

Cytokine release from a human mast cell line (HMC-1) in response to stimulation with anti-IgE and other secretagogues

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Introduction

The role of mast cells as primary effector cells in IgE-dependent immediate hypersensitivity is well established [1]. The discovery that mast cells are also a potential source of cytokines suggests an additional role of mast cells in modulating late-phase reactions and other chronic inflammatory processes. Cytokines such as IL-4, IL-5, IL-8, IL-13 and TNF- α are produced by rodent mast cells [2]. Whereas the proinflammatory cytokines IL-5, IL-8 and TNF- α mediate the infiltration and activation of leukocytes into the affected tissues, IL-4 and IL-13 not only upregulate vascular adhesion molecule expression and support B cell IgE production but also control Th2 development and function. However, comparatively little is known about the cytokine release from human mast cells [3]. We therefore investigated the release of these cytokines from the human mast cell line HMC-1 following stimulation with immunological and non-immunological stimuli.

Materials and methods

The human leukaemic mast cell line (HMC-1), kindly provided by J. H. Butterfield, Minneapolis, MN, was cultured in RPMI 1640 and sensitized for 48 hours with monoclonal IgE (0.5 μ g/ml). Following several washes, HMC-1 cells ($1-5 \times 10^5$ per tube) were incubated at 37 °C with either buffer (control), anti-IgE (1/200 dilution), compound 48/80 (c48/80; 10 μ g/ml), calcium ionophore A23187 (1 μ M), SCF (10 ng/ml) or a combination of SCF and anti IgE (preincubated with SCF for 10 min before addition of anti-IgE). Following stimulation for 30 min to 6 h cytokine levels in the supernatants were measured by ELISA (Promocell, Heidelberg, Germany). In addition, histamine release was determined (spectrofluometrically) following 30 min incubation.

Results and discussion

Spontaneous histamine release from HMC-1 cells was consistently high ($21.6 \pm 1.9\%$, $n = 5$) but histamine release was significantly increased following stimulation with anti-IgE ($32.6 \pm 3.3\%$, $n = 5$, $p = 0.03$) or the calcium ionophore A23187 ($43.2 \pm 1.5\%$, $n = 5$, $p = 0.0004$). However, there was

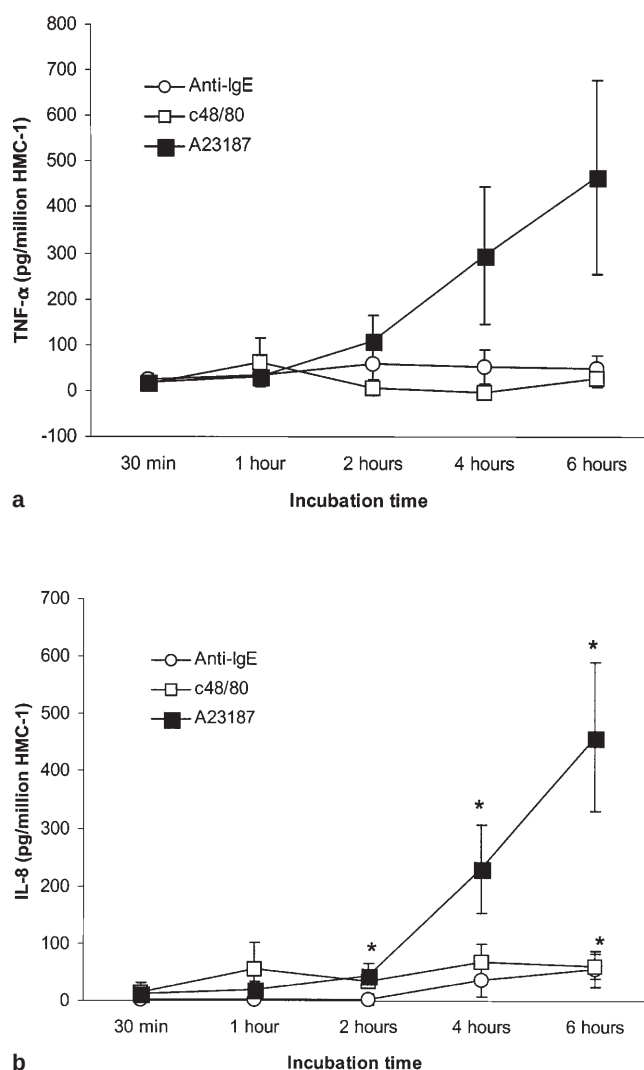


Fig. 1. TNF- α (a) and IL-8 (b) release from HMC-1 cells following stimulation with anti-IgE (1/200 dilution), compound 48/80 (c48/80, 10 μ g/ml) and A23187 (1 μ M). Results are expressed as mean net releases $\pm$ SEM ($n = 7$). All data are corrected for spontaneous secretions of TNF- α (< 30 pg/ 10^6 HMC-1) and IL-8 (< 40 pg/ 10^6 HMC-1). * Represents significant ($p < 0.05$) differences from spontaneous release as determined by the Student's t-test.

no significant net release of histamine to either compound 48/80 ($35.0 \pm 5.7\%$, $n = 5$) or SCF ($33.1 \pm 4.3\%$, $n = 5$) and preincubation of the cells with SCF did not enhance IgE-dependent histamine secretion ($35.2 \pm 6.1\%$, $n = 5$).

Regarding cytokine release, HMC-1 cells produced both TNF- α and IL-8 in response to A23187 but not to anti-IgE or to compound 48/80 following 6 h stimulation (Fig. 1a and b). The cells failed to reproducibly release these cytokines following treatment with SCF (either alone or in combination with anti-IgE, results not shown). In contrast, HMC-1 cells did not release IL-4, IL-5 or IL-13 regardless of the stimulus or incubation period employed. In addition to the above, we were able to detect both IL-8 (136 ± 115 pg/ 10^6 HMC-1) and TNF- α (407 ± 275 pg/ 10^6 HMC-1) in lysed, unstimulated HMC-1 cells indicating the presence of preformed stores of these factors. Messenger RNA for IL-8 and TNF- α in unstimulated HMC-1 cells was also observed (data not shown).

These results indicate that human mast cells may participate in initiating the chronic phase of allergic inflammation, due to rapid release of IL-8 and TNF- α and the resulting recruitment and activation of granulocytes. They also confirm our observations from human skin mast cells, where a rapid secretion of both IL-8 and TNF- α was detected [manuscript in preparation]. However, in contrast to skin mast cells, where the release of TNF- α and IL-8 to various stimuli is complete within 4 h, we observed that the kinetics of release of these factors from HMC-1 cells is much slower and had not reached

maximum levels after 6 h stimulation. Furthermore, and in contradicition to previous studies [4, 5], our data suggests that human mast cells (either skin or HMC-1) do not release IL-4 and IL-13, which amongst human histaminocytes appear to be derived from basophils [6].

Overall, these results show that human mast cells may play an important role in mediating chronic inflammatory processes due to the actions of TNF- α and IL-8 but they do not produce the wide panel of immunomodulatory cytokines ascribed to murine mast cells.

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