Syk deficiency in human non-releaser lung mast cells

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Abstract:

Human lung mast cells, like peripheral blood basophils, can have a non-releaser phenotype characterized by a failure to respond to FcεRI-IgE-mediated stimulation [1]. Previous studies on human basophils demonstrated that approximately 10–15% of donors fail to release histamine in response to FcεRI crosslinking [2], [3], [4], [5] and that depressed protein levels of the tyrosine kinase Syk correlated with this non-releaser status [6], [7]. Here we present evidence for diminished Syk protein in a second preparation of lung mast cells with a non-releasing phenotype.

Keywords: letter to the editor | lung mast cells | non-releaser phenotype | Syk | IgE

Article:

To the Editor,

Human lung mast cells, like peripheral blood basophils, can have a non-releaser phenotype characterized by a failure to respond to FcεRI-IgE-mediated stimulation [1]. Previous studies on human basophils demonstrated that approximately 10–15% of donors fail to release histamine in response to FcεRI crosslinking [2], [3], [4], [5] and that depressed protein levels of the tyrosine kinase Syk correlated with this non-releaser status [6], [7]. Here we present evidence for diminished Syk protein in a second preparation of lung mast cells with a non-releasing phenotype.

Mast cells were isolated from discarded surgical lung obtained within 24 h of surgery as approved by the VCU IRB. Mast cells were dispersed by mincing the lung and digesting the pieces with collagenase and hyaluronidase, enriched by Percoll density-dependent sedimentation, and purified to > 95% purity by positive selection using mouse IgG anti-Kit mAb and bead-conjugated anti-mouse Ab.

Because initial activation experiments with anti-FcεRI mAb did not cause degranulation (not shown), these putative non-releaser mast cells were examined in greater detail. Skin derived mast cells, obtained as described [8], were examined in parallel with the lung mast cells as a positive control. Skin and lung mast cells were challenged with various concentrations of anti-FcεRI mAb and examined for degranulation (β-hexosaminidase, Fig. 1A) and cytokine (GM-CSF, Fig. 1B) release. As expected, skin mast cells degranulated in response to each dose of anti-FcεRI mAb stimulation. In contrast, the lung mast cells failed to degranulate in response to any concentration of anti-FcεRI mAb. While incubation with IL-3 has been shown to revert non-releasing basophils to releasing basophils [5] with concomitant recovery in Syk levels [9], incubation of the lung mast cells with this cytokine (50 ng/ml for 4 days) did not convert them to a releasing phenotype (not shown). The FcεRI-mediated release of GM-CSF was also impaired. However, the abilities of the lung mast cells to degranulate and release GM-CSF in response the calcium ionophore, A23187, a non-FcεRI-dependent stimulus, were intact. Thus, these lung mast cells had a defect that was selectively manifested in their FcεRI-initiated signal transduction pathway.

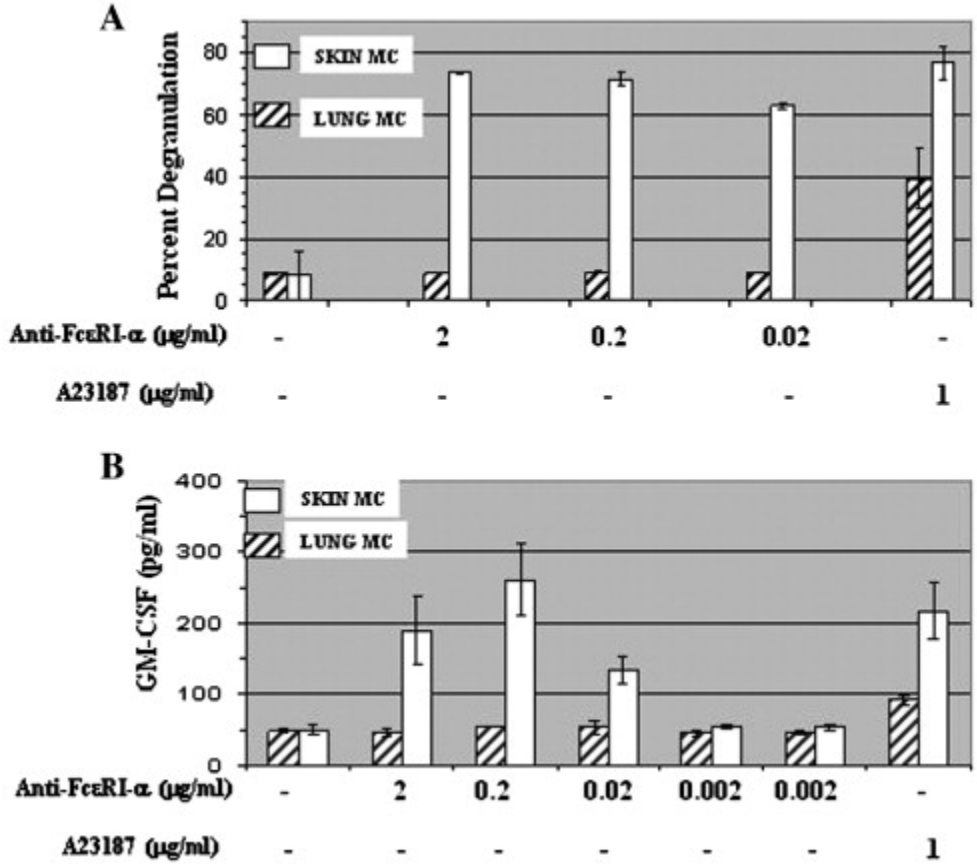

Figure 1. Specific impairment of FcεRI-dependent degranulation in lung mast cells. Lung mast cells or releasing skin mast cells were challenged with different concentrations of anti-FcεRI mAb, calcium ionophore (A23187; 1 μg/ml), or with buffer alone (spontaneous release) for 30 min (degranulation) or overnight (cytokine analysis) at 37 °C. The supernatants were collected as examined for β-hexosaminidase and GM-CSF release as described previously [10]. Data are from 1 experiment that is representative of 4 (degranulation) or 2 (cytokine) separate experiments, each done in duplicate (± SEM). The total amounts of β-hexosaminidase activity in skin and lung mast cells were comparable, 0.90 and 0.86 OD, respectively.

This non-releaser phenotype was not due to absent FcεRI. After 21 days in culture, as seen in Fig. 2A, these mast cells expressed high amounts of FcεRI and Kit by flow cytometry (Fig. 2—top panel) and tryptase by immunocytochemistry (Fig. 2—bottom panel). No obvious morphological abnormalities were apparent.

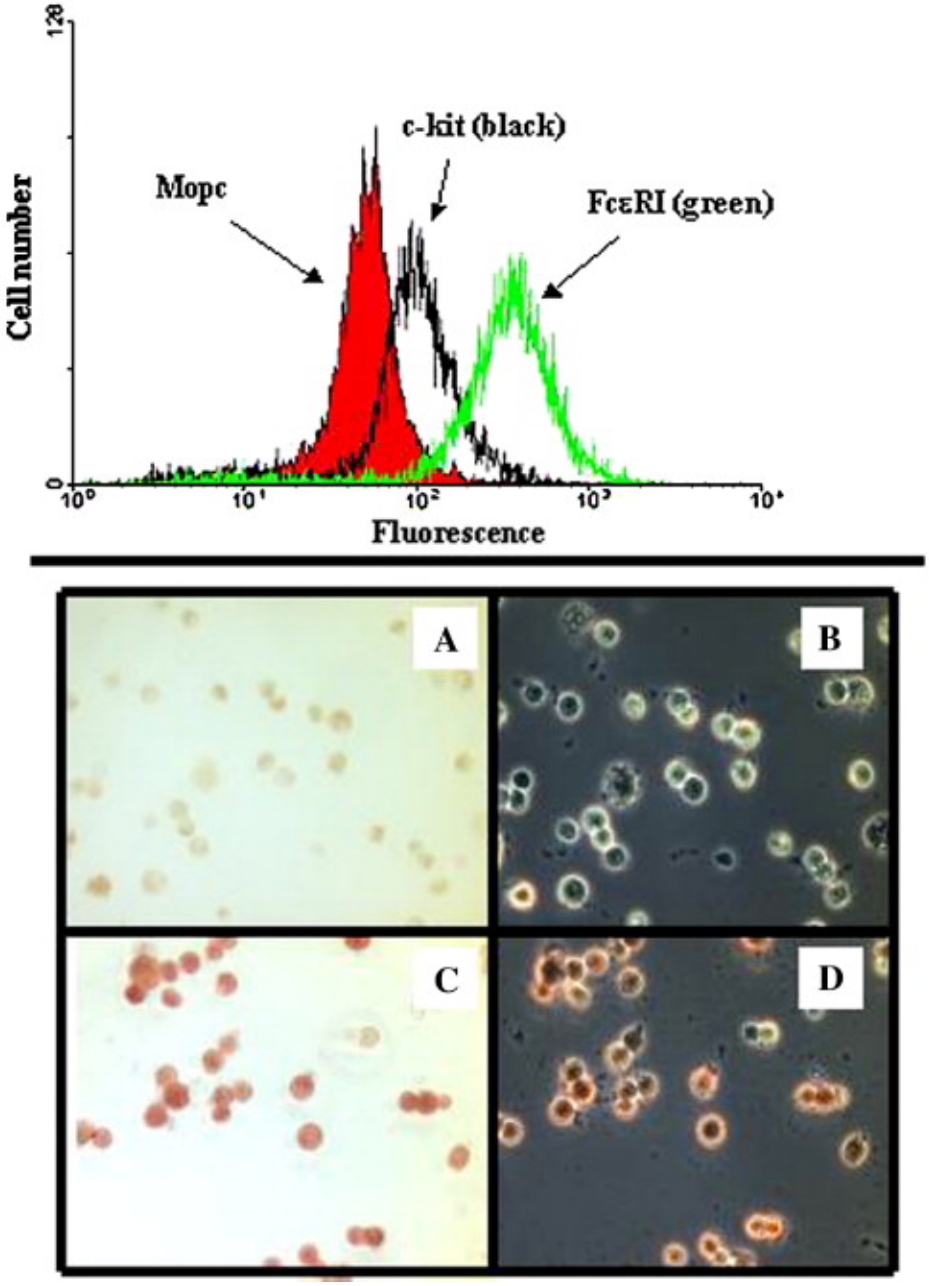

Figure 2. Characterization of non-releaser human lung mast cells. **Top panel**. Expression if FcεRI and Kit on non-releasing human lung mast cells. Lung mast cells were incubated at 4 °C with mouse IgG mAbs against FcεRI-α and Kit (5 μg/ml) followed by FITC-labeled goat anti-mouse IgG Ab. An irrelevant mouse IgG (MOPC) was substituted as a negative control. **Bottom panel**. Tryptaseimmunocytochemistry. Cytocentrifuge preparations of lung mast cells were fixed and incubated with an irrelevant isotype match (MOPC; A, B) or anti-tryptase (5 μg/ml, C, D) overnight. The next day cell cytospins were washed with TTBS and incubated with peroxidase-conjugated anti-mouse Abs followed by detection with AEC as described previously [11]. The photomicrographs in A and B as well as C and D are of identical fields visualized under light and phase contrast microscopy, respectively.

We next tested these lung mast cells for protein levels of Syk, Lyn, and Fyn, molecules previously associated with signaling after FcεRI aggregation. As seen in Fig. 3, the anti-FcεRI-stimulated non-releasing lung mast cells do not express detectable levels of Syk protein by Western blotting after 14 to 30 days of culture, in contrast to a releaser lung mast cell preparation. However, Lyn and Fyn expression was readily detected in non-releaser lung mast cells. Longer exposure times did not reveal Syk protein expression. We conclude that the lung mast cells in these experiments were defective in their IgE-mediated signaling, most likely related to a deficiency in Syk protein, analogous to the IgE non-releaser phenotype described for basophils [6], [7].

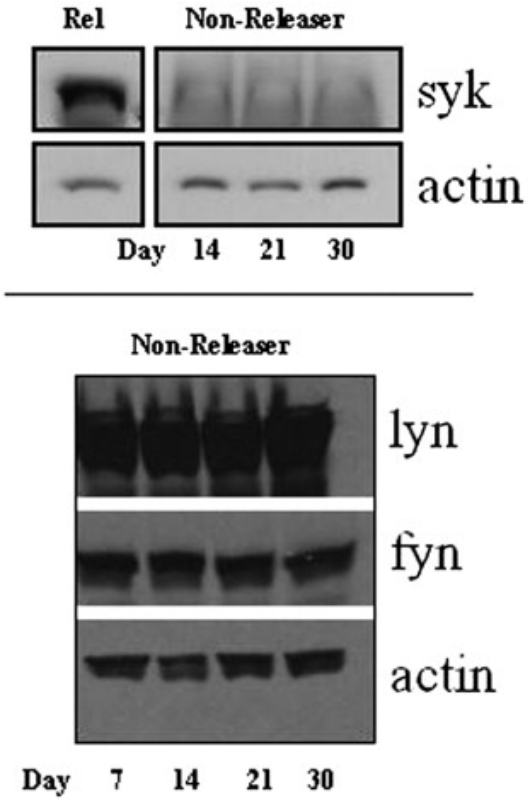

Figure 3. Non-releaser lung mast cells lack Syk protein. Non-releasing lung mast cells (~ 200,000 cell-equivalents/lane) after the indicated days in culture were collected and then lysed and subjected to Western blotting as described [6], [9]. Actin was used as a protein loading control. In the top panel a lysate from a previous preparation of lung mast cells that had shown their ability to degranulate in response to FcεRI aggregation (Releaser) is compared to that of the non-releaser mast cells.

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