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Meglumine gadoterate induces immunoglobulin-independent human mast cell activation via MRGPRX2

To the Editor,

Gadolinium-based contrast agents (GBCA) are intravenous drugs used to enhance resolution in magnetic resonance imaging. They can induce immediate hypersensitivity reactions, yet their pathogenic mechanisms remain poorly characterized. This hampers the ability to predict which patients are at risk of developing them.[1] In fact, affected patients usually show negative skin tests and can react upon the first known GBCA exposure, which implies that IgE-independent mechanisms might be driving this inflammatory response.

The Mas-related G protein-coupled receptor member X2 (MRGPRX2) has been recently associated with non-IgE-mediated immediate hypersensitivity reactions.[2] Some drugs, such as fluoroquinolones, vancomycin, neuromuscular-blocking agents, icatibant, morphine, leuprolide, and iodinated contrast media, have been reported to activate MRGPRX2, which is highly expressed in mast cells (MCs).[3]

To assess the ability of GBCA to induce non-IgE-mediated hypersensitivity reactions, we stimulated the human MC line LAD2

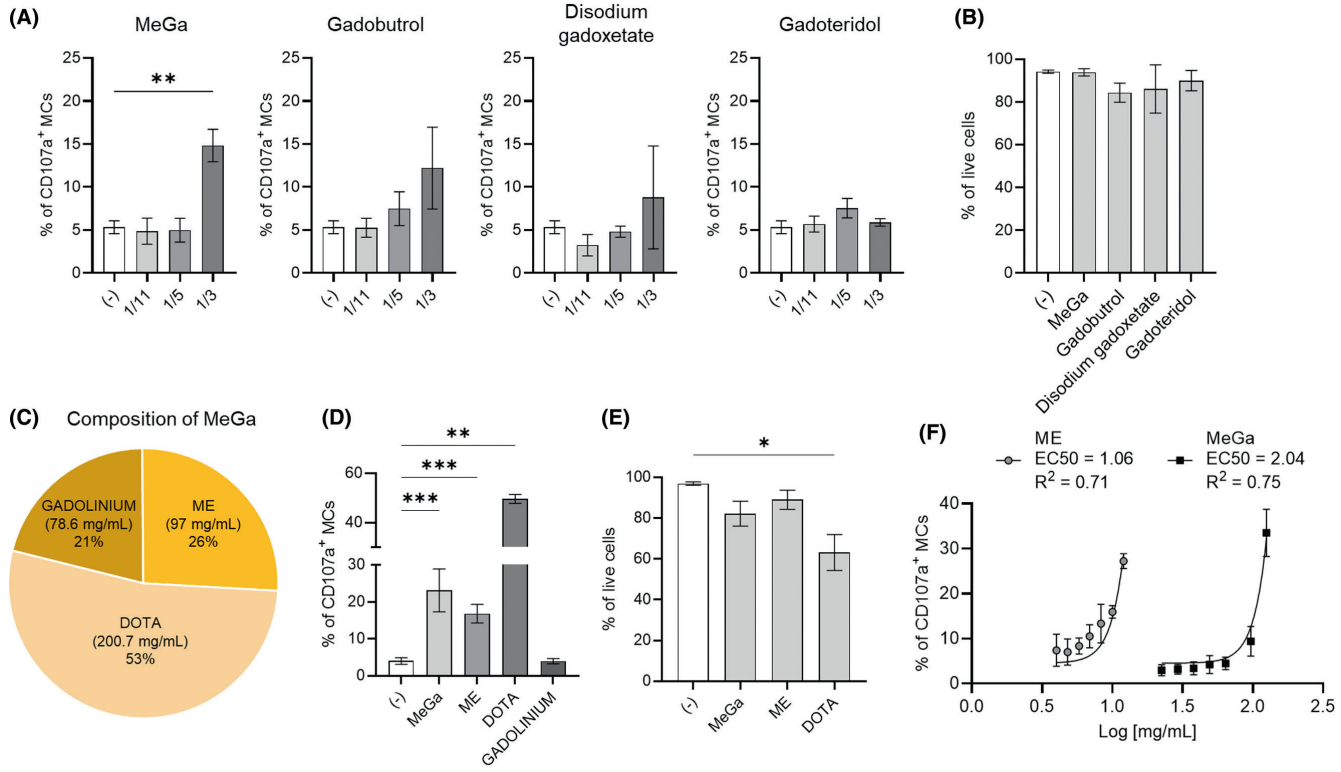

FIGURE 1 Meglumine gadoterate (MeGa) and its component meglumine (ME) induce mast cell (MC) activation without affecting cell viability. LAD2 cells were stimulated with different concentrations of gadolinium-based contrast agents (MeGa, gadobutrol, gadoxetate disodium, and gadoteridol) or vehicle control (−). Activation (A) and cell viability (B) of LAD2 cells stimulated with gadolinium-based contrast agents at 1/3 dilution from stock were assessed by flow cytometry. Circular diagram showing the composition of MeGa (C). LAD2 cell activation (D) and viability (E) assessed by flow cytometry following stimulation with MeGa, ME, DOTA, or gadolinium. Vehicle control (−) was used as negative control. Half maximal effective concentration (EC_{50}) of LAD2 cells challenged with increasing doses of MeGa and ME (F). Pooled data from, at least, three independent experiments in triplicates (A–F). Data are represented as mean ± SEM. One-way ANOVA with Dunnett multiple comparison correction to vehicle (−) condition (A). Kruskal–Wallis test with Dunn's correction was used when normality could not be assumed (B–E). Data are represented as mean ± SEM, $^{*}p \leq .05$; $^{**}p \leq .01$; $^{***}p \leq .001$.

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with several commercial GBCA, namely, meglumine gadoterate, gadobutrol, gadoxetate disodium, and gadoteridol. Then, we determined cell viability and degranulation by flow cytometry[4] (see a detailed material and methods section in this article's Appendix S1).

Of the GBCA tested, meglumine gadoterate was able to induce significant MC activation as compared to unstimulated MCs (Figure 1A; Figure S1A). It did it without reducing cell viability (Figure 1B), which can induce nonspecific activation. Moreover, we confirmed the ability of meglumine gadoterate to induce MC activation without affecting viability in LADR cells, a different MC line (Figure S1B).

Meglumine gadoterate is an ionic macrocyclic paramagnetic contrast media. It is composed of gadolinium, which together with the chelating agent tetraxetan (also known as DOTA), yields gadoteric acid. The base meglumine and gadoteric acid form the salt meglumine gadoterate (Figure 1C). We ascertained the ability of the different components of meglumine gadoterate to induce MC activation. Meglumine and DOTA induced MC activation significantly (Figure 1D), but only meglumine did it without affecting cell viability, as compared to untreated cells (Figure 1E). Interestingly, meglumine caused MC activation at lower concentrations than meglumine gadoterate, according to the half maximal effective concentration (EC_{50}) of both substances (Figure 1F). The logarithmically transformed EC_{50} for meglumine gadoterate was 2.04 ($R^2=0.75$), and for meglumine was about one order of magnitude lower (1.06; $R^2=0.71$).

Next, we decided to explore the involvement of MRGPRX2 in meglumine gadoterate-mediated MC activation. We assessed its expression on LAD2 cells by flow cytometry following stimulations with either meglumine gadoterate or vancomycin (a known MRGPRX2 agonist).[5] Under basal conditions, LAD2 cells expressed high levels of MRGPRX2 (Figure 2A). Following vancomycin

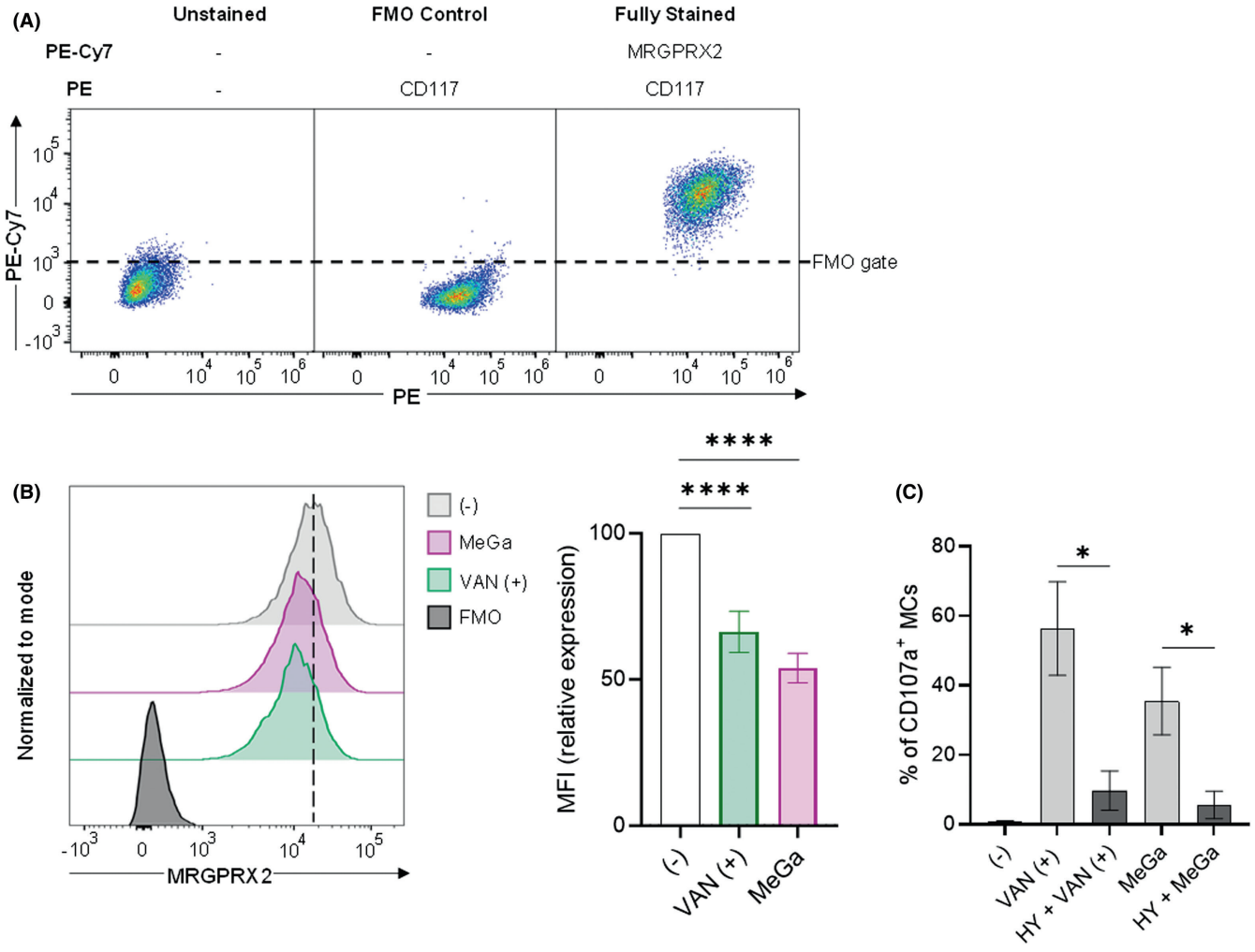

FIGURE 2 Meglumine gadoterate (MeGa)-mediated mast cell activation is dependent on MRGPRX2 signalling. Gating strategy and fluorescent minus one (FMO) control used to determine MRGPRX2 expression by flow cytometry (A). Representative histogram and geometric median fluorescence intensity (MFI) of MRGPRX2 following LAD2 cell stimulation with vancomycin (VAN) (+), or MeGa, represented as the percentage of MRGPRX2 expressed by unstimulated LAD2 cells (B). CD107a expression in VAN (+) and MeGa stimulated LAD2 cells pretreated, or not, with HY-145191 (HY) (C). Pooled data from, at least, three independent experiments (B-C). One-way ANOVA with Dunnett multiple comparison correction to vehicle (−) condition (B). Comparisons between two groups were made with an unpaired two-tailed t-student. Welch correction was used when equal SD between data could not be assumed. Data are shown as mean ± SEM. *$p \leq .05$; ****$p \leq .0001$.

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provocation, MRGPRX2 expression levels were reduced, as compared to untreated LAD2 cells (Figure 2B), while the levels of FcεRI, used as a control, remained unchanged (Figure S1C). Interestingly, we observed a similar decrease in MRGPRX2 expression levels upon meglumine gadoterate challenges, suggestive of both the signaling and the internalization of this receptor (Figure 2B).[6] On the contrary, a reduction in MRGPRX2 expression following meglumine stimulation was not detected (data not shown), which implies that meglumine induces MC activation by a different mechanism. Finally, to better understand the role of MRGPRX2 in MC activation by meglumine gadoterate, we pretreated MCs with a MRGPRX2 antagonist-1 (HY-145191) prior stimulation with meglumine gadoterate or vancomycin, which prevented MC activation (Figure 2C).

In conclusion, our study demonstrates the ability of meglumine gadoterate to induce immunoglobulin-independent MC activation via MRGPRX2. Furthermore, we have delved into the meglumine gadoterate components that may be involved in MC activation and identified meglumine as a potential causative of non-IgE mediated hypersensitivity reactions. Further studies should be performed to define clinically relevant interactions between diverse radiological contrast media and MRGPRX2.

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AUTHOR CONTRIBUTIONS

FV, RJS, and CB conceived and designed the study. PHR, CLS, and RJS performed the experiments and analyzed and discussed the data. PHR and CLS prepared figures. RJS wrote the paper. RJS and CB oversaw the project and raised funding. All the authors read, provided comments, and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

All the authors have no significant conflicts of interest to declare in relation to this manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Vega F, Lopez-Raigada A, Mugica MV, Blanco C. Fast challenge tests with gadolinium-based contrast agents to search for an alternative contrast media in allergic patients. *Allergy*. 2022;77(10):3151-3153.
2. Kolkhir P, Ali H, Babina M, et al. MRGPRX2 in drug allergy: what we know and what we do not know. *J Allergy Clin Immunol*. 2022;151:410-412.
3. Foer D, Wien M, Karlson EW, Song W, Boyce JA, Brennan PJ. Patient characteristics associated with reactions to Mrgprx2-activating drugs in an electronic health record-linked biobank. *J Allergy Clin Immunol Pract*. 2022;11:492-499.e2.
4. Lopez-Sanz C, Sanchez-Martinez E, Jimenez-Saiz R. Protocol to desensitize human and murine mast cells after polyclonal IgE sensitization. *STAR Protoc*. 2022;3(4):101755.

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5. Navines-Ferrer A, Serrano-Candelas E, Lafuente A, Munoz-Cano R, Martin M, Gastaminza G. MRGPRX2-mediated mast cell response to drugs used in perioperative procedures and anaesthesia. *Sci Rep*. 2018;8(1):11628.
6. Raj S, Hlushak S, Arizmendi N, Kovalenko A, Kulka M. Substance P analogs devoid of key residues fail to activate human mast cells via MRGPRX2. *Front Immunol*. 2023;14:1155740.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Annexin A5 inhibits mast cell activation via Allergin-1 immunoreceptor

To the Editor,

Mast cells (MCs) play a central role in type I allergic reactions mediated by allergen-IgE-bound FcεRI signaling. Allergin-1 inhibits FcεRI-mediated signaling in MCs and basophils and plays a vital role in suppressing systemic, cutaneous, and food anaphylaxis and airway hyperresponsiveness,[1–3] but the ligand for Allergin-1 has not yet been identified. This study aimed to identify the functional ligand for Allergin-1.

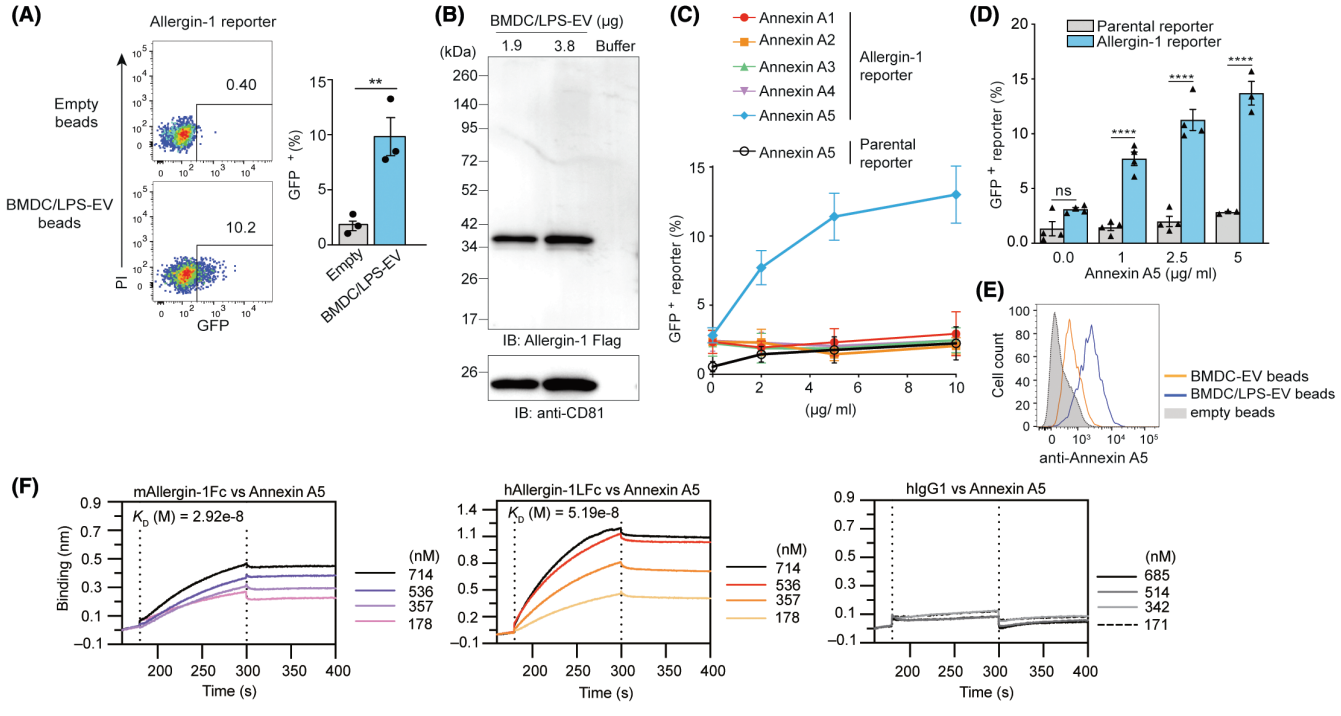

FIGURE 1 Annexin A5 binds to Allergin-1 and mediates signaling. (A) Flow cytometric analysis of GFP expression by Allergin-1 reporter cells upon stimulation with LPS-stimulated BMDC-EV-bearing beads. Empty beads were used as a negative control. Means ± SD of three independent experiments. $^{**}p < .01$ by Student's unpaired *t*-test. (B) Immunoblotting of LPS-stimulated BMDCs-derived EVs with Flag-tagged soluble Allergin-1 or anti-CD81 mAb. (C, D) Flow cytometric analysis of GFP expression by Allergin-1 reporter cells or parental reporter cells upon stimulation with recombinant Annexin proteins. Means ± SD of three (C) or four (D) independent experiments. ns, not significant; $^{****}p < .001$ by one-way ANOVA followed by Tukey's *t*-test. (E) Flow cytometric analysis of Annexin A5 expression on EVs. EVs were purified from culture supernatants of BMDC before and after stimulation of BMDC with LPS using EV-capture beads. Empty beads were used as a negative control. (F) Biotinylated human Annexin A5 was coated onto a streptavidin biosensor and reacted with the analytes indicated. Their affinity was measured in the global analysis mode of the BLItz system. Quantified affinities (K_D) are shown in the graph. Data are representative of two (E) or three (B and F) independent experiments.

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