Evidence for human mast cell nonreleaser phenotype

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Abstract:

Mast cells and basophils express high amounts of FcεRI. In basophils the concentration of anti-IgE or antigen required for maximal histamine release through FcεRI varies among donors, a concept termed "releasability." Some donors fail to release histamine in response to any concentration of IgE-mediated stimulus (nonreleaser)[1, 2, 3, 4] and were subsequently found to lack the protein tyrosine kinase Syk.[5] Although the nonreleaser phenoytpe is common (ie, present in 10% to 20% of all donors), it is not known whether mast cells exhibit a similar IgE-mediated nonresponsiveness. We report here a lung mast cell preparation that did not respond to IgE-mediated stimuli.

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Article:

To the Editor:

Mast cells and basophils express high amounts of FcεRI. In basophils the concentration of anti-IgE or antigen required for maximal histamine release through FcεRI varies among donors, a concept termed "releasability." Some donors fail to release histamine in response to any concentration of IgE-mediated stimulus (nonreleaser)[1, 2, 3, 4] and were subsequently found to lack the protein tyrosine kinase Syk.[5] Although the nonreleaser phenoytpe is common (ie, present in 10% to 20% of all donors), it is not known whether mast cells exhibit a similar IgE-mediated nonresponsiveness. We report here a lung mast cell preparation that did not respond to IgE-mediated stimuli.

Mast cells were dispersed from human lung tissue and enriched as described previously.[6] This donor was undergoing a thoracotomy for lung cancer and was not receiving steroid therapy. These studies were approved by the Human Studies Internal Review Board at Virginia Commonwealth University. Mast cells were positively selected with mouse anti-c-kit receptor

antibodies (YB5B8, a gift from Leonie Ashman) and bead-conjugated anti-mouse antibodies, as per the manufacturer's instructions (Miltenyi, Auburn, Calif). A total of 3.2×10^7 cells were isolated from 30 g of tissue. The purity of mast cells was 76%, as assessed with toluidine blue staining. The mast cells were cultured at 1×10^6 cells/mL in RPMI medium supplemented with 10% CPSR-1 (Sigma, St Louis, Mo), 10 µmol/L HEPES, 50 µmol/L 2-mercaptoethanol, 4 mmol/L L-glutamine, 1× MEM amino acids, 1× vitamin solution, 100 U/mL penicillin, 100 µg/mL streptomycin (Gibco, Gaithersburg Md), and 100 ng/mL recombinant human stem cell factor (Biosource, Camarillo, Calif). Nitrophenacetyl-specific IgE (NP-IgE; 5 µg/mL) was added on day 2 of culture. After 3 days, we performed FACS analysis by using the mouse anti-human FcεRI α chain (22E7, gift from Dr J. Kochan) and c-kit receptor antibodies. These mast cells expressed high amounts of FcεRI and the c-kit receptor (data not shown). No obvious morphologic differences were observed by means of light microscopy when comparing toluidine-stained mast cells from cells responsive to IgE-mediated stimuli (not shown).

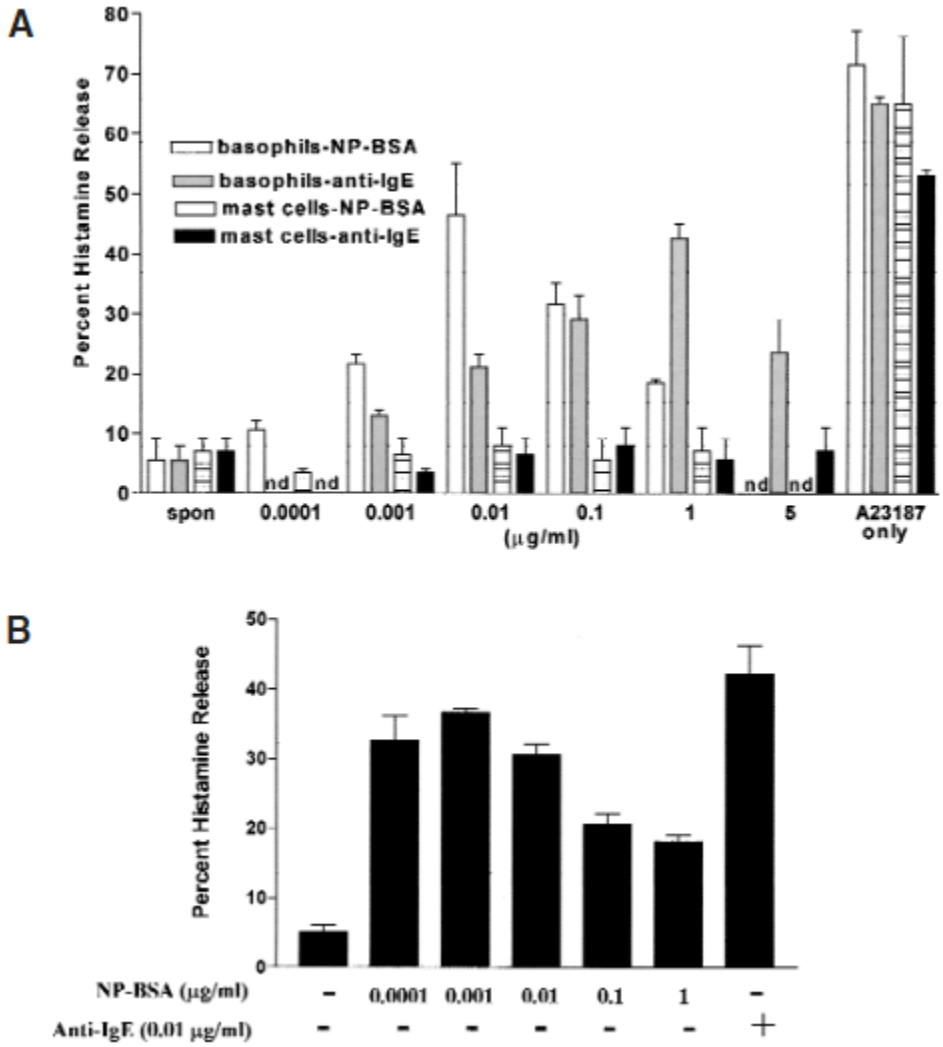

Fig. 1. A, Specific impairment of FcεRI-dependent degranulation in lung mast cells. Data are from 1 experiment and are representative of 2 other experiments, each done in duplicate and performed with mast cells (mean ± SEM, n = 3). *ND,* Not done. **B,** Isolation procedure for lung mast cells does not induce FcεRI nonresponsiveness.

Initial degranulatory experiments (routinely performed in our laboratory)[5, 6, 7, 8] with IgE-sensitized cells cultured for 3 days showed this preparation did not respond to IgE stimulus (not shown). We hypothesized that these mast cells were defective in their ability to degranulate in response to IgE-mediated stimulus. To test this hypothesis, we used peripheral blood basophils from a separate known releaser donor in parallel with the lung mast cells to ensure all reagents were working properly. IgE-sensitized lung mast cells or freshly isolated basophils (sensitized with NP-IgE for 4 hours)[7] were washed and challenged with different concentrations of anti-IgE or NP-BSA, A23187 (500 ng/mL), or no stimulus (spontaneous) for 30 minutes. The supernatants were measured for histamine release by using a kit from Beckman-Coulter (Marseille, France), and the percentage of histamine release was calculated as described previously.[8] As expected, basophils released histamine in response to antigen or anti-IgE antibodies. However, by using identical reagents and experimental conditions, the lung mast cells failed to release histamine in response to any concentration of antigen or anti-IgE (Fig 1, *A*).

In addition, no β-hexosiminidase release was detected in the supernatants of the mast cells (data not shown). Although FcεRI-induced secretion was absent, the mast cells secreted normally when challenged with Ca^{2+}ionophore, which induces secretion in part by direct effects on cytoplasmic Ca^{2+} levels. The methods for mast cell purification from the tissue did not cause IgE nonresponsiveness because a previous mast cell preparation (84% purity; isolated, sensitized, and activated as above) released histamine when challenged with varying concentrations of antigen (Fig 1, *B*). Therefore these mast cells had a specific defect in their IgE-mediated signal transduction pathway because their ability to degranulate remained intact.

We have not detected other mast cell preparations that did not degranulate in response to FcεRI-mediated stimulus (n = 4). We conclude that the mast cells in these experiments were defective in their IgE-mediated signaling and might reflect a heretofore unknown mast cell nonreleaser phenotype. More studies are needed to confirm these preliminary findings.

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