Multiple Cytokine mRNA Expression in Human Mast Cells Stimulated via FcεRI

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Key Words

Human mast cells
Cytokine
FcεRI

Abstract

Cross-linkage of FcεRI on human lung mast cells purified by affinity magnetic selection with monoclonal antibody YB5.B8 against **c-kit** (purity > 90%) expressed mRNA for multiple cytokines. There was no constitutive expression of interleukin (IL)-4 mRNA. Mast cell stimulation with **anti-IgE** induced IL-4 mRNA expression which appeared maximal at 2 h and waned slowly over the next 24 h. IL-5, IL-6, IL-8 and tumour necrosis factor (TNF)-α mRNA were constitutively expressed. Mast cell activation with **anti-IgE** led to an increase of IL-5 and TNF-α mRNA signals within 2 h and which persisted for at least 24–48 h. On the other hand, IL-6 and IL-8 mRNA expression were not affected by **anti-IgE** challenge.

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Introduction

Mast cells are the major initiating cell of allergic reactions. In the airways, interaction of allergen with superficial mast cells results in the release of histamine, proteases, prostaglandin D2 and leukotriene C4. These are the major mediators of the immediate phase of bronchoconstriction, oedema and mucus secretion which characterise asthma. However, the ability of the mast cell to initiate and support chronic inflammation is unclear. Rodent mast cells and mast cell lines have demonstrated both mRNA and multiple immunoreactive cytokines [1]. In the last several years, it has become clear that human cells which express FcεRI can produce multiple cytokines including interleukin (IL)-4, IL-5, IL-6, IL-8 and tumour necrosis factor (TNF)-α [2-8].

However, IgE-dependent regulation of human mast cells has not been extensively studied and the roles and significance of cytokine production by human mast cells is not known.

Cytokine Gene Expression in Human Mast Cells

Published studies on human mast cell cytokines have been confined to TNF-α, with demonstrations of cytokine-specific mRNA in bone-marrow-derived mast cells/basophils in culture [3] and in dispersed skin mast cells [7]. Recently, mRNA for IL-4 and IL-5 has been demonstrated in nasal mucosal mast cells, 24 h after nasal allergen challenge [9,10].

We have investigated the expression of mRNA for cytokines in lung mast cells using RT-PCR and in situ hybridisation. Lung mast cells were purified by affinity magnetic selection with monoclonal antibody YB5.B8 against c-kit to achieve a final mast cell purity > 90%. Human lung mast cells were precultured with stem cell factor (SCF) (10ng/ml) and myeloma IgE (3

µg/ml) for 16 h, washed and then challenged immunologically by incubation with anti-IgE (1 µg/ml) in the presence of SCF (50 ng/ml). Control cells were incubated similarly except that anti-IgE was omitted.

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Cells were harvested at 0, 2, 4, 12, 24 and 48 h for RT-PCR analysis of cytokine mRNA. There was no constitutive expression of IL-4 mRNA. Mast cell activation with anti-IgE induced the expression of IL-4 mRNA which appeared maximal at 2 h, waning slowly over the next 24 h. Analysis of IL-5 mRNA by RT-PCR showed weak constitutive expression. Mast cell stimulation with anti-IgE led to an increase of IL-5 mRNA within 2 h which persisted for at least 48 h. IL-6, IL-8 and TNF-α mRNA were constitutively expressed in the lung mast cell enrichment. Mast cell activation with anti-IgE led to an increase of TNF-α, but not IL-6 and IL-8 mRNA expression.

The Role of Human Mast Cell Cytokine Production in vitro

Information on the role of human mast cell cytokines is limited. Klein et al. [11] and Walsh et al. [7] demonstrated that induction of ELAM-1 expression is a consequence of the release of mast cell-derived TNF-α. Since the coordinated expression of cytokines and adhesion molecules is essential for the development of immune and inflammatory responses, the spectrum and biological activity of mast cell cytokines indicate that they play a major role. However, the direct evidence for this is sparse. Using an ELISA, immuno-reactive IL-5 and TNF-α were detected in the supernatant of human lung mast cells. IL-5 was detectable 8 h after activation with anti-IgE and it was maximal at 24-48 h. The total release of IL-5 over 24 h was 400 pg – 1.2 ng/10⁶ mast cells. IL-5 was not detectable in the supernatant of mast cells incubated with SCF alone. TNF-α was detectable within 2 h after activation and it was maximal at 12 h. Interestingly, TNF-α was detectable in the supernatant from cells incubated with SCF alone. These findings are in agreement with the hypothesis of Gordon et al. [1]. Soon after activation, mast cell cytokines which mediate proinflammatory events may exert their influence. Later, cytokines with autocrine or paracrine effects on the proliferation or function of inflammatory cells may exert their influence.

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