

Respiratory viral infection, epithelial cytokines, and innate lymphoid cells in asthma exacerbations

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RECEIVED MARCH 2, 2014; REVISED MAY 14, 2014; ACCEPTED MAY 14, 2014. DOI: 10.1189/jlb.3RI0314-129R

ABSTRACT

Exacerbations of asthma are most commonly triggered by viral infections, which amplify allergic inflammation. Cytokines released by virus-infected AECs may be important in driving this response. This review focuses on accumulating evidence in support of a role for epithelial cytokines, including IL-33, IL-25, and TSLP, as well as their targets, type 2 innate lymphoid cells (ILC2s), in the pathogenesis of virus-induced asthma exacerbations. Production and release of these cytokines lead to recruitment and activation of ILC2s, which secrete mediators, including IL-5 and IL-13, which augment allergic inflammation. However, little information is currently available about the induction of these responses by the respiratory viruses that are strongly associated with exacerbations of asthma, such as rhinoviruses. Further human studies, as well as improved animal experimental models, are needed to investigate appropriately the pathogenetic mechanisms in virus-induced exacerbations of asthma, including the role of ILCs. *J. Leukoc. Biol.* 96: 000–000; 2014.

Introduction

Asthma is one of the most common chronic illnesses in economically developed nations. Clinically, it is typified by episodic breathlessness and wheezing, together with hyper-responsiveness of the airways to a variety of stimuli. Underlying these manifestations is inflammation of the conducting airways, although the relationship between inflammation and airway hyper-responsiveness remains controversial [1]. In Australia, the prevalence of doctor-diagnosed asthma is ~10% overall, whereas in the United States, it is ~7% [2, 3]. The prevalence is lower in Asia but is expected to rise in parallel with economic development and urbanization [4]. Children are particularly affected by asthma, with an estimated prevalence as high

as 16% in Australian children aged 8–9 years [2]. Asthma is heterogeneous in terms of pathologic and immunologic features [1]. However, childhood asthma is strongly linked to atopy and a Th2-biased allergic response [5].

Acute exacerbations of asthma are defined clinically as events that require a change in treatment because of a change from the patient's previous status [6]. They are the main contributor to direct healthcare costs associated with the illness [7]. Viral infections are important triggers of exacerbations of allergic asthma. At least 50% of exacerbations in adults and an even larger proportion in children is associated with viral infections [8], of which RV appear to be the most common. Human RVA and -B have long been recognized as playing a role in exacerbations [9], whereas recent studies have similarly identified the novel RVC as being strongly associated [10]. However, numerous other viruses also contribute to worsening of asthma, including RSV, influenza and parainfluenza viruses, human metapneumovirus, and adenoviruses [11].

Viral infection and allergen exposure appear to be synergistic triggers for asthmatic exacerbations [12, 13], and children hospitalized for severe asthma are typically markedly atopic [14]. In patients with acute severe asthma, there is enhanced secretion of a variety of proinflammatory cytokines, with evidence of elevated levels of the Th2 cytokines IL-4, IL-5, and IL-13. This is paralleled by increased recruitment of eosinophils, together with numerous neutrophils, into the bronchial mucosa [15–18]. Exacerbations lead to a marked diminution in airflow, which correlates with the presence of increased concentrations of inflammatory mediators, such as leukotrienes [19].

ILCs, TH2-PROMOTING CYTOKINES, AND ASTHMA

ILCs are a newly characterized population of innate immune effector cells. Although they have lymphoid morphology and

Abbreviations: AEC=airway epithelial cell, ILC1–3=type 1–3 innate lymphoid cell, PRR=pattern recognition receptor, PVM=pneumonia virus of mice, RSV=respiratory syncytial virus(es), RV=rhinovirus(es), TSLP=thymic stromal lymphopoietin

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secrete what have been regarded as mediators characteristic of lymphocytes, they have no recombinase-dependent B or T cell receptors nor do they express myeloid or lymphoid cell-surface markers. Several recent reviews consider these cells, as well as the recently defined subgroups of ILC1–3, in considerable detail [20–22].

Here, we focus on ILC2s, which are a subgroup that express the cell-surface antigens ST2 (IL-33R subunit) [23], IL-17RB (IL-25R subunit), and CD127 (IL-7R α). These cells respond to cytokines IL-33 and IL-25 by releasing Th2 cytokines, including IL-5 and IL-13 (reviewed in refs. [20, 24]). Accumulating evidence suggests that they have a crucial role in the generation and regulation of Th2 allergic responses and may play an important role in the induction of asthma [25–27]. Moreover, as ILC2s are capable of eliciting responses from Th2 lymphocytes, it appears likely that they are also important in the setting of established allergic inflammatory disease of the airways [28–30] and that they can augment the Th2-mediated recruitment of eosinophils, neutrophils, and macrophages [31].

Recent reports provide direct evidence for accumulation of IL-5- and IL-13-secreting ILC2s in response to the epithelial Th2-promoting cytokines IL-33, IL-25, and TSLP [31–34]. IL-33 is particularly effective in expanding the ILC2 population [35] and inducing IL-13-producing ILC2s [36]. Amphiregulin, another secretory product of ILC2s, serves to promote airway repair in mouse models of allergic asthma [32]. Similarly, arginase-1, implicated in wound-healing and tissue fibrosis, is produced constitutively by this population of cells [35].

Current thinking emphasizes the role of TSLP, IL-25, and IL-33 in the initiation of allergic responses. These cytokines can drive Th2 polarization of CD4⁺ T cells via several distinct cellular pathways, important among which appears to be activation of dendritic cells [37–41]. IL-25 has been associated with the initiation of experimental allergic inflammation of the airways [42, 43] and is expressed in patients with chronic asthma. Recent studies suggest that IL-33 may also contribute to the induction phase of allergic asthma [44]. Although usually described as a Th2-promoting cytokine, IL-33 behaves in many respects like a damage-associated molecule or alarmin [45]. For example, extracellular ATP released from damaged cells induces translocation of newly synthesized IL-33 from the nucleus to the cytoplasm, followed by release of the full-length, biologically active molecule [46]. However, as a member of the IL-1 cytokine family, IL-33 signals via a specific receptor (ST2) [47] rather than via a PRR.

Expression of IL-25, IL-33, and TSLP is increased in asthmatics [26, 48], but the role of the Th2-promoting cytokines TSLP and IL-25 in asthmatic exacerbations is currently unclear. Expression of TSLP may be related to disease severity [49], and Petersen et al. [50] have suggested that IL-25 contributes to inflammation in a mouse model of RSV and allergen challenge. The evidence in support of a potentially important role for IL-33 in the pathogenesis of asthma exacerbations is more convincing. Immunohistochemical data suggest that the extent of expression of IL-33 by AECs is related to the severity of asthma [51]. We have shown that IL-33 plays a key

role in driving airway inflammation in an experimental exacerbation [52]. Specifically, we examined the role of this cytokine in airway inflammation in a model of an acute exacerbation of chronic asthma and found that neutralization of IL-33 significantly decreased both airway inflammation and the expression of proinflammatory cytokines by alveolar macrophages. Others have provided similar evidence in support of the importance of IL-33 in models of allergic inflammation of the airways [53, 54].

VIRAL INFECTION OF AECs AND TH2-PROMOTING CYTOKINES

In acute exacerbations of asthma triggered by viruses, allergic inflammatory responses, including to “bystander” (i.e., immunologically unrelated) aeroallergens, may be enhanced [55, 56]. This raises questions about the underlying mechanisms, given that most viral infections do not themselves provoke a substantial Th2 response.

A major target of the viral infections associated with exacerbations of asthma is the epithelium of the conducting airways. As has been shown in other circumstances, injury to epithelial surfaces triggers release of a variety of proinflammatory and Th2-promoting cytokines, the latter including IL-25, IL-33, and TSLP [57, 58]. In vitro infection of AEC with RV or RSV, or exposure to dsRNA to simulate infection, induces production of TSLP [59, 60]. Similarly, in response to RSV and PVM (the latter is a natural rodent pathogen that belongs to the same family/genus as RSV) [61], IL-25 is induced and is associated with the initiation of allergic inflammation of the airways [42, 43].

Given that IL-33 is released in response to cellular injury, it is also reasonable to hypothesize that respiratory viral infection would induce production of this cytokine by AEC. Consistent with this hypothesis, Le Goffic and colleagues [62] found that influenza infection of cells of the mouse type II pneumocyte MLE-15 and human bronchial epithelial A549 lines, as well as infection in C57BL/6 mice, all resulted in expression and release of IL-33. Similarly, we have shown that primary PVM infection of mice also induces the expression and release of IL-33 [63]. Chang and colleagues [64] found that in BALB/c mice, influenza H3N1 infection elicited IL-33 production by alveolar macrophages rather than AECs; however the IL-33 did activate ILC2s and promoted airway hyper-responsiveness.

To date, there are no reports that respiratory viral infection leads to increased production of IL-33 in human asthmatics. Nevertheless, it seems plausible that virus-induced injury, specifically to AECs and leading to enhanced release of IL-33, IL-25, and TSLP, might drive an acute exacerbation of allergic asthma via activation of ILC2s as type 2 cytokine-producing effector cells (**Fig. 1**). We believe that the links among virus-induced epithelial injury, release of epithelial cytokines, notably IL-33, and viral infection-associated acute exacerbations of asthma definitely merit further investigation. Such studies need to focus specifically on those viruses known to be important in promoting exacerbations of asthma.

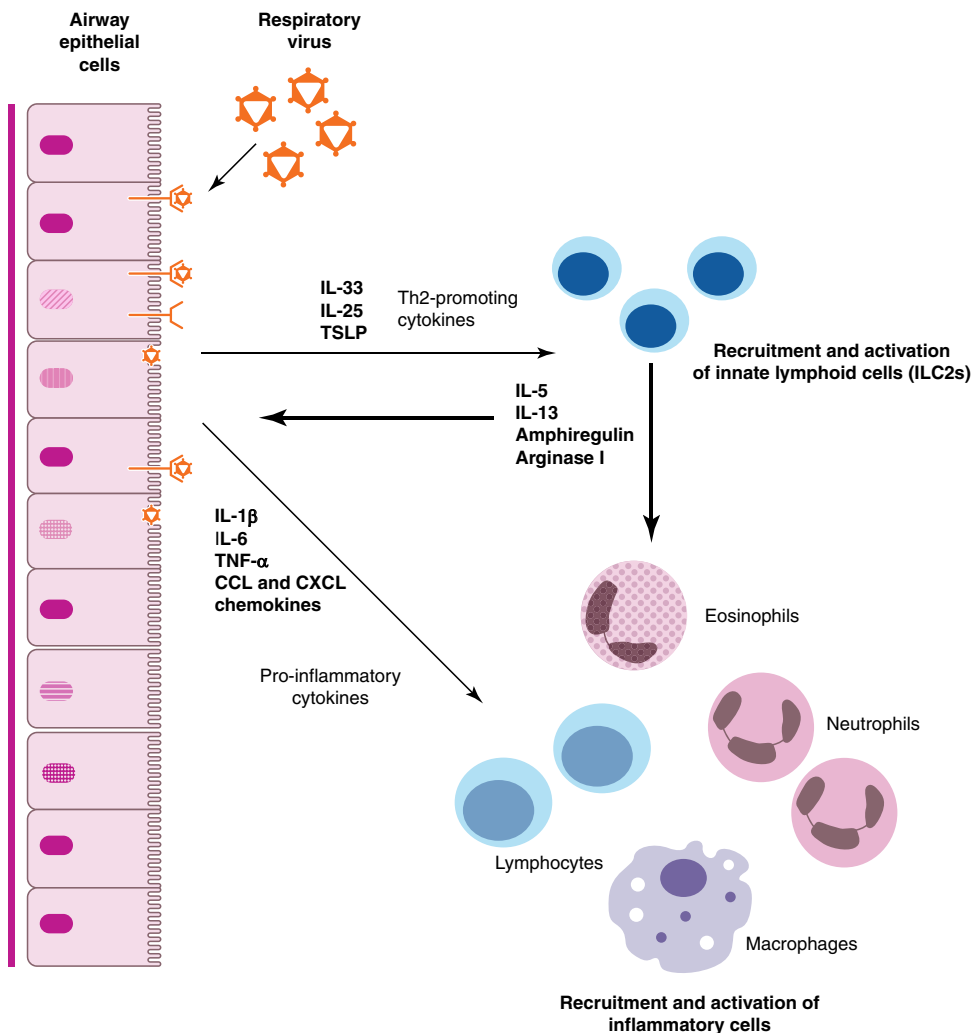


Figure 1. Infection of respiratory epithelial cells elicits inflammation via the activation of ILC2s. Respiratory viruses interact with one or more PRRs, replicate within, and are released from AECs, with resultant cellular injury. This is likely to lead to release of the Th2-promoting cytokines IL-33, IL-25, and TSLP, in turn recruiting ILC2s, which amplify inflammation (via release of IL-5 and IL-13) and promote airway repair/fibrosis (via amphiregulin, IL-13, and arginase-1). Although IL-33 has been detected in the airways in response to primary infection with influenza and PVM [62, 63] and specifically from alveolar macrophages in response to influenza [64], the links among viral infection, release of Th2-promoting cytokines from epithelial cells, and exacerbation of allergic asthmatic inflammation remain to be explored.

EXPERIMENTAL APPROACHES

Although macrophage-derived IL-33 has been implicated directly in a virus-induced model of airway hyper-responsiveness [64], we do not yet have a clear understanding of the role of epithelial cytokines in the response to viral infection in asthma. As such, it would be valuable to consider *in vitro* studies to assess factors that might promote enhanced expression and release of IL-33 and other Th2-promoting cytokines by human AECs infected with relevant respiratory viral pathogens. However, it is crucial to recognize that airway epithelium from asthmatics differs significantly from normal epithelium. For example, several studies indicate that AECs derived from asthmatics exhibit impaired IFN responses, in particular, IFN- α 2, IFN- β 1, and IFN- λ 2/3, when stimulated with RV [65–67]. Moreover, asthmatic AECs exhibit enhanced secretion of the Th2-promoting cytokine TSLP, in response to dsRNA as a surrogate for RV infection [68]. Various studies [66, 67, 69] have also reported increased viral replication in cultures of asthmatic AECs, although others have been unable to confirm this finding [70]. It remains unclear whether such altered responses are

inherent to AECs from asthmatics, are induced in these patients by the Th2 environment, or are a consequence of the effects of drug treatment. Therefore, characterization of the IL-33 response will need to include comparison of asthmatic and nonasthmatic airway epithelium.

The investigation of the role of activation of ILC2s by IL-33 and IL-25 in the pathogenesis of asthmatic exacerbations will require *in vivo* experiments. Whereas cytokine and receptor gene-deleted mice are, of course, instrumental in conducting such studies [71–73], there is also a need for suitable animal experimental models of a virus-induced acute exacerbation of allergic inflammation of the airways, preferably in the setting of chronic allergen challenge, to reproduce the features of human asthma in the most realistic possible manner [74]. Although several mouse models of virally enhanced allergic inflammation of the airways have been described, most of these have significant limitations.

With respect to RV-triggered exacerbations of asthma, there are no known native murine RV. The major group of human RV does not replicate in murine AECs, because of the lack of homology between the human and mouse ICAM-1 RV cell-

surface receptor [75]. Therefore, the most commonly reported models have used the minor group RV1B, which does replicate in mice, albeit in limited fashion, in the setting of acute allergic inflammation in animals sensitized and challenged with ovalbumin over 1–2 weeks [75–78]. Of note, no exacerbations were observed in mice chronically challenged with house dust mite and then challenged with RV1B [79]. Because of these issues, an attenuated mengovirus, which is a natural mouse pathogen of the same family (*Picornaviridae*) as RV, has been suggested as an alternative for the study of disease pathogenesis in rodent hosts [77].

There are also several reports of models of an exacerbation of asthma based on challenging mice with the human pneumovirus pathogen RSV. However, this approach is also not reliably associated with enhancement of the allergic inflammatory response [80, 81], which might again be related to the fact that there is minimal replication of human RSV in mice [82]. Still others have attempted to bypass the use of a virus altogether and have instead used dsRNA as a surrogate. Reported results are, however, not entirely consistent. Some authors have described neutrophil recruitment in response to dsRNA administered to naive mice and correspondingly, successful induction of inflammation resembling an acute exacerbation in asthma in previously sensitized, allergen-challenged animals [78, 83]. Others have suggested that dsRNA elicits only a lymphocytic response [84] and have demonstrated inflammation dominated by lymphocytes and eosinophils in response to administration of dsRNA in sensitized and challenged mice [85]. The latter has also been our experience using dsRNA delivered to chronically challenged mice (unpublished results).

Ideally, a model of a virally induced acute exacerbation would use a respiratory virus able to replicate in mouse airway epithelium and specifically, in animals that had previously been sensitized and chronically challenged with an appropriate model antigen. As noted above, PVM is a rodent-specific pathogen of the same family (*Paramyxoviridae*) and genus (pneumovirus) as RSV, which provides a realistic model of human RSV disease. In mice, low inocula of PVMs lead to a self-limiting infection, whereas higher inocula cause a severe and potentially fatal bronchiolitis [86, 87]. In a short-term model of allergic disease of the airways, PVM enhances pulmonary inflammation in mice [88] and promotes eosinophil degranulation in vivo [89], the latter being a feature of asthma exacerbations that is not usually reproduced in murine models [90]. However, PVM infection has not been tested in the setting of prior chronic challenge. Development of such a model would allow assessment of the independent and/or interconnecting roles of IL-33, IL-25, and TSLP, both by treatment with cytokine-neutralizing antibodies, as well as in studies involving depletion of responding cells or use of gene-deleted animals. Furthermore, by taking advantage of the availability of reporter mice for cells expressing IL-5 or IL-13 [29, 91], it would be possible to identify the unique contribution of ILC2s to the inflammatory response. Such studies are the focus of current work in our laboratories.

CONCLUDING REMARKS

The molecular and cellular mechanisms involved in virus-induced exacerbations of allergic asthma are starting to be unraveled, with increasing recognition of the potentially important role of cytokines produced by AECs. As yet, relatively little is known about the involvement of ILC2s, which may be recruited and activated by cytokines derived from airway epithelium, such as IL-33, IL-25, and TSLP. Experimental studies in appropriately designed mouse models, using relevant respiratory viruses appropriate to the species to trigger an exacerbation of airway inflammation, may help to improve our understanding of pathogenetic mechanisms and to identify novel targets for therapeutic intervention.

ACKNOWLEDGMENTS

Work in the authors' laboratories is supported by grants from National Health and Medical Research Council Australia and the National Institute of Allergy and Infectious Diseases Division of Intramural Research.

DISCLOSURES

The authors declare that they have no conflict of interest.

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KEY WORDS:

Th2 · interleukin-33 · allergy · animal models