Interleukin-18 Is Associated With Protection Against Rhinovirus-Induced Colds and Asthma Exacerbations

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Rhinoviruses cause the common cold and exacerbations of asthma. Animal models of infection have identified a protective role for interleukin-18 (IL-18). Following experimental rhinovirus infection, we observed increased respiratory symptoms in healthy and asthmatic subjects with low nasal and bronchial IL-18 levels.

Keywords. interleukin-18; inflammasome; rhinovirus infection; asthma.

The nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) are pattern-recognition receptors that function as intracellular pathogen sensors. Several members of the NLR family assemble to form large protein complexes known as inflammasomes. These activate caspase 1 to regulate the processing and release of interleukin (IL) 1β and the IL-1 family member IL-18 [1]. In recent years the inflammasome/caspase 1/IL-18 pathway has been shown to play a critical role in mucosal immune responses, including in the host response to bacterial and viral infections [1–7]. A fascinating finding of these studies is the identification of a protective role for IL-18. For example, mouse models of influenza infection have demonstrated increased mortality in IL-18 knockout (KO) mice [2] as well as in caspase 1 KO mice compared with wild-type mice [5, 7]. Ceballos-Olvera et al reported that IL-18 was protective following infection with the bacterium *Burkholderia pseudomallei*, demonstrating greatly increased susceptibility to infection in IL-18–deficient mice [6], whereas IL-18 alone was shown to limit the respiratory infection caused by both *Mycobacterium tuberculosis* [8] and Legionnaires' disease [9].

Rhinoviruses are the predominant cause of the common cold and are the leading precipitant for acute asthma exacerbations. The respiratory epithelium is considered the principal site of rhinovirus infection, and recent studies have reported increased IL-18 in response to rhinovirus infection of human bronchial epithelial cells in vitro [10, 11]. In addition, a large genomewide association study identified the IL-18 receptor as one of 6 loci most associated with asthma [12]. However, it remains unknown whether rhinovirus infection leads to increased IL-18 secretion in vivo, and whether the protective role for IL-18 suggested by animal models can be demonstrated in humans.

Using a human model of experimental rhinovirus infection and novel airway sampling methodology, we set out to measure IL-18 in nasal and bronchial mucosal lining fluid of healthy and asthmatic volunteers at baseline and during rhinovirus infection. We have previously reported clinical outcomes of experimental infection in association with augmented type 2 immune pathways in the same study subjects [13, 14].

METHODS

The methods and study design of the human experimental model have been previously reported [13], but are repeated in brief here for clarity. Twenty-eight asthmatic (15 treated with inhaled corticosteroids and 13 steroid-naive) and 11 healthy volunteers with negative rhinovirus 16 (RV-16) serum antibodies were inoculated with RV-16 via a nasal atomizer. Nasal and bronchial mucosal lining fluid was sampled at baseline and on day 4 after inoculation using a synthetic absorptive matrix placed on the airway mucosa. Mucosal lining fluid was then eluted from the synthetic matrix by spin filter centrifugation and frozen at −80°C [13]. Additional nasal sampling was performed on days 2, 3, 5, 7, and 10. Daily upper (cold) and lower (chest) respiratory symptoms were recorded. Levels of IL-18, IL-1β, interferon gamma (IFN-γ), IL-12, and CXCL10/IFN-γ-induced protein 10 (IP-10) were analyzed using the

Received 24 November 2014; accepted 13 January 2015; electronically published 2 February 2015.

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Clinical Infectious Diseases® 2015;60(10):1528–31

DOI: 10.1093/cid/civ062

Meso-Scale Discovery platform (limit of detection, 0.6 pg/mL except for CXCL10/IP-10 [10 pg/mL]). Clinical characteristics are available online (Supplementary Table 1).

Statistical Analysis

Statistical analysis was performed using SPSS version 22.0 (IBM Corporation). The distributions of cytokine measurements are nonparametric and results shown are medians. Differences between groups were analyzed by Mann–Whitney test and within-group comparisons were analyzed by Wilcoxon signed-rank test. Subjects were divided into "high" and "low" baseline IL-18 groups around the median for each group. Correlations were examined using Spearman correlation test. Differences were considered significant at P values <.05. All P values are 2-sided.

RESULTS

Baseline levels of IL-18 in nasal mucosal fluid did not differ significantly between healthy (median, 509 pg/mL [interquartile range {IQR}, 271–1036 pg/mL]) and asthmatic (median, 418.4 pg/mL [IQR, 171–887 pg/mL]) subjects. Rhinovirus infection led to increased IL-18 in both asthmatic ($P = .003$) and healthy ($P = .006$) subjects, with a greater induction seen in healthy vs asthmatic subjects (peak nasal IL-18: 1188 pg/mL [IQR, 862–2456 pg/mL] in healthy subjects vs 767 pg/mL [IQR, 350–1421 pg/mL] in asthmatic subjects; $P = .018$; Figure 1*A*).

Baseline levels of bronchial IL-18 correlated strongly with baseline levels in the nose ($r = 0.56$, $P < .001$; Figure 1*B*). However, bronchial sampling during infection (limited to day 4 due to invasiveness of bronchoscopy) failed to identify evidence of induction (Supplementary Figure 1). Additionally, levels of bronchial IL-18 did not differ significantly between asthmatic subjects treated with and those naive to inhaled corticosteroid therapy. At baseline, median bronchial IL-18 was 197 pg/mL (IQR, 103–293 pg/mL) in steroid-naive asthmatic subjects vs 130 pg/mL (IQR, 77–228 pg/mL) in steroid-treated asthmatic subjects ($P = .23$); day 4 bronchial IL-18 was 165 pg/mL (IQR, 130–287 pg/mL) in steroid-naive asthmatic subjects vs 124 pg/mL (IQR, 78–164 pg/mL) in steroid-treated asthmatic subjects, respectively ($P = .13$).

As reported previously [13], infection with rhinovirus resulted in increased cold symptoms in both healthy and asthmatic subjects, with additional lower respiratory symptoms (eg, breathlessness and wheeze) experienced by asthmatic subjects only [13]. However, when we categorized subjects purely on the basis of their basal IL-18 levels into "high" (equal to or greater than the median IL-18 level of the group) and "low" (below the median), we observed significantly more severe colds in subjects with low baseline nasal IL-18 vs those with high baseline IL-18 (Figure 1*C*). This finding was not disease specific, being equally apparent in healthy and asthmatic subjects. Combining groups, the total upper respiratory symptom score (total of daily scores between day 0 and 14)—akin to the overall severity of the cold—correlated inversely with baseline nasal IL-18 ($r = -0.45$, $P = .005$) (Figure 1*D*). Furthermore, the observed relationships between nasal IL-18 and cold symptoms corresponded in an identical manner to relationships between baseline bronchial IL-18 levels and the severity of virus-induced lower respiratory symptoms in asthma (Figure 1*E*).

We next performed an analysis of virus load according to baseline IL-18 status. Interestingly, in the healthy subjects a trend toward increased virus load was observed in subjects with low IL-18 levels; however, differences did not reach statistical significance, and the same trend was not apparent in the asthmatic group (Figure 1*F*).

In view of the shared inflammasome-directed activation pathway, we next measured IL-1β levels in airway samples. In accordance with IL-18, baseline nasal IL-1β levels were similar between healthy and asthmatic subjects and were significantly induced by rhinovirus in both healthy ($P = .013$) and asthmatic ($P < .001$) subjects; however, in contrast to IL-18, no significant differences were observed between groups during infection (Supplementary Figure 2*A*). Furthermore, despite the common requirement for caspase 1 processing, IL-1β and IL-18 levels did not correlate at baseline or during infection (Supplementary Figure 2*B* and 2*C*), and no inverse relationship with infection severity could be identified for IL-1β (Supplementary Figure 2*D*). Last, we investigated whether IL-18 correlated with mediators of helper T-cell 1 (Th1) inflammation including IFN-γ and IL-12, observing that IL-18 failed to relate to these cytokines despite strong relationships of these mediators with each other (Supplementary Table 2).

DISCUSSION

Rhinovirus-induced activation of the inflammasome/IL-18 pathway has recently been observed in vitro [4, 10]; however, to date, this is the first report of rhinovirus-induced IL-18 in vivo in humans. Furthermore, we provide the first human data in support of a protective role for IL-18. Specifically, we show that levels of airway mucosal IL-18 prior to infection predicts the severity of the rhinovirus-induced infection, with an inverse relationship demonstrable in both the nasal and bronchial airways. Moreover, this relationship is not disease specific, being present in both healthy and asthmatic individuals.

Our human in vivo findings are consistent with those of animal models in which IL-18–deficient mice experience increased disease severity and mortality following bacterial and viral infections, with a restoration of the normal host response following IL-18 replacement [2, 3, 5–7]. Interestingly, our data are also consistent with animal models of colitis in which a

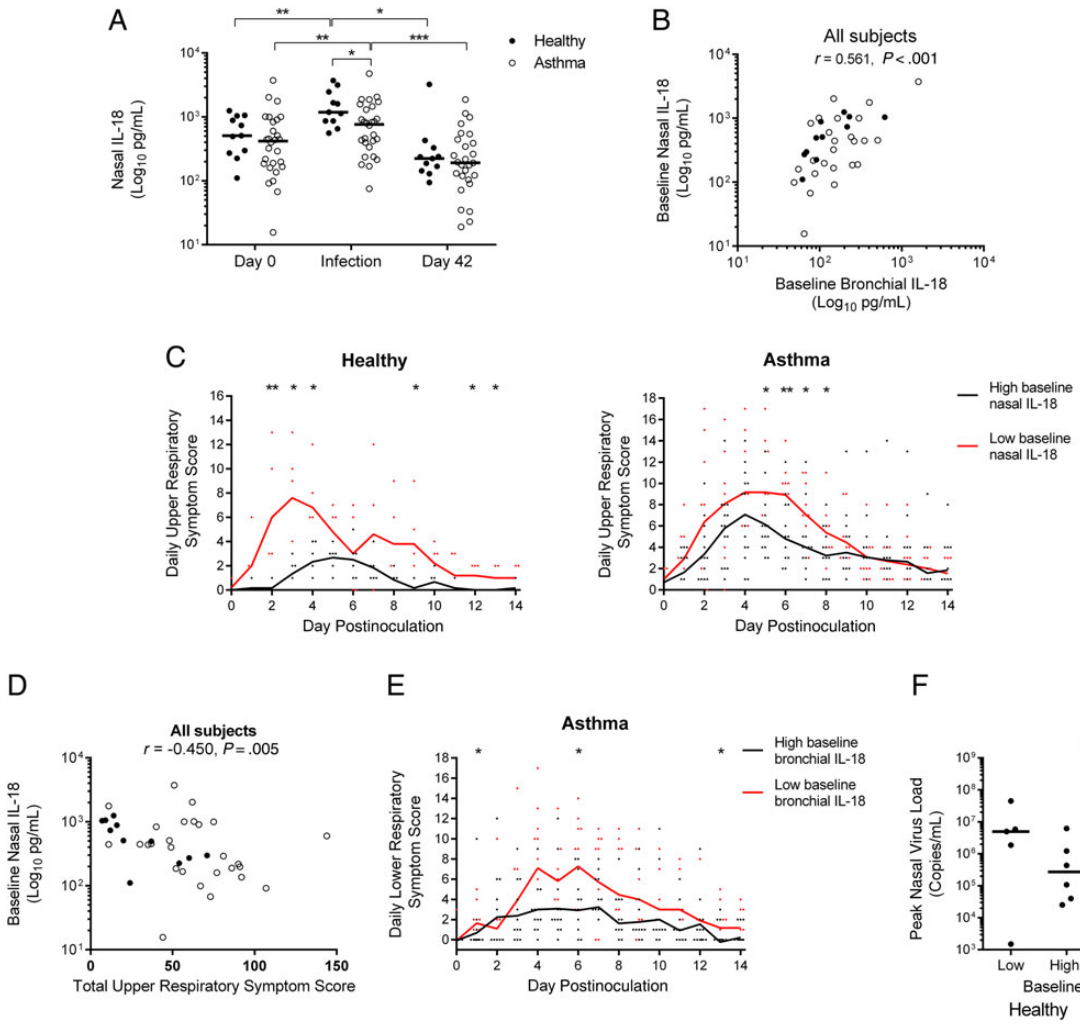

Figure 1. *A*, Interleukin-18 (IL-18) is induced by rhinovirus, with a more marked induction seen in healthy (closed circles, n = 11) vs asthmatic (open circles, n = 28) subjects. *B*, Levels prior to infection in the nose relate to levels in the lung. *C* and *D*, Healthy (n = 6) and asthmatic (n = 14) individuals with high baseline nasal IL-18 levels (black dots) experience significantly milder cold symptoms following rhinovirus infection than subjects with low baseline levels (red dots). *E*, Asthmatic subjects with high baseline bronchial IL-18 (n = 13) experience milder virus-induced asthma exacerbations than asthmatic subjects with low baseline bronchial levels (n = 11). *F*, No statistically significant (NS) differences in virus load were identified according to IL-18 group. Bars (A and *F*) and lines (*C* and *E*) represent median levels, and all correlations are nonparametric (Spearman correlation test). The "high" and "low" IL-18 categories are divided around the medians specific to the healthy and asthmatic groups. Baseline bronchoscopy (*B* and *E*) could not be performed in 3 subjects. Symptom scores (*C*–*E*) are missing for an additional 1 asthmatic subject. The infection level (*A*) and peak level (*F*) represent the greatest (maximal) level during the infection for each subject. **P* < .05; ***P* < .01; ****P* < .001.

key protective role for IL-18 in gastrointestinal mucosal barrier function [15] reminds us of the common embryologic origin of the respiratory and alimentary tracts. Indeed, it is noteworthy that expression of IL-18 and the IL-18 receptor is concentrated within the epithelium of both the respiratory and intestinal tracts [16].

The precise mechanism(s) through which IL-18 achieves its protective function remains debated. Using a model of dextran sulphate sodium–induced colitis, NLRP3$^{-/-}$ and caspase 1$^{-/-}$ mice developed increased intestinal barrier permeability and mortality compared with wild-type mice—an outcome that could be reversed by the administration of IL-18 [15]. Impaired respiratory epithelial integrity in the context of viral infection may similarly lead to increased disease severity.

It is also possible that the protective role of IL-18 may instead relate to its ability to induce IFN-γ (and therefore promote a pro-Th1, antiviral environment). Administration of exogenous IFN-γ in a mouse model of influenza infection rescued the survival of IL-18 KO infected mice [6]; however, other studies have shown that IL-18 administration was sufficient to significantly

improve severe vaccinia-induced symptoms even in mice treated with anti–IFN-γ antibody [17]. Interestingly, we were unable to demonstrate any relationships between IL-18 and IFN-γ levels in vivo, and the protective association of IL-18 was not apparent for IFN-γ. Taken together, it is likely that IL-18–induced upregulation of IFN-γ may be part of, but not solely responsible for, the IL-18-mediated antiviral effects.

In summary, we report the first human data demonstrating rhinovirus-induced IL-18 and suggesting a protective role for IL-18 in the mucosal host defense against virus infection. Although we were unable to demonstrate bronchial IL-18 induction on the single sampling time point of day 4, it is possible that earlier or later sampling may have identified an induction. Although our in vivo findings are limited to a report of relationships, when considered in the light of published animal models of respiratory infection and colitis, we believe our observations of IL-18–associated protection are likely functionally relevant. The relationships we observed were present in both healthy and asthmatic individuals, and in both nasal and bronchial airways, adding strength to this argument. Further studies are now required to explore whether the mucosal protection that IL-18 may confer relates to enhancement of pro-Th1/antiviral immunity or more specifically to structural epithelial integrity. However, what remains tantalizing is the therapeutic potential of IL-18 for patients with chronic respiratory diseases such as asthma, for whom a common cold can potentially be a life-threatening event.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Financial support. This work was supported by the European Research Council (ERC FP7 grant number 233015); a Chair from Asthma UK (number CH11SJ); the Medical Research Council Centre (grant number G1000758); National Institute of Health Research Biomedical Research Centre (grant number P26095); Predicta FP7 Collaborative Project (grant number 260895); and the National Institute for Health Research Biomedical Research Centre at Imperial College London. Novartis Institute for Biomedical Research funded the cytokine assays.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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