

REVIEW

Human mast cells and basophils as sources of cytokines

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Introduction

Mast cells and basophils express the high affinity immunoglobulin (Ig) E receptor (FcεRI) on the surface, and the cross-linking of cell bound IgE-FcεRI complex with specific antigens leads to the release of inflammatory mediators such as histamine and eicosanoids. These mediators induce contraction of smooth muscle, tissue oedema and vascular hyperpermeability. Therefore, mast cells and basophils are important effector cells in the early phase allergic reactions. Recently it has become apparent that mast cells and basophils are the sources of several cytokines. These cytokines regulate functions of inflammatory cells, suggesting that these cells may be involved in the pathogenesis of late phase of allergic responses. Despite some similarities to mast cells, human basophils are developmentally, phenotypically and functionally most closely related to eosinophils. Mast cells and basophils similarly derive from precursors that originate in the bone marrow, however, the human mast cells mature under local influences following migration into the tissue. On the other hand, basophils complete the maturation in the bone marrow and enter the circulation as mature granulated cells. Therefore, the profiles of cytokine production by these cells are different, suggesting that the roles of mast cells and basophils may be different in the allergic inflammation. In this review, the profile and characteristics of cytokines produced by human mast cells and basophils are examined, with an emphasis on the role of the cytokines produced by these cells.

Evidence of human mast cell cytokine production in human allergic diseases

Using immunocytochemistry, Bradding *et al.* [1–4] reported that interleukin (IL)-4, IL-5, IL-6, and tumour necrosis factor (TNF)-α were present within mast cells in nasal and bronchial mucosa taken from both normal subjects and patients with allergic perennial rhinitis, allergic seasonal

rhinitis, and allergic asthma. In both asthma and rhinitis, cells expressing immunoreactivity for IL-4 were increased in number. In asthmatics, there was a disease-related increase in the number of cells expressing immunoreactivity for TNF-α. Ying *et al.* [5] reported the expression of IL-4 and IL-5 mRNA and protein in bronchial mucosal mast cells obtained from atopic and nonatopic (intrinsic) asthmatics. Horsmanheimo *et al.* [6] demonstrated that in biopsies from normal skin and those from patients with atopic dermatitis, mast cells contain the majority of IL-4 immunoreactivity. Pawankar *et al.* [7] reported that IL-13 positive cells were detected in the nasal mucosa of perennial allergic rhinitic patients by immunohistochemistry, and the major source of IL-13 appeared to be mast cells and lymphocytes morphologically. IL-13 mRNA expression of mast cells and lymphocytes was detected in the epithelial compartment of the nasal mucosa of 18 out of 19 patients with perennial rhinitis but it was undetected in normal subjects and chronic infective rhinitics. IL-5 and GM-CSF were present within mast cells of the bronchial mucosa of asthmatics [8] and within intestinal mucosal mast cells of asthmatics [9]. Thus, these findings indicate that the production of specific cytokines from mast cells, including IL-4, IL-5, IL-13, GM-CSF and TNF-α, may be up-regulated in the allergic inflammation.

Mast cell phenotypes and cytokine expression

It is still not clear whether one mast cell produces several cytokines at the same time or whether there may be phenotypes of mast cells with regard to cytokine production. From immunohistochemical studies, some mast cells contained combinations of cytokines, and others appeared to produce only one cytokine [4]. It is well known that mast cells can be classified based on the proteases, namely those that express only tryptase, MC(T), and those that express both chymase and tryptase, MC(TC). Previous studies demonstrated that more than 90% of intraepithelial mast cells are MC(T), whereas mast cells in the superficial lamina propria are comprised of MC(TC) and MC(T) [10,11]. The relationship between mast cell protease expression and

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cytokine content was examined using immunocytochemistry. In the nasal and bronchial mucosal biopsy specimens, IL-4 was distributed in both mast cell phenotypes although predominantly the MC(TC), while IL-5 and IL-6 were localized almost exclusively to the MC(T) [12]. It was reported that 60% of mast cells distributed in the deep lamina propria (mainly MC(TC)) and superficial lamina propria (MC(TC) and MC(T)) of the nasal mucosa expressed IL-4, but less than 20% of mast cells in the epithelial compartment expressed IL-4 [13]. On the other hand, majority of IL-5, IL-6 and IL-7 expressing mast cells were distributed in the epithelial compartment and in the superficial lamina propria (mainly MC(T)), but not in the deep lamina propria. IL-8 and IL-10 expressing mast cells were relatively more in the deep lamina propria than in the epithelial and superficial lamina propria compartments [13]. These observations suggest heterogeneity of human mast cells with regard to cytokine expression in addition to protease production.

Cytokine production from human lung mast cells *in vitro*

We examined cytokine mRNA expression and cytokine production from highly purified human lung mast cells in response to IgE-mediated stimulation. In our studies, human lung mast cells were purified by affinity magnetic selection with monoclonal antibody (mAb) YB5.B8 against *kit* to achieve a final mast cell purity of 93–99% [14]. Purified mast cells were precultured with human myeloma IgE for 16 h before challenge with stem cell factor (SCF) and anti-IgE. Using RT-PCR, ELISA and immunocytochemistry, we evaluated the capacity of human lung mast cells to express and produce various cytokines. Messenger RNA for IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, TNF α , GM-CSF was expressed on human lung mast cells following IgE-dependent activation [15–21]. Jaffe *et al.* [22,23] reported IL-5 and IL-13 production from human lung mast cells following IgE-mediated activation. It is also reported that constitutive synthesis of SCF mRNA was seen in human lung and skin mast cells, but cross-linking of IgE receptor on the lung mast cells did not alter SCF mRNA expression [24]. In our experiments, IL-3 mRNA was expressed 2 h after stimulation and waned at 8 h [15]. IL-4 mRNA expression was detected in only six of 13 experiments and the level of its expression peaked at 2 h after activation and lessened over the next 12 h [18]. IL-5 mRNA was constitutively expressed and peaked at 2–4 h and persisted for up to 48–72 h in all of 12 experiments [18]. IL-10 mRNA was constitutively expressed, up-regulated after stimulation and peaked at 8–12 h [20]. IL-13 mRNA was constitutively expressed, up-regulated at 2–4 h and persisted for up to 12–24 h after activation [21]. Constitutive expression of TNF α and GM-CSF mRNA was observed and this peaked at 2 h after

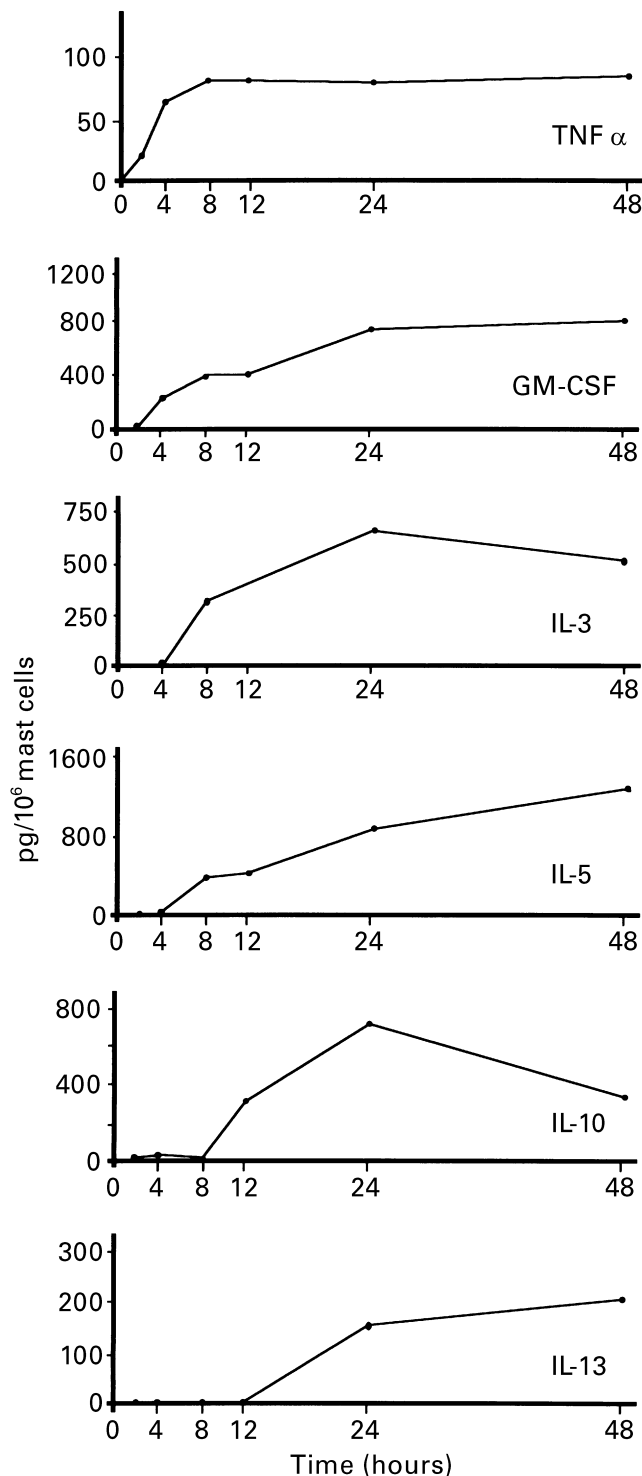
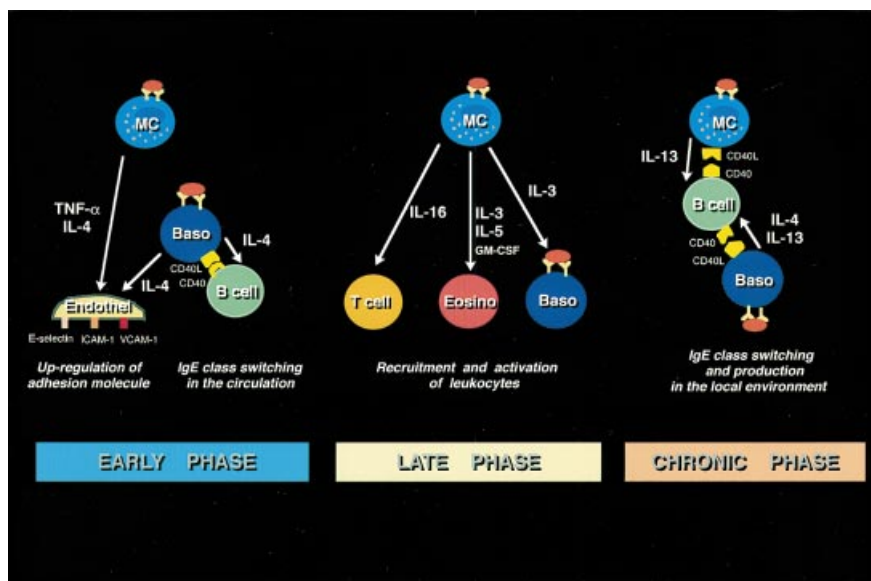


Fig. 1. Kinetics of cytokine release from purified human lung mast cells. Human lung mast cells were precultured with 3 μ g/mL IgE for 16 h, and then washed, and challenged with SCF (50 ng/mL) and anti-IgE (1 μ g/mL). Cytokines in cell supernatants were measured by ELISA. Because the levels of cytokine production were somewhat variable, representative data were shown in this figure.

Fig. 2 Biological effects of cytokines from human mast cells (MC) and basophils (Baso). In the early phase allergic reactions, $\text{TNF}\alpha$ and IL-4 from mast cells and IL-4 from basophils up-regulate the expression of E-selectin, ICAM-1 and VCAM-1 on endothelial cells (Endothel). Basophils may induce IgE production from B cells in the circulation. In the late phase, IL-3 secreted by mast cells activates basophils, and cytokines such as IL-3, IL-5 and GM-CSF stimulate eosinophils (Eosino). IL-16 from mast cells is the CD4 + T cell chemoattractant factor. Thereafter, IL-4 and IL-13 secreted by basophils and mast cells together with CD40L-CD40 physical contact induce B cells to proliferate and to switch IgE-producing cells in the inflammatory site.



stimulation and almost undetectable by 24–48 h [19]. Neither IL-2 nor $\text{IFN}\gamma$ mRNA was detectable in human lung mast cells following IgE-mediated stimulation (unpublished data).

Next, we performed the kinetic study of cytokine production from human lung mast cells (Fig. 1). $\text{TNF}\alpha$ was released within 2 h after stimulation and began to steady at 4–8 h (unpublished data). GM-CSF was released 4–8 h after stimulation and steadied at 24–48 h [19]. IL-3 and IL-5 release were observed 8 h after stimulation [15,18]. IL-10 and IL-13 release were obviously detected 4–12 h and 24 h after stimulation, respectively [20–21]. IL-4 protein was not detected by ELISA in our study [18]. Thus, the specific cytokine production is up-regulated by IgE-dependent stimulation. Human mast cells first produce $\text{TNF}\alpha$ which is reported to stimulate vascular endothelial cells to induce adhesion molecules on the cell surface [25–26] and then produce GM-CSF, IL-3 and IL-5, which mainly stimulate granulocytes. Thereafter, IL-10 and IL-13, which are mainly associated with lymphocyte functions, are released. These

findings suggest that each cytokine mRNA expression and secretion may be regulated by distinct pathways and activated mast cells may orchestrate various inflammatory cells through cytokines released at various time points (Table 1).

In parallel with IL-13, we measured IL-4 protein from human lung mast cells by ELISA, however, IL-4 protein was not detectable in the supernatants from four donors in which IL-13 protein was detected at 24 h after SCF and anti-IgE activation [21]. This is consistent with our report [18] and that of Jaffe *et al.* which showed that IL-4 mRNA was barely expressed in purified human lung mast cells after IgE-mediated stimulation [22]. It was reported that stimulation of human cord blood-derived human cultured mast cells (HCMCs) by cross-linking of the high-affinity IgE receptor induced the expression of IL-13 mRNA and protein, but not IL-4. In addition, IL-13 production from HCMCs was remarkably increased after preculture with IL-4, whereas IL-4 protein was still undetectable [27]. These findings suggest that IL-4 production from human mast cells is very restricted.

Table 1. Characteristics of cytokine mRNA expression and protein production from purified human mast cells following IgE-mediated stimulation in our series of examination. Kinetics of release means kinetics of cytokine release after IgE-mediated stimulation.

	mRNA expression	Immunostaining	Protein release	Kinetics of release
IL-3	Yes	Yes	Yes	After 8 h
IL-4	Yes	Yes	Not detectable	Not detectable
IL-5	Yes	Yes	Yes	After 8 h
IL-6	Yes	Yes	Not done	Not done
IL-10	Yes	Yes	Yes	After 4–12 h
IL-13	Yes	Yes	Yes	After 24 h
GM-CSF	Yes	Yes	Yes	After 4 h
$\text{TNF}\alpha$	Yes	Yes	Yes	Within 2 h

The interaction between mast cells and endothelial cells

The expression of adhesion molecules on endothelial cells is known to mediate the infiltration of inflammatory cells at the site of inflammation. Some studies focused on the regulation of adhesion molecules by cytokines from mast cells. Walsh *et al.* [25] found that human skin mast cells contained TNF α as a preformed mediator within granules and released TNF α was capable of endothelial leucocyte adhesion molecule 1 (ELAM-1 or E-selectin) expression on endothelial cells. Meng *et al.* [26] reported that activated mast cells or supernatant from activated mast cells up-regulated intercellular and vascular cell adhesion molecules (ICAM-1 and VCAM-1) expression on human dermal microvascular endothelial cells and human umbilical vein endothelial cells. The ICAM-1 up-regulation was almost completely neutralized by anti-TNF α antibody, while VCAM-1 expression was partly neutralized by anti-TNF α antibody. In addition, it is reported that IL-4 [28] and IL-13 [29] selectively induced endothelial cell expression of VCAM-1 which binds to eosinophil and basophil VLA-4, although these two cytokines did not affect the expression of E-selectin and ICAM-1. These findings suggest that mast cell cytokines such as TNF α , IL-4 and IL-13 may participate in the regulation of inflammatory cell recruitment.

The interaction between mast cells and eosinophils

An association between activated eosinophils and mast cells have been described in the pathology of bronchial asthma and urticaria [30–31]. Morphological features of mast cells and eosinophil degranulation were observed in asthmatics by electron microscopy [32]. We evaluated the effects of cytokines from human lung mast cells on eosinophil functions. Highly purified human lung mast cells were precultured with SCF (50 ng/mL) and anti-IgE (1 μ g/mL) for 24 h. Next, highly purified peripheral blood eosinophils were incubated with various concentrations of the culture supernatant of mast cells. Then, eosinophil cationic protein (ECP), which was released from activated eosinophils, was measured by RIA. The supernatant caused a dose-dependent release of ECP from eosinophils and anti-IL-5 neutralizing mAb, anti-TNF α neutralizing mAb, and anti-GM-CSF neutralizing mAb significantly inhibited the supernatant-induced ECP release. The combination of anti-TNF α and anti-GM-CSF mAb did not enhance the inhibitory effect of any mAb, whereas the combination of anti-TNF α and anti-IL-5 mAb slightly enhanced the inhibitory effect. These results indicate that cytokines released from activated mast cells such as IL-5, TNF α and GM-CSF synergistically induce the eosinophil degranulation [33]. Note that IL-3, IL-5, and GM-CSF are reported to support

the survival of eosinophils [34]. Indeed, Tachimoto *et al.* [35] reported that the supernatant from activated human cord blood-derived mast cells enhanced eosinophil survival and the effect was blocked by anti-GM-CSF neutralizing mAb.

The interaction between mast cells and B lymphocytes

Gauchat *et al.* [36] first reported that human mast cell lines (HMC-1) and freshly isolated purified human mast cells can provide the cell contact signals, CD40 ligand that are required for IgE synthesis. Pawankar *et al.* [37] demonstrated that nasal mast cells from patients with perennial allergic rhinitis expressed significantly greater levels of IL-4, IL-13 and CD40 ligand when compared with those from patients with chronic infective rhinitis. Indeed, nasal mast cells from patients with perennial allergic rhinitis induced IgE synthesis by purified B cells in the presence of mite antigen, even without the addition of exogenous IL-4 or IL-13. Furthermore, this IgE induction by mast cells was partially inhibited by anti-IL-4 mAb, whereas the anti-IL-13 mAb completely inhibited the synthesis of IgE [37]. These observations indicate that mast cells can directly induce IgE production from B cells independently of T cells and IL-13 from mast cells may play a more important role in the maintenance of IgE synthesis.

The interaction between mast cells and T lymphocyte

Bone marrow-derived cultured mast cells (BMMC) have shown to be the antigen-presenting cell to CD4 + T cells through expressing human major histocompatibility antigen (MHC) class II [38]. Frandji *et al.* [39] reported that antigen presentation of BMMC to T cells was performed through CD80 and CD86. Rumsaeng *et al.* [40] demonstrated that BMMC and HMC-1 produce IL-16, a CD4 + T cell chemoattractant cytokine, and using immunohistochemistry, human lung mast cells were shown to be a source of IL-16. The capacity of human mast cells to synthesize and release biologically active IL-16 provides a possible link between mast cell activation and the accumulation of T cells. In other *in vitro* studies, histamine has been shown to cause IL-16 release from CD8 + T cells and bronchial epithelial cells [41,42]. Therefore, the release of histamine from activated mast cells may cause further IL-16 release from other sources in the tissue microenvironment.

Cytokine production from human basophils

Basophils are circulating leucocytes and rarely seen in the tissues, but they were found in bronchial submucosa and lumen of fatal asthmatic patients [43]. Furthermore, it was

reported that the number of basophils in the allergic inflammatory sites correlated with the severity of the disease [44]. Therefore, basophils play an important role in allergic inflammation. The recent evidence indicated that peripheral basophils isolated from normal individuals have the capacity to produce of IL-4 [45–48] and IL-13 [49–51] in response to IgE-dependent or IgE-independent activation. Human bone marrow-derived cultured basophils also produce IL-4 and IL-13 [52]. It was reported that CXC chemokine IL-8 mRNA was expressed in human basophils [53]. In addition, Li *et al.* [54] reported that basophils produced macrophage inflammatory protein-1 α (MIP-1 α), a chemotactic cytokine belonging to the CC chemokine family, in response to IgE-dependent activation.

Gibbs *et al.* [51] demonstrated that IL-4 release from human basophils activated by anti-IgE was biphasic, namely, preformed IL-4 was released within 5–10 min and IL-4 generated *de novo* was released 4 h after stimulation. On the other hand, IL-13 secretion was generally more sustained than IL-4, and detected within 24 h following stimulation [49–51]. With regard to gene expression, IL-4 mRNA was constitutively expressed [47] and IL-13 mRNA was expressed at lower levels than IL-4 mRNA in unstimulated purified human basophils, however, they were upregulated similarly after anti-IgE activation [50]. In addition to IgE-mediated activation, IL-3 alone was capable of inducing IL-13 [49,50,55], and enhanced anti-IgE mediated IL-4 [45–48,50] and IL-13 production [49,50]. Ochensberger *et al.* [56] demonstrated that basophils produced neither Th1-type cytokines, such as IL-2 and IFN γ , nor Th2-type cytokines, such as IL-3, IL-5, IL-10, and GM-CSF. While mast cells have the ability to produce various Th2-type cytokines, basophils express a very restricted cytokine profile.

It is very interesting to see how the difference between atopic and nonatopic patient concerning basophil cytokine production. Ochi *et al.* [57] examined the capacity of IL-4 production from lymphocytes and basophils after stimulation with mite extract. Lymphocytes and basophils from patients with elevated serum IgE specific to mite allergen could produce detectable levels of IL-4, whereas those from patients with low RAST score and normal volunteers could not. In mite-sensitive atopic asthmatics, more IL-4 and IL-13 were produced by basophils in response to mite antigens in proportion to the concentration of mite-specific IgE [58].

Induction of IgE synthesis by basophils

When human blood basophils activated by phorbol myristate acetate (PMA) and ionomycin incubated with B cells, IgE was produced even in the absence of exogenous IL-4. Further immunocytochemical examination confirmed the expression of CD40 ligand on human basophils [36].

When cord blood-derived cultured basophils were stimulated with anti-IgE, significant amount of immunoreactive IL-4 and IL-13 was secreted, and CD40 ligand was expressed. The induction of IgE synthesis by activated cultured basophils was completely abrogated by two neutralizing mAb against IL-4 and IL-13 and by a soluble form of CD40 [52]. These findings suggest that like mast cells, basophils may induce B cells to proliferate and to switch IgE-producing cells.

Regulation of cytokine production by human basophils

The intracellular mechanisms of the generation of cytokines from basophils are not apparent yet. PMA, an activator of protein kinase C, was a potent stimulator for IL-13 secretion from basophils, however, it induced little to no IL-4 secretion, despite being a potent secretagogue of histamine from basophils [55]. In fact, the secretion of IL-4 by basophils stimulated with ionomycin alone was down-regulated with simultaneous addition of PMA [59]. FK506, an immunosuppressant, had no effect on the generation of IL-13 in response to both IL-3 and anti-IgE, while strikingly inhibiting the accumulation of IL-4 mRNA and protein secretion. When basophils were activated with anti-IgE alone, FK506 inhibited the secretion of both IL-4 and IL-13 [55]. These data suggest that IL-4 and IL-13 secretion from basophils may be regulated by differential intracellular mechanisms.

Summary

The specific cytokines from human mast cells and basophils are up-regulated in human allergic diseases. Mast cells produce a broad range of cytokines, mainly Th2 type, following IgE-mediated stimulation. The production of specific cytokines from mast cells including IL-4, IL-5, IL-13, GM-CSF and TNF α is up-regulated in the allergic inflammation. There may be heterogeneity of human mast cells with regard to cytokine expression. We confirmed *in vitro* that the production of IL-3, IL-5, IL-10, IL-13, GM-CSF and TNF α was up-regulated by aggregation of Fc ϵ RI. Cytokine release from human mast cells activated by anti-IgE was triphasic and each cytokine produced by mast cells may play important roles such as up-regulation of adhesion molecules on endothelial cells, leucocyte recruitment, eosinophil activation, and local IgE synthesis in the allergic inflammation (Fig. 2). On the other hand, the cytokine profile of basophils is restricted to IL-4, IL-8, IL-13 and MIP-1 α . IL-4 and IL-13 produced by basophils induce local IgE synthesis by the interaction with B cells. The release of IL-4 from basophils is very rapid, within 5–10 min following IgE-dependent activation, suggesting that IL-4 from basophils induce IgE class switching in the circulation (Fig. 2). These findings indicate that basophils and mast

cells may regulate various inflammatory cells through cytokine production at various time points in allergic inflammation.

References

- Bradding P, Feather IH, Howarth PH, Mueller R, Roberts JA, Britten K, Bews JP, Hunt TC, Okayama Y, Heusser CH, Bullock GR, Church MK, Holgate ST. Interleukin 4 is localized to and released by human mast cells. *J Exp Med* 1992; 176:1381–6.
- Bradding P, Feather IH, Wilson S, Bardin PG, Heusser CH, Holgate ST, Howarth PH. Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects. The mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. *J Immunol* 1993; 151:3853–65.
- Bradding P, Roberts JA, Britten KM, Montefort S, Djukanovic R, Mueller R, Heusser CH, Howarth PH, Holgate ST. Interleukin-4, -5 and -6 and tumor necrosis factor- α in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am J Respir Cell Mol Biol* 1994; 10:471–80.
- Bradding P. Human mast cell cytokines. *Clin Exp Allergy* 1996; 26:13–9.
- Ying S, Humbert M, Barkans J, Corrigan CJ, Pfister R, Menz G, Larche M, Robinson DS, Durham SR, Kay AB. Expression of IL-4 and IL-5 mRNA and protein product by CD4+ and CD8+ T cells, eosinophils, and mast cells in bronchial biopsies obtained from atopic and nonatopic (intrinsic) asthmatics. *J Immunol* 1997; 158:3539–44.
- Horsmanheimo L, Harvima I, Jarvikallio A, Harvima R, Naukkarinen A, Horsmanheimo M. Mast cells are one major source of interleukin 4 in atopic dermatitis. *Br J Dermatol* 1994; 131:348–53.
- Pawankar RU, Okuda M, Hasegawa S, Suzuki K, Yssel H, Okubo K, Okumura K, Ra C. Interleukin-13 expression in the nasal mucosa of perennial allergic rhinitis. *Am J Respir Crit Care Med* 1995; 152:2059–67.
- Sousa AR, Lams BE, Pfister R, Christie PE, Schmitz M, Lee TH. Expression of interleukin-5 and granulocyte-macrophage colony-stimulating factor in aspirin-sensitive and non-aspirin-sensitive asthmatic airways. *Am J Respir Crit Care Med* 1997; 156:1384–9.
- Wallaert B, Desreumaux P, Copin MC, Tillie I, Benard A, Colombel JF, Gosselin B, Tonnel AB, Janin A. Immunoreactivity for interleukin 3 and 5 and granulocyte/macrophage colony-stimulating factor of intestinal mucosa in bronchial asthma. *J Exp Med* 1995; 182:1897–904.
- Irani AM, Schelter S, Craig G, Deblois G, Schwartz LB. Two types of mast cells that have distinct neutral protease compositions. *Proc Natl Acad Sci USA* 1986; 83:4464–8.
- Irani AM, Schwartz LB. Mast cell heterogeneity. *Clin Exp Allergy* 1989; 19:143–55.
- Bradding P, Okayama Y, Howarth PH, Church MK, Holgate ST. Heterogeneity of human mast cells based on cytokine content. *J Immunol* 1995; 155:297–307.
- Pawankar R, Ra C. IgE-Fc ϵ R1-mast cell axis in the allergic cycle. *Clin Exp Allergy* 1998; 28 (Suppl. 3):6–14.
- Okayama Y, Hunt TC, Kassel O, Ashman LK, Church MK. Assessment of the anti-c-kit monoclonal antibody YB5.B8 in affinity magnetic enrichment of human lung mast cells. *J Immunol Methods* 1994; 169:153–61.
- Ishizuka T, Okayama Y, Kobayashi H, Mori M. Interleukin-3 production by mast cells from human lung. *Inflammation* 1999; 23:25–35.
- Okayama Y, Bradding P, Tunon de Lara JM, Holgate ST, Church MK. Cytokine production by human mast cells. *Chem Immunol* 1995; 61:114–34.
- Okayama Y, Semper A, Holgate ST, Church MK. Multiple cytokine mRNA expression in human mast cells stimulated via Fc ϵ R1. *Int Arch Allergy Immunol* 1995; 107:158–9.
- Okayama Y, Petit-Frere C, Kassel O, Semper A, Quint D, Tunon de Lara MJ, Bradding P, Holgate ST, Church MK. IgE-dependent expression of mRNA for IL-4 and IL-5 in human lung mast cells. *J Immunol* 1995; 155:1796–808.
- Okayama Y, Kobayashi H, Ashman LK, Dobashi K, Nakazawa T, Holgate ST, Church MK, Mori M. Human lung mast cells are enriched in the capacity to produce granulocyte-macrophage colony-stimulating factor in response to IgE-dependent stimulation. *Eur J Immunol* 1998; 28:708–15.
- Ishizuka T, Okayama Y, Kobayashi H, Mori M. Interleukin-10 is localized to and released by human lung mast cells. *Clin Exp Allergy* 1999; 29:1424–32.
- Kobayashi H, Okayama Y, Ishizuka T, Pawankar R, Ra C. Production of IL-13 by human lung mast cells in response to Fc ϵ receptor cross-linkage. *Clin Exp Allergy* 1998; 28:1219–27.
- Jaffe JS, Glaum MC, Raible DG, Post TJ, Dimitry E, Govindarao D, Wang Y, Schulman ES. Human lung mast cell IL-5 gene and protein expression: temporal analysis of upregulation following IgE-mediated activation. *Am J Respir Cell Mol Biol* 1995; 13:665–75.
- Jaffe JS, Raible DG, Post TJ, Wang Y, Glaum MC, Butterfield JH, Schulman ES. Human lung mast cell activation leads to IL-13 mRNA expression and protein release. *Am J Respir Cell Mol Biol* 1996; 15:473–81.
- Zhang S, Anderson DF, Bradding P, Coward WR, Baddeley SM, MacLeod JD, McGill JI, Church MK, Holgate ST, Roche WR. Human mast cells express stem cell factor. *J Pathol* 1998; 186:59–66.
- Walsh L, Trinchieri G, Waldorf H, Whitaker D, Murphy G. Human dermal mast cells contain and release tumor necrosis factor α , which induces endothelial leukocyte adhesion molecule 1. *Proc Natl Acad Sci USA* 1991; 88:4220–4.
- Meng H, Tonnesen MG, Marchese MJ, Clark RA, Bahou WF, Gruber BL. Mast cells are potent regulators of endothelial cell adhesion molecule ICAM-1 and VCAM-1 expression. *J Cell Physiol* 1995; 165:40–53.
- Toru H, Pawankar R, Ra C, Yata J, Nakahata T. Human mast cells produce IL-13 by high-affinity IgE receptor cross-linking: enhanced IL-13 production by IL-4-primed human mast cells. *J Allergy Clin Immunol* 1998; 102:491–502.
- Schleimer R, Sterbinsky S, Kaiser J, Bickel C, Klunk D, Tomioka K *et al.* IL-4 induces adherence of human

- eosinophils and basophils but not neutrophils to endothelium. Association with expression of VCAM-1. *J Immunol* 1992; 148:1086–92.
- 29 Sironi M, Sciacca FL, Matteucci C, Conni M, Vecchi A, Bernasconi S, Minty A, Caput D, Ferrara P, Colotta F *et al.* Regulation of endothelial and mesothelial cell function by interleukin-13: selective induction of vascular cell adhesion molecule-1 and amplification of interleukin-6 production. *Blood* 1994; 84:1913–21.
 - 30 Beasley R, Roche WR, Roberts JA, Holgate ST. Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis* 1989; 139:806–17.
 - 31 Nathony SF, Phillips ME, Elias JM, Godfrey HP, Kaplan AP. Histologic studies of chronic idiopathic urticaria. *J Allergy Clin Immunol* 1983; 71:177–83.
 - 32 Djukanovic R, Wilson JW, Britten KM, Wilson SJ, Walls AF, Roche WR, Howarth PH, Holgate ST. Quantitation of mast cells and eosinophils in the bronchial mucosa of symptomatic atopic asthmatics and healthy control subjects using immunohistochemistry. *Am Rev Respir Dis* 1990; 142:863–71.
 - 33 Okayama Y, Kobayashi H, Ashman LK, Holgate ST, Church MK, Mori M. Activation of eosinophils with cytokines produced by lung mast cells. *Int Arch Allergy Immunol* 1997; 114 (Suppl. 1):75–7.
 - 34 Weller PF. The immunobiology of eosinophils. *N Engl J Med* 1991; 324:1110–8.
 - 35 Tachimoto H, Ebisawa M, Iikura Y, Kimata M, Mori K, Sakaguchi N, Akasawa A, Nakajima T, Saito H. Activated human mast cells release factors supporting eosinophil survival in vitro. *Int Arch Allergy Immunol* 1997; 113:293–4.
 - 36 Gauchat JF, Henchoz S, Mazzei G, Aubry JP, Brunner T, Blasey H, Life P, Talabot D, Flores Romo L, Thompson J *et al.* Induction of human IgE synthesis in B cells by mast cells and basophils. *Nature* 1993; 365:340–3.
 - 37 Pawankar R, Okuda M, Yssel H, Okumura K, Ra C. Nasal mast cells in perennial allergic rhinitis exhibit increased expression of the FcεRI, CD40L, IL-4, and IL-13, and can induce IgE synthesis in B cells. *J Clin Invest* 1997; 99:1492–9.
 - 38 Galli SJ, Costa JJ. Mast-cell-leukocyte cytokine cascades in allergic inflammation. *Allergy* 1995; 50:851–62.
 - 39 Frandji P, Tkaczyk C, Oskertizian C, David B, Desaymard C, Mecheri S. Exogenous and endogenous antigens are differentially presented by mast cells to CD4+ T lymphocytes. *Eur J Immunol* 1996; 26:2517–28.
 - 40 Rumsaeng V, Cruikshank WW, Foster B, Prussin C, Kirshenbaum AS, Davis TA, Kornfeld H, Center DM, Metcalfe DD. Human mast cells produce the CD4+ T lymphocyte chemoattractant factor, IL-16. *J Immunol* 1997; 159:2904–10.
 - 41 Laberge S, Cruikshank W, Kornfeld H, Center D. Histamine-induced secretion of lymphocyte chemoattractant factor from CD8+ T cells is independent of transcription and translation. *J Immunol* 1995; 155:2902–10.
 - 42 Bellini A, Yoshimura H, Vittori E, Marini M, Mattoli S. Bronchial epithelial cells of patients with asthma release chemoattractant factors for T lymphocytes. *J Allergy Clin Immunol* 1993; 92:412–24.
 - 43 Koshino T, Teshima S, Fukushima N, Takaishi T *et al.* Identification of basophils by immunohistochemistry in the airways of postmortem cases of fatal asthma. *Clin Exp Allergy* 1993; 23:919–25.
 - 44 Knol EF, Mul FP, Lie WJ, Verhoeven AJ, Roos D. The role of basophils in allergic disease. *Eur Respir J Suppl* 1996; 22:126s–31s.
 - 45 Brunner T, Heusser CH, Dahinden CA. Human peripheral blood basophils primed by interleukin 3 (IL-3) produce IL-4 in response to immunoglobulin E receptor stimulation. *J Exp Med* 1993; 177:605–11.
 - 46 Arock M, Merle Beral H, Dugas B, Ouaz F, Le Goff L, Vouldoukis I, Mencia Huerta JM, Schmitt C, Leblond Misenard V, Debre P *et al.* IL-4 release by human leukemic and activated normal basophils. *J Immunol* 1993; 151:1441–7.
 - 47 MacGlashan D Jr, White JM, Huang SK, Ono SJ, Schroeder JT, Lichtenstein LM. Secretion of IL-4 from human basophils. The relationship between IL-4 mRNA and protein in resting and stimulated basophils. *J Immunol* 1994; 152:3006–16.
 - 48 Schroeder JT, MacGlashan DW Jr, Kagey Sobotka A, White JM, Lichtenstein LM. IgE-dependent IL-4 secretion by human basophils. The relationship between cytokine production and histamine release in mixed leukocyte cultures. *J Immunol* 1994; 153:1808–17.
 - 49 Li H, Sim TC, Alam R. IL-13 released by and localized in human basophils. *J Immunol* 1996; 156:4833–8.
 - 50 Ochensberger B, Daepf GC, Rihs S, Dahinden CA. Human blood basophils produce interleukin-13 in response to IgE-receptor-dependent and -independent activation. *Blood* 1996; 88:3028–37.
 - 51 Gibbs BF, Haas H, Falcone FH, Albrecht C, Vollrath IB, Noll T, Wolff HH, Amon U. Purified human peripheral blood basophils release interleukin-13 and preformed interleukin-4 following immunological activation. *Eur J Immunol* 1996; 26:2493–8.
 - 52 Yanagihara Y, Kajiwaru K, Basaki Y, Ikizawa K, Ebisawa M, Ra C, Tachimoto H, Saito H. Cultured basophils but not cultured mast cells induce human IgE synthesis in B cells after immunologic stimulation. *Clin Exp Immunol* 1998; 111:136–43.
 - 53 Markvardsen P, Bjerke T, Rudiger N, Schiotz PO, Gregersen N, Justesen J, Paludan K. A polymerase chain reaction-based method for the semiquantitative study of interleukin-8 mRNA in human basophil leukocytes. *Scand J Clin Lab Invest* 1995; 55:487–93.
 - 54 Li H, Sim T, Grant J, Alam R. The production of macrophage inflammatory protein-1α by human basophils. *J Immunol* 1996; 157:1207–12.
 - 55 Redrup AC, Howard BP, MacGlashan DW Jr, Kagey Sobotka A, Lichtenstein LM, Schroeder JT. Differential regulation of IL-4 and IL-13 secretion by human basophils: their relationship to histamine release in mixed leukocyte cultures. *J Immunol* 1998; 160:1957–64.
 - 56 Ochensberger B, Rihs S, Brunner T, Dahinden CA. IgE-independent interleukin-4 expression and induction of a late phase of leukotriene C4 formation in human blood basophils. *Blood* 1995; 86:4039–49.

- 57 Ochi H, Tanaka T, Katada Y, Naka T, Aitani M, Hashimoto S, Maeda K, Toyoshima K, Igarashi T, Suemura M, Kishimoto T. Peripheral blood T lymphocytes and basophils, freshly isolated from house-dust-mite-sensitive patients, produce interleukin-4 in response to allergen-specific stimulation. *Int Arch Allergy Immunol* 1996; 111:253–61.
- 58 Shimizu Y, Shichijo M, Hiramatsu K, Takeuchi M, Nagai H, Takagi K. Mite antigen-induced IL-4 and IL-13 production by basophils derived from atopic asthma patients. *Clin Exp Allergy* 1998; 28:497–503.
- 59 Schroeder JT, Howard BP, Jenkins MK, Kagey Sobotka A, Lichtenstein LM, MacGlashan DW Jr. IL-4 secretion and histamine release by human basophils are differentially regulated by protein kinase C activation. *J Leukoc Biol* 1998; 63:692–8.