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IL-13 directly promotes esophagus production of CCL11 and CCL24 and the migration of eosinophils

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Abstract

Background—Eosinophilic esophagitis (EE) is a clinico-pathological defined esophageal disorder that is characterized by eosinophil migration into esophageal tissues. There is growing support for EE being an allergic disease and for a contribution of Th2-associated cytokines in disease pathogenesis. The respiratory system has been shown to be critical in driving the development of EE in animal models. However, the mechanisms underlying the recruitment of eosinophils into the esophagus remain unclear.

Objective—We sought to investigate the influence of Th2-associated cytokines on the production of eosinophil-specific chemokines from the esophagus directly.

Methods—In order to eliminate the potential involvement of the lung, we utilized isolated esophageal rings. These were treated in vitro with IL-4 or IL-13 and the expression and production of CCL11 and CCL24 determined.

Results—Our data demonstrates that IL-13 is a potent and direct inducer of both CCL11 and CCL24 production from the esophagus, as is IL-4 also. The expression of CCL11 precedes CCL24 by several hours but is then diminished over time, as well as at high concentrations of IL-13. We demonstrate that there is an up-regulation of the inhibitory IL-13 receptor, IL-13Rα2 but that IL-13Rα1 remains unaltered. Esophagus rings isolated from STAT6$^{-/-}$ mice were unable to produce CCL11 or CCL24 upon IL-13 treatment. Lastly, we demonstrate that esophageal production of CCL11 and CCL24 upon IL-13 stimulation is sufficient to promote eosinophil migration.

Conclusions—IL-13 is capable of directly stimulating esophageal tissue to produce eosinophil-attracting chemokines and drive eosinophil migration.

Keywords

Esophagus; eosinophil; IL-13; chemokine; eotaxin; STAT6; esophagitis

Introduction

Eosinophilic esophagitis (EE) is an inflammatory gastrointestinal disease of the esophagus that is increasing in prevalence in both children and adults [1]. A critical aspect of clinical diagnosis of EE, versus reflux-associated gastroesophageal disease, is a determination of the presence and frequency of eosinophils within the esophageal tissues [1]. There is an increased incidence of EE in atopic individuals [2,3] and EE patients demonstrate frequent

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reactivity to both food and aeroallergens [2-5]. As such, there is a growing appreciation that EE represents an allergic disorder, similar to asthma, eczema or food allergy. Early investigations into the nature of the immune responses underlying EE in patients utilized candidate gene expression profiles to demonstrate that the expression of IL-4 and IL-13 were unchanged within the esophageal tissue [6]. Instead, this study demonstrated an increase in IL-5, as well as mast cell tryptase. In addition, CCL11 (eotaxin-1) was decreased, CCL24 (eotaxin-2) was increased and CCL26 (eotaxin-3) was unaltered. The authors concluded that EE was predominantly mediated via an IL-5-selective, CCL24-driven mechanism. However, more recent studies have reported significant up-regulation of CCL26 at the gene and protein level [7], as well as a unique expression profile of mast cell and chemotactic genes that included modest increases in both CCL11 and CCL24. Interestingly, while previous work using peripheral blood mononuclear cells from EE patients had shown IL-13 to be enhanced upon stimulation [8], the mRNA for IL-13 was not elevated in this study. One possibility is that IL-13 is not expressed directly within the esophagus but is aspirated from the lung or nasal tissues, or alternatively, is produced by an early infiltrating cell, as has been shown to occur for eosinophils themselves [9].

Experimental models of EE have demonstrated an important requirement for antigen exposure in the respiratory tract. Mishra et al. demonstrated that eosinophil accumulation and epithelial cell hyperplasia occurred after respiratory antigen exposure but not by oral or gastric exposure [10]. Similarly, administration of recombinant mouse IL-13 to the trachea was sufficient to promote a dose-dependent increase in esophageal eosinophils [11]. Human IL-13 also elicited similar effects in mice and specific blockade of IL-13, using an anti-human IL-13 antibody, ablated responses [12]. The effects of IL-13 have been suggested to be mediated via IL-5 since IL-5$^{-/-}$ mice were refractory to IL-13 induced experimental EE [11]. Despite the evidence supporting the lung as an important site of antigen-triggered crosstalk to the esophagus, the mechanisms through which eosinophils might be specifically recruited to the esophagus tissue in EE remain unclear.

In this study, we investigated the ability of the esophagus to produce eosinophil attractant chemokines, namely CCL11 and CCL24 in the mouse, in the absence of respiratory influences. Our findings show that IL-13 can potently and directly promote the esophagus to express and release CCL11 and CCL24. This occurs via a STAT6 dependent mechanism, since tissues from STAT6$^{-/-}$ mice are unable to respond. Importantly, we demonstrate that IL-13 activation of the esophagus is sufficient to promote eosinophil migration, and so we propose that IL-13 may be directly contributing to the chemokine-mediated attraction of eosinophils to the esophagus in EE.

Methods

Animals

4-8 week old BALB/c (female, Taconic Farms), C57Bl/6 (female, Jackson Labs), STAT6$^{-/-}$ (female, Jackson Labs) or NJ.1638 mice (female, obtained from Dr James Lee, Mayo Clinic, Arizona) were housed under specific-pathogen free conditions. The Northwestern University Animal Care and Use Committee approved all procedures and treatments.

Esophagus Ring Preparation

Mice were euthanized and the esophagus from mouth to stomach was carefully dissected under sterile conditions. The tissue was cut into three 1cm long segments that were cultured in 1mL of culture media (RPMI-1640 media containing 10% fetal calf serum, 1 mmol/L sodium pyruvate, 2 mmol/L L-glutamine, 0.05 mmol/L 2-mercaptoethanol, 100 U/mL

penicillin and 100 g/mL streptomycin) in a 24 well tissue culture plate. Each well represented the esophagus from an individual animal.

Real-time RT-PCR

Total RNA was isolated using QIAGEN RNeasy kit and cDNA was synthesized using an iScript cDNA synthesis kit (Bio-Rad). Gene expression was determined by real-time PCR using an ABI 7500 Thermal cycler (Applied Biosystems) and specific Taqman probes (Applied Biosystems) for each gene of interest. Expression of β-actin was utilized as a housekeeping gene for analysis of changes in cycle threshold values. The fold induction above control was determined based upon changes in the delta cycle threshold values.

ELISA

The production of CCL11 and CCL24 were determined using ELISA (R&D Systems), according to the manufactures instructions. The levels of IL-13Rα2 were determined using anti-mouse IL-13Rα2 antibodies and mIL-13Rα2-Fc (R&D Systems) as a standard, according to protocols provided by Dr Gurjit Hershey at Cinicinatti Childrens Hospital, OH [13]

Eosinophil Chemotaxis

Purified eosinophils were isolated from the blood of IL-5 transgenic mice (NJ.1638), as previously described [14], and assessed in vitro using transwell migration by chemotaxis. 2×10^5 eosinophils were placed above wells containing esophagus rings treated with or without IL-13 for 48 hours. The number of eosinophils that migrated into the lower chamber was determined after 30 minutes. In order to confirm the migrated cells as eosinophils, cytospins of the cells were performed and stained with DiffQuick (Baxter Healthcare). Antibodies against CCL11 or CCL24 were obtained from R&D Systems and administered to the lower chambers 1h before addition of eosinophils to the upper chamber.

Statistics

Statistics were performed on GraphPad Prism 4 software using Mann-Whitney rank sum test or 2-tailed Student's *t* test to determine significance, as appropriate.

Results

IL-13 promotes esophageal production of CCL11 and CCL24

The concept that responses to antigens or Th2-cytokines in the lung could promote the accumulation of eosinophils into the esophagus is well supported experimentally [10,11,15] but fails to explain the specific localization of these cells to the esophagus. We hypothesized that the esophagus itself may be responsible for producing eosinophil-specific chemoattractants and so investigated the expression and production of CCL11 (eotaxin-1) and CCL24 (eotaxin-2) in response to IL-13, an important Th2-associated mediator of downstream allergic responses, such as mucus production in the airway [16]. Importantly, IL-13-associated genes have been shown to be specifically up-regulated in esophageal biopsies from eosinophilic esophagitis patients [17]. In order to eliminate any possible involvement of the nasal tissues or the lung, we utilized eosinophilic rings that were exposed to IL-13 in vitro. We also utilized the whole esophagus from each individual animal in each well, in order to avoid potential differences between proximal or distal portions of the tissue. The expression of CCL11 and CCL24 were determined by real-time RT-PCR. ELISA was used to also determine the secretion of the chemokines. Figure 1 shows that expression of both CCL11 (Figure 1A) and CCL24 (Figure 1B) at 48 hours were statistically increased by IL-13 stimulation. The enhanced gene transcription was also associated with increased

secretion of CCL11 (Figure 1C) and CCL24 (Figure 1D), although there was evidence of a down-regulation at high doses of IL-13 and the peak of responsiveness appeared to be approximately 5ng/mL. The up-regulation in both chemokines was at doses of IL-13 greater than 0.1-0.5ng per esophagus, which is more than 1000 fold less than the doses that were needed to induce eosinophilic esophagitis in vivo when administered via the trachea [11].

CCL11 and CCL24 expression exhibit different kinetics

We next performed a time course analysis to determine the rate of expression for CCL11 and CCL24 in response to IL-13. 5ng/mL of IL-13 was used to stimulate the expression of CCL11 and CCL24. Interestingly, expression of CCL11 (Figure 2A) occurred rapidly, with a peak of expression within 6 hours while CCL24 (Figure 2B) was considerably slower and did not peak until 48 hours after IL-13 stimulation. However, the expression of CCL11 was sustained and remained elevated at 48 hours also.

IL-13 promotes specific expression of IL-13Rα2 but not IL-13Rα1 in the esophagus

IL-13 is known to regulate it's effects via the IL-4Rα/IL-13Rα1/IL-13Rα2 family of receptors where IL-4Rα and IL-13Rα1 form a binding and signaling-competent complex and IL-13Rα2 acts as either a membrane form that is incapable of activation, in part due to its truncated cytoplasmic tail, or as a soluble inhibitor of IL-13 [13]. We investigated if there was evidence of regulation of the IL-13 receptors within the esophagus upon IL-13 exposure. Figure 3 demonstrates that IL-13Rα1 is unchanged by any of the IL-13 doses used (Figure 3A) but that IL-13Rα2 expression is enhanced by relatively low doses of IL-13 and reaches a peak at 5ng/mL (Figure 3B). IL-13Rα2 was also elevated within the supernatants from IL-13 treated esophagus rings (Figure 3C) This indicates that there is both activation and regulation of the IL-13-driven pathways occurring within the esophagus.

IL-4 also induces up-regulation of CCL11 and CCL24

IL-4 and IL-13 share many similarities in terms of receptor signaling but can often promote difference responses at the biological function level (reviewed by Wills-Karp and Finkelman [18]). Therefore, we next sought to investigate if IL-4 was also sufficient to promote the eosophagus to express and secrete CCL11 or CCL24. Figure 4 demonstrates that IL-4 does promote statistically significant expression of CCL11 (Figure 4A) and CCL24 (Figure 4B). The secretion of CCL11 (Figure 4C). and CCL24 (Figure 4D) were also significantly altered by IL-4. This data indicates that, in addition to IL-13, IL-4 is also able to increase the expression of CCL11 and CCL24 and secretion of CCL11 and CCL24 protein.

IL-13-driven production of esophageal CCL11 and CCL24 is STAT6 dependent

STAT6 has been shown to be an important downstream regulator in modulating the effects of IL-13 [16]. However, an alternative pathway, whereby early growth response-1 (Egr-1) can mediate effects of IL-13 via an ERK1/2-dependent but STAT6-independent pathway has been shown [19,20]. We sought to investigate the transcriptional pathway through which IL-13 enhanced CCL11 and CCL24 by utilizing $STAT6^{-/-}$ mice. The eosophagus from these mice did not show any enhancement in expression of CCL11 (Figure 5A) or CCL24 (Figure 5B) in response to IL-13. Similarly, the secretion of CCL11 was statistically lower in $STAT6^{-/-}$ esophagus cultures (Figure 5C) while CCL24 was also lower, albeit below significance ($p=0.11$ by Students t-test). Oddly, while CCL24 secretion was enhanced to statistical significance in the experiments shown in Figure 1D, this was not reached in the case of the wildtype tissues in these experiments. This may reflect differences in responsiveness between BALB/c and C57/BL6, the genetic background control strain for the $STAT6^{-/-}$ mice, since differences in the IL-13-driven signaling events between these two strains have been shown [21].

Esophageal stimulation with IL-13 supports eosinophil migration

In order to test if the response of the esophageal tissue to IL-13 was functionally capable of attracting eosinophils, we utilized transwell migration assays. Using eosinophils from the NJ.1638 mice, which transgenically overexpress IL-5, we were able to obtain a highly purified population of activated eosinophils. These were used to investigate migration towards chambers containing IL-13-stimulated esophagus rings. As shown in Figure 6, esophagus rings that were stimulated with 5ng/mL IL-13 for 48 hours were able to significantly support the attraction of the eosinophils into the lower chamber while chambers containing only IL-13 did not. Importantly, treatment with neutralizing antibodies (5μg/ml) against either CCL11 or CCL24 has only a small effect on the degree of eosinophil migration while a combination of both significantly inhibited the response. This data demonstrates that IL-13 supports eosinophil migration by stimulating the production of eosinophilic chemoattractants by the esophagus tissue. In particular, our data shows that CCL11 and CCL24 are both important in promoting eosinophil movement.

Discussion

EE is an increasingly prevalent disease and the mechanisms that regulate the pathophysiology remain elusive. Significantly, EE is being recognized as an allergic disease and both children and adults patients have been shown to have reactivity towards both aeroallergens and food allergens [3,4]. There is also convincing evidence that EE is associated with Th2-type cytokine responses, including IL-4 and IL-13 [8]. As such, the pathogenesis of EE appears to be divergent from other esophageal disorders, such as gastrointestinal reflux. In this regard, it has shown that the responsiveness of patients to gastrointestinal reflux therapy actually negatively correlates with the eosinophil frequency in the esophagus [22].

Experimental models of eosinophilic esophagitis have demonstrated a close connection between the respiratory system and the esophagus. In studies comparing antigen exposure via the lung, mouth or intestine, only the antigen exposure in the lung was able to promote increases in the numbers of eosinophils within the esophagus [10]. Similarly, the same group of researchers were able to show that intratracheal IL-13 was sufficient to induce esophageal eosinophilia [11]. This compelling evidence, supported by clinical observations, suggests that the lung may be an important site of allergen triggered responses but fails to explain how eosinophils are recruited into the esophagus. While the trachea and esophagus are closely situated within the body, there is no evidence to support an actual cooperative sharing of mediators between the tissues. With this concept in mind, we sought to investigate whether the esophagus was capable of producing eosinophil chemoattractants. Since patients with EE have been shown to exhibit an IL-13-associated pattern of gene expression within their esophagus tissue [17], we hypothesized that IL-13 might be an important stimuli. Our data now demonstrates that the esophagus itself is able to rapidly and potently respond to IL-13 by producing the eosinophilic chemokines CCL11 and CCL24. While CCL26 has also been shown to be an important chemoattractant in humans and is up-regulated in the tissues of EE patients [7], mice lack CCL26 and yet still exhibit eosinophil recruitment to the esophagus. As such, CCL11 and CCL24 are the critical signals for recruitment of eosinophils in mice. Indeed, $CCL11^{-/-}$ mice show significantly reduced esophageal eosinophil numbers in experimental EE [11]. These chemokines may be produced by several possible cells within the structure of the esophagus, including epithelial cells, endothelial cells and smooth muscle cells, as has been shown in the airway [23].

Interestingly, it has been previously shown that the generation of IL-13-induced esophageal eosinophilia is actually dependent upon IL-5, since $IL\text{-}5^{-/-}$ mice failed to mount eosinophilic responses to intratracheal IL-13 [11]. Our data demonstrates that IL-13 alone can promote

esophageal production of eosinophilic chemokines and that this does not require IL-5. We were unable to detect any IL-5 production from the IL-13-stimulated esophagus (data not shown). IL-5 has been shown to be vital for the maturation of eosinophils from bone marrow, their activation and for their ability to respond and migrate towards chemoattractants [24]. As such, the absence of esophageal eosinophils in the IL-5$^{-/-}$ mice may relate to the inability of eosinophils to be sufficiently mature or be activated to respond to chemoattracts. Our data would support an alterative model whereby IL-13 regulates the production of the chemoattractants from the esophageal tissue and IL-5 is necessary for priming the eosinophils for responsiveness to these signals.

We have demonstrated that higher doses of IL-13 actually suppress the production of CCL11 and that this might be regulated via up-regulation of IL-13Rα2. Conversely, the production of CCL11 is dependent upon STAT6, which is a critical regulator of the downstream signaling cascade from the IL-4Rα/IL-13Rα1 complex [25]. It has been shown that increases in gene expression of IL-13Rα2 directly increase the release, without affecting the membrane bound levels [13] and we also show that IL-13 increased the levels of IL-13Rα2 within the supernatants from esophagus rings. As such, our data would support an IL-13 dependent up-regulation of eosinophil chemoaattractants at low doses via IL-4Rα/IL-13Rα1 and STAT6 but then a transition towards suppression at high doses due to increases in the inhibitory form of its own receptor that may then reduce the cellular responses. One of the important clinical features of EE is that there are frequent periods of relapse and remittance and it is an intriguing concept to imagine that such relapses and remittance episodes may be, at least in part, due to the esophageal responses directly, including up-regulation of IL-13Rα2. Recently, recombinant IL-13Rα2 has been shown to reduce mucus production from human bronchial epithelial cells, while anti-IL-13Rα2 actually increased this response [26]. Therefore, this receptor appears to be a vital component in the responsiveness to IL-13 and a possible therapeutic target for allergic airway disease. Our results suggest that such therapeutics may be also useful in treating EE.

Finally, our data demonstrates that IL-13-treated esophagus is sufficient to promote eosinophil recruitment in the absence of any respiratory tract involvement. As such, we have defined the esophagus as being capable of directly regulating eosinophil recruitment, rather than eosinophils being driven into that tissue as a result of respiratory responses. The lung or nasal tissue could be a critical source of the IL-13, in part due to the presence of resident or recruited Th2 T cells and, in support of this, recent studies have demonstrated that T cells are required for the development of experimental EE [27].

Importantly, it has been demonstrated that allergic diseases, particularly airway disease in adults but also elevated sensitivity to foods in children, may actually precedes the development of EE [28], implying that allergic sensitization may be occurring at distant sites. This sensitization could explain the initial events that generate IL-13 producing T cells. However, several other cell types have been shown to also produce IL-13, including mast cells[29] and eosinophils themselves [9]. Eosinophils have also been shown to express IL-4 under inflammatory conditions [30]. Therefore, the initial recruitment of activated eosinophils to the esophagus could be setting in place an amplification loop whereby local production of IL-4 and IL-13 support production of more eosinophilic chemoattractants.

One caveat to our studies is that, despite observing robust eosinophil migration towards the esophageal rings, we failed to detect eosinophilic recruitment into the tissues. It is possible that, while the esophagus is capable of producing the chemoattractants, other processes are required to facilitate eosinophil entry. The eosinophils migrating in response to the esophageal rings do not experience endothelial adhesion and migration, which may be one necessary event for portal to the tissues.

In conclusion, we have described a previously unappreciated direct role of the esophagus in regulating the expression of eosinophilic chemoattractants upon exposure to Th2 cytokines that supports eosinophil migration. In contrast to previous conclusions, this occurs in the absence of any respiratory influence. Mechanistically, the esophagus produces both CCL11 and CCL24 in response to low amounts of IL-13 via a STAT6 dependent process. In support of this mechanism, IL-4, which also drives STAT6-dependent responses, also elicits similar effects to IL-13. We demonstrate that CCL11 precedes CCL24 in production but that high doses of IL-13 inhibit the production of either chemokine. We propose that this regulation is likely the result of changes in the balance between the STAT6-activating IL-4Rα/IL-13Rα1 complex and the inhibitory IL-13Rα2 molecule. This work demonstrates that the esophagus is an important and direct regulator of eosinophil-associated responses.

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References

1. Furuta GT, Liacouras CA, Collins MH, Gupta SK, Justinich C, Putnam PE, Bonis P, Hassall E, Straumann A, Rothenberg ME. Eosinophilic esophagitis in children and adults: a systematic review and consensus recommendations for diagnosis and treatment. Gastroenterology. 2007; 133:1342–63. [PubMed: 17919504]
2. Assa'ad A. Eosinophilic esophagitis: association with allergic disorders. Gastrointestinal endoscopy clinics of North America. 2008; 18:119–32. x. [PubMed: 18061106]
3. Roy-Ghanta S, Larosa DF, Katzka DA. Atopic characteristics of adult patients with eosinophilic esophagitis. Clin Gastroenterol Hepatol. 2008; 6:531–5. [PubMed: 18304887]
4. Plaza-Martin AM, Jimenez-Feijoo R, Andaluz C, Giner-Munoz MT, Martin-Mateos MA, Piquer-Gibert M, Sierra-Martinez JI. Polysensitization to aeroallergens and food in eosinophilic esophagitis in a pediatric population. Allergologia et immunopathologia. 2007; 35:35–7. [PubMed: 17338901]
5. Sugnanam KK, Collins JT, Smith PK, Connor F, Lewindon P, Cleghorn G, Withers G. Dichotomy of food and inhalant allergen sensitization in eosinophilic esophagitis. Allergy. 2007; 62:1257–60. [PubMed: 17711545]
6. Gupta SK, Fitzgerald JF, Kondratyuk T, HogenEsch H. Cytokine expression in normal and inflamed esophageal mucosa: a study into the pathogenesis of allergic eosinophilic esophagitis. Journal of pediatric gastroenterology and nutrition. 2006; 42:22–6. [PubMed: 16385249]
7. Blanchard C, Wang N, Stringer KF, Mishra A, Fulkerson PC, Abonia JP, Jameson SC, Kirby C, Konikoff MR, Collins MH, Cohen MB, Akers R, Hogan SP, Assa'ad AH, Putnam PE, Aronow BJ, Rothenberg ME. Eotaxin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. J Clin Invest. 2006; 116:536–47. [PubMed: 16453027]
8. Straumann A, Bauer M, Fischer B, Blaser K, Simon HU. Idiopathic eosinophilic esophagitis is associated with a T(H)2-type allergic inflammatory response. J Allergy Clin Immunol. 2001; 108:954–61. [PubMed: 11742273]
9. Schmid-Grendelmeier P, Altznauer F, Fischer B, Bizer C, Straumann A, Menz G, Blaser K, Wuthrich B, Simon HU. Eosinophils express functional IL-13 in eosinophilic inflammatory diseases. J Immunol. 2002; 169:1021–7. [PubMed: 12097410]
10. Mishra A, Hogan SP, Brandt EB, Rothenberg ME. An etiological role for aeroallergens and eosinophils in experimental esophagitis. J Clin Invest. 2001; 107:83–90. [PubMed: 11134183]
11. Mishra A, Rothenberg ME. Intratracheal IL-13 induces eosinophilic esophagitis by an IL-5, eotaxin-1, and STAT6-dependent mechanism. Gastroenterology. 2003; 125:1419–27. [PubMed: 14598258]

12. Blanchard C, Mishra A, Saito-Akei H, Monk P, Anderson I, Rothenberg ME. Inhibition of human interleukin-13-induced respiratory and oesophageal inflammation by anti-human-interleukin-13 antibody (CAT-354). Clin Exp Allergy. 2005; 35:1096–103. [PubMed: 16120093]
13. Daines MO, Tabata Y, Walker BA, Chen W, Warrier MR, Basu S, Hershey GK. Level of expression of IL-13R alpha 2 impacts receptor distribution and IL-13 signaling. J Immunol. 2006; 176:7495–501. [PubMed: 16751396]
14. Pero RS, Borchers MT, Spicher K, Ochkur SI, Sikora L, Rao SP, Abdala-Valencia H, O'Neill KR, Shen H, McGarry MP, Lee NA, Cook-Mills JM, Sriramarao P, Simon MI, Birnbaumer L, Lee JJ. Galphai2-mediated signaling events in the endothelium are involved in controlling leukocyte extravasation. Proc Natl Acad Sci U S A. 2007; 104:4371–6. [PubMed: 17360531]
15. Mishra A, Hogan SP, Brandt EB, Rothenberg ME. IL-5 promotes eosinophil trafficking to the esophagus. J Immunol. 2002; 168:2464–9. [PubMed: 11859139]
16. Kuperman DA, Huang X, Koth LL, Chang GH, Dolganov GM, Zhu Z, Elias JA, Sheppard D, Erle DJ. Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. Nature medicine. 2002; 8:885–9.
17. Blanchard C, Mingler MK, Vicario M, Abonia JP, Wu YY, Lu TX, Collins MH, Putnam PE, Wells SI, Rothenberg ME. IL-13 involvement in eosinophilic esophagitis: transcriptome analysis and reversibility with glucocorticoids. J Allergy Clin Immunol. 2007; 120:1292–300. [PubMed: 18073124]
18. Wills-Karp M, Finkelman FD. Untangling the complex web of IL-4- and IL-13-mediated signaling pathways. Science signaling. 2008; 1:pe55. [PubMed: 19109238]
19. Cho SJ, Kang MJ, Homer RJ, Kang HR, Zhang X, Lee PJ, Elias JA, Lee CG. Role of early growth response-1 (Egr-1) in interleukin-13-induced inflammation and remodeling. The Journal of biological chemistry. 2006; 281:8161–8. [PubMed: 16439363]
20. Lee PJ, Zhang X, Shan P, Ma B, Lee CG, Homer RJ, Zhu Z, Rincon M, Mossman BT, Elias JA. ERK1/2 mitogen-activated protein kinase selectively mediates IL-13-induced lung inflammation and remodeling in vivo. J Clin Invest. 2006; 116:163–73. [PubMed: 16374521]
21. Hirota JA, Ask K, Fritz D, Ellis R, Wattie J, Richards CD, Labiris R, Kolb M, Inman MD. Role of STAT6 and SMAD2 in a model of chronic allergen exposure: a mouse strain comparison study. Clin Exp Allergy. 2009; 39:147–58. [PubMed: 19032363]
22. Ruchelli E, Wenner W, Voytek T, Brown K, Liacouras C. Severity of esophageal eosinophilia predicts response to conventional gastroesophageal reflux therapy. Pediatr Dev Pathol. 1999; 2:15–8. [PubMed: 9841701]
23. Li D, Wang D, Griffiths-Johnson DA, Wells TN, Williams TJ, Jose PJ, Jeffery PK. Eotaxin protein and gene expression in guinea-pig lungs: constitutive expression and upregulation after allergen challenge. Eur Respir J. 1997; 10:1946–54. [PubMed: 9311484]
24. Wardlaw AJ. Eosinophil trafficking in asthma. Clinical medicine (London, England). 2001; 1:214–8.
25. Kaplan MH, Schindler U, Smiley ST, Grusby MJ. Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. Immunity. 1996; 4:313–9. [PubMed: 8624821]
26. Tanabe T, Fujimoto K, Yasuo M, Tsushima K, Yoshida K, Ise H, Yamaya M. Modulation of mucus production by interleukin-13 receptor alpha2 in the human airway epithelium. Clin Exp Allergy. 2008; 38:122–34. [PubMed: 18028464]
27. Mishra A, Schlotman J, Wang M, Rothenberg ME. Critical role for adaptive T cell immunity in experimental eosinophilic esophagitis in mice. Journal of leukocyte biology. 2007; 81:916–24. [PubMed: 17194734]
28. Simon D, Marti H, Heer P, Simon HU, Braathen LR, Straumann A. Eosinophilic esophagitis is frequently associated with IgE-mediated allergic airway diseases. J Allergy Clin Immunol. 2005; 115:1090–2. [PubMed: 15867873]
29. 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; 33:1711–6. [PubMed: 14656359]
30. Nonaka M, Nonaka R, Woolley K, Adelroth E, Miura K, Okhawara Y, Glibetic M, Nakano K, O'Byrne P, Dolovich J, et al. Distinct immunohistochemical localization of IL-4 in human

inflamed airway tissues. IL-4 is localized to eosinophils in vivo and is released by peripheral blood eosinophils. J Immunol. 1995; 155:3234–44. [PubMed: 7673736]

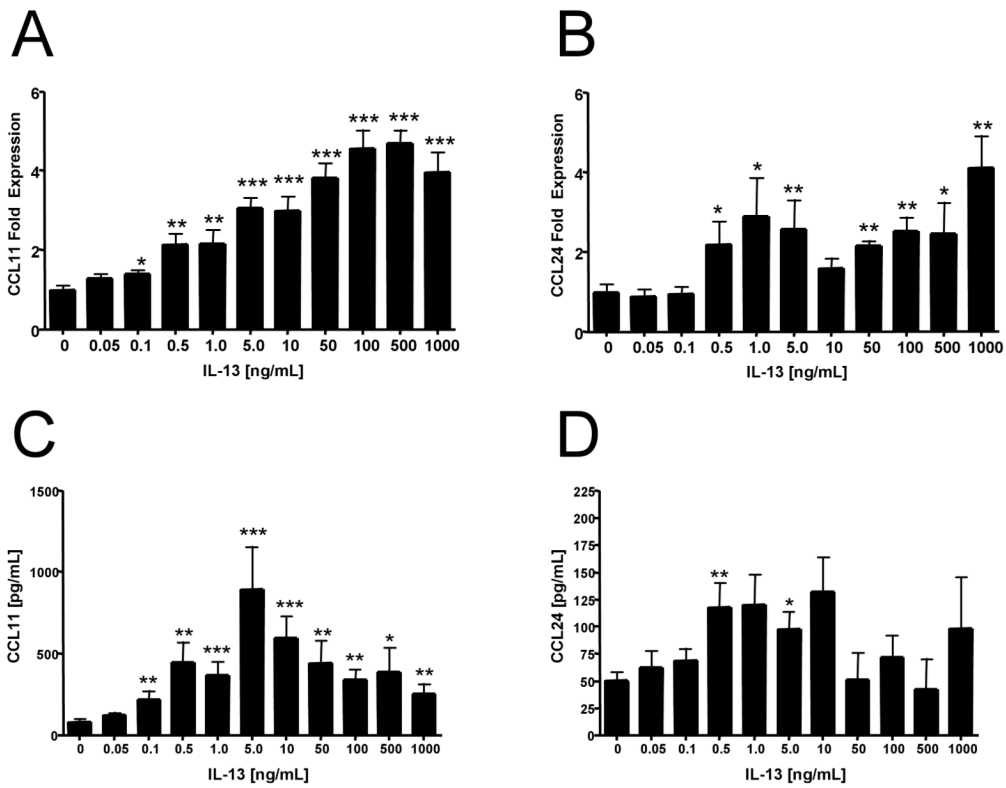

Figure 1. IL-13 Promotes Esophageal Production of CCL11 and CCL24

The expression of CCL11 (A) or CCL24 (B) was determined by RT-PCR in esophageal rings cultures with or without IL-13 for 48 hours. The production of CCL11 (C) or CCL24 (D) in the supernatants from these cultures was also determined by ELISA. Data represents the mean± SEM. * indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p<0.005$ by Students *t* test, n=6-9.

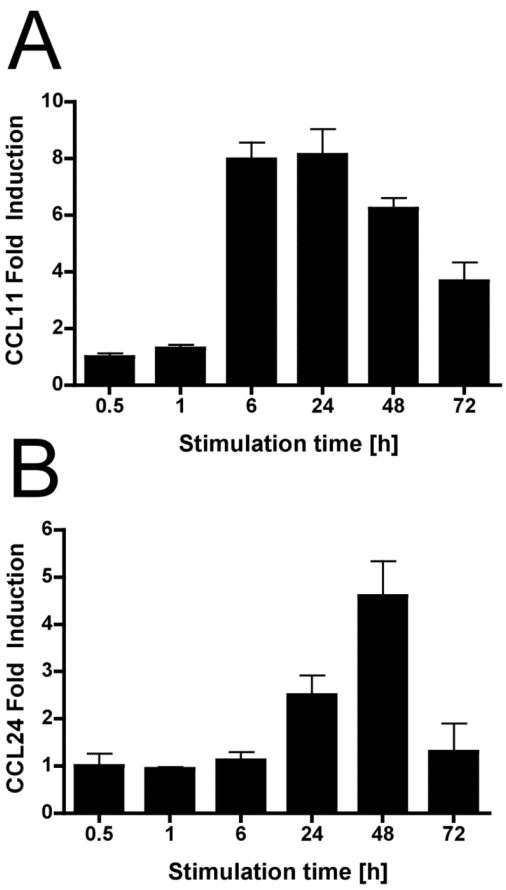

Figure 2. Different Kinetics of CCL11 and CCL24 Expression

The expression of CCL11 (A) or CCL24 (B) was determined by RT-PCR in esophageal rings cultures after stimulation with 5ng/mL IL-13 for the times shown. Data represents the mean± SEM. n=3 individual mice per time point.

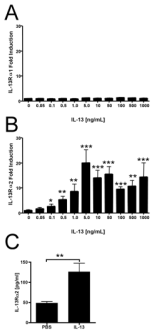

Figure 3. IL-13 Enhances the Expression of IL-13Rα2 but not IL-13Rα1

The expression of IL-13Rα1 (A) or IL-13Rα2 (B) was determined by RT-PCR in esophageal rings cultures with or without IL-13 for 48 hours. The levels of IL-13Rα2 protein in the supernatants were determined by ELISA in esophageal rings treated with 5μg/ml IL-13 (C). Data represents the mean± SEM. * indicates $p<0.05$, ** indicates $p<0.01$*** indicates $p<0.005$ by Mann-Whitney U test, n=6-9.

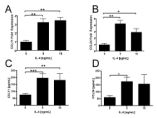

Figure 4. IL-4 Promotes Esophageal Production of CCL24

The expression of CCL11 (A) or CCL24 (B) was determined by RT-PCR in esophageal rings cultures with or without IL-4 for 48 hours. The production of CCL11 (C) or CCL24 (D) in the supernatants from these cultures was also determined by ELISA. Data represents the mean± SEM. * indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p<0.005$ by Mann-Whitney U test, n=6.

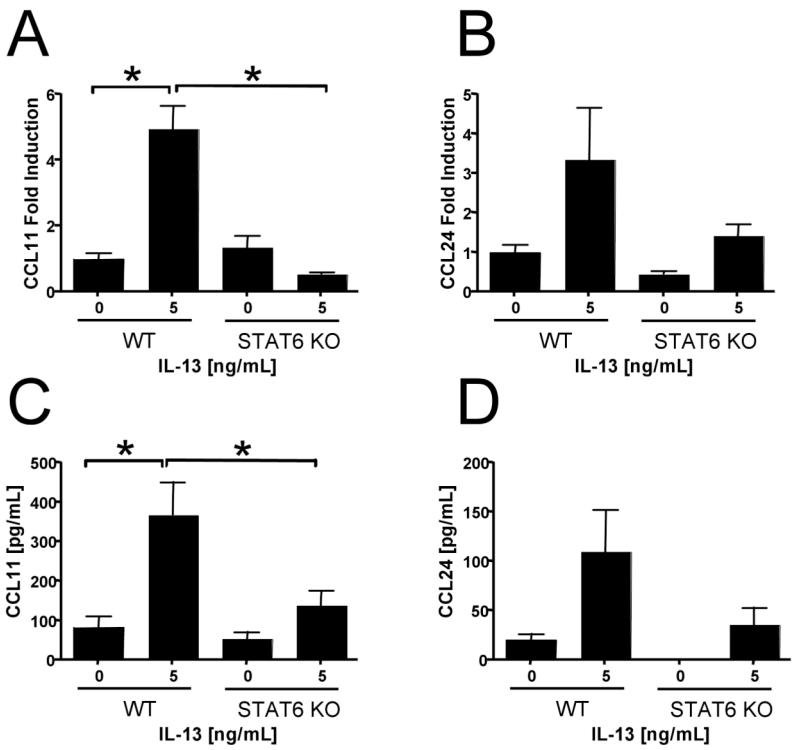

Figure 5. IL-13-Driven Production of CCL11 and CCL24 is STAT6 Dependent

The expression of CCL11 (A) or CCL24 (B) was determined by RT-PCR in esophageal rings cultures from C57/BL6 or STAT6$^{-/-}$ mice with or without 5ng/mL IL-13 for 48 hours. The production of CCL11 (C) or CCL24 (D) in the supernatants from these cultures was also determined by ELISA. Data represents the mean± SEM. * indicates $p<0.05$ by Mann-Whitney U test, n=3.

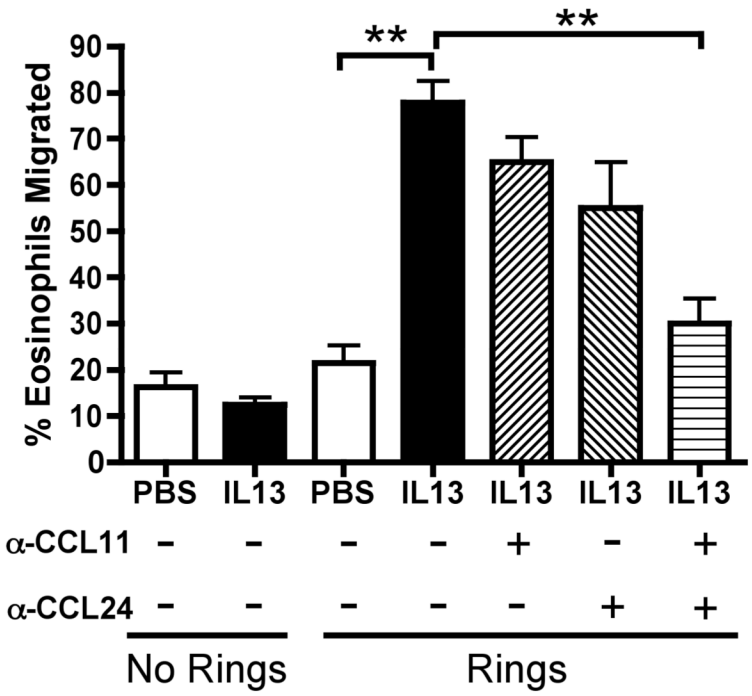

Figure 6. IL-13 Treatment of Esophagus Supports Eosinophil Migration

The migration of eosinophils from NJ.1638 mice were used to study eosinophil migration towards esophageal ring cultures stimulated with or without 5ng/mL IL-13 or phosphate buffered saline (PBS) for 48 hours in the lower chamber of transwell tissue culture plates. 2×10^5 eosinophils were added to the upper chamber and the numbers of cells migrated determined after 30 minutes. Where indicated, neutralizing antibodies against CCL11 or CCL24 were added 1 hour prior to addition of eosinophils. Data represents the mean± SEM. ** indicates $p<0.01$, by Mann-Whitney U test, n=3-7.