

Localization of interleukin (IL) -4 but not IL-5 to human mast cell secretory granules by immunoelectron microscopy

S. J. WILSON, J. K. SHUTE, S. T. HOLGATE, P. H. HOWARTH and P. BRADDING*

University Medicine, Southampton University, Southampton, UK and *Department of Respiratory Medicine, Glenfield Hospital, Leicester, UK

Summary

Background Human mast cells synthesize and secrete many cytokines of relevance to the pathogenesis of allergic diseases such as asthma and rhinitis. In particular, interleukin (IL) -4 and IL-5 are likely to play key roles in the development of the inflammatory response that characterizes these diseases. Immunohistochemical studies on human nasal and bronchial mucosal biopsies suggest that IL-4 and IL-5 may be stored preformed in mast cells.

Objective To identify whether IL-4 and IL-5 are stored within mast cell secretory granules.

Methods We used immunogold electron microscopic analysis on bronchial mucosa and lung parenchyma from resected lung specimens, and a nasal mucosal biopsy from a patient with active allergic rhinitis. Samples were fixed in 4% paraformaldehyde plus 0.5% glutaraldehyde and processed into Lowicryl K4M resin by the 'Progressive Lowering of Temperature' technique. Ultrathin sections were stained immunohistochemically by an indirect immunogold method.

Results Immunoreactivity for IL-4, but not IL-5, was localized to the granules of mast cells in all tissue samples. IL-5 was localized to the matrix of eosinophil granules in these samples, but neither cytokine was detected in T cells. IL-4 immunoreactivity increased in the granules of mast cells 24 h after immunoglobulin (Ig) E-dependent activation (mean 17.5 ± 1.4 gold particles per granule) compared with nonactivated mast cells (mean 6.8 ± 0.8 gold particles per granule, $P < 0.001$), suggesting replenishment of stores by newly generated protein. Immunoreactive IL-5 remained undetectable in mast cells 24 h after activation, a time point at which they are known to secrete large quantities of this cytokine.

Conclusion Human mast cells store IL-4 within the matrix of their granules. Very few, if any, lung or nasal mast cells store IL-5. A store of preformed IL-4 within mast cell granules is likely to have an important influence during the initiation and maintenance of the allergic immunological response.

Keywords: electron microscopy, human mast cells, interleukin-4, interleukin-5, immunohistochemistry

Clinical and Experimental Allergy, Vol. 30, pp. 493–500. Submitted 6 October 1998; revised 29 June 1999; accepted 7 July 1999.

Introduction

Asthma and rhinitis are both characterized by the presence of a specific type of mucosal inflammation comprising increased numbers of tissue eosinophils, accompanied by

mast cell and T-cell activation [1]. This inflammatory response is considered to cause much of the disordered airway function in these diseases. Two cytokines, interleukin (IL) -4 and IL-5 are selectively upregulated in asthma, and serve to orchestrate the inflammatory process. IL-4 promotes the synthesis of immunoglobulin (Ig) E by B cells, and induces eosinophilic inflammation and tissue

Correspondence: P. Bradding, Department of Respiratory Medicine, Glenfield Hospital, Groby Rd, Leicester LE3 9QP, UK.

damage when overexpressed *in vivo* in mice [2,3]. IL-5 is the major growth, differentiation and priming factor for eosinophils, and prolongs their survival by inhibiting apoptosis [4]. The importance of these two cytokines in human allergic disease has been confirmed by the demonstration of increased transcription of IL-4 and IL-5 in bronchial mucosal biopsies from allergic asthmatic subjects [5,6], in nasal biopsies following allergen challenge [7] and in cutaneous biopsies from atopic subjects after intradermal challenge [8]. Additionally, increased concentrations of these cytokines have been found in the bronchoalveolar lavage fluid of asthmatics [9].

Until recently it was believed that the TH2 subset of T lymphocytes was the only source of these cytokines in asthma. However, it is now well established that human mast cells synthesize and secrete many pro-inflammatory cytokines including IL-4, IL-5, IL-6, IL-8, IL-13, IL-16, granulocyte-macrophage cell stimulating factor (GM-CSF), stem cell factor, basic FGF, and tumour necrosis factor- α (TNF α) (for review see [10]). Using immunohistochemistry, we have reported the presence of intracellular IL-4, IL-5, IL-6, and TNF α in mast cells present in both bronchial and nasal mucosal biopsies from subjects with asthma and allergic rhinitis, respectively [11,12]. In addition, mast cells express mRNA for these cytokines within the allergic airway mucosa suggesting that there is ongoing mast cell cytokine synthesis in allergic disease [11–13]. Eosinophils also express mRNA and protein for IL-4 and IL-5 in asthma, but interestingly, no studies have been able to identify cytokine product within T cells. This may be because mast cells and eosinophils contain the apparatus necessary to store cytokines, namely secretory granules, in contrast with T cells which do not. Further evidence that mast cells store preformed cytokines comes from the observations that they express IL-4 and IL-5 protein but not mRNA in normal subjects [5,11] and that both TNF α and IL-4 are released within minutes from IgE-triggered human mast cells [14–16].

Based on the above evidence, we hypothesized that mast cells store IL-4 and IL-5 within their secretory granules. We tested this hypothesis directly by employing immunoelectron microscopy on airway mucosal tissue using monoclonal antibody (mAb) -directed to epitopes on IL-4 and IL-5, enabling us to localize these cytokines to specific intracellular structures.

Materials and methods

Immunoelectron microscopic analysis for IL-4 and IL-5 was performed on bronchial and nasal mucosal samples, fragments of lung parenchyma, and also on peripheral blood eosinophils.

Samples

Bronchial mucosal samples, > 1 mm in diameter, were taken using alligator biopsy forceps, from fresh lung lobectomy specimens ($n = 3$) removed surgically for management of carcinoma. Only macroscopically normal tissue distant from the tumour was used. To assess the effect of IgE-dependent activation on mast cell cytokine expression, fragments of lung parenchyma from resected lung samples were also studied with or without IgE-dependent activation ($n = 3$). For these experiments, resected lung tissue was finely chopped into 0.5 mm³ pieces and sensitized with 2 μ g/mL human myeloma IgE (Calbiochem-Novabiochem, Nottingham, UK) in DMEM containing 2% penicillin/streptomycin (Sigma, Poole, UK) and 10% fetal calf serum (Gibco, Paisley, UK) for 18 h at 37 °C. The parenchymal fragments were then washed and challenged with 10% anti-IgE (Serotec, Kidlington, UK) for 24 h before processing.

Nasal mucosal tissue was obtained from a symptomatic subject with seasonal rhinitis during the grass pollen season. This nasal biopsy was taken from the inferomedial edge of the inferior turbinate using 2 mm cupped Hartman's forceps following local anaesthesia with 1% tetracaine containing 1 : 10000 adrenaline.

To assess whether peripheral blood eosinophils also store IL-5, eosinophils were isolated from blood of an atopic donor by removal of mononuclear cells on Percoll, followed by immunomagnetic selection and removal of neutrophils from the granulocyte fraction using the MACS system [17]. Isolation by this procedure provided a yield with 99.5% purity. These cells were embedded in 4% agarose (Sigma, Poole, UK) and treated as tissue blocks.

Patients gave written informed consent, and the study was approved by the Southampton University and Hospitals Joint Ethics Committee.

Processing

Samples of bronchial mucosa, lung parenchyma, nasal mucosa, and peripheral blood eosinophils were fixed in 4% paraformaldehyde plus 0.5% glutaraldehyde in cacodylate buffer pH 7.4 for 2 h. Tissue was then treated with ammonium chloride and processed by the Progressive Lowering of Temperature technique into Lowicryl K4M resin [18].

Monoclonal antibodies

Each mAb used in this study was mouse antihuman IgG1 isotype, and they have all been well characterized and used in previous studies. AA1, a mAb directed to human mast cell tryptase (Dako, High Wycombe, UK) [19] and EG₂ which recognizes the cleaved form of eosinophil cationic

protein (ECP; Pharmacia, Milton Keynes, UK) [20] were used as positive controls at concentrations of 50 and 5 µg/mL, respectively. The antihuman IL-4 mAb 4D9 (C. Heusser, Ciba Geigy, Basel, Switzerland) [14] and antihuman IL-5 mAb MAB7 (P. Hissey, Glaxo, Greenford, UK) [12,21,22] were used at concentrations of 100 and 114 µg/mL, respectively. Control sections were treated as test sections but with either the primary antibody omitted or in the presence of a matched isotype control used at the same immunoglobulin concentration (114 µg/mL) (Sigma). These antibody concentrations are standard for this type of work, in contrast with the lower concentrations required for immunohistochemistry using light microscopy. All specimens were stained in duplicate.

Immunolabelling

Ultrathin sections (90 nm) were cut and then labelled by an indirect immunohistochemical technique. Sections were treated with blocking media (20% FCS and 1% BSA in DMEM) for 30 min to reduce non-specific staining, then incubated with primary mouse mAb at appropriate dilutions in TBS for 1 h at room temperature. After washing with TBS containing 0.05% Tween 20, sections were incubated with a gold-conjugated (10 nm) goat antimouse (Fab')₂ fragment (Biocell, Cardiff, UK) at room temperature for 1 h. Sections were then counter-stained with saturated aqueous uranyl acetate and Reynolds lead citrate. All labelling experiments were performed in duplicate.

Electron microscopy

All sections were examined in an Hitachi H7000 transmission electron microscope and representative photographs taken.

Quantification and statistical analysis

Mast cells, eosinophils and T cells within tissue samples were identified using well-described morphological characteristics [23]. All mast cells in each section were analysed (range 3–7 mast cells per section in lung tissue, 2 mast cells per section in the nasal sample). The number of colloidal gold particles were counted in the substructures in which they were observed in all the test and control sections. These counts are expressed as mean $\pm$ SEM gold particles per granule. In addition, to allow for variation in granule size, granule area was calculated using computerized image analysis with Colourvision software (Improvision, Coventry, UK) and counts expressed as mean $\pm$ SEM gold particles per µm² of granule. The data were analysed statistically using SPSS (SPSS, Woking, UK). The paired Student's *t*-test was used to compare the data from the test and control

sections for each tissue, and between IgE-activated lung fragments and nonactivated controls.

Results

Immunogold labelling with positive control mAbs was appropriate with AA1 (antimast cell tryptase) confined to the granules of mast cells, and EG₂ (antieosinophil cationic protein) restricted to the matrix of eosinophil granules (Fig. 1a,b). In all samples analysed, no significant colloidal gold

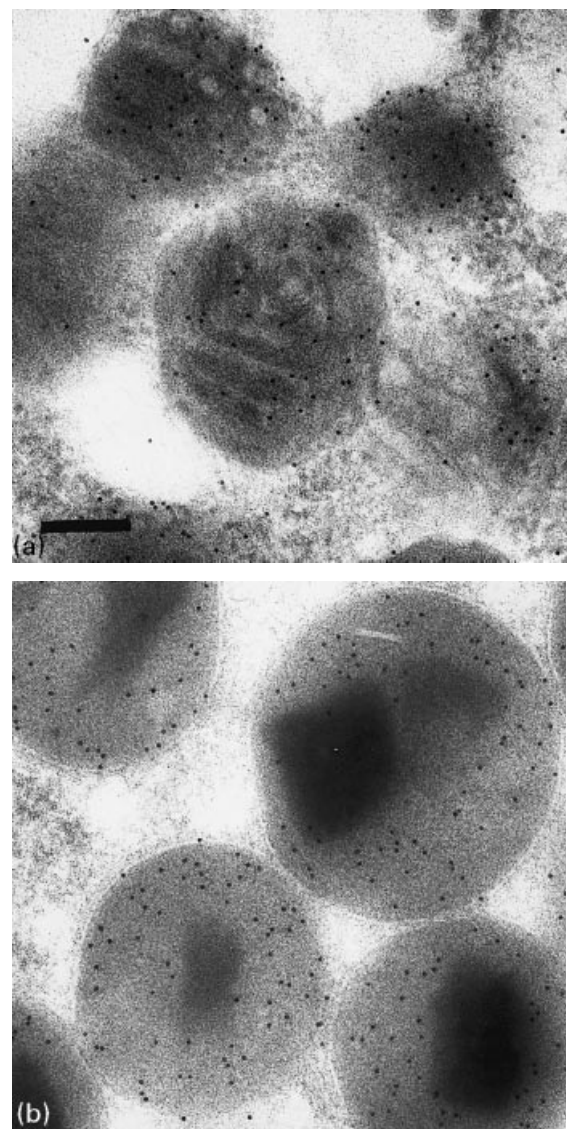


Fig. 1. Examples of positive control sections showing immunolabelling with (a) the mAb AA1 directed against mast cell tryptase, labelling is seen within the mast cell granules. (b) The mAb EG₂ directed against ECP, labelling is confined to the matrix of these eosinophil granules (bar = 200 nm)

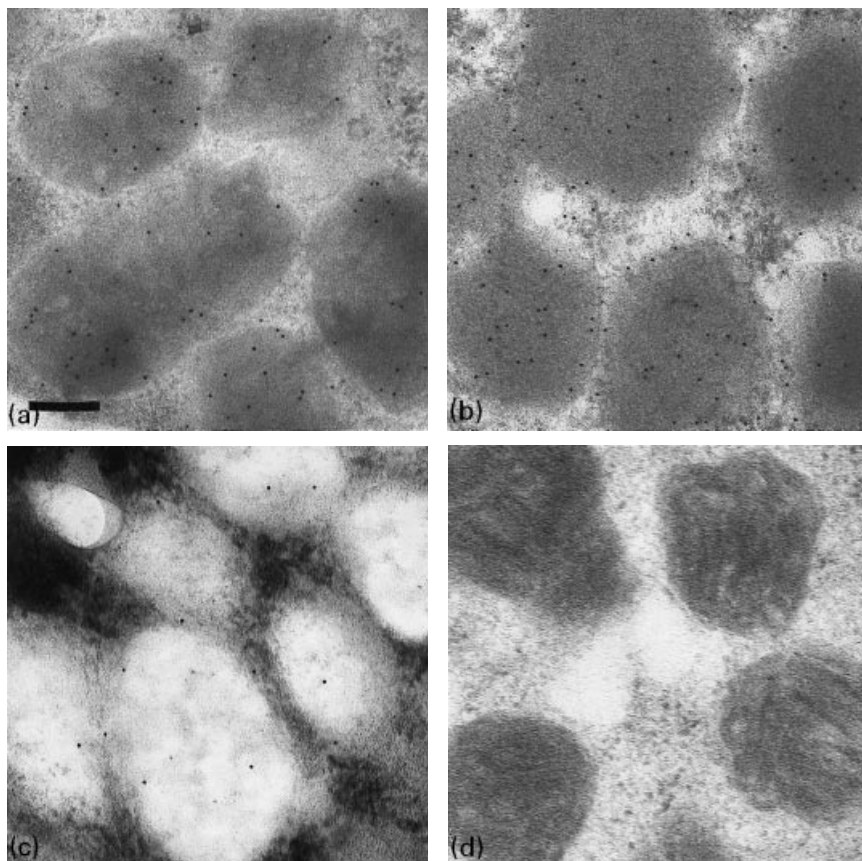


Fig. 2. Immunogold staining with mAb 4D9 directed against IL-4. Labelling is observed in the granules of the mast cell in (a) bronchial tissue from a resected lung, (b) IgE-stimulated lung fragments, and (c) nasal biopsy tissue from a symptomatic seasonal rhinitic. (d) Isotype control section, no significant labelling is evident (bar = 200 nm)

labelling was observed in the absence of primary antibody or with the IgG1 isotype control mAb (see below).

Immunogold labelling with the anti-IL-4 mAb 4D9 was localized to the secretory granules of mast cells in all tissues sampled (Fig. 2a–c). Labelling was present in all mast cell granules in the lung tissues examined (mean 67 granules per section of bronchial mucosa, 36 granules per section of lung parenchyma), and 61% of mast cell granules ($n = 18$ granules) in the nasal mucosal sample. With this anti-IL-4 mAb, a mean ($\pm$ SEM) 16.6 ± 2.2 , 6.84 ± 0.8 , and 5.0 ± 1.6 colloidal gold particles per granule were observed, respectively, in mast cells in the bronchial mucosa, unchallenged lung fragments, and nasal mucosa, vs <0.2 in all of the isotype control sections from the same tissue sources ($P < 0.001$). Interestingly, there was a highly significant increase in IL-4 immunoreactivity in the granules of mast cells resident within IgE-activated lung parenchyma (mean 17.5 ± 1.4 gold particles per granule) compared with mast cells in unchallenged tissue (mean 6.8 ± 0.8 gold particles per granule, $P < 0.001$) (see Fig. 2a,b). No IL-4 immunoreactivity was evident in mast cell mitochondria, on the cell surface of mast cells, nor in the granules or cytoplasm of eosinophils or lymphocytes. Occasional gold particles (not quantified) were seen apparently between mast cell granules

in IgE-challenged lung fragments (but not in unchallenged fragments or bronchi), and may therefore be localized to the cytoplasm, or alternatively, inter-digitating granule matrix (see Fig. 2b). No IL-4 labelling was seen in resin adjacent to cells.

Immunoreactivity for IL-5 was confined to eosinophils in the lung and nasal tissues studied, an observation which was confirmed in purified peripheral blood eosinophils. Within these eosinophils labelling was restricted to the matrix of the granules (Fig. 3a–c). No significant immunolabelling was observed in the eosinophils of the isotype control sections (Fig. 3d). A mean 9.2 ± 2.7 , 7.4 ± 1.5 and 9.2 ± 1.0 anti-IL-5 colloidal gold particles per granule were seen in the matrix of eosinophil granules in the bronchial mucosa, lung parenchyma, and isolated peripheral blood eosinophils, respectively. Values from the control sections were <0.2 gold particles per granule ($P < 0.001$). No labelling for IL-5 was observed in eosinophil mitochondria nor in resin adjacent to cells. Nor was such labelling seen in mast cells or lymphocytes in any of the tissues examined, and was not seen in these cells after IgE-dependent activation of lung parenchyma.

In case variability in granule area affected the above data, we also expressed the results in terms of gold particles per

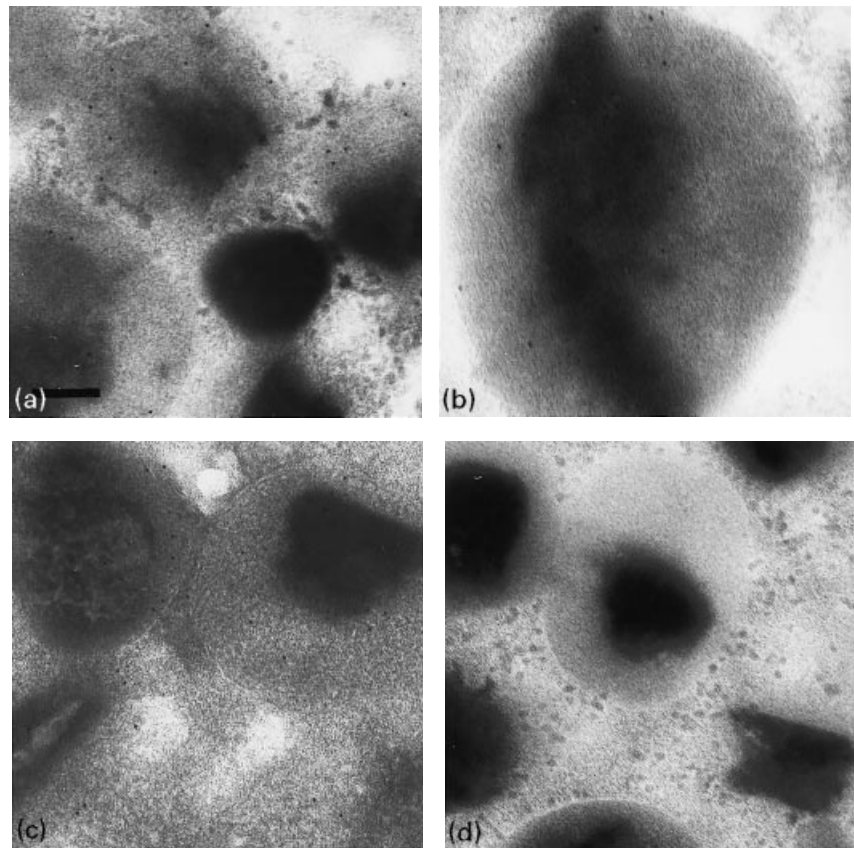


Fig. 3. Immunostaining with the mAb MAB 7 directed against IL-5. Immunogold labelling is confined to the matrix of the eosinophil granules in (a) bronchial tissue from a resected lung, (b) nasal biopsy tissue from a symptomatic seasonal rhinitic, and (c) peripheral blood eosinophils from an atopic donor. No immunoreactivity is observed in the core of the eosinophil granules or in the cell cytoplasm. (d) Isotype control section, no significant labelling is evident (bar = 200 nm)

granule area. Fold differences between groups remained the same, with similar levels of statistical significance for comparisons between groups (data not shown).

Discussion

This is the first study to investigate the sub-cellular localization of IL-4 and IL-5 in human mast cells. We have confirmed our hypothesis that airway mast cells store preformed IL-4 in the matrix of their secretory granules. However, while mast cell-associated IL-4 was abundant, we failed to detect mast cell-associated IL-5.

The absence of IL-5 colocalization to mast cells was initially surprising in view of our previous observations [11,12]. In these studies, approximately 60% of bronchial mucosal biopsies contained IL-5 immunoreactivity, and in these IL-5 was identified in about 10% of mast cells. The ultrathin sections used for immunoelectron microscopy are 20 times thinner than the glycol methacrylate sections used for immunohistochemistry and have only a quarter of the area. Thus the probability of sampling an IL-5⁺ mast cell by immunoelectron microscopy will be considerably less than by immunohistochemistry at the light microscope level. It is therefore likely that we have not sampled enough mast cells

in order to identify IL-5-immunoreactive cells, although IL-5 immunostaining was clearly present within eosinophil granules. It should also be noted, however, that IL-5 cannot be detected in human lung mast cell supernatants until 4 h after IgE-dependent activation [24,25]. This latter observation, coupled with the evidence in this study, suggests that if any lung mast cells do store preformed IL-5, the number must be small, and/or the concentration per mast cell very low. Previous studies have demonstrated that 24 h after IgE-dependent activation of lung parenchyma, over 90% of mast cells express IL-5 mRNA and secrete large quantities of IL-5 [24,25]. The failure in this study to detect intracellular IL-5 within mast cells even under these circumstances again suggests that a large proportion of mast cell-derived IL-5 following cell activation is synthesized *de novo*, secreted rapidly and that secretion does not involve the secretory granules.

During the course of this study Dubucquoi *et al.* [26] demonstrated the presence of immunoreactive IL-5 in the matrix of eosinophil granules, an observation which we have confirmed. IL-5 appears to localize within the granule in a similar pattern to ECP, that is, to the matrix [27] and not the electron-dense crystalloid core which is comprised of major basic protein. These findings indicate that this

cytokine is likely to be released in conjunction with other eosinophil granule products. The precise contribution of granule-derived IL-5 in the allergic tissue response is not known, but is likely to serve an autocrine function since IL-5 is a powerful inhibitor of apoptosis [28] and is able to prime eosinophils for chemotaxis and mediator release [29,30].

The failure to detect stored preformed cytokines in lymphocytes is not unexpected. T cells do not contain storage granules, unlike the mast cell or eosinophil, and it is most likely that cytokines are synthesized and exported immediately. Recently, T-lymphocyte immunostaining, using FACS analysis of stimulated airway T cells which have been treated with monensin to block golgi transport, has demonstrated accumulation of intracellular IL-2 and IFN γ [31], consistent with the synthesis of cytokines but absence of storage mechanisms under normal circumstances.

This study adds to an increasing body of evidence that mast cells are a potentially important source of IL-4. Mast cells clearly express IL-4 mRNA and intracellular IL-4 protein, with evidence of decreased intracellular immunoreactivity immediately after mast cell activation by anti-IgE [5,11,12,14,24,32–36]. There is also evidence of mast cell IL-4 release following IgE-dependent activation as measured by ELISA, but we and others have found this to be highly variable between mast cell preparations [14,15, 24,37]. At least two factors are thought to explain this. Firstly, IL-4 is rapidly degraded by mast cell chymase which is very active in this respect [37]. Secondly, Jones *et al.* [38] have shown that IL-4 binds tightly to the glycosaminoglycan side chains of heparin. The observation in this study that IL-4 is stored in the mast cell granule matrix makes it very likely that it is heparin-bound in this sub-cellular location. Thus IL-4 may well be released attached to heparin, which would serve to localize its biological effects, but may well mask immunoreactive epitopes which are required for successful detection in an ELISA. This is supported by the recent observation that human skin mast cell-derived IL-4 induces fibroblast proliferation when the two cell types are in contact, although it cannot be detected in the culture supernatant [39].

A further interesting feature of this study was the apparent increase in mast cell granule IL-4 protein expression 24 h after IgE-dependent cell activation of lung parenchyma. It is likely that new protein synthesis has occurred by this time point, which fits with the known time-course of IL-4 mRNA expression in purified human lung mast cells. This peaks at 2 h after IgE-dependent activation and wanes by 12 h [24]. Increased amounts of immunoreactive IL-4 in mast cell granules 24 h after cell activation suggests that newly generated IL-4 (unlike IL-5) is targeted to the secretory granules, presumably as a means of replenishing depleted stores. Whether secretion of newly synthesized

IL-4 also occurs through a pathway that bypasses the secretory granules cannot be elucidated from the present study.

The confirmation that mast cells contain a store of preformed IL-4 has important implications for their role at the onset of immune responses. Human mast cells are known to induce IgE synthesis in B cells through the release of IL-4 and IL-13 [40], and in rodents, present antigen to T cells [41], and may induce TH2 lymphocyte differentiation [42]. Conventionally, mast cell activation in asthma and rhinitis has been interpreted as an IgE-dependent event, but recent studies indicate that common allergens, many of which are serine proteases, can induce mast cell histamine and IL-4 secretion directly through an IgE-independent mechanism [43,44]. The release of preformed IL-4 at the onset of an immune response to these allergens would clearly have the potential to bias the ensuing immune reaction towards a TH2 response. Thus, the mast cell may not only contribute to the chronic airway inflammatory response and symptomatology of asthma and rhinitis, but may have a central role in the initiation of the immune response to allergens and parasites.

In summary, we have shown definitively that human mast cells store preformed IL-4 within their secretory granules, but probably not IL-5. We suggest that there may be distinct pathways of secretion for these two cytokines following IgE-dependent activation. These observations have important implications for the role of the mast cell in immunological responses.

Acknowledgement

This work was supported by a grant-in-aid from Ciba-Geigy, Basel, Switzerland.

References

- 1 Djukanovic R, Roche WR, Wilson JW et al. Mucosal inflammation in asthma. *Am Rev Respir Dis* 1990; 142:434–57.
- 2 Del Prete G, Maggi E, Parronchi P et al. IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cell clones and their supernatants. *J Immunol* 1988; 140:4193–8.
- 3 Tepper RI, Levinson DA, Stanger BZ, Campos-Torres J, Abbas AK, Leder P. IL-4 induces allergic-like inflammatory disease and alters T cell development in transgenic mice. *Cell* 1990; 62:457–67.
- 4 Sanderson CJ. Interleukin-5, eosinophils, and disease. *Blood* 1992; 79:3101–9.
- 5 Ying S, Durham SR, Corrigan CJ, Hamid Q, Kay AB. Phenotype of cells expressing mRNA for TH2-type (interleukin 4 and interleukin 5) and TH1-type (interleukin 2 and interferon gamma) cytokines in bronchoalveolar lavage and bronchial biopsies from atopic asthmatic and normal control subjects. *Am J Respir Cell Mol Biol* 1995; 12:477–87.

- 6 Humbert M, Durham SR, Ying S et al. IL-4 and IL-5 mRNA and protein in bronchial biopsies from patients with atopic and nonatopic asthma: evidence against 'intrinsic' asthma being a distinct immunopathologic entity. *Am J Respir Crit Care Med* 1996; 154:1497–504.
- 7 Durham SR, Ying S, Varney VA et al. Cytokine messenger RNA expression for IL-3, IL-4, IL-5, and granulocyte/macrophage-colony-stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. *J Immunol* 1992; 148:2390–4.
- 8 Kay AB, Ying S, Varney V et al. Messenger RNA expression of the cytokine gene cluster, interleukin 3 (IL-3), IL-4, IL-5, and granulocyte/macrophage colony-stimulating factor, in allergen-induced late-phase cutaneous reactions in atopic subjects. *J Exp Med* 1991; 173:775–8.
- 9 Walker C, Bode E, Boer L, Hansel TT, Blaser K, Virchow JC Jr. Allergic and nonallergic asthmatics have distinct patterns of T-cell activation and cytokine production in peripheral blood and bronchoalveolar lavage. *Am Rev Respir Dis* 1992; 146:109–15.
- 10 Bradding P, Holgate ST. The mast cell as a source of cytokines in asthma. *Ann New York Acad Sci* 1996; 796:272–81.
- 11 Bradding P, Roberts JA, Britten KM et al. 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.
- 12 Bradding P, Feather IH, Wilson S et al. 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.
- 13 Suzumura Y, Ohasi M. Immunoelectron microscopic localization of HLA-DR antigen on mast cells and vessels in normal and tuberculin-reactive skin. *Am J Dermatopathol* 1991; 13:568–74.
- 14 Bradding P, Feather IH, Howarth PH et al. Interleukin 4 is localized to and released by human mast cells. *J Exp Med* 1992; 176:1381–6.
- 15 Gibbs BF, Arm JP, Gibson K, Lee TH, Pearce FL. Human lung mast cells release small amounts of interleukin-4 and tumour necrosis factor- α in response to stimulation by anti-IgE and stem cell factor. *Eur J Pharmacol* 1997; 327:73–8.
- 16 Walsh LJ, Trinchieri G, Waldorf HA, Whitaker D, Murphy GF. 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.
- 17 Hansel TT, De Vries IJ, Iff T et al. An improved immunomagnetic procedure for the isolation of highly purified human blood eosinophils. *J Immunol Methods* 1991; 145:105–10.
- 18 Carlemalm E, Villiger W, Hobot JA, Acetarin JD, Kellenberger E. Low temperature embedding with Lowicryl resins: two new formulations and some applications. *J Microsc* 1985; 140:55–63.
- 19 Walls AF, Bennett AR, McBride HM, Glennie MJ, Holgate ST, Church MK. Production and characterization of monoclonal antibodies specific for human mast cell tryptase. *Clin Exp Allergy* 1990; 20:581–9.
- 20 Tai PC, Spry CJ, Peterson C, Venge P, Olsson I. Monoclonal antibodies distinguish between storage and secreted forms of eosinophil cationic protein. *Nature* 1984; 309:182–4.
- 21 McNamee LA, Fattah DI, Baker TJ, Bains SK, Hissey PH. Production, characterisation and use of monoclonal antibodies to human interleukin-5 in an enzyme-linked immunosorbent assay. *J Immunol Methods* 1991; 141:81–8.
- 22 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.
- 23 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.
- 24 Okayama Y, Petit-Frere C, Kassel O et al. IgE-dependent expression of mRNA for IL-4 and IL-5 in human lung mast cells. *J Immunol* 1995; 155:1796–808.
- 25 Jaffe JS, Glaum MC, Raible DG et al. 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.
- 26 Dubucquoi S, Desreumaux P, Janin A et al. Interleukin 5 synthesis by eosinophils: association with granules and immunoglobulin-dependent secretion. *J Exp Med* 1994; 179:703–8.
- 27 Ackerman SJ, Loegering DA, Venge P et al. Distinctive cationic proteins of the human eosinophil granule: major basic protein, eosinophil cationic protein, and eosinophil-derived neurotoxin. *J Immunol* 1983; 131:2977–82.
- 28 Yamaguchi Y, Suda T, Ohta S, Tominaga K, Miura Y, Kasahara T. Analysis of the survival of mature human eosinophils: interleukin-5 prevents apoptosis in mature human eosinophils. *Blood* 1991; 78:2542–7.
- 29 Warringa RA, Schweizer RC, Maikoe T, Kuijper PH, Bruijnzeel PL, Koendermann L. Modulation of eosinophil chemotaxis by interleukin-5. *Am J Respir Cell Mol Biol* 1992; 7:631–6.
- 30 Kita H, Weiler DA, Abu-Ghazaleh R, Sanderson CJ, Gleich GJ. Release of granule proteins from eosinophils cultured with IL-5. *J Immunol* 1992; 149:629–35.
- 31 Krug N, Madden J, Redington AE et al. T-cell cytokine profile evaluated at the single cell level in BAL and blood in allergic asthma. *Am J Respir Cell Mol Biol* 1996; 14:319–26.
- 32 Ying S, Humbert M, Barkans J et al. 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.
- 33 Ying S, Durham SR, Jacobson MR et al. T lymphocytes and mast cells express messenger RNA for interleukin-4 in the nasal mucosa in allergen-induced rhinitis. *Immunology* 1994; 82:200–6.
- 34 Horsmanheimo L, Harvima IT, Jarvikallio A, Harvima RJ, Naukkarinen A, Horsmanheimo M. Mast cells are one major source of interleukin-4 in atopic dermatitis. *Br J Dermatol* 1994; 131:348–53.
- 35 Cook EB, Stahl JL, Graziano FM, Barney NP. Human conjunctival mast cells stain positive for the cytokines IL-4, IL-5,

- and TNF α by intracellular FACS analysis. *J Allergy Clin Immunol* 1997; 99:S91. (Abstract).
- 36 Okayama Y, Bradding P, Tunon-De-Lara JM, Holgate ST, Church MK. Cytokine production by human mast cells. *Chem Immunol* 1995; 61:114–34.
- 37 Tunon Lara JM, Okayama Y, McEuen AR, Heusser CH, Church MK, Walls AF. Release and inactivation of interleukin-4 by mast cells. *Ann New York Acad Sci* 1994; 725:50–8.
- 38 Jones CA, Williams KA, Finlay-Jones JJ, Hart PH. Interleukin 4 production by human amnion epithelial cells and regulation of its activity by glycosaminoglycan binding. *Biol Reprod* 1995; 52:839–47.
- 39 Trautmann A, Krohne G, Brocker EB, Klein CE. Human mast cells augment fibroblast proliferation by heterotypic cell-cell adhesion and action of IL-4. *J Immunol* 1998; 160:5053–7.
- 40 Pawankar R, Okuda M, Yssel H, Okumura K, Ra C. Nasal mast cells in perennial allergic rhinitis exhibit increased expression of the Fc epsilonRI, CD40L, IL-4, and IL-13, and can induce IgE synthesis in B cells. *J Clin Invest* 1997; 99:1492–9.
- 41 Frandji P, Tkaczyk C, Oskeritzian C et al. Presentation of soluble antigens by mast cells: upregulation by interleukin-4 and granulocyte/macrophage colony-stimulating factor and downregulation by interferon-gamma. *Cell Immunol* 1995; 163:37–46.
- 42 Huels C, Germann T, Goedert S et al. Co-activation of naive CD4+ T cells and bone marrow-derived mast cells results in the development of Th2 cells. *Int Immunol* 1995; 7:525–32.
- 43 Machado DC, Horton D, Harrop R, Peachell PT, Helm BA. Potential allergens stimulate the release of mediators of the allergic response from cells of mast cell lineage in the absence of sensitization with antigen-specific IgE. *Eur J Immunol* 1996; 26:2972–80.
- 44 Dudler T, Machado DC, Kolbe L et al. A link between catalytic activity, IgE-independent mast cell activation, and allergenicity of bee venom phospholipase A2. *J Immunol* 1995; 155:2605–13.