

Original article

Mast cells express IL-13R α 1: IL-13 promotes human lung mast cell proliferation and Fc ϵ RI expression

Background: The Th2 cytokine interleukin (IL)-13 is implicated in the development of various allergic diseases including asthma. The IL-13 receptor, IL-13R α 1, is expressed on most leukocytes, except T-cells. Evidence to support IL-13R α 1 expression on mast cells is limited.

Methods: We investigated: (i) IL-13R α 1 expression by human lung mast cells (HLMC); (ii) the number of IL-13R α 1 + bronchial submucosal mast cells in subjects with asthma and normal controls and (iii) the effect of IL-13 priming on HLMC expression of high-affinity IgE receptor (Fc ϵ RI), stem cell factor receptor (CD117), histamine release, proliferation, and survival.

Results: Human lung mast cell expressed IL-13R α 1 mRNA. IL-13R α 1 was highly expressed on the surface HLMC ($82 \pm 9\%$). Bronchial submucosal mast cell IL-13R α 1 expression was higher in asthmatics ($86 \pm 2\%$) than normal controls ($78 \pm 2\%$; $P = 0.015$). IL-13 priming for 30 min did not increase HLMC histamine release, in the presence or absence of SCF or in response to IgE/anti-IgE activation. IL-13 priming for 5 days upregulated HLMC Fc ϵ RI expression (22% increase in fluorescent intensity; $P = 0.003$), increased histamine release following IgE/anti-IgE activation by 56% ($P = 0.03$) and increased proliferation by 50% ($P = 0.003$) without affecting cell survival or CD117 expression. The IL-13 specific neutralizing antibody CAT-354 inhibited all IL-13 mediated effects.

Conclusion: Human lung mast cell express IL-13R α 1 and activation by IL-13 for 5 days increased Fc ϵ RI expression and proliferation. Histamine release was not affected by short-term priming with IL-13, but was upregulated by priming for 5 days suggesting that this effect was mediated by the increased Fc ϵ RI expression. These data support the view that targeting IL-13 may be beneficial in the treatment of asthma.

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The Th2 cytokine interleukin (IL)-13 has been implicated in the immunopathogenesis of various allergic diseases including asthma (1). IL-13 has pleiotropic effects on a wide variety of cell types relevant to allergic disease, playing important roles in the regulation of IgE synthesis (2), induction of adhesion molecules (3, 4) and chemokine expression (1). In particular, animal models of allergic lung inflammation have provided compelling evidence that IL-13 plays a pivotal role in the development of cardinal features of allergic asthma including airway hyperresponsiveness, remodelling and eosinophilic inflammation (1).

Interleukin-13 mediates its effects predominately through activation of a receptor complex comprised of

IL-4R α and an IL-13 binding subunit IL-13R α 1 (5). IL-13R α 1 is expressed on the surface of most leukocytes, except T cells, as well as airway smooth muscle (ASM) (6, 7), fibroblasts (6, 8) and bronchial epithelial cells (6, 9). Mast cells play a significant role in the pathophysiology of asthma through their immunomediator-secretory activities (10). However, there are no reports of IL13R α 1 mRNA or protein expression by primary human mast cells. In asthma mast cells are microlocalized within the ASM bundle. Interactions between mast cells and ASM are likely to be pivotal in the development of airway hyperresponsiveness (11). Hence it is important to determine whether mature human mast cells express functional IL-13R α 1 as mast cell activation by IL-13 may have important implications for airway hyperresponsiveness in asthma.

In this study we have investigated the expression of IL-13R α 1 by mast cells in bronchial biopsies and *ex vivo*

Abbreviations: HLMC, human lung mast cell; HMC-1, human mast cell line; HASM, human airway smooth muscle; SCF, stem cell factor.

HLMC and examined the effects of IL-13 priming on HLMC FcεRI expression, histamine release, proliferation and survival.

Materials and methods

Bronchial tissue samples

Subjects were recruited from patients and staff at Glenfield Hospital, Leicester. Subjects with asthma (*n* = 7) gave an appropriate history and had one or more of methacholine airway hyperresponsiveness (PC20FEV1 < 8 mg/ml), >15% improvement in FEV1 10 min after 200 µg inhaled albuterol and PEF >20% maximum within day amplitude from PEF recordings over 2 weeks. Asthmatics were treated with β2 agonists alone. Normal subjects (*n* = 7) were asymptomatic with normal airway physiology. All subjects had either never smoked or were ex-smokers for at least 6 months with <5 pack/year history. Clinical characteristics are shown in Table 1. The study was approved by the Leicestershire Ethics Committees and patients gave written informed consent.

Subjects had spirometry, methacholine challenge test and allergen skin prick tests to *Dermatophagoides pteronyssinus*, cat and dog fur, grass pollen and *Aspergillus fumigatus*. One-week later, subjects underwent fibreoptic bronchoscopy and biopsies were immediately transferred into ice-cooled acetone containing the protease inhibitors iodoacetamide (20 mM) and phenylmethylsulphonyl fluoride (PMSF) (2 mM) for fixation (Sigma, Dorset, UK), stored at -20°C for 24 h, and processed into the water-soluble resin, glycolmethacrylate (GMA) (Polysciences, Northampton, UK) (12).

Mast cell isolation

Human lung mast cells (HLMC) were dispersed from macroscopically normal lung obtained within 1 h of resection for lung cancer (16 donors) and purified using immunomagnetic affinity selection with antimouse IgG1 magnetic beads coated with the mouse anti-kit mAb YB5.B8 (BD, Oxfordshire, UK) as described previously (13). A typical lung sample provided a yield of 0.1–0.5 × 10⁶ HLMC/g of lung. The HLMC purity ranged between 98% and 100%. Cells were cultured in DMEM (Invitrogen Life Technologies, Scotland), 10% FBS supplemented with stem cell factor (SCF) (100 ng/ml), IL-10 (10 ng/ml) and IL-6 (50 ng/ml, all supplied by R&D Systems, Oxfordshire, UK).

Human airway smooth muscle cells

Pure ASM bundles were dissected free from airway obtained at surgery for carcinoma and grown as explants in DMEM, 10% FBS.

Table 1. Subject Characterisation

	Normal	Asthma
Number	7	7
Age*	37 ± 4	42 ± 5
Male	3	4
Atopy	2	5
PC20FEV1 (mg/ml)†	>16	2.0 (0.6–4.4)
FEV1 percentage predicted*	98 ± 3	94 ± 3
FEV1/FVC %*	82 ± 2	78 ± 2

*Mean ± SEM.

†Geometric mean (range).

ASM characteristics were confirmed by immunofluorescence. Cells were stained with mAb α-smooth muscle actin-FITC (clone 1A4 Sigma). Human airway smooth muscle cells (HASM) was used as a positive control for IL-13Rα1 expression (passages 3–6).

Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of IL-13Rα1 expression by *ex vivo* HLMC and HASM cells. Total cellular RNA was extracted from HLMC (1–1.5 × 10⁶) or HASM (2–2.5 × 10⁶) following lysis with Trizol according to the manufacturer's instructions (Invitrogen). RNA was further purified using DNase treatment according to Invitrogen protocol. RT-PCR was performed using the Omniscript RT Kit (100 ng RNA per reaction) (Qiagen, Sussex, UK). First strand DNA (20 ng) was then amplified using Taq PCR Master mix Kit (Qiagen, 5 µl cDNA, 2.5 U Taq DNA Polymerase, 50 pmol primer mix, 200 µM dNTP). Amplification of cDNA encoding IL-13Rα1 was performed over 35 cycles with 1-min cycles of denaturation at 96°C, 1 min of annealing at 60°C and 1 min extension at 72°C; forward-primer 5'-CTCC TTCCACAATGATGACC-3'; reverse primer, 5'-GGAATTGCG CTTCTTACCTA-3'. Beta-actin was used as a positive control. PCR products were subjected to electrophoresis on a 2% (w/v) agarose gel (Invitrogen) and visualized by ethidium bromide (Sigma) staining.

Sequencing IL-13Rα1 expression by *ex vivo* HLMC and HASM cells purified from PCR gel

The PCR products of the predicted size (319 bp) for IL-13Rα1 underwent DNA sequencing (Lak Technologies, Essex, UK).

Analysis of IL-13Rα1 cell surface expression by HLMC and HASM

HLMC IL-13Rα1 expression was examined in three donors. HLMC (0.5–1 × 10⁵/tube) were resuspended in PBS containing bovine serum albumin (0.5%). Cells were stained with mAb against IL-13Rα1 (clone 116708, R&D) indirectly labelled with FITC (Dako, Hamburg, Germany) and then stained directly with a conjugated mAb against CD117-RPE (clone 104 D2, Dako). Nonspecific antibody binding was blocked using mouse IgG (Sigma). HASM cells (three donors) were stained indirectly with the same mAb against IL-13Rα1 as described above for HLMC. Receptor expression was analysed by one or two colour flow cytometry on a FACScan (BD, Oxford, UK).

IL-13Rα1 expression by submucosal mast cells

Bronchial mucosal biopsies from seven asthmatics and seven normal controls were cut into 2 µm sections, floated on ammonia in water (0.2% v/v) for 1 min and dried at RT for 14 h. The technique of immunostaining applied to GMA embedded tissue has been described previously (12). To assess co-localization of IL-13Rα1 with mast cells in the bronchial submucosa sequential sections were stained with IL-13Rα1 (goat polyclonal antibody, R&D) or tryptase (clone A11, mAb, Dako) as described previously (14).

Effect of IL-13 priming on mast cell function

The following conditions were used to assess the effect of IL-13 priming on HLMC (six donors). HLMC cultured in DMEM, containing FBS (10%) and SCF (100 ng/ml) were treated for 5 days with IL-13 (10 ng/ml) (R&D) alone or in combination with CAT-354 (100 nM) (IgG4 neutralizing mAb IL-13) or CAT-251 (100 nM) (isotypecontrol). Both CAT-354 and CAT-251 were provided by Cambridge Antibody Technology (UK). The IL-13 concentration

used was based on previous reports establishing IL-4, -5 and -13 priming to be optimal between 5 and 20 ng/ml (15–17). On day 5, cell culture supernatants were removed and stored for later analysis.

Fc ϵ RI and CD117 expression. Human lung mast cells at day 0 and after 5 days of priming using the conditions described above, were stained with antibodies to Fc ϵ RI (clone 3G6; Upstate Biotechnology, NY, USA), indirectly labelled with FITC and then directly CD117-RPE. Receptor expression was assessed by two-colour flow cytometry.

Spontaneous IL-6 release. Cell supernatant IL-6 concentration from the IL-13 priming experiments was measured by commercial ELISA according to the manufacturer's protocol (Caltag Systems, Burlingame, CA, USA). The limit of detection was 1.6 pg/ml.

Cell number, viability and nuclear morphology assessment. The number of HLMC present at day 0 and 5 days were determined by Kimura staining using a haemocytometer; viability was assessed by trypan blue. Cytospins were prepared from treated HLMC samples; fixed with methanol, stained with DAPI (0.5 μ g/ml) (Sigma) for 1 min washed, mounted and then visualized under a fluorescent microscope (Olympus, BX-70, Olympus Optical Corporation, Ltd, Japan).

Histamine release after IgE/anti-IgE activation. In separate experiments the effect of IL-13 on HLMC histamine release was assessed using the following conditions: (i) HLMC ($n = 3$) incubated with IL-13 (0.1–100 ng/ml) for 30 min at 37°C in the presence and absence of SCF (100 ng/ml), (ii) HLMC ($n = 3$) were sensitized overnight with human myeloma IgE (2.5 μ g/ml) (Calbiochem-Novabiochem, Nottingham, UK), washed, then incubated with IL-13 (0.1–100 ng/ml) for 30 min and activated with goat polyclonal anti-human IgE (1 : 1000 dilution) (Sigma) for 30 min at 37°C and (iii) HLMC ($n = 3$) incubated with IL-13 (10 ng/ml) and SCF (100 ng/ml) for 5 days alone or in combination with CAT-354 (100 nM) (IgG4 neutralizing mAb IL-13) or CAT-251 (100 nM) (isotype control) and then sensitized and activated with IgE/anti-IgE as for condition 2. Cells were centrifuged, supernatant removed and pellets lysed in sterile de-ionized water for total histamine content. Histamine was measured by radioenzymatic assay as previously described (13). The amount of histamine released after activation was corrected for spontaneous histamine release and expressed as a percentage of the total histamine content for each condition.

Cell proliferation assays. Human lung mast cells proliferation assays were performed in 96-well plates using four further HLMC donors. In brief, HLMC were cultured using the conditions described above and incubated for 24 h ($8\text{--}10 \times 10^4$ cells/well in 200 μ l). [^{3} H]-thymidine (37 kBq) was then added to each well, plates cultured for a further 48 h, and cells harvested. Thymidine incorporation was determined using the 1500 Tri Carbo, Liquid Scintillation analyser (Tri Carbo, Berks, UK).

Statistical analysis

The number of mast cells that expressed IL-13R α 1 on their surface, the number of cells in the bronchial biopsies that expressed IL-13R α 1 and the proportion of these cells that were colocalized to mast cells, were normally distributed and therefore described as the mean $\pm$ SEM. Fc ϵ RI expression, cell number and thymidine incorporation were log normally distributed and

described as geometric mean $\pm$ log SEM. Comparisons were made across groups of conditions by ANOVA and between conditions by *t*-tests. A value of $P < 0.05$ was taken as being statistically significant.

Results IL-13R α 1 expression

RT-PCR. A PCR product of the expected size, based on the published IL-13R α 1 (319 bp) coding sequence, was generated by RT-PCR from RNA purified from HLMC and HASM (Fig. 1A, B). DNA sequencing confirmed that these 319 bp PCR products encoded IL-13R α 1.

Flow cytometry. The mean $\pm$ SEM proportion of cells that expressed IL-13R α 1 on their surface was $82 \pm 9\%$ for HLMC ($n = 3$) and $29 \pm 16\%$ for HASM ($n = 4$) (Fig. 1C–E).

Immunohistochemistry. Examples of bronchial submucosal mast cells and ASM immunostained with IL13R α 1 and tryptase are shown in Fig. 2A–D. The number of IL-13R α 1+ cells in the bronchial submucosa was significantly increased in subjects with asthma vs controls (46 ± 4 vs 35 ± 2 cells/mm ^{2} , respectively; $P = 0.03$; both $n = 7$; Fig. 2E). The number of mast cells was very similar between asthmatic and control subjects (31 ± 3 vs 28 ± 1 cells/mm ^{2} , respectively; $P = 0.4$; both $n = 7$). The proportion of mast cells that expressed IL-13R α 1 was significantly increased in the asthmatic compared with controls subjects ($86 \pm 2\%$ vs $78 \pm 2\%$, respectively; $P = 0.015$; both $n = 7$). The number of IL-13R α 1+ cells in the bronchial submucosa of atopic subjects was not

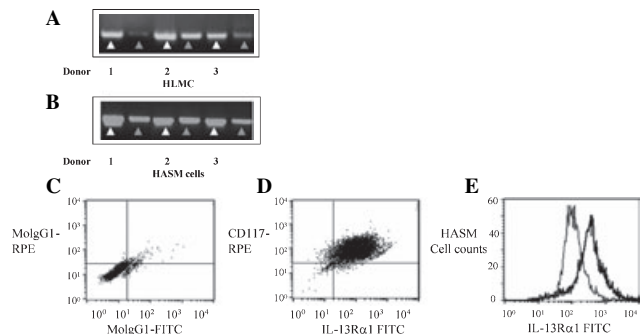


Figure 1. Interleukin-13R α 1 mRNA and surface expression by *ex vivo* human lung mast cells (HLMC) and human airway smooth muscle (HASM) PCR products generated by RT-PCR using IL-13R α 1 specific primers from purified RNA (A) HLMC (three donors) and (B) HASM (three donors). The white arrow refers to the positive control β -actin (350 bp) and the grey arrows refer to IL-13R α 1 (319 bp). Cell surface IL-13R α 1 expression by HLMC and HASM cells was assessed by flow cytometry. The example dot plots illustrate (C) HLMC dot plot for the mouse IgG1 double negative and (D) HLMC that were CD117+ and IL-13R α 1+. The example histogram represent (E) HASM that were IL-13R α 1+ (black line) plotted with the corresponding isotype control (grey line).

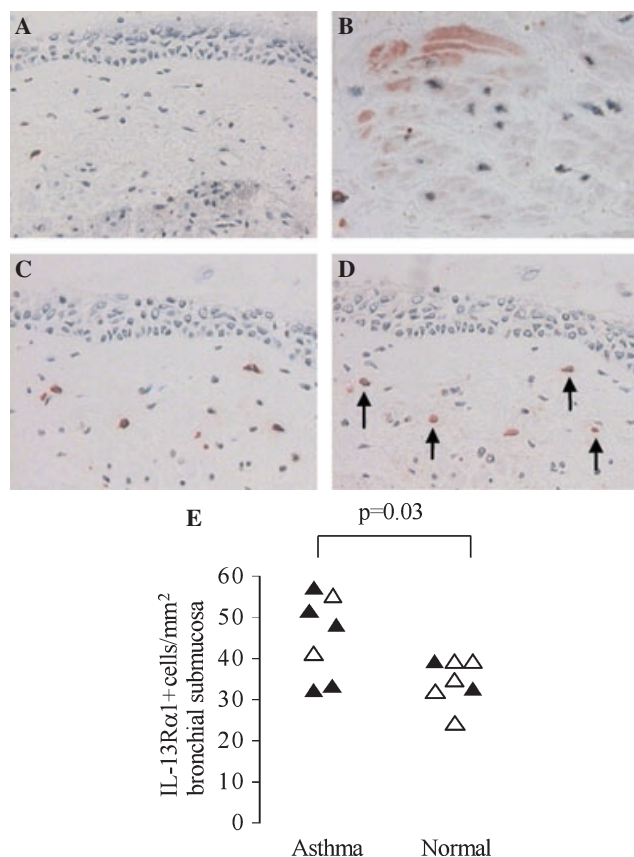


Figure 2. Interleukin-13R α 1 expression by mast cells in bronchial biopsies. Representative photomicrographs of a bronchial biopsy from a subject with asthma stained with (A) goat immunoglobulins as the control ($\times 200$) and (B) IL-13R α 1 illustrating expression by airway smooth muscle ($\times 400$). The sequential sections demonstrate in (C) tryptase + mast cells colocalized to (D) IL-13R α 1 + cells ($\times 200$). (E) The number of IL-13R α 1 + cells in the bronchial submucosa in subjects with asthma and healthy controls. Closed symbols – atopic; open symbols – nonatopic.

increased compared with nonatopic (43 ± 4 vs 39 ± 4 cells/mm ^{2} ; $P = 0.5$; both $n = 7$; Fig. 2E).

The Fc ϵ RI and CD117 expression and IL-6 release, following IL-13 priming IL-13 priming of HLMC for 5 days upregulated cell surface expression of Fc ϵ RI. HLMC Fc ϵ RI fluorescence intensity was significantly increased in IL-13 primed cells compared with those cultured in SCF alone (79 ± 0.09 vs 65 ± 0.08 , respectively; 22% increase; $P = 0.003$; $n = 6$; Fig. 3A). This IL-13-induced response was significantly inhibited by IL-13 neutralizing but not isotype control antibody ($P = 0.001$, $n = 4$; Fig. 3A). In contrast, CD117 expression was not upregulated by IL-13 priming and fluorescence intensity was similar in IL-13 primed cells compared with those cultured in SCF alone (144 ± 0.1 vs 139 ± 0.11 , respectively) and was not affected by either IL-13 neutralizing or isotype control antibody.

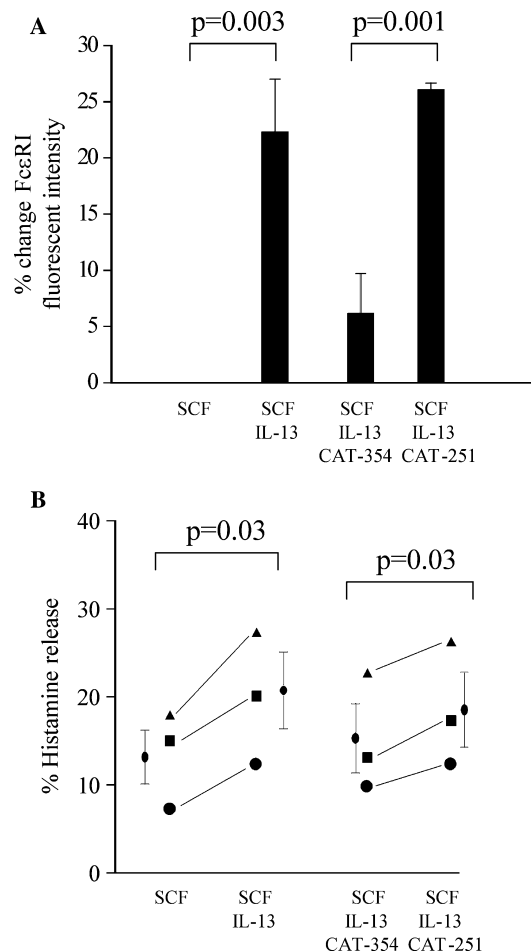


Figure 3. Human lung mast cells (HLMC) Fc ϵ RI upregulation and increased histamine release after interleukin-13 priming. (A) Bar graph of change in HLMC (six donors) Fc ϵ RI expression presented as geometric mean $\pm$ SEM percentage change in fluorescence intensity after IL-13 priming with SCF, in the presence or absence of IL-13 neutralizing antibody CAT-354 or isotype control CAT-251 compared with SCF alone. (B) Individual data points for percentage histamine release by HLMC following IgE/anti-IgE activation (three donors, each donor is represented by a different symbol) after IL-13 priming with the same conditions as for (A).

Human lung mast cells release of the mast cell survival factor IL-6 was not upregulated by IL-13 priming. Total IL-6 secretion was similar between IL-13 primed and SCF-alone treated HLMCs (14 ± 8 ng vs 19 ± 11 ng/ 10^6 HLMC, respectively; $P = 0.2$, $n = 4$) and was not affected by IL-13 neutralizing or isotype control antibody (20 ng/ 10^6 HLMC, respectively; $P = 0.3$).

Histamine release by IL-13 primed HLMC after IgE/anti-IgE activation

Histamine release was not increased above spontaneous secretion ($4.5 \pm 2.2\%$; $n = 3$) following incubation for 30 min with IL-13 (0.1–100 ng/ml) alone ($P = 1.0$) or in

combination with SCF ($P = 0.99$) for any of the concentrations of IL-13 tested. As expected IgE/anti-IgE activation significantly increased histamine release above spontaneous secretion ($14 \pm 1.0\%$; $P = 0.049$; $n = 3$), but this was not affected by incubation with IL-13 ($P = 0.53$). Histamine release in response to IgE/anti-IgE activation was significantly higher in HLMC primed for 5 days with IL-13 in combination with SCF than in cells treated with SCF-alone suggesting that IL-13 mediated increase in Fc ϵ RI expression had functionally relevant consequences ($13 \pm 3\%$ vs $20 \pm 4\%$, respectively; $56 \pm 10\%$ increase; $P = 0.03$; $n = 3$). IL-13 mediated enhanced histamine release was significantly inhibited by IL-13 neutralizing but not isotype control antibody ($15 \pm 4\%$ vs $19 \pm 4.3\%$; $P = 0.03$) (Fig. 3B).

IL-13 priming promotes HLMC proliferation, but not survival

Culture in media with SCF for 5 days did not induce HLMC proliferation [$(18 \pm 0.05) \times 10^3$ cells at day 0 vs $(22 \pm 0.13) \times 10^3$ cells at day 5; $P = 0.5$; $n = 6$]. In contrast, IL-13 priming induced HLMC proliferation [$(33 \pm 0.12) \times 10^3$ cells vs $(22 \pm 0.13) \times 10^3$ cells cultured in SCF alone; 50% increase; $P = 0.003$; $n = 6$; Fig. 4A]. The proliferative effect of IL-13 priming was reduced by IL-13 neutralizing antibody but not isotype control antibody ($P = 0.021$; $n = 3$; Fig. 4B). Proliferation was also assessed by measurement of thymidine incorporation compared with SCF alone (2143 ± 0.4 cpm vs 1936 ± 0.1 cpm, respectively; 11% increase; $P = 0.04$; $n = 4$). This increased thymidine incorporation induced by IL-13 priming could be decreased by IL-13 neutralizing but not isotype control antibody (1746 ± 0.4 cpm vs 2198 ± 0.4 cpm, respectively; $P = 0.032$; $n = 4$). In all of the proliferation experiments the purity of the HLMC were $>98\%$ before and after stimulation.

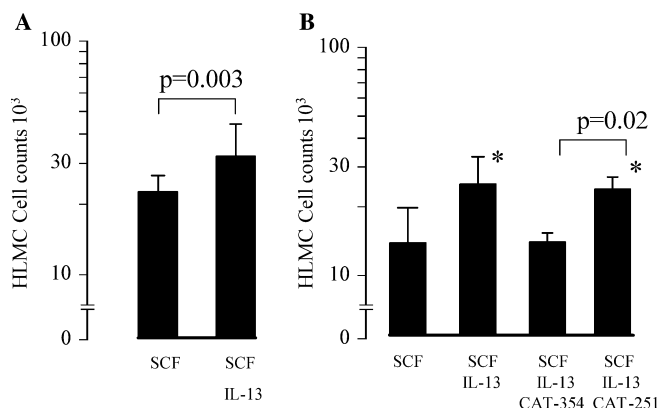


Figure 4. Human lung mast cells (HLMC) proliferation mediated by interleukin (IL)-13. HLMC number after (A) IL-13 priming (six donors) and (B) inhibition of this priming effect by the IL-13 neutralizing antibody (CAT-354) compared with isotype control (CAT-251) (three donors). Bar graphs represent the geometric mean $\pm$ SEM. * $P < 0.05$ (ANOVA).

IL-13 priming did not affect the proportion of live HLMC as measured by trypan blue (data not shown) and did not affect the proportion of HLMC exhibiting features of apoptosis compared with SCF alone ($9.5 \pm 2\%$; vs $10 \pm 1\%$; $P = 0.8$; $n = 3$ (data not shown)).

Discussion

We report for the first time that *ex vivo* HLMC and human mast cells in bronchial biopsies express the IL-13 receptor IL-13R α 1. IL-13 priming of mast cells for 5 days upregulated expression of the high affinity IgE receptor, Fc ϵ RI and enhanced HLMC proliferation without affecting mast cell survival. IL-13 did not result in an immediate increase in histamine release, but interestingly after IL-13 priming for 5 days histamine release in response to IgE/anti-IgE activation was upregulated suggesting that this functional response may have been mediated by increased Fc ϵ RI expression. An IL-13 specific neutralizing antibody, CAT-354, but not isotype control, inhibited all IL-13 induced responses. Together these findings demonstrate that HLMC express functional IL-13R α 1 and that secretion of IL-13 by mast cells or other cells may be an important cofactor in the activation and proliferation of lung mast cells.

In asthma IL-13 is elevated in bronchoalveolar lavage, bronchial biopsies and sputum (18–20). Preclinical studies show that IL-13 plays a central role in many of the pathophysiological abnormalities characterizing asthma (1) including development of eosinophilic airway inflammation, mucus hypersecretion and airway hyperresponsiveness. Several cell types have been described as important sources of IL-13 including T-lymphocytes (18, 19, 21), mast cells (14, 22), eosinophils (20, 23) and basophils (24). Importantly in asthma mast cells are the only inflammatory cell localized within the ASM bundle (11). The number of mast cells in the ASM bundle is positively correlated with the degree of airway hyperresponsiveness. Therefore IL-13 is upregulated in the airways of subjects with asthma and this cytokine has the potential to modulate the function of many cell types including the mast cell.

Prior to this study there was no direct evidence that primary human mast cells express IL-13R α 1. There is evidence to suggest that the human mast cell line (HMC-1) has a functional IL-13 receptor as IL-13 activation leads to increased expression of c-fos and intercellular adhesion molecule-1 (16, 17). However, HMC-1 is an immature mast cell line that does not express Fc ϵ RI limiting its value in investigating the effects of IL-13 priming on mast cell function (25). Literature on IL-13R α 1 expression by mast cells from other species is conflicting. IL-13R α 1 mRNA expression has been seen in one of two dog mastocytoma cell line (26) but not in murine bone marrow-derived mast cells

(27). Here we report several lines of evidence that demonstrate, for the first time, that human mast cells express IL-13R α 1. We found that HLMC express IL-13R α 1 mRNA. Moreover, IL-13R α 1 was highly expressed on the surface of HLMC. In bronchial biopsies we found that the proportion of mast cells that were co-localized to IL-13R α 1 was similar to the proportion of *ex vivo* human lung mast cells that expressed IL-13R α 1 on their surface. Interestingly the number of IL-13R α 1+ cells and the proportion of mast cells that expressed IL-13R α 1 was increased in asthma. Although atopy was more common in the group with asthma, IL-13R α 1 expression was not different in those subjects with or without atopy suggesting that the increased expression is likely to be related to presence of disease rather than atopy alone. Importantly in asthma this increase number of IL-13R α 1+ mast cells occurs in a microenvironment characterized by increased IL-13 expression. Therefore it is likely that mast cell priming by IL-13 may play an important role in asthma.

In this study we have explored the functional consequences of priming mast cells with IL-13. We found that IL-13 promotes proliferation, but not survival, of HLMC as evidenced by an increase in number and thymidine incorporation following IL-13 priming but without a change in the proportion of apoptotic mast cells or the concentration of IL-6 a cytokine that promotes mast cell survival (28). In addition to changes in proliferation we found that IL-13 priming of HLMC led to a 22% increase in Fc ϵ RI expression. Although this increase in Fc ϵ RI expression was relatively small it is likely to be biologically relevant as it was associated with a 56% increase in histamine release following Fc ϵ RI cross-linking with IgE/anti-IgE. Our view that these effects were mediated by IL-13 was strengthened by our ability to inhibit these effects using a specific IL-13 neutralizing antibody. Importantly, IL-13 did not result in an immediate increase in histamine release supporting the view that the increased histamine release observed after IL-13 priming for 5 days was mediated by increased Fc ϵ RI expression. In asthma mast cell expression of Fc ϵ RI in the bronchial submucosa is increased (29). Therefore this increased expression of Fc ϵ RI may in part be mediated by IL-13 either derived from other inflammatory cells or in an autocrine manner. Similarly, it is possible that IL-13 may be important in local proliferation of mast cells within the airway. One intriguing possible explanation for the increased number of mast cells within the ASM bundle and increased

hyperresponsiveness in asthma is that IL-13 released by these mast cells may promote their own local proliferation and activation, in concert with other local factors, through an autocrine mechanism.

The immunomodulatory effects of IL-13 on HLMC described here are broadly similar to the reported effects of IL-4. HLMC express both IL-13 and IL-4 (14). We found that IL-13 priming upregulated Fc ϵ RI expression in HLMC. This is consistent with the observation that priming of mature peripheral blood derived mast cells with IL-4 both led to induction of Fc ϵ RI expression and increased Fc ϵ RI mediated cytokine release (15, 30, 31). Indeed, Ochi *et al.* found that IL-4 priming increased IgE/anti-IgE mediated histamine release by 50% (15), which was similar to the effect we observed with IL-13. We found that IL-13 priming resulted in increased proliferation of HLMC. Similarly, IL-4 increased mast cell division of mature peripheral blood derived mast cells (31) and the number of gut derived mast cells (32). Our findings support the view that both IL-4 and IL-13 may exert important functional effects on mast cells in the asthmatic airway.

In conclusion, IL-13R α 1 expression was increased in asthma. Bronchial submucosal mast cells and *ex vivo* HLMC expressed IL-13R α 1. Activation of IL-13R α 1 with IL-13 mediated upregulation of Fc ϵ RI, and consequently Fc ϵ RI cross-linking led to induced histamine release and promoted cell proliferation. The IL-13 neutralizing antibody CAT-354 attenuated all IL-13 mediated effects. These findings support the view that targeting IL-13 may be beneficial in the treatment of allergic disease such as asthma.

Acknowledgments

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Conflict of interest

CB has received research grants (250 000) and consultancy fees (1500) from Cambridge Antibody Technology. Two of the authors (PM & RM) are employed by Cambridge Antibody Technology whose (potential) product was studied in the present work.

References

1. Wills-Karp M. Interleukin-13 in asthma pathogenesis. *Immunol Rev* 2004;**202**:175–190.
2. Oettgen H, Geha R. IgE regulation and roles in asthma pathogenesis. *J Allergy Clin Immunol* 2001;**107**:429–440.
3. Bochner BS, Klunk DA, Sterbinsky SA, Coffman RL, Schleimer RP. IL-13 selectively induces vascular cell adhesion molecule-1 expression in human endothelial cells. *J Immunol* 1995;**154**:799–803.
4. Woltmann G, McNulty CA, Dewson G, Symon FA, Wardlaw AJ. Interleukin-13 induces PSGL-1/P-selectin-dependent adhesion of eosinophils, but not neutrophils, to human umbilical vein endothelial cells under flow. *Blood* 2000;**95**:3146–3152.

5. Hershey G. IL-13 receptors and signaling pathways: An evolving web. *J Allergy Clin Immunol* 2003;**111**:677–690.
6. Wang I-M, Lin H, Goldman SJ, Kobayashi M. Stat-1 is activated by IL-4 and IL-13 in multiple cell types. *Molecular Immunology* 2004;**41**:873–884.
7. Laporte JC, Moore PE, Baraldo S, Jouvin MH, Church TL, Schwartzman IN et al. Direct Effects of Interleukin-13 on Signaling Pathways for Physiological Responses in Cultured Human Airway Smooth Muscle Cells. *Am J Respir Crit Care Med* 2001;**164**:141–148.
8. Murata T, Husain SR, Mohri H, Puri RK. Two different IL-13 receptor chains are expressed in normal human skin fibroblasts, and IL-4 and IL-13 mediate signal transduction through a common pathway. *Int Immunol* 1998;**10**:1103–1110.
9. Lordan JL, Bucchieri F, Richter A, Konstantinidis A, Holloway JW, Thorner M et al. Cooperative Effects of Th2 Cytokines and Allergen on Normal and Asthmatic Bronchial Epithelial Cells. *J Immunol* 2002;**169**:407–414.
10. Brightling CE, Bradding P. The re-emergence of the mast cell as a pivotal cell in asthma pathogenesis. *Curr Allergy Asthma Rep* 2005;**5**:130–135.
11. Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. *N Engl J Med* 2002;**346**:1699–1705.
12. Britten KM, Howarth PH, Roche WR. Immunohistochemistry on resin sections: a comparison of resin embedding techniques for small mucosal biopsies. *Biotech Histochem* 1993;**68**:271–280.
13. Sanmugalingam D, Wardlaw AJ, Bradding P. Adhesion of human lung mast cells to bronchial epithelium: evidence for a novel carbohydrate-mediated mechanism. *J Leukoc Biol* 2000;**68**:38–46.
14. Brightling CE, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID, Bradding P. Interleukin-4 and -13 expression is co-localized to mast cells within the airway smooth muscle in asthma. *Clin Exp Allergy* 2003;**33**:1711–1716.
15. Ochi H, De Jesus N, Hsieh F, Austen K, Boyce J. IL-4 and 5 prime human mast cells for different profiles of IgE-dependent cytokine production. *Proc Natl Acad Sci* 2000;**97**:10509–10513.
16. Nilsson G, Nilsson K. Effects of interleukin (IL)-13 on immediate-early response gene expression, phenotype and differentiation of human mast cells. Comparison with IL-4. *Eur J Immunol* 1995;**25**:870–873.
17. Tsang C, Wong C, IP W, Lam C. Synergistic effect of SCF and TNF-(alpha) on the upregulation of cell-surface expression of ICAM-1 on human leukemia mast cell line (HMC)-1 cells. *J Leukoc Biol* 2005;**78**:239–247.
18. Huang SK, Xiao HQ, Kleine-Tebbe J, Paciotti G, Marsh DG, Lichtenstein LM et al. IL-13 expression at the sites of allergen challenge in patients with asthma. *J Immunol* 1995;**155**:2688–2694.
19. Humbert M, Durham SR, Kimmitt P, Powell N, Assoufi B, Pfister R et al. Elevated expression of messenger ribonucleic acid encoding IL-13 in the bronchial mucosa of atopic and nonatopic subjects with asthma. *J Allergy Clin Immunol* 1997;**99**:657–665.
20. Berry MA, Parker D, Neale N, Woodman L, Morgan A, Monk P et al. Sputum and bronchial submucosal IL-13 expression in asthma and eosinophilic bronchitis. *J Allergy Clin Immunol* 2004;**114**:1106–1109.
21. Kotsimbos TC, Ernst P., Hamid QA. Interleukin-13 and interleukin-4 are co-expressed in atopic asthma. *Proc Assoc Am Physicians* 1996;**108**:368–373.
22. Burd PR, Thompson WC, Max EE, Mills FC. Activated mast cells produce interleukin-13. *J Exp Med* 1995;**181**:1373–1380.
23. Schmid-Grendelmeier P, Altnauer F, Fischer B, Bizer C, Straumann A, Menz G et al. Eosinophils Express Functional IL-13 in Eosinophilic Inflammatory Diseases. *J Immunol* 2002;**169**:1021–1027.
24. Li H, Sim TC, Alam R. IL-13 released by and localized in human basophils. *J Immunol* 1996;**156**:4833–4838.
25. Nilsson G, Blom T, Kusche-Gullberg M, Kjellen L, Butterfield JH, Sundstrom C et al. Phenotypic characterization of the human mast-cell line HMC-1. *Scand J Immunol* 1994;**39**:489–498.
26. Tang L. Molecular cloning of canine IL-13 receptor [alpha] chain ([alpha]1 and [alpha]2) cDNAs and detection of corresponding mRNAs in canine tissues. *Vet Immunol Immunopath* 2001;**79**:181–195.
27. Suzuki K, Nakajima H, Watanabe N, Kagami SI, Suto A, Saito Y et al. Role of common cytokine receptor gamma chain (gamma c)- and Jak3-dependent signaling in the proliferation and survival of murine mast cells. *Blood* 2000;**96**:2172–2180.
28. Yanagida M, Fukamachi H, Ohgami K, Kuwaki T, Ishii H, Uzumaki H et al. Effects of T-helper 2-type cytokines, interleukin-3 (IL-3), IL-4, IL-5, and IL-6 on the survival of cultured human mast cells. *Blood* 1995;**86**:3705–3714.
29. Humbert M, Grant JA, Taborda-Barata L, Durham SR, Pfister R, Menz G et al. High-affinity IgE receptor (Fcepsilon RI)-bearing cells in bronchial biopsies from atopic and nonatopic asthma. *Am J Respir Crit Care Med* 1996;**153**:1931–1937.
30. 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.
31. Kulka M, Metcalfe DD. High-resolution tracking of cell division demonstrates differential effects of TH1 and TH2 cytokines on SCF-dependent human mast cell production in vitro: correlation with apoptosis and Kit expression. *Blood* 2005;**105**:592–599.
32. Lorentz A, Bischoff SC. Regulation of human intestinal mast cells by stem cell factor and IL-4. *Immunol Rev* 2001;**179**:57–60.