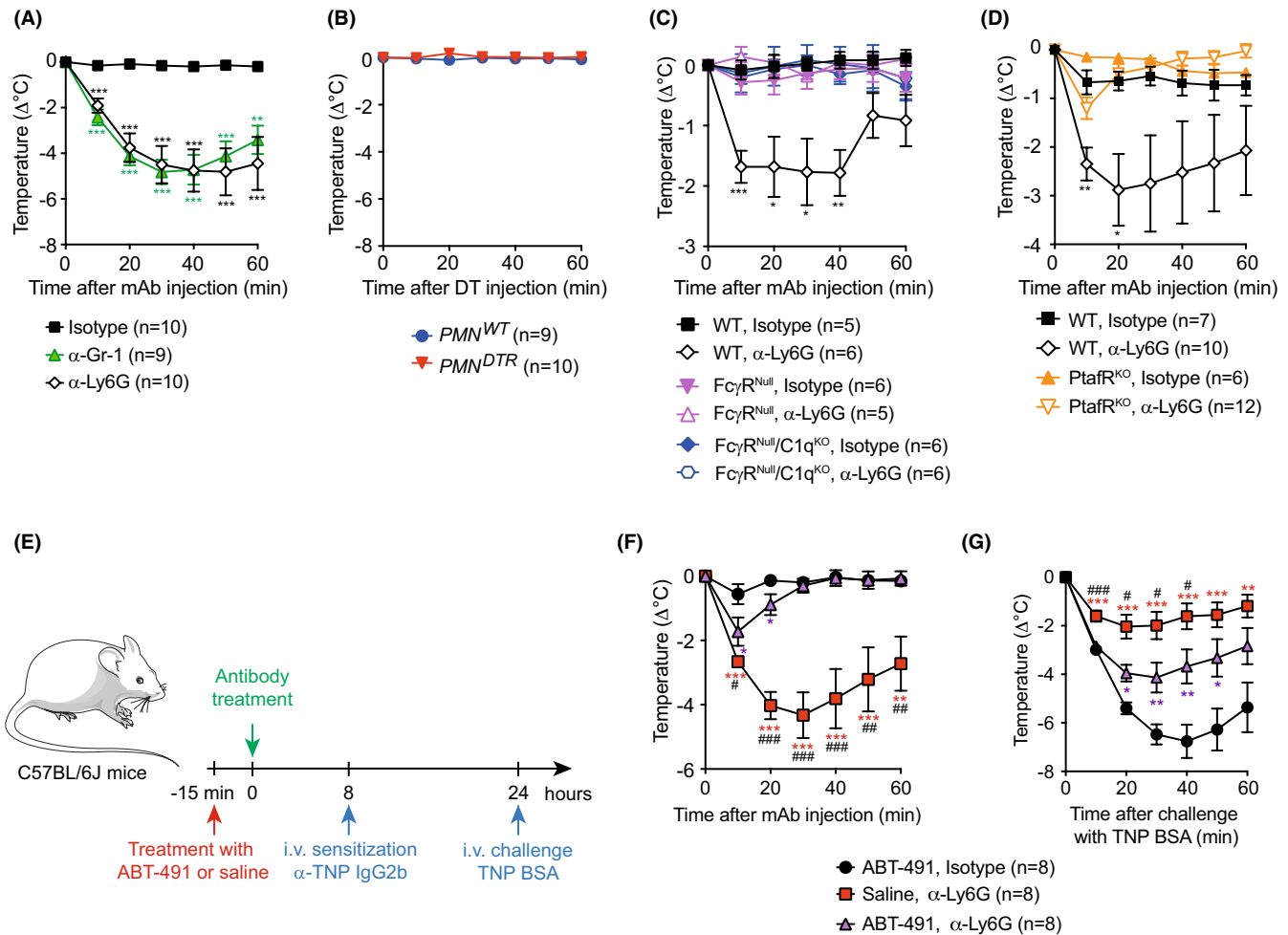
Our data demonstrate that IgG-anaphylaxis can still occur in neutropenic mice and that neutrophil-targeting mAbs likely protect from anaphylaxis through their capacity to desensitize effector cells by engaging FcγRs and inducing the release of PAF, rather than via neutrophil depletion *per se*. Neutrophils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

In addition, we used *PMN<sup>DTR</sup>* mice to confirm that neutrophils do play a pivotal role in two other models of IgG-mediated inflammation: immune complex-induced airway inflammation (Figure S3A–E) and

†Co-senior authorship.



**FIGURE 1** Neutrophil depletion using antibodies, but not the *PMN<sup>DTR</sup>* model, protects mice from active or passive anaphylaxis. (A–E) Active systemic anaphylaxis (ASA) in WT mice pretreated with neutrophil-depleting  $\alpha$ -Gr-1 or  $\alpha$ -Ly6G mAbs (or an isotype control), and in *PMN<sup>DTR</sup>* and *PMN<sup>WT</sup>* mice pretreated with DT. (A) Protocol outline. (B, D) Changes in body temperature ( $\Delta^{\circ}\text{C}$ ) and (C, E) survival after challenge with BSA. (F) IgG2a- or IgG2b-mediated passive systemic anaphylaxis (PSA) protocol outline. (G–J) Changes in body temperature ( $\Delta^{\circ}\text{C}$ ) after challenge with TNP-BSA. Data are pooled from at least two independent experiments, means  $\pm$  SEM. \* $p < .05$ , \*\* $p < .01$ , \*\*\* $p < .005$ , using Mann-Whitney test (two-tailed) or Mantel-Cox log-rank test.



**FIGURE 2**  $\alpha\text{-Ly6G}$  mAbs induce  $Fc\gamma R$ - and PAF-dependent hypothermia which protects mice from subsequent IgG anaphylaxis. (A, B) Changes in body temperature ( $\Delta^{\circ}\text{C}$ ) after injection of (A) neutrophil-depleting  $\alpha\text{-Ly6G}$  or  $\alpha\text{-Gr-1}$  mAbs, or a rat IgG2b isotype control (Isotype) in C57BL/6J mice, or (B) DT into  $PMN^{WT}$  or  $PMN^{DTR}$  mice. (C, D) Changes in body temperature following injection of  $\alpha\text{-Ly6G}$  mAbs or a rat IgG2b isotype control (Isotype) in (C) WT,  $Fc\gamma R^{Null}$ , and  $Fc\gamma R^{Null}/C1q^{KO}$ , or (D) controls versus  $Ptafr^{KO}$  mice. (E–G) Mice were pretreated with ABT-491 or saline 15 minutes prior to injection of  $\alpha\text{-Ly6G}$  mAbs or isotype control. Mice were then sensitized with  $\alpha\text{-TNP IgG2a}$  and challenged 16 h later with TNP-BSA (E). Changes in body temperature were monitored after injection of  $\alpha\text{-Ly6G}$  mAbs (F) and after challenge with TNP-BSA (G). Data are pooled from at least two different experiments, means  $\pm$  SEM. \* $p < .05$ , \*\* $p < .01$ , \*\*\* $p < .005$  versus respective isotype control group; # $p < .05$ , ## $p < .01$ , ### $p < .005$  between saline,  $\alpha\text{-Ly6G}$  and ABT-491,  $\alpha\text{-Ly6G}$  groups (in F and G) using Mann-Whitney test (two-tailed).

autoantibody-induced arthritis (Figure S3F–H). This extends the validation of  $PMN^{DTR}$  mice as a meaningful model in neutrophil research.

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

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We are grateful to Lynn E. MacDonald and Andrew L. Murphy, working at Regeneron Pharmaceuticals, for providing VG1505 ( $Fc\gamma R1^{only}$ ) and VG598 ( $C1q^{KO}$ ) mouse strains.

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**CONFLICT OF INTEREST STATEMENT**

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

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