Effects of Interleukin-4 or Stem Cell Factor on Mast Cell Mediator Release and Cytokine Gene Expression

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Key Words

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Abstract

We have investigated the capacity of interleukin (IL)-4 or stem cell factor (SCF) to induce direct mediator release from rodent peritoneal mast cells, and also to induce or regulate cytokine gene expression in the human HMC-1 mast cell line. SCF, but not IL-4, induced low levels of serotonin release from mouse or rat peritoneal mast cells; rat mast cells acquired enhanced responsiveness to SCF during culture. IL-4, but not SCF, enhanced ionomycin-induced transcription and secretion of several genes, including the cytokines IL-3, IL-4, granulocyte/macrophage-colony-stimulating factor, IL-8 and the receptor for IL-6 in the human HMC-1 mast cell line.

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Introduction

Studies in rodents have shown that the secretory behaviour of tissue mast cells is subject to in vivo regulatory influences independently of the IgE response [1, 2]. In vitro studies provide evidence that cytokines may be the agents responsible for this regulation [3-6]. For example, interferon (IFN)- γ generated by splenic T cells inhibits IgE/anti-gen-dependent histamine and serotonin release from isolated mouse peritoneal mast cells [3, 4] while interleukin (IL)-3 and IL-4 enhance mast cell mediator release [5, 6]. IFN- γ and IL-4 also reciprocally control release of arachid-onate from mast cells [6].

As well as T-cell-derived cytokines, the fibroblast- and stromal-cell-derived cytokine, stem cell factor (SCF), the ligand at the receptor encoded by the proto-oncogene c-kit, up-regulates IgE/antigen-induced serotonin release from mouse peritoneal mast cells [5].

In this article we report further studies examining the effects of IL-4 or SCF on mast cells; first as direct agonists of serotonin release from rodent peritoneal mast cells, and second, as inducers and regulators of cytokine gene expression in a human mast cell line.

SCF, but not IL-4,

Induces Mast Cell Serotonin Release

Freshly isolated purified mouse or rat mast cells released low levels of serotonin (< 15%) in response to 30 min challenge with SCF at concentrations up to 500 ng/ml, but failed to respond to IL-4 [for methods see ref. 5]. Rat mast cells maintained in culture progressively acquired responsiveness to SCF such that by 48 h the cells released > 30% net serotonin after challenge with 100 ng/ml SCF. This increase in responsiveness to SCF was accompanied by increased re-

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sponsiveness to anti-IgE but not compound 48/80 or calcium ionophore A23187 [Taylor et al., unpubl. results].

IL-4, but not SCF, Up-Regulates Induced Cytokine Expression in HMC-1 Mast Cells

Incubation of HMC-1 human mast cells with IL-4, but not SCF (each at 100 ng/ml), for 24 h enhanced expression of mRNA encoding several genes that were induced when the cells were stimulated with ionomycin, including IL-4, granulocyte/macrophage-colony-stimulating factor, (GM-CSF), IL-6 receptor (fig. 1), IL-3 and IL-8 [7], but not tumour necrosis factor- α or β -actin. Quantitation of the relative levels of IL-3, IL-4 and IL-8 mRNA by competitive PCR [7] revealed that IL-4 enhanced ionomycin-induced IL-3 mRNA expression 6-fold, IL-4 2-fold, and IL-8 4-fold. HMC-1 cells activated by ionomycin for 1 h and then cultured for 24 h released detectable levels of IL-3 and IL-8 product (measured by ELISA). Pretreatment of the cells with IL-4 for 24 h led to a 15- to 20-fold increase in ionomycin-induced IL-3 release (peaking at 24 h), and a doubling of ionomycin-induced IL-8 release (peaking at 8 h) [7].

The effects of IL-4 on gene expression were not accompanied by any increase in HMC-1 cell number or proliferation rate, and thus represent a true gene regulatory effect.

Conclusions

Fig. 1. Effects of IL-4 on HMC-1 cell mRNA expression. Duplicate cultures of HMC-1 cells (2×10^6 /ml in 2 ml) were incubated for 24 h with 100 ng/ml IL-4 (lanes B, D) or culture medium alone (A, C). The cells were then washed and challenged for 4 h with either ionomycin (lanes C, D) or medium alone (A, B). The cDNA generated from these cells was amplified by PCR using primers for IL-4, GM-CSF and IL-6 receptor (IL-6R). M indicates molecular size markers. Arrows indicate the predicted position of the PCR products. β -Actin mRNA levels were constant for all treatments.

cells, whereas IL-4, but not SCF, enhances the induced expression of some cytokine genes and their secreted products, and some cytokine receptors in the HMC-1 human mast cell line.

Although IL-4 and SCF are both able to up-regulate IgE/ antigen-induced mediator release from mouse peritoneal mast cells, they exert quite distinct effects as direct mediator-releasing or cytokine gene regulatory agents. SCF, but not IL-4, induces degranulation of rodent peritoneal mast

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