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The anti-Fc ϵ RI antibody MAR-1 depletes basophils and cross-reacts with myeloid cells through its Fc portion

To the Editor,

In humans, the high-affinity IgE receptor Fc ϵ RI is expressed as a tetramer in mast cells (MCs) and basophils, and as a trimer in additional myeloid cell populations, including monocytes, macrophages, dendritic cells (DCs), and Langerhans cells.¹ In mice, the expression profile of Fc ϵ RI is more debatable. Some studies report Fc ϵ RI⁺ macrophages and DCs.² However, recent data indicate that the anti-Fc ϵ RI mAb (clone MAR-1) used in all these studies can recognize myeloid cells in an Fc ϵ RI-independent manner, and cross-reacts with Fc γ RI and Fc γ RIV.^{3,4} Since MAR-1 has been extensively used *in vivo*

to assess the role of Fc ϵ RI and to deplete basophils,⁵ the off-target effects of this mAb raise concerns regarding some of the conclusions made in prior studies. We thus decided to assess by which mechanism MAR-1 cross-reacts with Fc γ Rs, and whether this cross-reactivity accounts for some of the functions previously attributed to Fc ϵ RI and/or basophils.

We confirmed that MAR-1 binds MCs derived from WT but not from Fc ϵ RI-deficient mice, and that it also binds transfected CHO cells expressing Fc γ RI or Fc γ RIV (Figure 1A and B and Figure S1A). It was suggested that such binding to Fc γ Rs results from a lack of

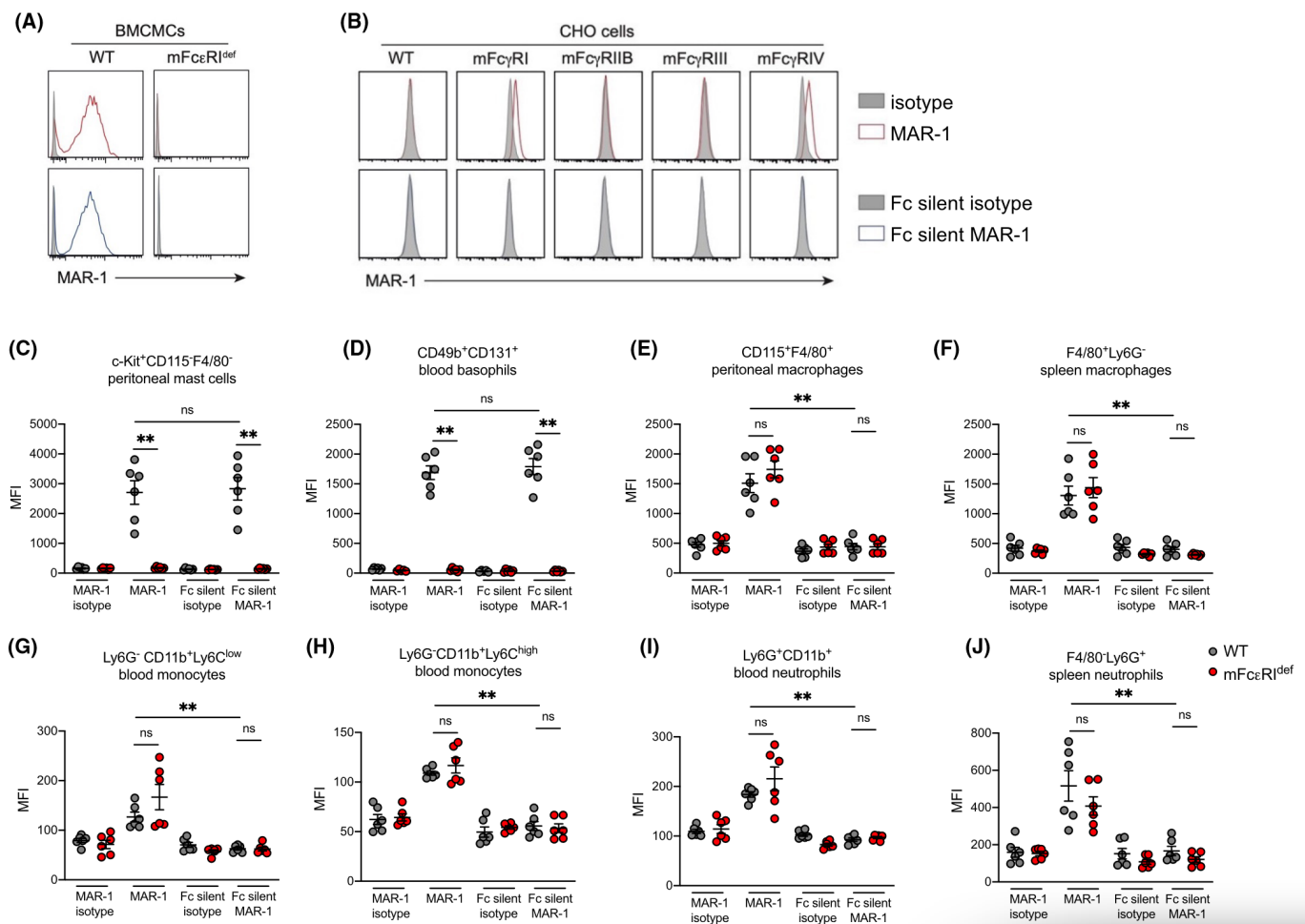


FIGURE 1 Fab portion of MAR-1 is specific for Fc ϵ RI, but MAR-1 binds Fc γ Rs and recognizes myeloid cells. Staining with MAR-1, Fc silent MAR-1 or their respective isotype controls in bone marrow-derived cultured mast cells or mFc ϵ RI^{def} mice (A), and in CHO cells stably transfected to express Fc γ Rs (B). Data are representative of two independent experiments. (C–J) Quantification [mean fluorescence intensity (MFI)] of staining with MAR-1, Fc silent MAR-1 or their respective isotype controls in the indicated myeloid cell populations. Error bars indicate means \pm SEM pooled from two independent experiments (test); ns, not significant

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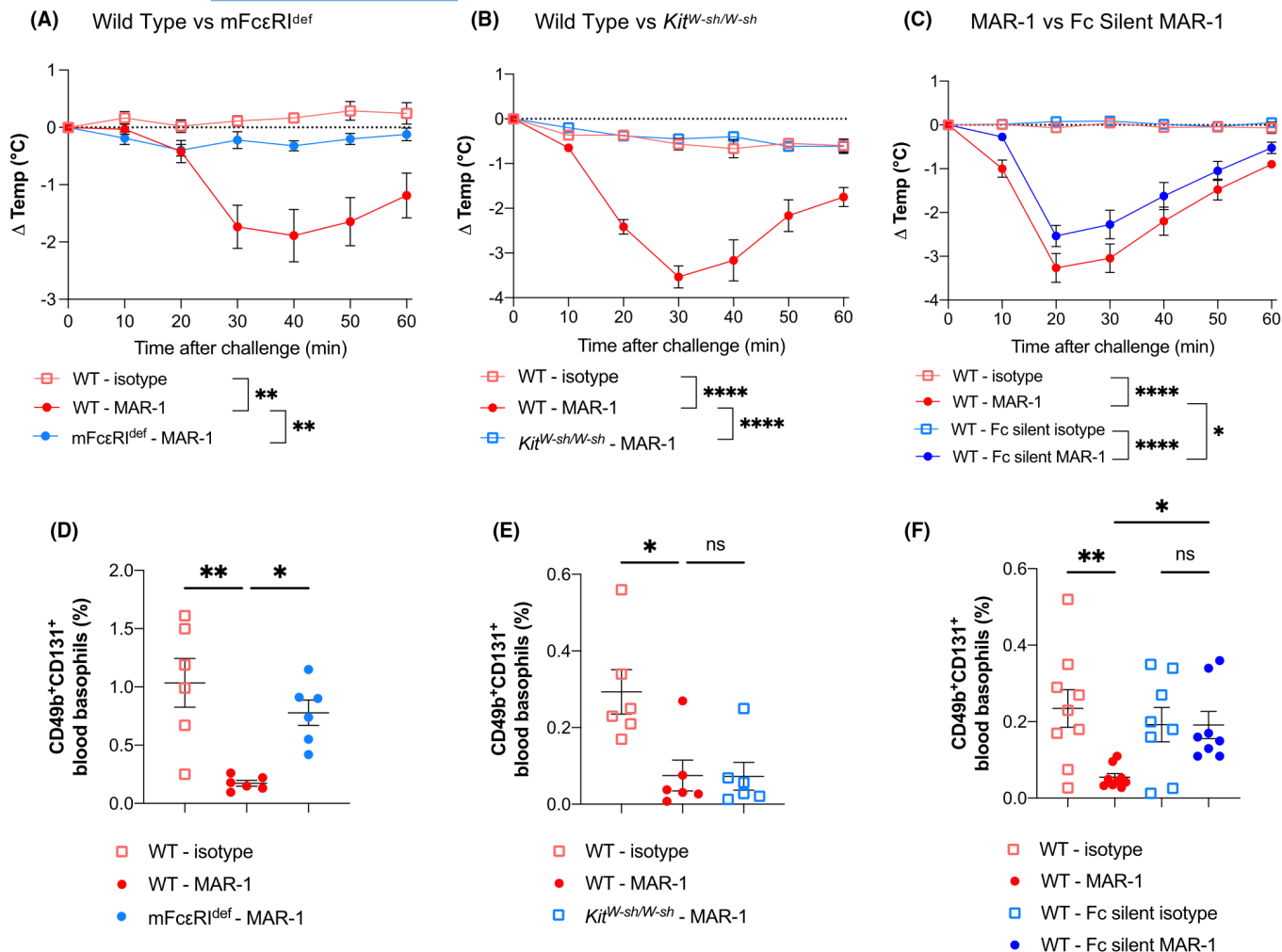


FIGURE 2 Fc portion of MAR-1 amplifies anaphylaxis and mediates basophil depletion. (A–C) Change in body temperature after injection of MAR-1, Fc silent MAR-1 or isotype controls in the indicated groups of mice. Data show means \pm SEM pooled from two independent experiments. * $p < .05$; ** $p < .01$; **** $p < .0001$ (one-way ANOVA, with Šidák's correction for multiple comparisons). (D–F) Percentage of blood basophils 24 h after injection of MAR-1, Fc silent MAR-1 or isotype controls. Data are values from individual mice; error bars indicate means \pm SEM pooled from two independent experiments. * $p < .05$; ** $p < .01$; ns: not significant ($p > .05$) (Kruskal–Wallis test, with Dunn's correction for multiple comparisons)

specificity of MAR-1.³ However, it is also possible that the Fab portion of MAR-1 is specific for FcεRI but that the mAb binds FcγRs through its Fc portion. To assess this, we used a recombinant version of MAR-1 produced as a mutant human IgG1 unable to engage FcγRs through its Fc portion ("Fc silent" format). As expected, the Fc silent MAR-1 recognizes FcεRI on MCs and basophils (Figure 1A, C–D and Figure S1B). By contrast, it does not bind FcγR-expressing CHO cells (Figure 1B), demonstrating that MAR-1 binds FcγRs through its Fc portion. IgG aggregation can dramatically increase the binding to FcγRs. However, we verified that MAR-1 is stable and soluble in solution using dynamic light scattering (DLS) (Figure S1C). Furthermore, we used MALDI-TOF mass spectrometry (Figure S1D) to confirm the absence of contaminants and degradation, further underlining MAR-1's quality in solution.

We then assessed the extent to which such binding to FcγRs accounts for the ability of MAR-1 to recognize myeloid cells. We confirmed that MAR-1 stains macrophages, monocytes, and neutrophils

from both WT and FcεRI-deficient mice. However, Fc silent MAR-1 did not stain these cells (Figure 1E–J), while retaining full capacity to stain MCs and basophils (Figure 1C and D). IgG glycosylation is required for binding of the Fc portion to FcγRs. Enzymatic deglycosylation of MAR-1 by endoglycosidase peptide-N-glycosidase F (PNGase F) also abrogated MAR-1's ability to stain monocytes, without modifying the staining of MCs and basophils (Figure S1E and F). Altogether, these data demonstrate that MAR-1 recognizes myeloid cells (other than MCs and basophils) through its Fc portion.

Injection of a high dose of MAR-1 induces anaphylaxis in mice.⁶ As expected, we found that this reaction is abrogated in FcεRI-deficient or MC-deficient (Figure 1A and B). However, we also observed reduced anaphylaxis severity upon injection of Fc silent MAR-1 compared to MAR-1 in WT mice (Figure 1C). Fc silent format prevents interaction with FcγRs C1q (Figure S1G), these latter results

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of FcγRs and/or the complement pathway may contribute to the increased severity of anaphylaxis by MAR-1, which predominantly relies on crosslinking of FcεRI.

MAR-1 is also used to deplete basophils *in vivo*.⁵ Several mechanisms could account for this, including activation-induced cell death, FcγR-mediated antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), or complement-dependent cytotoxicity (CDC). As expected, we found that MAR-1 depletes blood basophils in WT and *Kit^{W-sh/W-sh}* but not FcεRI-deficient mice (Figure 2D–F). Although this mAb does not deplete peritoneal MCs 24 h post-injection (Figure S2A), we observed that IgE levels were markedly reduced at the surface of MCs (Figure S2B). This reduction in surface IgE levels lasted for at least one week (Figure S2C) and might reflect the fact that MAR-1 competes with IgE for binding to FcεRI.⁶ Strikingly, Fc silent MAR-1 did not deplete basophils (Figure 2F). Thus, our data demonstrate that MAR-1's Fc portion is essential to induce basophil depletion, likely through FcγR-mediated ADCC, ADCP, and/or CDC.

Overall, we show that the Fab portion of MAR-1 is specific for FcεRI but the mAb recognizes FcγRI and FcγRIV through its Fc portion. This explains why MAR-1 stains some myeloid cells in an FcεRI-independent manner. Thus, prior results on FcεRI expression and function in these cells, which were obtained using MAR-1, should be re-evaluated in light of our findings. We propose that Fc silent MAR-1 should be used to assess FcεRI expression. Moreover, this Fc silent format cannot be used for basophil depletion experiments. Because of the multiple and compounding off-target effects of MAR-1 use *in vivo* we have shown here, we suggest that results on the role of basophils in disease models obtained with MAR-1 should be confirmed by additional approaches, such as genetic models of constitutive or inducible basophil deficiency.⁵

KEYWORDS

anaphylaxis, basophil, IgE, mast cells

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CONFLICT OF INTEREST

The authors declare no competing interests.

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FcεRI- and MRGPRX2-evoked acute degranulation responses are fully additive in human skin mast cells

To the Editor,

Mast cell (MC) activation by FcεRI-aggregation is the root of hypersensitivity reactions. However, the recent discovery of MRGPRX2 (Mas-Related G Protein-Coupled Receptor-X2) as a major IgE-independent stimulation pathway has also attracted much interest.¹⁻⁴ Indeed, MRGPRX2 is activated by a plethora of ligands, which can be constantly present in tissues, but the low-affinity nature of MRGPRX2 (requiring micromolar agonist concentrations) likely impedes its permanent stimulation; it is, therefore, controversial whether clinically significant reactions can be triggered by MRGPRX2 (alone).^{2,3} Combinatorial scenarios are an interesting option, but the sequels following concurrent activation of FcεRI and MRGPRX2 remain unresolved.

We tested this possibility in skin MCs stimulated by FcεRI-aggregation or the MRGPRX2 agonists compound 48/80 (c48/80), Substance P (SP), or codeine, individually or jointly. MRGPRX2 agonists were also combined. Degranulation was assessed by histamine or β-hexosaminidase release. Activated MCs were quantified by CD107a exteriorization (detailed methods can be found in Appendix S1).

Using cultured skin-derived MCs, more responsive to FcεRI-aggregation than to MRGPRX2 ligation,⁵ we found that histamine release on combinatorial stimulation surpassed the one elicited by each receptor individually (Figure 1A). Still more, joint stimulation of the routes was fully additive since measured release by the two stimuli was at least equal to the calculated secretion when both were applied separately (i.e., sum of "stimulus 1 + stimulus 2") (Figure 1B). Results were the same for β-hexosaminidase (Figure S1).

For CD107a upregulation, barely any increase was noted after 2 min in cells stimulated via the FcεRI-route, while MRGPRX2 ligation led to exteriorization at this point (Figures 1C and S2a). Conversely, FcεRI-aggregation resulted in a well-separated CD107a⁺ population after 30 min (Figures 1E and S2b). Differential kinetics

SUPPORTING INFORMATION

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were as expected since signaling by MRGPRX2 is more rapid.^{1,3,6} Importantly, the proportions of CD107⁺ cells were additive on combined stimulation (Figure 1D, F).

In *ex vivo* skin MCs, simultaneous activation of MRGPRX2 and FcεRI likewise elevated the response over each stimulus alone (Figure 2A-C). Additivity was ascertained by contrasting the sum of individual stimuli against the measured response on joint stimulation (Figure 2D-F). Activating the routes at different stimulus concentrations revealed additivity also at suboptimal conditions, even trending towards synergism (Figure S4a-d).

Collectively, in skin MCs, signals from the two receptor systems unite to produce strong and, indeed, additive responses, a finding of substantial clinical relevance. For example, allergens reportedly contain stimuli activating both receptors (e.g., hymenoptera venoms); moreover, drug classes (opiates, antibiotics), that activate MRGPRX2, can also elicit IgE responses in susceptible individuals. When co-activated, the anaphylactic threshold may be more readily exceeded.

We tested whether low concentrations of MRGPRX2 agonists can amplify each other. Indeed, SP and codeine at suboptimal concentrations resulted in increased responsiveness over each individual stimulus (Figure S3a). In contrast, joining SP and codeine at saturating concentrations did not enhance degranulation *vis-à-vis* each substance (Figure S3b).^{6,7} Accordingly, the sum was equivalent to the measured value in the suboptimal setting (Figure S3c), but far above under optimal conditions (Figure S3d).

Collectively, we demonstrate that IgE-dependent and IgE-independent pathways unite to boost exocytosis of human skin MCs. The same degree of mediator release and clinical response may thus be achieved by strong activation of FcεRI or MRGPRX2 individually or by weaker activation of both. The latter likely the common scenario in

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