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SUPPORTING INFORMATION

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DOI: 10.1111/all.15157

Monensin induces selective mast cell apoptosis through a secretory granule-mediated pathway

To the Editor,

Mast cells (MCs) are known to have an aggravating impact on a range of debilitating human diseases, including allergic asthma,[1,2] and strategies to dampen their harmful activities are therefore highly demanded.[3,4] Conceptually, selective depletion of MCs would constitute an efficient regimen to accomplish this. To identify candidate drugs for this purpose, we screened the Prestwick compound library (containing 1,200 approved drugs). This led to the identification of monensin as a drug with potent cytotoxic activity on MCs vs. primary fibroblasts, primary airway epithelial cells, and human embryonic kidney 293 cells (Figures S1 and S2), causing apoptotic cell death in various populations of both mouse and human MCs (Figure S3).

Previous studies have shown that MCs are remarkably sensitive to cell death by mechanisms targeting their secretory granules.[3] To assess whether monensin acts through a granule-mediated pathway, we gated MC populations for high and low granule maturity and assessed if these subpopulations were differentially sensitive to monensin. This showed that MCs with high granularity were excessively sensitive to monensin (Figure 1A). A requirement of intact granule content was supported by experiments revealing that MCs lacking serglycin (a granule-restricted proteoglycan) underwent necrotic rather than apoptotic cells death (Figure 1B). As an additional sign of granule involvement, monensin caused a reduction in granule acidity (Figure 1C–D), suggesting that monensin resulted in granule permeabilization, and we also found that interference with granule acidification (using bafilomycin-A1) blocked the effect of monensin on MCs (Figure 1E). Granule permeabilization would lead to leakage of protons into the cytosol, and, in support of this, treatment of MCs with monensin caused a significant drop in the cytosolic pH (Figure 1F). As further evidence for a mechanism involving granule permeabilization, monensin caused translocation of tryptase (a granule marker) into the cytosol (Figure 1G). Monensin-induced cell death was caspase-independent (Figures S1B and S4) and monensin did not cause MC activation (degranulation), as assessed by calcium flux and β-hexosaminidase release (Figure S5).

Next, we assessed the selectivity of monensin for MC vs. other immune cell types. This analysis revealed that mouse peritoneal B-, T-lymphocytes, and macrophages were largely resistant to monensin

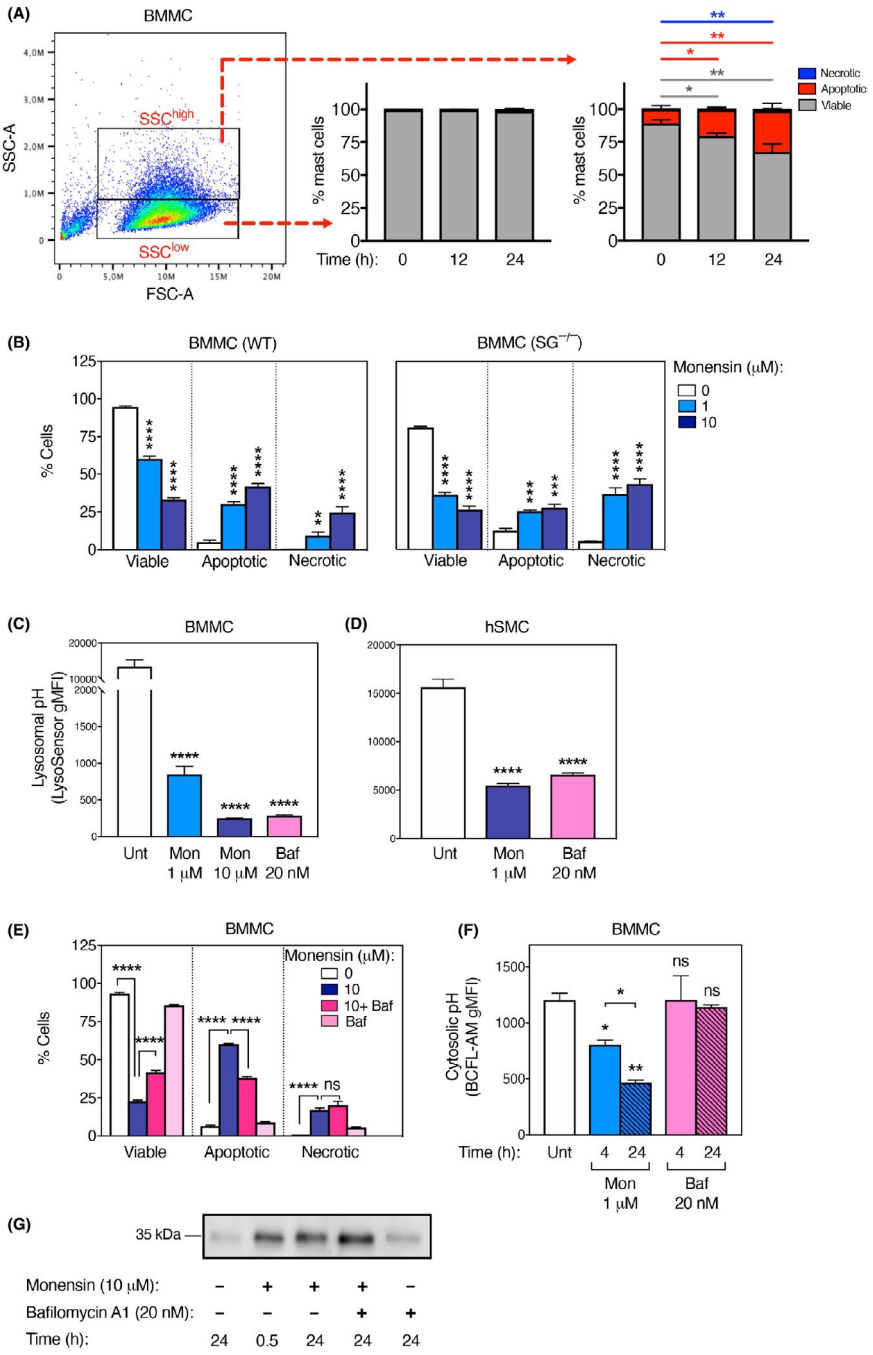

FIGURE 1 MC death induced by monensin is dependent on acidic MC secretory granules. (A) Bone marrow–derived MCs (BMMCs) were treated with 1 μM monensin for 12 and 24 h, followed by Annexin V/DRAQ7 staining to measure cell death. MCs were gated into cells having high and low maturity, as assessed by side scatter analysis for granularity. The right panels depict the quantification of apoptotic (Annexin V^+/$DRAQ7^-$) and necrotic (Annexin V^+/$DRAQ7^+$) cell death in the respective populations ($n = 3$). (B) BMMCs (0.5×10^6 cells) were developed from WT or serglycin$^{-/-}$ ($SG^{-/-}$) mice and were treated with monensin at the indicated concentrations for 24 h, followed by cell death assessment (Annexin V/DRAQ7 staining). (C–D) BMMCs (C; 0.5×10^6 cells) or human skin MCs (hSMC) (D; 0.1×10^6 cells) were treated with monensin or bafilomycin A1 at the indicated concentrations for 30 min, followed by staining with LysoSensor Blue DND-167 (a lysosome probe sensitive to pH change) ($n = 4$–5). (E) BMMCs (0.5×10^6 cells) were treated with either monensin (10 μM) or bafilomycin A1 (20 nM) alone or in combination for 24 h, followed by measurement of cell death (Annexin V/DRAQ7 staining) ($n = 3$). (F) BMMCs (0.5×10^6 cells) were treated with monensin or bafilomycin A1 for 4 or 24 h, followed by assessment of cytosolic pH by BCFL-AM ($n = 4$). (G) Monensin causes translocation of tryptase (mMCP-6) from granules to the cytosol. BMMCs (2×10^6 cells) were treated for the indicated time periods ±monensin (10 μM) and ±bafilomycin A1 (20 nM), as indicated. Cytosolic extracts were prepared and analyzed by Western blot for levels of the tryptase mMCP-6. The animal experiments were approved (Uppsala djurförsöksetiska nämnd Dnr 5.8.18-05357/2018; Swedish animal experimentation ethical review board (N143/14 and 10973–2019))

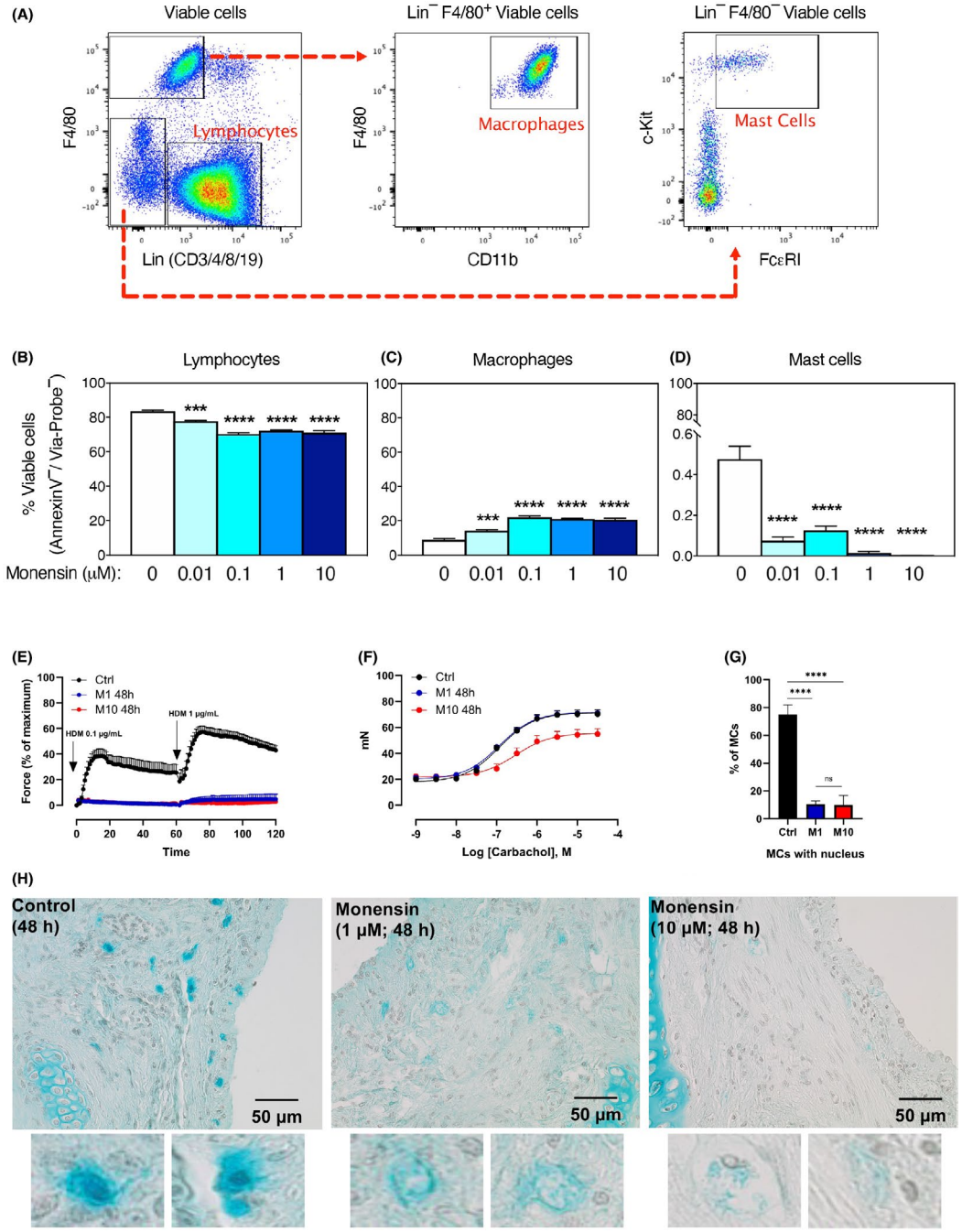

FIGURE 2 Monensin induces selective apoptosis of mouse MCs and abrogates airway reactivity in trachea isolated from house dust mite (HDM)-sensitized guinea pigs. Peritoneal cell populations were recovered by peritoneal lavage of mice. (A) Gating strategy to identify viable cells including lymphocytes (Lin^+ $F4/80^-$), macrophages (Lin^- $F4/80^+$ $CD11b^+$), and MCs (Lin^- $F4/80^-$ c-Kit^+ $Fc\varepsilon RI^+$) within the peritoneal cell populations. Doublets were excluded prior to analysis. (B–D) Peritoneal cells were subjected to monensin at the indicated concentrations for 24 h, followed by flow cytometric analysis. The panels show quantification of viable (Annexin V^-/ Via-$Probe^-$) cells within the lymphocyte (B), macrophage (C), and MC (D) populations ($n = 3$). (E–H) Tracheal segments isolated from guinea pigs that were sensitized by a single i.p. injection of HDM extract with aluminum hydroxide (100 μg/100 mg HDM/ aluminum hydroxide). The segments were cultured for 48 h in the absence or presence of monensin (1 (M1) and 10 μM (M10)). After the culture period, the segments were mounted in tissue baths to examine the contractile response to (E) HDM (measured as percentage of maximal contraction to carbachol) and to (F) carbachol (measured in mN). These experiments were performed in the presence of 3 μM indomethacin to exclude the involvement of prostaglandin E_2 that mediate spontaneous tone in the guinea pig trachea. The segments were subjected to histological analysis using double staining of tracheal tissue with Astra Blue (visualizes MCs by staining their granules) and hematoxylin (nuclear staining). (G) Percentage of MCs with intact nucleus. (H) Typical images of tracheal tissues. Below each image, further magnification of mast cells is shown ($n = 6–8$)

whereas peritoneal MCs were highly sensitive (Figure 2A–D). In agreement, human blood monocytes, lymphocytes, and neutrophils were minimally affected by monensin (Figure S6).

To evaluate whether monensin has an impact on a pathophysiological response where MCs are implicated, we investigated its effect on allergic responses in airways. For this, we employed a guinea pig model for antigen-induced bronchoconstriction.[5] Tracheal segments from guinea pigs, sensitized with house dust mite (HDM) extract, were excised and cultured for 48 h in the absence or presence of monensin (1 or 10 μM). HDM extract induced a strong tracheal contraction, which at the highest concentration (1 μg/mL) reached almost 60% of maximal contraction induced by carbachol (Figure 2E). Notably, the HDM-induced tracheal contraction was almost completely abolished by monensin—both at 1 and 10 μM. At 1 μM, monensin did not affect the carbachol-induced contraction, indicating that monensin at 1 μM did not cause tissue damage (Figure 2F). Histological analysis showed that monensin caused a profound reduction in the proportion of MCs having a defined nucleus (Figure 2G,H), suggesting that monensin causes apoptosis of MCs populating the guinea pig tracheal tissue.

Altogether, our findings indicate that monensin induces selective apoptotic MC cell death by targeting the secretory granules. Notably, MCs have a markedly higher content of acidic granules than any other cell type,[6] which could explain why monensin shows selectivity for MCs. Potentially, monensin and similarly acting drugs can thereby be developed for therapeutic purposes in diseases in which MCs have a detrimental impact, as exemplified by allergic asthma.

FUNDING INFORMATION

Cancerfonden; Vetenskapsrådet; Welander-Finsen Foundation; Knut och Alice Wallenbergs Stiftelse; Hjärt-Lungfonden

ACKNOWLEDGEMENTS

This study was supported by grants from The Swedish Heart and Lung Foundation, The Swedish Research Council, The Swedish Cancer Foundation, The Knut & Alice Wallenberg Foundation, and The Welander-Finsen Foundation.

CONFLICT OF INTEREST

The authors have no conflict of interest in relation to this work. The concept of using monensin as an anti-MC agent is under patenting.

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