

Inhibition of human interleukin-13-induced respiratory and oesophageal inflammation by anti-human-interleukin-13 antibody (CAT-354)

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Summary

Background Allergic asthma is a complex disorder characterized by local and systemic T helper type 2-cell responses such as the production of IL-13, a cytokine associated with the induction of airway hyper-responsiveness (AHR), chronic pulmonary eosinophilia, airway mucus overproduction and eosinophilic oesophagitis.

Objective Our study aimed to address the therapeutic potential of a human anti-human IL-13 IgG4 monoclonal antibody (CAT-354) in a murine model of respiratory and oesophageal inflammation induced by intratracheal human IL-13.

Methods BALB/c mice were treated on days 1 and 3 with CAT-354 (intraperitoneal injection), and human IL-13 was injected intratracheally on days 2 and 4. AHR to methacholine, airway eosinophilia in bronchoalveolar lavage fluid, histologic analysis of goblet cell metaplasia and oesophageal eosinophilia were evaluated.

Results Human IL-13 induced airway eosinophilia and goblet cell metaplasia in mice in a dose-dependent manner. Moreover, intratracheal dosing with 25 µg of human IL-13 was sufficient to induce AHR, goblet cell metaplasia and oesophageal eosinophilia. Pretreatment with CAT-354 significantly reduced AHR, airway eosinophilia and oesophageal eosinophilia.

Conclusion These results demonstrate that anti-human IL-13 (CAT-354) is a potential therapeutic treatment for allergic airway and oesophageal diseases.

Keywords allergy, asthma, cytokines, eosinophils, oesophagitis, IL-13

Submitted 2 February 2005; revised 14 April 2005; accepted 24 May 2005

Introduction

The incidence of allergic asthma in the western world is increasing. Airway hyper-responsiveness (AHR) and excessive mucus production by goblet cells are two of the principal causes of airway obstruction observed in asthma patients [1–4]. Atopic asthma is also characterized by local and systemic allergic inflammation, elevated serum IgE and chronic pulmonary eosinophilia. In a subset of patients and experimental systems in animals, allergic respiratory responses are associated with allergic eosinophilic inflammation in the oesophagus (termed eosinophilic oesophagitis, EE) [5]. Consequently, EE has recently been described as 'asthma of the oesophagus' [6].

Allergic inflammation is mediated by CD4⁺ T lymphocytes producing a type 2 cytokine repertoire [7–10]. Correspondingly, CD4⁺ T helper type 2 (Th2) cells are necessary for the induction of allergic asthma in murine models and Th2 cells, and their associated cytokines are increased in patients with allergic disorders [11–13].

Glucocorticoids remain the most effective anti-inflammatory therapeutics available in asthma treatment. The central mechanism of the efficacy of glucocorticoids is the inhibition of the synthesis of inflammatory mediators such as Th2 cytokines and chemokines [14]. Because glucocorticoids are often only partially effective and often have side-effects, research has focused on alternative therapeutic approaches targeting specific mediators [15]. Of the Th2 cytokines examined in preclinical trials, studies have convincingly demonstrated that IL-13 is a key mediator of allergic respiratory and oesophageal inflammation, and, as such, IL-13 represents an attractive target for intervention [16–18]. For example, intratracheal administration of murine IL-13 is sufficient to induce asthma-like changes in mice (AHR, goblet cell hyperplasia and leucocyte infiltration in the lungs) [17, 19–21]. Moreover, intratracheal IL-13 induces EE in a time frame that correlates with lung inflammation [18]. Furthermore, neutralization of IL-13 by the administration of a soluble IL-13 receptor (R) (e.g. IL-13Rα2-IgFc fusion protein) blocks the cardinal features of experimental asthma induced by ovalbumin (OVA) in mice [16, 19]. In other OVA-induced asthma models, treatment with a mutated IL-4 variant, able to compete with both IL-4 and IL-13 signalling, inhibits several aspects of allergic lung inflammation [22, 23].

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In this study, we aimed to first determine whether intratracheal delivery of human IL-13 was able to induce experimental respiratory and oesophageal disease. Second, we aimed to study the efficacy of CAT-354, a human monoclonal antibody directed against human IL-13, isolated and optimized using antibody display technologies [24], in an *in vivo* model of human IL-13-induced inflammatory responses. Our results establish that intratracheal administration of human IL-13 is sufficient to induce leucocyte infiltration in the lung, AHR, goblet cell metaplasia and EE in mice. Furthermore, we demonstrate that CAT-354 significantly reduces many of these parameters. Thus, CAT-354 may have potential therapeutic utility for human allergic diseases.

Materials and methods

Intratracheal cytokine delivery and antibody treatment

BALB/c mice (8–12 weeks old) were obtained from the Jackson Laboratory (Bar Harbor, ME, USA) and maintained under specific pathogen-free conditions. Cytokines were delivered intratracheally as described [18]. Briefly, mice (20–25 g) were anaesthetized by intraperitoneal (i.p.) injection of 400 µg of Ketaject (Ketamine HCL; Phoenix Pharmaceutical Inc., St Joseph, MO, USA). The tongue was gently extended, and a pipette tip was inserted directly into the mouth of anaesthetized mice, followed by the delivery of the cytokines. Recombinant murine IL-13 (10 µg) or human IL-13 (Peptrotech; Rock Hill, NJ, USA) (10 or 25 µg) in 25 µL of saline or saline alone was delivered. Mice were pretreated on days 1 and 3 with antibodies, and human IL-13 was administered intratracheally on days 2 and 4. The human monoclonal anti-human IL-13 IgG4 antibody CAT-354 (Cambridge Antibody Technology, Granta Park, Cambridge, UK) and isotype control antibody CAT-001 (Cambridge Antibody Technology) were delivered i.p. at doses of 1, 10 or 30 mg/kg. Airway responsiveness was assessed on day 5 and mice were killed on day 6.

Airway hyper-responsiveness

Airway responsiveness to methacholine was assessed 24 h after the final IL-13 delivery (day 5) in conscious, unrestrained mice by barometric plethysmography as previously reported [25], using apparatus and software supplied by Buxco (Troy, NY, USA). This system yields a dimensionless parameter termed enhanced pause (Penh) that can be used to monitor airway function empirically. Briefly, mice were placed in the chamber, and exposed initially to aerosolized saline to establish a baseline responsiveness, and then sequentially to increasing concentrations of methacholine (concentrations in solution ranging from 3.125 to 50 mg/mL) delivered through an inlet into the chamber for 2 min. The average Penh was determined over a period of 5 min after each dose was administered.

Bronchoalveolar lavage fluid collection and analysis

Mice were killed by carbon dioxide inhalation 48 h after the final IL-13 delivery (Day 6). Immediately thereafter, the trachea was cannulated. The lungs were lavaged two times

with 0.75 mL phosphate-buffered saline (PBS) containing 1% fetal calf serum (FCS) and 0.5 mmol/L ethylenediamine tetraacetic acid. Lysis of red blood cells was carried out using RBC lysis buffer (Sigma, St Louis, MO, USA) according to the manufacturer's recommendations. The bronchoalveolar lavage fluid (BALF) recovered was centrifuged, and cell pellets were resuspended in 200 µL PBS 1% FCS. Total cell numbers per lung were counted with a haemocytometer. Differential cell counts were determined from cytopspins stained with Giemsa-Diff-Quick (Dade Diagnostics of P.R. Inc., Aguada, PR, USA).

Histological analysis

Following BALF collection, the lung and oesophagus were fixed in formalin solution 10% neutral buffer (Sigma) and then embedded in paraffin. Lung sections (2–3 µm) were stained with periodic acid-Schiff (PAS) for goblet cell metaplasia analysis. The histologic mucus index (HMI) provides a percentage of epithelial cells that are PAS-positive cells. Oesophageal sections were also immunostained with antiserum against mouse eosinophil major basic protein (anti-MBP) [18]. Briefly, endogenous peroxidases in the tissues were quenched with 0.3% hydrogen peroxide in methanol followed by pepsin digestion for 5 min and by non-specific protein blocking with normal goat serum for 30 min at room temperature. Tissue sections were then incubated with rabbit anti-MBP (a kind gift from Dr James Lee, Mayo Clinic, Scottsdale, AZ, USA) (1 : 10 000) for 1 h at 4 °C, followed by a 1 : 1000 dilution of biotinylated goat anti-rabbit IgG secondary antibody for 30 min at room temperature. Negative controls were assessed by replacing the primary antibody with normal rabbit serum to check endogenous biotin and peroxidase activity. Then an avidin–peroxidase complex (Vector Laboratories, Burlingame, CA, USA) was used for 30 min. Quantification of the immunoreactive cells was carried out by using a video-assistant integrated computer software program (Image Pro Plus 4.1; Media Cytometrics, Silver Spring, MD, USA). The eosinophil levels are expressed as cells/mm². In the lung, eosinophil levels were assessed in the peribronchial and perivascular regions.

Statistical analysis

The significance of differences between experimental groups was analysed using ANOVA or Student *t*-test. Values are reported as mean ± SEM. *P*-values <0.05 were considered statistically significant.

Results

Intratracheal human interleukin-13 induces lung inflammation

To determine whether human IL-13 could induce pulmonary responses in mice, inflammatory cell recruitment to the lung in response to human IL-13 (10 or 25 µg) was compared with that elicited by murine IL-13 (10 µg). Repeat administration of murine IL-13 to the lungs of BALB/c mice on days 1 and 3 significantly increased the total cell recovery from BALF 48 h after the second IL-13 challenge. In contrast, modest in-

creases in total cell numbers in human IL-13-treated animals did not reach statistical significance (Fig. 1a). Differential cell counts indicated that the changes in the total cell number were accounted for by increases in the representation of neutrophils and eosinophils as a proportion of total cells in IL-13-treated relative to saline-treated animals. The percentages of eosinophils and neutrophils were $1.2 \pm 0.3\%$ and $1.6 \pm 1\%$, respectively, in saline-treated mice, $11 \pm 6\%$ and $22.8 \pm 16\%$, respectively, in murine IL-13-treated mice and $9.6 \pm 6\%$ and $9.6 \pm 8\%$, respectively, in mice receiving $25 \mu\text{g}$ of human IL-13. Administration of $10 \mu\text{g}$ of human IL-13 did not modify BALF cell composition (0.3% and 0.6% , respectively) (Fig. 1b). Correspondingly, the total number of eosinophils in BALF in mice treated with murine-IL-13 (9472 ± 2948) or $25 \mu\text{g}$ of human IL-13 (5221 ± 2614) was significantly elevated relative to the saline-treated animals

(242 ± 142). However, increases in the numbers of neutrophils did not reach statistical significance (Fig. 1c). There was no significant difference between eosinophil or neutrophil numbers in mice treated with $10 \mu\text{g}$ of human IL-13 (Fig. 1c). Total numbers of macrophages or lymphocytes were not significantly affected in any of the IL-13 treatment groups when compared with the saline-treated mice. Together, these data suggest that human IL-13 may elicit similar pulmonary responses to murine IL-13 in the mouse, albeit at a lower potency with respect to inflammatory cell recruitment.

Human interleukin-13-induced lung eosinophilia is blocked by anti-human interleukin-13 (CAT-354)

This model of human IL-13-dependent lung inflammation allowed the investigation of the therapeutic potential of an

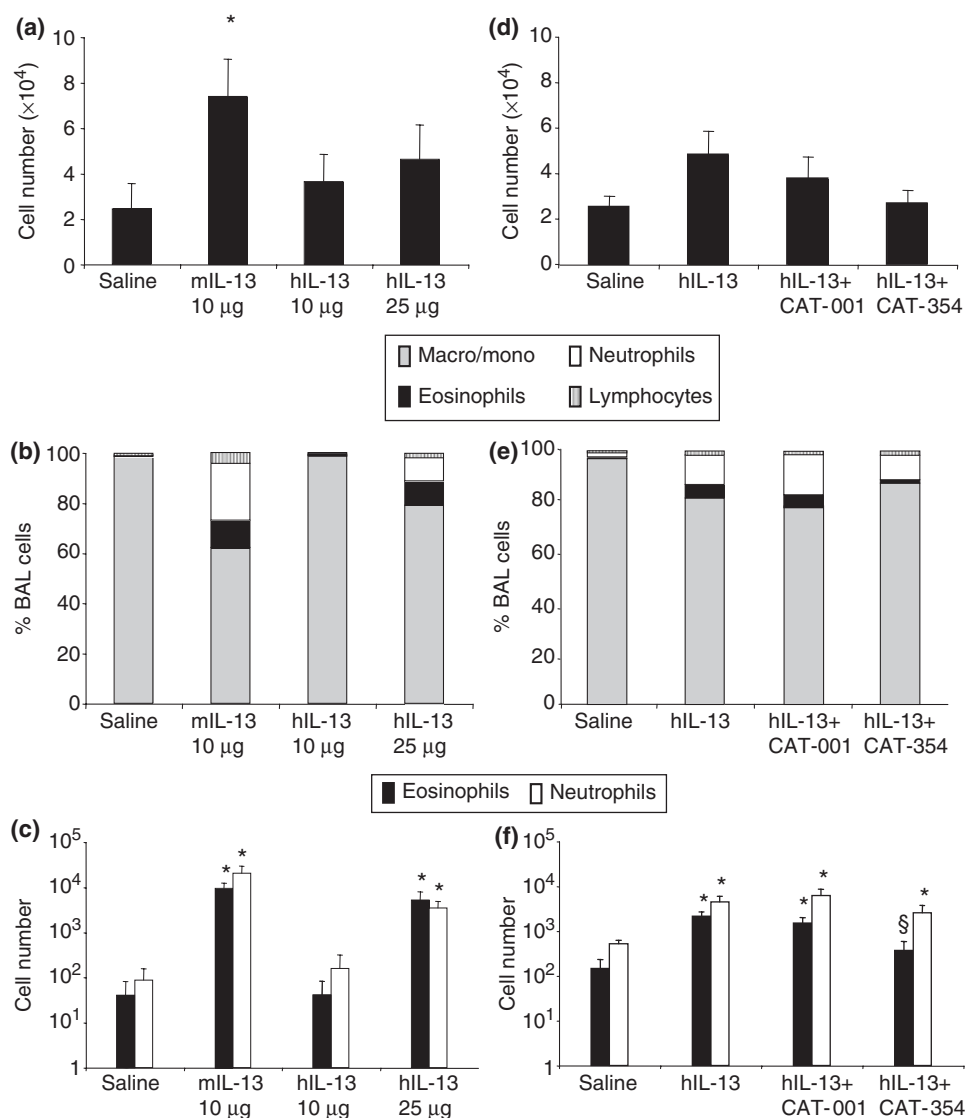


Fig. 1. Effect of anti-human-IL-13 antibody on human IL-13-induced eosinophil recruitment to the lungs. Mice were treated with either two doses of intratracheal saline, mL-13 ($10 \mu\text{g}$) hIL-13 ($10 \mu\text{g}$) or hIL-13 ($25 \mu\text{g}$) 48 h apart (left panel), or were pretreated on days 0 and 2 with saline, control antibody CAT-001 (1 mg/kg) or anti-human-IL-13 CAT-354 (1 mg/kg) by intraperitoneally followed by two doses of intratracheal saline or hIL-13 at $25 \mu\text{g}$ (right panel). Bronchoalveolar lavage (BAL) cells were collected and analysed 48 h after the last intratracheal exposure. The total cell numbers are shown (a and d). The percentage of each cell type is shown (b and e). Total BAL fluid numbers of eosinophils (black) and neutrophils (white) are presented (c and f). In these representative experiments, error bars present the mean $\pm$ SEM; $n = 5-8$ mice (* $P < 0.05$ vs. saline group, § $P < 0.05$ vs. control antibody (CAT-001) group).

anti-human IL-13 monoclonal antibody (CAT-354) that does not recognize murine IL-13. Using a similar protocol, 25 µg of human IL-13 was administered on days 1 and 3. CAT-354 and/or an isotype control antibody (CAT-001) were delivered i.p. at 1, 10 and 30 mg/kg on days 2 and 4. In preliminary studies, we found that the doses of 10 and 30 mg/kg of control antibody decreased the asthma parameters in part (data not shown). Therefore, we studied CAT-354 at a dose of 1 mg/kg. Figure 1d–f shows the effect of CAT-354 treatment on BALF cell numbers and composition. In agreement with the previous experiment, increases in total cell numbers in BALF recovered 48 h after the second human IL-13 challenge in human IL-13-treated relative to saline-treated animals did not reach statistical significance (Fig. 1d). However, as before there was a significant increase in total eosinophil numbers (Fig. 1f) and elevated representation of eosinophils and neutrophils as a proportion of total cells (Fig. 1e). Systemically administered CAT-354 (1 mg/kg), but not the isotype control antibody, significantly inhibited eosinophil recruitment to the lung in response to human IL-13 (Fig. 1f). Decreases in neutrophil numbers were not significant (Fig. 1f). Together, these results indicate that CAT-354 treatment significantly reduces eosinophil recruitment to the lung.

Airway hyper-responsiveness is significantly decreased by anti-human-interleukin-13 antibody (CAT-354) treatment

We first established that human IL-13 could induce AHR in the mouse. Airway responsiveness to inhaled methacholine was measured 24 h after the second administration of human IL-13 (25 µg) and compared with that of mice receiving murine IL-13. Repeated administration of both human ($AUC_{0-50} = 3.44 \pm 0.62$; $P < 0.05$) and murine ($AUC_{0-50} = 3.68 \pm 0.58$; $P < 0.01$) IL-13 significantly increased airway reactivity to methacholine, as determined by the area under the log methacholine dose Penh response curve (AUC_{0-50}) when compared with saline-treated animals ($AUC_{0-50} = 1.25 \pm 0.31$) (Fig. 2a). Furthermore, there was no significant difference between the responsiveness of human IL-13-treated animals when compared with those mice receiving murine IL-13 (10 µg).

We next investigated the efficacy of the anti-human IL-13 monoclonal antibody against human IL-13-dependent AHR. Using a similar protocol, CAT-354 and/or an isotype control antibody (CAT-001) were delivered i.p. at 1 mg/kg 24 h prior to each IL-13 challenge (Fig. 2b). As in the pilot experiment, administration of human IL-13 induced significant AHR ($AUC_{0-50} = 3.07 \pm 0.37$; $P < 0.01$) compared with saline-treated animals ($AUC_{0-50} = 1.23 \pm 0.13$). CAT-354 (1 mg/kg) ($AUC_{0-50} = 1.74 \pm 0.28$; $P < 0.05$) but not the isotype control antibody ($AUC_{0-50} = 3.55 \pm 0.51$) treatment significantly inhibited the response to human IL-13, reducing AHR to levels similar to saline-treated mice (Fig. 2b). These results indicate that pretreatment with an anti-human IL-13 significantly decreases hIL-13-induced AHR in mice.

Anti-human-interleukin-13 antibody (CAT-354) does not affect human interleukin-13-induced goblet cell metaplasia in the lungs

To determine whether human IL-13 could induce goblet cell hyperplasia in the mouse, mucus-secreting cells were identi-

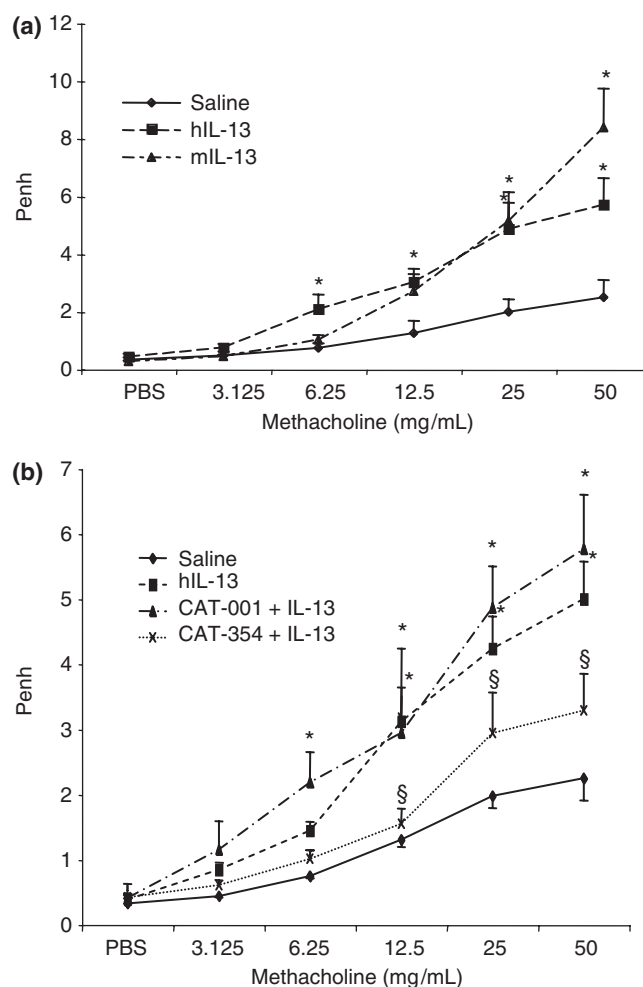


Fig. 2 Effect of an anti-human-IL-13 treatment on intratracheal human IL-13-induced airway hyper-responsiveness (AHR). (a) Intratracheal human IL-13-induced AHR. Mice were treated with two doses of intratracheal saline, mL-13 (10 µg) or hIL-13 (25 µg) separated by 48 h. (b) Mice were pretreated on days 0 and 2 with saline, control antibody CAT-001 (1 mg/kg) or anti-human-IL-13 CAT-354 (1 mg/kg) intraperitoneally followed by two doses of intratracheal saline or hIL-13 (25 µg). Methacholine sensitivity (Penh) was assessed 24 h following the last intratracheal treatment using non-invasive barometric whole-body plethysmography. In these two representative experiments, error bars present the mean $\pm$ SEM $n = 7-8$ mice (* $P < 0.05$ vs. saline group, § $P < 0.05$ vs. control antibody group).

fied in lung sections from the studies described above by histological analysis. In human IL-13-treated mice, the number of mucus-producing cells in large airways was dramatically increased (Figs 3b–d). In contrast, mucus-secreting cells were difficult to find in lung sections from saline-treated control mice (Fig. 3a). HMI, equivalent to the linear percent of epithelium positive for mucus, in saline, murine IL-13 (10 µg), human IL-13 (25 µg) and human IL-13 (10 µg)-treated mice was 0.6 ± 0.3 , 53.5 ± 6.8 , 33.6 ± 9.8 and 19.4 ± 6.0 , respectively (Fig. 3e). These data indicate that human IL-13 can induce goblet cell metaplasia in the mouse lung in a dose-dependent manner. We next investigated the ability of CAT-354 treatment to reduce human IL-13-induced goblet cell metaplasia. A decrease in HMI in animals receiving CAT-354 (1 mg/kg) treatment did not reach statistical significance (Figs 3f–i). The HMI scores in saline, human IL-13 (25 µg), human IL-13 25 µg with CAT 001 or

human IL-13 25 µg and CAT-354 were 0.7 ± 0.4 , 42.0 ± 6.2 , 41.5 ± 8.6 and 27.3 ± 9.5 , respectively (Fig. 3j). These results indicate that CAT-354 pretreatment does not significantly reduce human IL-13-dependent goblet cell metaplasia in this model.

Oesophageal eosinophilia is significantly improved by treatment with CAT-354

To determine whether human IL-13 could induce eosinophil recruitment to the oesophagus, eosinophil numbers were compared in oesophageal sections from the studies described above by histological analysis. While eosinophils were difficult to find in the oesophagus of saline-treated mice, mice that received murine IL-13 (10 µg) or human IL-13 (10 and 25 µg) showed clear oesophageal eosinophilia (Figs 4a–d). Quantification revealed a significant increase in eosinophil numbers in oesophageal sections of mice treated with murine IL-13 (10 µg) (7.5 ± 2.1) or human IL-13 (25 µg) (9.1 ± 2.4) compared with saline-treated mice (1.0 ± 0.4). The apparent increase in cell numbers in mice receiving 10 µg of human IL-13 (2.7 ± 0.9) was not significant (Fig. 4e). These results indicate that intratracheal administration of human IL-13 can induce eosinophil recruitment to the oesophagus.

We next investigated the ability of CAT-354 to reduce eosinophil recruitment to the oesophagus in response to

repeated administration of human IL-13 (25 µg). CAT-354 (1 mg/kg) treatment reduced eosinophil recruitment, whereas the isotype control antibody CAT-001 had no effect (Figs 4f–i). The numbers of oesophageal eosinophils following CAT-354 treatment were significantly reduced (1.7 ± 0.6) when compared with CAT-001 (6.0 ± 1.8) (Fig. 4j). These results indicate that CAT-354 significantly inhibits eosinophil recruitment to the oesophagus.

Discussion

In this report, we establish that intratracheal administration of human IL-13 to the airways of mice results in an asthma-like phenotype and oesophagitis, recapitulating previous studies in which murine IL-13 was administered. Using this model, we then demonstrate the powerful prophylactic activity of CAT-354, an anti-human IL-13 monoclonal antibody that does not recognize murine IL-13. CAT-354 (at a dose of 1 mg/kg) specifically inhibited IL-13-induced eosinophil recruitment to the lung and oesophagus, as well as the development of AHR.

This study was designed to test the potential effects of an anti-human IL-13 antibody in a mouse model; as such, the quantity of human IL-13 administered was a first concern. We thus injected the lowest dose of human IL-13 needed to

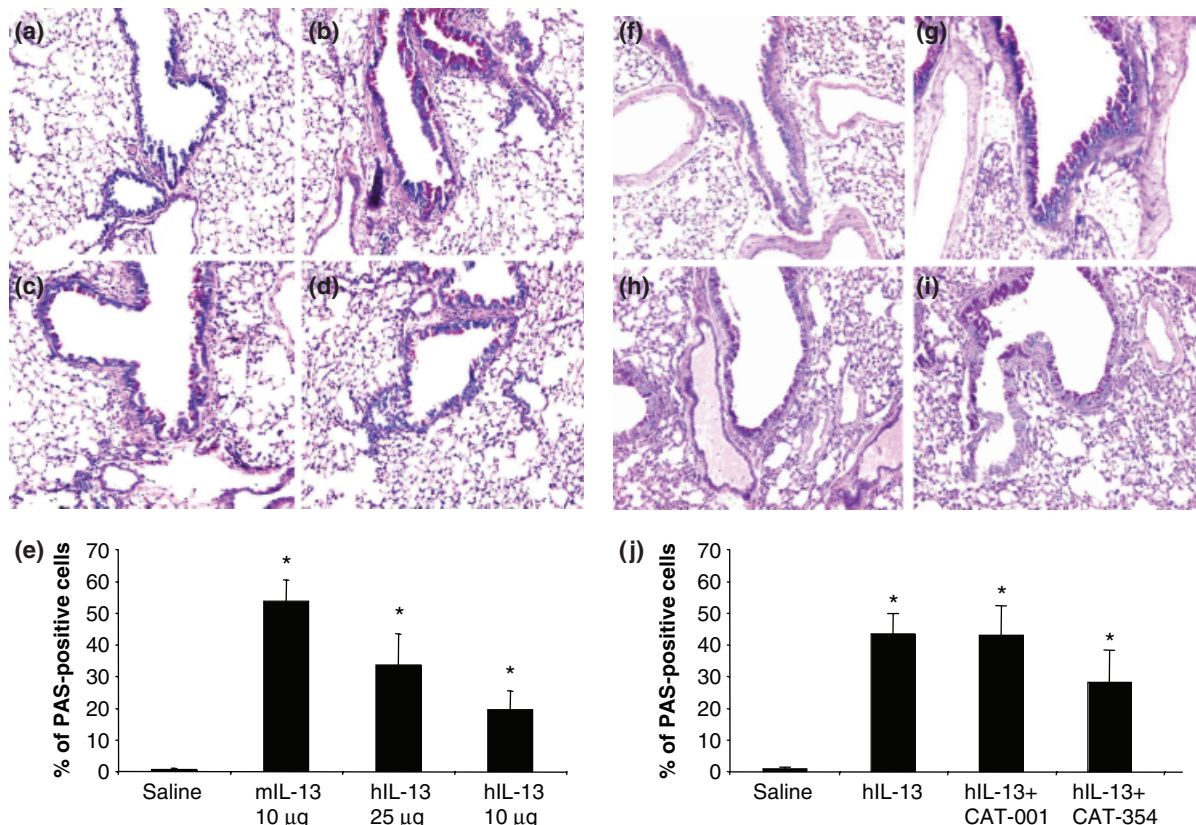


Fig. 3. Effect of human anti-IL-13 antibody on IL-13-induced goblet cell metaplasia in large airways. Lungs were collected 48 h after the last intratracheal IL-13 treatment. Lung sections were stained with periodic acid-Schiff (PAS) to detect neutral mucins. Mice were treated with saline (a), mL-13 10 µg (b), hIL-13 25 µg (c) or hIL-13 10 µg (d). Mice were treated intraperitoneally and intratracheally with saline (f), saline intraperitoneally and hIL-13 25 µg intratracheally (g); mice were treated with intraperitoneal CAT-001 (1 mg/kg) and hIL-13 25 µg (h), with CAT-354 (1 mg/kg) and hIL-13 25 µg (i). Results represent the percentage of PAS-positive epithelial cells (e and j). In these representative experiments, data are expressed as mean $\pm$ SEM; $n = 7$ –8 mice (* $P < 0.05$ vs. saline group).

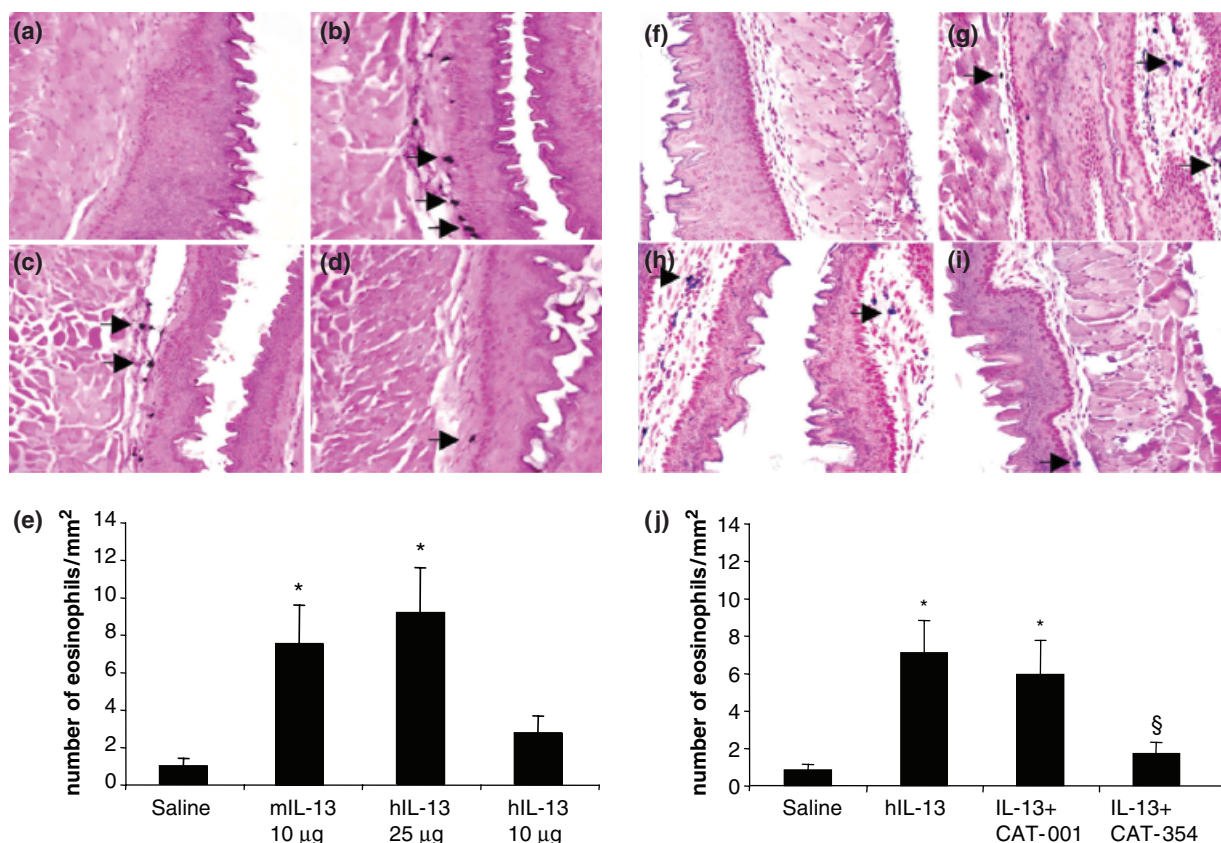


Fig. 4. Effect of anti-human IL-13 antibody on human IL-13-induced eosinophilic oesophagitis. The oesophagus was harvested 48 h after the last intratracheal treatment and oesophageal sections were stained with anti-MBP. Mice were treated with saline (a), mIL-13 10 µg (b), hIL-13 25 µg (c) or hIL-13 10 µg (d). Mice were treated with intraperitoneal (IP) and intratracheal saline (f), with i.p. saline and hIL-13 (25 µg) intratracheally (g), i.p. CAT-001 (1 mg/kg) and hIL-13 (25 µg) (h) or with i.p. CAT-354 (1 mg/kg) and intratracheal hIL-13 (25 µg) (i). Results represent the number of eosinophils (mean ± SEM $n = 7-8$) present in the oesophagus per mm² of representative experiments ($n = 3$) (e and j). * $P < 0.05$ vs. saline group, § $P < 0.05$ vs. control antibody group.

induce disease pathology. A dose of 25 µg was found to be sufficient to significantly induce a number of pathological endpoints (eosinophilia, AHR, goblet cell metaplasia and EE). However, IL-13 (25 µg) was not sufficient to increase the total cell count significantly. Indeed, higher doses of human IL-13 may increase the total cell count and improve the statistical significance of the total leucocyte levels.

Traditionally, the inflammatory component of asthmatic disease, specifically lung eosinophilia, has been associated with the development of AHR [5, 21, 26]. However, the association between eosinophils and AHR is not clear; AHR has been associated with numerous phenomena other than eosinophilia. For example, inflammatory mediators and their receptors expressed by other activated leucocytes, epithelial cells, fibroblasts and sensory neurons have been associated with AHR in mice [27–37]. Moreover, the failure of anti-IL-5 antibodies to significantly alter AHR in asthmatic subjects, despite marked reductions in both blood and sputum eosinophil levels, has brought the functional association into question [38, 39]. However, more recent analysis has shown that the level of lung eosinophils was only modestly affected by anti-IL-5 treatment [40]. Using a model similar to that described in this paper, Kibe et al. [14] found that glucocorticoids administration ablated murine IL-13-dependent lung eosinophilia, but it did not affect the development

of AHR, suggesting that IL-13 may play a key role in other, steroid-unresponsive mechanisms that contribute to the development of AHR. A further study demonstrating that AHR but not eosinophilia were blocked in a rodent model of allergen challenge by administration of an IL-13 antagonist supports this hypothesis [16].

While glucocorticoids remain the most effective anti-inflammatory therapeutics available in asthma treatment [15], the magnitude of reduction in AHR varies significantly among patients. Furthermore, dosing is limited by mechanism-associated side-effects. If our model reflects steroid-insensitive mechanisms of AHR in human disease, inhibition of IL-13 signalling, for example with CAT-354 represents an attractive therapeutic approach.

An unanticipated feature of this study was the failure of CAT-354 to inhibit IL-13-dependent goblet cell hyperplasia significantly. Significantly, goblet cell metaplasia was the only feature of asthma, observed following repeated administration of the lower dose of human IL-13 (10 µg) in this model. The direct effects of IL-13 (25 µg) on epithelial cells may explain the fact that CAT-354 systemically does not reverse the metaplasia of the goblet cells induced by the direct contact of the cytokine on the IL-13 receptor-bearing epithelial cells of the lung [41]. Muc5AC expression and goblet cell metaplasia develop in epithelial airways cells following

IL-13 treatment [42]. Additionally, in primary bronchial epithelial cell cultures, IL-13 induces effects on goblet cell differentiation and MUC5AC expression [41, 43]. Thus, IL-13 appears capable of acting directly on epithelial cells of the airways (inducing goblet cell metaplasia and hyperplasia). However, it has also been shown that IL-13 induces mucin production indirectly by stimulating epidermal growth factor receptors and by activating neutrophils *in vivo* [44]. In our model, it is likely that the indirect effects of IL-13-induced goblet cell metaplasia are blocked by the CAT-354 antibody. However, the direct effect of IL-13 on epithelial cells may not be completely blocked when the antibody is administered systematically. It is also important to consider that CAT-354 does not block the endogenous mIL-13 that may be induced by human IL-13, either directly or indirectly.

Interestingly, in our model, high concentrations of the isotype control antibody CAT-001 (10 and 30 mg/kg) inhibited hIL-13-mediated lung inflammation. It is interesting to note that high-dose intravenous γ -globulin has been shown to be useful for the treatment of asthma [45]. Fc γ R and IFN- γ have been implicated in the repression of allergic inflammation following high-dose IgG treatment [46]. IgG may increase the IFN- γ production (via Fc γ R) and thus Th1 reactivity [46, 47]. Moreover, Th1-promoting antigen-presenting cell activity of lung macrophages has been shown to inhibit airway eosinophilia by an IFN- γ -dependent mechanism [47]. In order to study the specific effect of human IL-13 blockade, we studied CAT antibody treatment at different doses. It is interesting to note that the decrease observed with the specific antibody (at 10 and 30 mg/kg) was similar (in magnitude) to that observed with the control antibody at the same concentration. In both cases, a residual inflammation was observed that was comparable with that observed with the specific antibody CAT-354 at the 1 mg/kg dose. These results suggest that the higher doses of CAT-354 antibody (2 or 5 mg) may reduce inflammation, but not more efficiently than the 1 mg/kg dose. As such, at the dose of 1 mg/kg, there was a specific effect of CAT-354 compared with control antibody CAT-001. We therefore considered the inhibition observed in CAT-354 (1 mg/kg)-treated mice as a specific inhibition of lung inflammation.

Taken together, we provide evidence that human IL-13 is active in mice and is able to induce AHR, goblet cell metaplasia and respiratory and oesophageal eosinophilia. The induction of experimental EE by human IL-13 extends previous studies demonstrating that intratracheal murine IL-13 can induce EE [18]. Combined with clinical studies showing elevated production of IL-13 in oesophageal tissue from human EE [48], these results draw increasing attention to the potential role of IL-13 in human EE. We also demonstrate that anti-human-IL-13 (CAT-354) blocks certain aspects of IL-13-induced inflammation and may have a potential role as a therapeutic agent for IL-13-associated disorders. It is interesting to note that while eosinophilia and AHR were inhibited, mucus production and neutrophilia were not significantly inhibited by CAT-354. This suggests that the dose of IL-13 required to induce these pathological features is significantly lower than that required to induce eosinophilia in AHR. As such, an anti-IL-13 therapeutic may have variable activity on diverse IL-13-induced responses.

Acknowledgements

The authors wish to thank Andrea Lippelman for assistance with the preparation of this paper, and Dominique Brandt for precious advice on statistical analysis. This work was supported in part by Cambridge Antibody Technology and by NIH grants R01 AI42242 (M. E. R.), R01 AI45898 (M. E. R.), P01 HL-076383-01 (M. E. R.) and R24 DK064403.

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