Published in final edited form as:

J Allergy Clin Immunol. 2012 July ; 130(1): 225–232.e4. doi:10.1016/j.jaci.2012.04.019.

Airway epithelial cells activate Th2 cytokine production in mast cells via IL-1 and thymic stromal lymphopoietin

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Abstract

Background—Airway epithelial cells are important regulators of innate and adaptive immunity. Although mast cells are known to play a central role in manifestations of allergic inflammation and are found in the epithelium in Th2-related diseases, their role is incompletely understood.

Objectives—The objective of this study was to investigate the role of airway epithelial cells in production of Th2 cytokines in mast cells.

Methods—Normal human bronchial epithelial cells (NHBE) were stimulated with TNF, IL-4, IFN- γ , IL-17A and dsRNA alone or in combination. Human mast cells were stimulated with epithelial cell-derived supernatants, or co-cultured with NHBE. Th2 cytokine responses were blocked with neutralizing antibodies.

Results—Supernatants from IL-4 and dsRNA stimulated NHBE significantly enhanced Th2 cytokine production from mast cells. The combination of IL-4 and dsRNA itself or supernatants from NHBE stimulated with other cytokines did not activate mast cells, suggesting that mast cell responses were induced by epithelial cell factors that were only induced by IL-4 and dsRNA. Epithelial supernatant-dependent Th2 cytokine production in mast cells was suppressed by anti-IL-1 and anti-TSLP, and was enhanced by anti-IL-1Ra. Similar results were observed in co-culture experiments. Finally, we found dsRNA-dependent production of IL-1, TSLP, and IL-1Ra in NHBE was regulated by Th cytokines, and their ratio in NHBE correlated with Th2 cytokine production in mast cells.

Conclusions—Pathogens producing dsRNA, such as respiratory viral infections, may amplify local Th2 inflammation in asthmatics via the production of TSLP and IL-1 by epithelial cells and subsequent activation of Th2 cytokine production by mast cells in the airways.

Keywords

Epithelial cells; Mast cells; Virus; Asthma; Th2 cytokine; IL-1; TSLP

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INTRODUCTION

Asthma is a chronic airway disease affecting 8.2% of the US population,¹ and is characterized by increased airway inflammation, mucus oversecretion, airway remodeling, and increased bronchoconstrictor responses to provoking agonists.² Respiratory viruses that bind to the airway epithelium and initiate airway inflammation are key triggers of asthma exacerbations in children and adults, although the precise triggering mechanisms are not known.^{3–5} Among the infiltrating inflammatory cells, eosinophils, Th2 cells, and mast cells are classical features of asthma.^{6–8} Of these, mast cells, are well known to express the high affinity IgE receptor, FcεRI.⁹ Upon cross-linking of FcεRI, mast cells degranulate and release a wide variety of mediators such as histamine and cysteinyl leukotrienes, promoting immediate allergic responses, and cytokines which induce late phase inflammation including eosinophilia and mucus production.^{9, 10} Mast cells can also be activated by non-IgE mediated mechanisms, including TLR ligands, neuropeptides and pro-inflammatory cytokines.^{11–13} Upon activation, these agents enhance the production of cytokines and mediators which can subsequently augment airway inflammation. Recently, mast cells have been documented to accumulate in the airway epithelium of Th2-high asthmatics and correlate with severity of the disease.^{14, 15} However, the interaction of mast cells with airway epithelial cells and their role in virus induced airway inflammation is not well understood.

Airway epithelial cells are among the first responders to invading allergens and viruses, and mount an immediate innate response to counter an infection.¹⁶ Respiratory virus-infected airway epithelial cells have been reported to produce IL-8, IL-6 and eotaxins,^{16–18} indicating that apart from being a natural barrier to pathogens, epithelial cells play a seminal role in the initiation and amplification of innate immune responses to viral infections. We have previously shown that cytokine thymic stromal lymphopoietin (TSLP) is synergistically induced by the combination of the Th2 cytokine IL-4, and either double stranded RNA (dsRNA), a TLR3 ligand and a viral mimic, or rhinovirus infection, in primary human bronchial epithelial cells, in a TLR3- and STAT6-dependent manner.¹⁹ TSLP is an IL-7-like cytokine, derived mainly from epithelial cells, which activates immature dendritic cells to express the OX40L ligand, and causes preferential differentiation of naïve T cells into Th2 cells.^{16, 20–22} TSLP expression has been associated with Th2 mediated diseases, including asthma, co-localizing to the bronchial epithelium of patients.^{20, 23} While TSLP alone cannot stimulate human mast cells *in vitro*, TSLP synergistically enhances IL-5 and IL-13 release in mast cells which are exogenously treated with IL-1 and TNF.¹¹ IL-1α and IL-1β are pro-inflammatory cytokines derived primarily from monocytes and macrophages, can be produced from airway epithelial cells, and bind to a common heterodimeric receptor consisting of the IL-1R1 and IL-1 receptor accessory protein (IL-1RAP).^{24–26} IL-1 receptor antagonist (IL-1Ra) negatively regulates the activity of IL-1α and IL-1β by binding IL-1R1 and preventing heterodimerization of the receptor, thus inhibiting signal transduction.²⁵

In light of the above studies, it is conceivable that epithelial cells can create a microenvironment that profoundly activates mast cells to produce cytokines. The specific role of the epithelium in the direct activation of mast cells in the context of a Th2 environment and a viral stimulus has not been investigated. In the current study, we demonstrate that epithelial cells can produce a combination of cytokines that directly activates human mast cells in an allergic (i.e. Th2) environment. Our findings aim to provide a possible mechanism for increased airway inflammation in asthmatics after exposure to a respiratory infection.

METHODS

Cell culture

Primary normal human bronchial epithelial cells (NHBE), purchased from Lonza (Walkersville, MD), were seeded in collagen coated plates, and were maintained in serum-free bronchial epithelial cell growth medium (BEGM, Lonza).¹⁹ Human peripheral blood-derived mast cells were obtained as described previously²⁷ and details can be found in the Methods section in the Online Repository. Submerged NHBE were stimulated with IL-4, TNF, IL-17A (100 ng/ml each), or with IFN- γ (10 ng/ml), alone or in combination with dsRNA (5 μ g/ml), for 72 hours. The cell free supernatants (20% volume) were overlaid on mast cells (5×10^5 cells/ml) for 48 hours. In some experiments, mast cells were stimulated with IL-1 α or IL-1 β (10 ng/ml each) alone or in combination with TSLP (10 ng/ml). In select experiments, supernatants from IL-4 and dsRNA (IL-4 + dsRNA)-stimulated NHBE (10% volume) were overlaid on mast cells along with neutralizing antibodies to IL-1 α (10 μ g/ml), IL-1 β (10 μ g/ml), TSLP (32 μ g/ml), IL-1Ra (10 μ g/ml), or control IgG for 48 hours. In co-culture experiments, mast cells were cultured with NHBE in medium containing 50% BEGM and 50% mast cell culture medium, and then stimulated with IL-4 alone or in combination with dsRNA or influenza A virus strain A/udorn/1972 (H3N2) (MOI of 0.1).

ELISA and cytometric bead array (CBA)

The concentrations of IL-1 α , IL-1 β , IL-5, and IL-13 in cell-free supernatants were measured by CBA flex sets from BD Biosciences (San Jose, CA). The limit of detection for each is 2.5 pg/ml. The concentrations of IL-1Ra and TSLP were assayed by ELISA (R&D systems), and the limit of detection is 39.1 pg/ml and 15.6 pg/ml respectively.

Statistical Analysis

All data are reported as the mean $\pm$ SEM unless otherwise noted. Differences between groups were analyzed using one-way ANOVA or with paired Student's *t* test where indicated, and considered to be significant if $p < 0.05$ unless otherwise indicated. Correlations were assessed using the Spearman's rank correlation.

RESULTS

Soluble factors from airway epithelial cells induce Th2 cytokine production in mast cells

NHBE were stimulated with cytokine alone or in combination with dsRNA for 72 hours. We found that only the supernatants from IL-4 + dsRNA-stimulated NHBE significantly enhanced production of IL-5 (87.1 ± 58.2 pg/ml) and IL-13 (16.3 ± 6.8 pg/ml) by human mast cells compared to medium controls (undetectable) (Fig 1, A and B, $p < 0.05$, $n=9$). It has previously been reported that TLR3 and IL-4R are expressed on human mast cells.^{12, 28} We therefore next examined whether IL-4 and dsRNA would directly promote Th2 cytokine production in mast cells. Interestingly, enhancement of IL-5 production in mast cells was only observed by IL-4 + dsRNA-stimulated NHBE supernatants but not by direct stimulation with IL-4, dsRNA, or the combination (Fig 1, C, $n=3-5$). We next examined whether IL-4 + dsRNA-stimulated NHBE supernatants also induced degranulation of mast cells and found that these supernatants did not induce β -hexosaminidase release ($n=3$, Fig E1). These results suggest that only soluble factors from NHBE directly enhance Th2 cytokine release by mast cells and hence we focused on cytokines produced by NHBE.

IL-1 is a critical factor for NHBE dependent Th2 cytokine production in mast cells

It has been reported that IL-1 α and IL-1 β , can amplify Th2 cytokine production in mast cells;¹¹ we therefore employed neutralizing antibodies to IL-1 α and IL-1 β . We found anti-IL-1 α significantly suppressed IL-5 production (2.3 ± 0.7 pg/ml) in mast cells compared to the IL-4 + dsRNA-stimulated NHBE supernatant alone (25.4 ± 10.5 pg/ml, not shown) and with the goat control IgG (79.7 ± 17.6 pg/ml), suggesting that IL-1 α from stimulated NHBE was required for the IL-5 response in mast cells (Fig 2, A, and Fig E2, $p < 0.05$, $n=7$). Similarly anti-IL-1 β antibody significantly suppressed NHBE supernatant-dependent IL-5 induction in mast cells (9.9 ± 2.0 pg/ml) compared to IL-4 + dsRNA-stimulated NHBE supernatant alone (14.2 ± 1.3 pg/ml, not shown) and the goat control IgG (55.9 ± 26.1 pg/ml) indicating that IL-1 β was also required for the epithelial cell induced IL-5 response (Fig 2, B, and Fig E2, $p < 0.05$, $n=6$). We also found that IL-4 + dsRNA-stimulated NHBE supernatant-dependent IL-13 production was significantly inhibited by anti-IL-1 α and anti-IL-1 β in mast cells (data not shown, $n=4$).

Although airway epithelial cells are known to release IL-1 α and IL-1 β , regulation of these cytokines is not well elucidated. We therefore determined whether IL-1 α and IL-1 β were induced by dsRNA and cytokines in NHBE. We found that production of IL-1 α and IL-1 β was not upregulated by dsRNA (IL-1 α ; 59.8 ± 11.9 pg/ml, IL-1 β ; 35.5 ± 10.1 pg/ml,) or induced by the individual cytokines tested compared to medium control (IL-1 α ; 61.2 ± 11.8 pg/ml, IL-1 β ; 4.1 ± 0.6 pg/ml) ($n=8$, Fig 3). Interestingly, TNF (370.6 ± 79.9 pg/ml), IL-4 (356.8 ± 49.2 pg/ml), IFN- γ (908.3 ± 177.8 pg/ml) and IL-17A (617.7 ± 63.8 pg/ml) significantly enhanced dsRNA-dependent IL-1 α production in NHBE (Fig 3, A, $p < 0.05$, $n=8$). In contrast to the induction of IL-1 α , only IL-17A + dsRNA significantly induced IL-1 β production (128.2 ± 38.3 pg/ml) in NHBE (Fig 3, B, $p < 0.05$, $n=8$).

TSLP enhances NHBE dependent Th2 cytokine response in mast cells

It has been previously reported that TSLP enhances IL-1-dependent Th2 cytokine production from mast cells (Fig 2, A).¹¹ In order to determine whether TSLP contributes to the IL-5 response in mast cells induced by supernatants from IL-4 + dsRNA-stimulated NHBE, we examined the effect of a neutralizing antibody against TSLP. We found that IL-4 + dsRNA-stimulated NHBE supernatant-dependent IL-5 production was significantly attenuated by anti-TSLP but not by mouse control IgG1 in mast cells (Fig 2, C, $p < 0.05$, $n=4$). This result suggests that TSLP is one of the factors amplifying the IL-5 response in mast cells. We confirmed our previous findings¹⁹ that IL-4 synergistically enhanced dsRNA-dependent TSLP production in NHBE (354.2 ± 39.6 pg/ml, $p < 0.05$, $n=6$) (Fig 3, D). Interestingly, IL-17A and IFN- γ significantly suppressed dsRNA-induced TSLP production.

The ratio of IL-1 and IL-1Ra in airway epithelial cells is a critical factor for Th2 cytokine production in mast cells

Based on the above results, we determined whether the concentration of IL-1 and TSLP in supernatants of IL-4 + dsRNA-stimulated NHBE correlated with IL-5 responses in mast cells. We found no individual correlation between the levels of TSLP, IL-1 α , or IL-1 β in NHBE and the levels of IL-5 production in mast cells (Fig 4, A–C). In addition, the observed increases in IL-1 α and IL-1 β in NHBE stimulated with dsRNA + IFN- γ , IL-17A, or TNF did not correlate with the corresponding levels of IL-5 in mast cells (Fig 1 and 3). We hypothesized that the activity of IL-1 may be regulated by an inhibitor, IL-1Ra, simultaneously produced by epithelial cells. We found that IL-1Ra production in NHBE was significantly induced by TNF + dsRNA (59.4 ± 7.3 ng/ml) and IFN- γ + dsRNA (45.2 ± 7.3 ng/ml) but not by dsRNA alone or the cytokines tested compared to medium control (3.9 ± 0.3 ng/ml) (Fig 3, C, $p < 0.05$, $n=9$). The concentration of IL-1Ra in IL-4 + dsRNA-stimulated NHBE supernatants significantly and inversely correlated with the IL-5 response

in mast cells (Fig 4, *D*, $p=0.015$, $r=-0.758$). These results suggest that the induction of IL-1Ra could be involved in negatively regulating the activity of IL-1 in NHBE supernatants, thus inhibiting IL-5 production in mast cells treated with dsRNA + IFN- γ or TNF-stimulated NHBE supernatants. Furthermore, the ratio of IL-1 α /IL-1Ra ($p < 0.001$, $r=0.951$) and IL-1 β /IL-1Ra ($p=0.016$, $r=0.733$) in NHBE correlated strongly and positively with IL-5 production in mast cells (Fig 4, *E* and *F*). This suggests that the ratio of IL-1 to IL-1Ra in NHBE supernatants is a key determinant and TSLP is a potent enhancer of the induction of IL-5 in mast cells, and only supernatants from IL-4 + dsRNA-stimulated NHBE contain the positive IL-1/IL1Ra ratio and sufficient TSLP to induce Th2 cytokine production in mast cells.

IL-1 and TSLP enhance IL-5 response in mast cells when co-cultured with NHBE

In order to verify whether mast cells could be activated by NHBE, we grew NHBE to confluence, added mast cells, and cultured them together for 72 hours. The combination of IL-4 and dsRNA significantly and synergistically enhanced IL-5 production (632.3 ± 152.8 pg/ml, $p < 0.05$, $n=6$) compared to medium (0.79 ± 0.26 pg/ml), IL-4 (140.1 ± 35.9 pg/ml) or dsRNA (34.8 ± 14.2 pg/ml) treatment (Fig 5, *A*). We also found that IL-4 + dsRNA dose dependently enhanced IL-5 production (Fig 5, *B*). Importantly, a concentration of IL-4 as low as 1 ng/ml was sufficient to enhance IL-5 production in the presence of dsRNA. In order to determine whether IL-1 and TSLP play an important role in the IL-5 response in the co-culture system, we employed neutralizing antibodies. As expected, we observed a significant reduction in the IL-5 response by neutralization of IL-1 α , IL-1 β and TSLP, and significant enhancement by anti-IL-1Ra, compared to the control IgG (Fig 6, $p < 0.05$, $n=4-5$). To test the effect of influenza virus (IV) infection, NHBE were infected with IV in the presence or absence of IL-4 and then cocultured with mast cells. We found that the combination of IL-4 and IV significantly enhanced IL-5 production in mast cells (Fig 7, *A*, $p < 0.05$, $n=5$). Since we found IV significantly induced IL-1Ra (Fig E3), we applied anti-IL-1Ra antibody and found that the combination of IL-4 and IV further enhanced IL-5 production in the presence of anti-IL-1Ra (Fig 7, *B*, $p < 0.05$, $n=5$). These data suggest that in the context of a viral infection in a Th2 rich environment typically prevalent in asthmatics, epithelial cells could directly activate mast cells through the production of factors including TSLP, IL-1 α and IL-1 β which could further exacerbate airway inflammation and that IL-1Ra is an important mediator in negatively regulating this response.

DISCUSSION

The current study demonstrates that exposure of epithelial cells to IL-4 and dsRNA, a mimic of viral infection in a Th2 environment, leads to production of factors that drive IL-5 and IL-13 secretion in mast cells (Fig 1). Our studies show that the mechanism of this response is via epithelial production of soluble factors including IL-1 α , IL-1 β and TSLP, which drive Th2 cytokine production in mast cells. We also found that the combination of IL-4 and IV enhanced IL-5 production in mast cells co-cultured with NHBE, suggesting a clinical situation in which this may occur (Fig 7). Finally, we show that the response is modulated by the production of IL-1Ra by the epithelial cells. The role of IL-5 in the accumulation, survival, and activation of eosinophils in the tissues and the roles of eosinophils in the pathophysiology of asthma are well known.²⁹⁻³¹ IL-13 also activates eosinophils, upregulates eotaxin expression, and plays a pivotal role in upregulating mucus secretion in the airways, one of the key features of exacerbations.³²⁻³⁴ Thus in the context of a Th2 cytokine environment in which a viral infection occurs, asthmatics may be highly susceptible to the exacerbation of allergic inflammation via the production of factors including IL-1 and TSLP, released from the airway epithelium, which further enhance IL-5 and IL-13 production in mast cells. We found that IL-5 production was 2.7 to 10 fold higher

in studies utilizing co-culture of mast cells and epithelial cells compared to exposure of mast cells to epithelial supernatants (Fig 1 and 5). These results can be explained by the fact that only 10–20% of the volume of NHBE supernatants was used to stimulate the mast cells. However, we cannot discount the effects of cell-to-cell contact, which could also result in the upregulation of receptors or mediators which may enhance IL-5 production.

In our studies, we investigated the role of IL-1 and IL-1Ra in the activation of IL-5 production in mast cells by epithelial supernatants. Blocking IL-1 significantly attenuated the mast cell IL-5 inducing ability of supernatants from IL-4 + dsRNA-stimulated NHBE (Fig 2 and 6). IL-1 α and IL-1 β are primarily derived from monocytes and to a smaller extent from epithelial and endothelial cells.²⁵ IL-1 β is expressed at higher levels in bronchoalveolar lavage fluid of patients with symptomatic asthma and is also detected in the bronchial epithelium of asthmatics.^{35, 36} Additionally, IL-1 β is also elevated in nasal secretions during rhinovirus induced colds and in airway epithelial cells.^{37, 38} Under normal conditions, IL-1 β synthesis and release are tightly controlled and cannot be detected in the circulation. In contrast to IL-1 β , the precursor to IL-1 α is constitutively expressed and serves as an autocrine growth factor.²⁵ During cellular injury such as occurs during viral infections, necrotic cells release mature IL-1 α which serves as a danger signal and promotes subsequent inflammation. Bronchial epithelial cells infected with respiratory viruses have been reported to release IL-1 α *in vitro*.^{24, 26} In our studies, IL-4 + dsRNA-stimulated NHBE showed significantly higher levels of IL-1 α compared to cells stimulated with either IL-4 or dsRNA alone (Fig 3). However, production of IL-1 α and IL-1 β in NHBE did not correlate with IL-5 production in mast cells (Fig 4). Thus we investigated the role of IL-1Ra, which negatively regulates the activity of IL-1 α and IL-1 β . IL-1Ra has been reported to be significantly downregulated at baseline in nasal lavages of asthmatics, suggesting that alterations of the ratio of IL-1 to IL-1Ra may occur in asthma.^{25, 38} Interestingly TNF and IFN- γ enhanced dsRNA induced IL-1Ra production in NHBE (Fig 3), and these supernatants did not induce IL-5 production in mast cells (Fig 1). In addition, a strong positive correlation of the IL-5 response in mast cells with the ratio of both IL-1 α /IL-1Ra and IL-1 β /IL-1Ra in IL-4 + dsRNA-stimulated NHBE was observed. Finally, application of the anti-IL-1Ra antibody significantly enhanced the IL-5 response in mast cells following exposure to IL-4 + dsRNA-stimulated NHBE supernatants, suggesting a role for endogenous IL-1Ra in NHBE in the regulation of IL-5 production by mast cells (Fig 6). This interplay between IL-1 and the IL-1Ra from epithelial cells and its role in regulation of Th2 cytokines from mast cells is a novel mechanism by which viruses may modulate airway inflammation in asthma.

The finding that TSLP plays a role in the epithelial activation of an IL-5 response in mast cells is also of clinical interest. TSLP has been associated with airway obstruction in asthmatics and correlates with disease severity.²³ We have shown previously that IL-4 synergistically enhances dsRNA- and rhinovirus-dependent TSLP expression in NHBE.¹⁹ Although mechanisms are still not clear, published data suggest that synergistic effects could be the result of upregulation of the IL-4 receptor by dsRNA and/or the influence of a composite binding element for STAT6 and NF- κ B in the TSLP promoter.^{19, 39} Bronchial epithelial cells from asthmatics produce higher amounts of TSLP in the response to dsRNA compared to cells from healthy subjects.⁴⁰ In our studies, blocking TSLP caused a significant attenuation of the IL-4 + dsRNA-stimulated NHBE-dependent IL-5 responses in mast cells (Fig 2 and 6). We note that low quantities (as little as 10% volume) of the stimulated NHBE supernatants, containing only 20–50 pg/ml TSLP, were sufficient to enhance Th2 cytokine production in mast cells. This suggests that a modest induction of TSLP by the combination of an allergic stimulus and a viral infection could have important clinical implications for symptom worsening. On the other hand, IL-17A and IFN- γ strongly suppressed dsRNA-induced TSLP production in NHBE, consistent with studies performed

in keratinocytes,⁴¹ and nasal epithelial cells⁴². Thus even with high levels of IL-1, the absence of TSLP production resulted in an insufficient activation of Th2 cytokine responses in mast cells.

As discussed above, TSLP expression is elevated in asthmatic bronchial epithelial cells, upon dsRNA stimulation,⁴⁰ and IL-1Ra has been reported to be downregulated at baseline in asthmatics with experimental rhinovirus infection.³⁸ It is therefore conceivable that bronchial epithelial cells in a Th2 microenvironment in patients with asthma may be able to induce stronger IL-5 responses in mast cells than cells in normal subjects during virus infection. Future studies will be required to determine the comparative effect of airway epithelial cells from patients with asthma in the interaction with mast cells. Our observations provide insight into possible therapeutic strategies to reduce allergic airways disease, including blockade of IL-1 α , IL-1 β or TSLP which are released from the airway epithelium in the presence of a viral stimulus and a Th2 environment or maneuvers to elevate IL-1Ra. Recombinant IL-1Ra (Anakinra) has been approved for treatment of autoinflammatory diseases.⁴³ However, as far as we are aware, there is no information about the utility of this drug in prevention of exacerbations of asthma. Our findings suggest that targeting IL-1, TSLP or its receptors clinically in the treatment of asthma will be worthy of detailed investigation.

The present study also underscores the importance of recent reports of mast cells located in the bronchial epithelium of asthmatics. Dougherty et al observed accumulation of mast cells in the bronchial epithelium of Th2-high asthmatics, while Balzar et al observed that the density of mast cells in epithelial cells correlated with severity of the asthma.^{14, 15} Importantly, Dougherty et al identified Th2-high asthma by using the levels of three IL-13 inducible genes, including periostin in epithelium, but did not identify the Th2 cytokine producing cells.¹⁵ Earlier studies showed that IL-5 mRNA⁺ mast cells were elevated in bronchial biopsies of atopic asthmatics.⁴⁴ The role of mast cells in Th2 cytokine production in asthma, and particularly in asthma exacerbations, is therefore worthy of future investigation.

In summary, we report here that human bronchial epithelial cells directly enhanced Th2 cytokine production in mast cells by secretion of factors including IL-1 and TSLP following stimulation with IL-4 and dsRNA (or respiratory virus). Because IL-1Ra competitively inhibits the activity of IL-1 α and IL-1 β , the ratio of IL-1 and IL-1Ra from epithelial cells also controlled Th2 cytokine production in mast cells. These results provide a novel mechanism for the susceptibility of asthmatics to viral infections and could point the way toward novel therapeutic interventions in the treatment of exacerbations.

Acknowledgments

Funding: This research was supported in part by NIH grants, R01 HL078860, R01 AI072570, R37 HL068546 and U01AI082984 and by the Ernest S. Bazley Trust.

Abbreviations

NHBE	Normal human bronchial epithelial cells
dsRNA	Double stranded RNA
IL-1Ra	IL-1 receptor antagonist
IL-1RAP	IL-1 receptor accessory protein
TSLP	Thymic stromal lymphopoietin

CBA Cytometric bead array
IV Influenza virus

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Clinical implications

Specific therapies that target TSLP or IL-1, such as antibodies, soluble receptors or IL-1Ra, may have value in the treatment or prevention of virus induced airway inflammation in Th2 airways diseases such as asthma.

Figure 1.

IL-4 + dsRNA-stimulated NHBE promote Th2 cytokine production in mast cells. NHBE were stimulated with 100 ng/ml TNF, 100 ng/ml IL-4, 10 ng/ml IFN- γ and 100 ng/ml IL-17A alone or in combination with 5 μ g/ml dsRNA for 72 hours. Mast cells were then stimulated with derived supernatants (20% volume) for 48 hours (A, B). Mast cells were stimulated directly with IL-4 and dsRNA or with NHBE supernatants (Sup) (C). IL-5 (A, C) and IL-13 (B) production was assessed by CBA. Results shown are mean $\pm$ SEM of 9 (A and B) or 3–5 (C) independent experiments using 3 NHBE donors and 3 mast cell donors. * p <0.05, compared to all other groups, one-way ANOVA.

Figure 2.

Neutralization of IL-1 and TSLP suppresses NHBE supernatant induced IL-5 production in mast cells. Mast cells were stimulated with 10 ng/ml IL-1 α , 10 ng/ml IL-1 β and 10 ng/ml TSLP, epithelial culture medium (BEGM, 10% volume) or with IL-4 + dsRNA-stimulated NHBE supernatants (NHBE Sup, 10% volume) for 48 hours along with neutralizing antibodies against IL-1 α (10 μ g/ml, A), IL-1 β (10 μ g/ml, B), TSLP (32 μ g/ml, C), or with respective isotype controls. IL-5 responses were measured by CBA. Results shown are mean $\pm$ SEM of 7 (A), 6 (B) or 4 (C) independent experiments using 2 NHBE donors and 6 mast cell donors. * $p < 0.05$, paired Student's t test.

Figure 3.

Induction of cytokines following NHBE stimulation. NHBE were stimulated with 100 ng/ml IL-4, 10 ng/ml IFN- γ , 100 ng/ml IL-17 and 100 ng/ml TNF, alone or in combination with 5 μ g/ml dsRNA for 72 hours. Concentrations of cytokines were measured by CBA (IL-1 α (A) and IL-1 β (B)) or ELISA (IL-1Ra (C) and TSLP (D)). Results shown are mean $\pm$ SEM of 8 (A and B), 9 (C) and 6 (D) independent experiments using 5 NHBE donors. * $p < 0.05$, Different from medium; † $p < 0.05$, different from dsRNA, one-way ANOVA

Figure 4. Correlation of cytokines in supernatants from IL-4 + dsRNA-stimulated NHBE with IL-5 production in mast cells. Data is representative of 2 mast cell donors and 3 NHBE donors (N=5). The correlations were assessed by using the Spearman rank correlation. NS, not significant.

Figure 5.

IL-4 and dsRNA dose-dependently and synergistically enhance IL-5 production in mast cells when co-cultured with epithelial cells. Mast cells were overlayed on NHBE and then were stimulated with 10 ng/ml IL-4 and 5 µg/ml dsRNA (A) or at indicated concentrations (B) for 72 hours. Production of IL-5 was measured by CBA. Results shown are mean $\pm$ SEM of 6 independent experiments using 4 NHBE donors and 3 mast cell donors. * $p < 0.05$, one-way ANOVA (A) and different from all groups; † different from medium, 0.1, 1, and 100 ng/ml IL-4, ** compared to medium and 0.1 ng/ml IL-4, ¶ compared to medium, and 1 ng/ml IL-4, Repeated Measures one-way ANOVA Newman-Keuls Test (B).

Figure 6.

IL-4 and dsRNA enhance IL-5 production in mast cells when co-cultured with NHBE in an IL-1 and TSLP dependent manner. Mast cells were overlayed on NHBE and then were stimulated with 10 ng/ml IL-4 and 5 μ g/ml dsRNA along with neutralizing antibodies against IL-1 α (A), IL-1 β (A), IL-1Ra (A), TSLP (B) or isotype controls for 72 hours. IL-5 response was measured by CBA. Results shown are mean $\pm$ SEM of 5 (A) or 4 (B) independent experiments using 2 NHBE donors and 3 mast cell donors. * $p < 0.05$, paired Student's t -test.

Figure 7.

The combination of IL-4 and influenza virus enhances IL-5 production from mast cells. NHBE were stimulated with IV at a MOI of 0.1 and 10 ng/ml IL-4 along with neutralizing antibody to IL-1Ra 20 μ g/ml or with 20 μ g/ml control goat IgG for 72 hours. Results shown are mean $\pm$ SEM of 5 independent experiments using 4 NHBE donors and 3 mast cell donors. * $p < 0.05$, one-way ANOVA.