# IL-13 dampens human airway epithelial innate immunity through induction of IL-1 receptor–associated kinase M

Qun Wu, MD, PhD,[a] Di Jiang, BS,[a] Sean Smith, BS,[a] Jyoti Thaikoottathil, PhD,[a] Richard J. Martin, MD,[a] Russell P. Bowler, MD, PhD,[a] and Hong Wei Chu, MD[a,b] *Denver, Colo*

**Background: Impaired airway mucosal immunity can contribute to increased respiratory tract infections in asthmatic patients, but the involved molecular mechanisms have not been fully clarified. Airway epithelial cells serve as the first line of respiratory mucosal defense to eliminate inhaled pathogens through various mechanisms, including Toll-like receptor (TLR) pathways. Our previous studies suggest that impaired TLR2 function in $T_H2$ cytokine–exposed airways might decrease immune responses to pathogens and subsequently exacerbate allergic inflammation. IL-1 receptor–associated kinase M (IRAK-M) negatively regulates TLR signaling. However, IRAK-M expression in airway epithelium from asthmatic patients and its functions under a $T_H2$ cytokine milieu remain unclear.**

**Objectives: We sought to evaluate the role of IRAK-M in IL-13–inhibited TLR2 signaling in human airway epithelial cells.**

**Methods: We examined IRAK-M protein expression in epithelia from asthmatic patients versus that in normal airway epithelia. Moreover, IRAK-M regulation and function in modulating innate immunity (eg, TLR2 signaling) were investigated in cultured human airway epithelial cells with or without IL-13 stimulation.**

**Results: IRAK-M protein levels were increased in asthmatic airway epithelium. Furthermore, in primary human airway epithelial cells, IL-13 consistently upregulated IRAK-M expression, largely through activation of phosphoinositide 3-kinase pathway. Specifically, phosphoinositide 3-kinase activation led to c-Jun binding to human IRAK-M gene promoter and IRAK-M upregulation. Functionally, IL-13–induced IRAK-M suppressed airway epithelial TLR2 signaling activation (eg, TLR2 and human β-defensin 2), partly through inhibiting activation of nuclear factor κB.**

**Conclusions: Our data indicate that epithelial IRAK-M overexpression in $T_H2$ cytokine–exposed airways inhibits TLR2 signaling, providing a novel mechanism for the increased susceptibility of infections in asthmatic patients. (J Allergy Clin Immunol 2012;129:825-33.)**

***Key words:*** *IL-13, IL-1 receptor–associated kinase M, Toll-like receptor 2, airway epithelial cells*

Asthma prevalence is predicted to increase globally by 50% every decade.[1] Approximately 300 million persons worldwide have asthma. Asthmatic patients have increased susceptibility to respiratory tract bacterial infections, which frequently cause acute exacerbations of the disease, significantly increase health care costs, and negatively affect the quality of life of the patients and their families.[2] Although several studies have suggested that impaired airway mucosal immunity might account for the frequent airway bacterial infections seen in asthmatic patients,[3,4] the involved molecular mechanisms have not been fully clarified.

Airway epithelial cells represent the first line of respiratory mucosal defense to eliminate inhaled pathogens through various mechanisms, including Toll-like receptor (TLR) pathways.[5,6] For example, human airway epithelial cells respond to bacterial lipopeptide with induction of the antimicrobial peptide human β-defensin 2 (hBD2) in a TLR2-dependent manner.[7] Our previous studies have demonstrated that ovalbumin-induced airway allergic inflammation or $T_H2$ cytokines (eg, IL-4 and IL-13) significantly impair TLR2 expression and IL-6 production in lung cells (eg, dendritic cells), which delays clearance of *Mycoplasma pneumoniae* from murine lungs.[8] Furthermore, adenovirus-mediated TLR2 gene transfer to airway epithelial cells of $Tlr2^{-/-}$ mice significantly primes host defense against *M pneumoniae*, indicating that therapy aimed at enhancing dampened airway epithelial TLR2 signaling in patients with chronic lung diseases (eg, asthma) would be beneficial in the eradication of airway pathogenic bacteria.[9] The above studies strongly suggest that reduction in or lack of TLR2 function in airways might contribute to acute asthma exacerbations by rendering hosts more susceptible to infections with pathogens containing TLR2 ligands.

The $T_H2$ response is a prominent feature of allergic diseases, including asthma. Among $T_H2$ cytokines, IL-13 is considered particularly critical to asthma immunopathogenesis.[10,11] Previous studies suggest that IL-4 and IL-13 impair the expression and function of hBD2 and hBD3 in human epidermal keratinocytes, which might account for the increased susceptibility to skin infections seen in patients with atopic dermatitis.[12,13] Furthermore, IL-4 and IL-13 directly reduce levels of the innate immune molecules TLR9 and hBD2 in human sinonasal epithelial cells and might contribute to microbial colonization in the nasal polyps of patients with chronic rhinosinusitis.[14] In addition, IL-4 and IL-13 downregulate TLR4 expression in a human intestinal epithelial cell line.[15] However, these publications did not provide detailed molecular mechanisms underlying $T_H2$ cytokine–mediated impairment of innate immunity.

IL-1 receptor–associated kinase M (IRAK-M), also known as IRAK-3, negatively regulates TLR signaling and associated

From the Departments of [a]Medicine and [b]Immunology, National Jewish Health and the University of Colorado Denver.

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Corresponding author: Hong Wei Chu, MD, Department of Medicine, National Jewish Health, 1400 Jackson St, Room A639, Denver, CO 80206. E-mail: chuhw@njhealth.org.

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*Abbreviations used*
ALI: Air-liquid interface
AP-1: Activator protein 1
BEGM: Bronchial epithelial cell growth medium
GAPDH: Glyceraldehyde-3-phosphate dehydrogenase
hBD2: Human β-defensin 2
IRAK-M: IL-1 receptor–associated kinase M
NF-κB: Nuclear factor κB
PI3K: Phosphoinositide 3-kinase
rhIL-13: Recombinant human IL-13
shRNA: Short hairpin RNA
TLR: Toll-like receptor

inflammation.[16] The IRAK-M gene is located to chromosome 12q14.2,[17] a region that is repeatedly shown to have linkage to asthma and IgE levels.[18,19] Recently, Balaci et al[20] have revealed that a variation within the IRAK-M gene is associated with early-onset persistent asthma. They have shown that IRAK-M protein is expressed in alveolar and bronchial epithelial cells of healthy human lungs. Their results challenge the previous findings that human IRAK-M is specifically expressed in monocytes and macrophages.[21,22] However, it remains unknown whether epithelial IRAK-M expression differs between healthy subjects and asthmatic patients. If so, does the aberrant IRAK-M expression in the airways of asthmatic patients contribute to impaired innate immune responses (eg, TLR2 signaling)?

In this study we hypothesized that IL-13 can upregulate IRAK-M expression and subsequently inhibits TLR2 signaling (eg, TLR2 and hBD2 expression) in human airway epithelial cells. First, we demonstrated upregulation of IRAK-M protein in epithelium from asthmatic patients versus that found in normal airway epithelium. Second, we verified that IL-13 could induce IRAK-M expression in air-liquid interface (ALI) cultures of primary human bronchial epithelial cells. Third, we revealed that c-Jun directly bound to the human IRAK-M gene promoter on IL-13 stimulation in human airway epithelial cells. Lastly, we defined a critical role of IRAK-M in IL-13–impaired TLR2 signaling in human airway epithelial cells.

## METHODS

For further details, see the Methods section in this article's Online Repository at www.jacionline.org.

### IRAK-M immunohistochemistry staining in human endobronchial biopsy specimens

Endobronchial biopsy specimens of healthy subjects (n = 4) or patients with mild-to-moderate asthma (n = 6) were obtained through bronchoscopy, and the subjects' characteristics have been described in our previous publications.[23-26] Our research protocol was approved by the Institutional Review Board at National Jewish Health. Tissue sections were stained with rabbit anti-human IRAK-M (Millipore, Temecula, Calif) or control rabbit IgG (Vector Laboratories, Burlingame, Calif), and the IRAK-M–stained area in airway epithelium was analyzed.[25]

### ALI cultures of human brushed bronchial epithelial cells

Human primary bronchial epithelial cells were obtained from endobronchial brushings from healthy nonsmoking subjects (n = 4) and patients with mild-to-moderate asthma (n = 6), and the clinical characteristics have been described in our previous publication.[27] Our research protocols were approved by the Institutional Review Board at National Jewish Health. Epithelial cell ALI cultures were performed as previously reported.[27] On day 10 of ALI culture, cells were treated with BSA or 10 ng/mL recombinant human IL-13 (rhIL-13; R&D Systems, Minneapolis, Minn) for 7 days and then lysed for IRAK-M and TLR2 Western blotting.

### ALI cultures of normal human tracheobronchial epithelial cells for mechanistic studies of IL-13–induced IRAK-M expression

Normal human tracheobronchial epithelial cells were obtained from tracheas and bronchi of deidentified organ donors by digesting the tissue with 0.2% protease solution and then subjected to ALI cultures, as described above. On day 10 of ALI culture, cells were treated with (1) BSA or 10 ng/mL rhIL-13 for up to 120 minutes. Then, cells were lysed for phospho-Akt and total Akt Western blotting or (2) BSA or 10 ng/mL rhIL-13 for 6 days. Thereafter, cells were treated with BSA or rhIL-13 in the absence or presence of the phosphoinositide 3-kinase (PI3K) inhibitor wortmannin (100 nmol/L; Cell Signaling, Danvers, Mass) for 2 hours to measure phospho–c-Jun levels or for 24 hours (a total of 7 days of IL-13 treatment) to quantify IRAK-M and TLR2 proteins by using Western blotting.

### c-Jun activity assay in normal human tracheobronchial epithelial cell cultures

Normal human tracheobronchial epithelial cells under submerged culture conditions were treated with BSA or 10 ng/mL rhIL-13 for up to 120 minutes. Nuclear c-Jun activity was analyzed by using an ELISA-based TransAM activator protein 1 (AP-1)/c-Jun activation assay (Active Motif, Carlsbad, Calif).

### Chromatin immunoprecipitation analysis

Normal human tracheobronchial epithelial cells under submerged culture conditions were treated with BSA or 10 ng/mL rhIL-13 for 2 hours. Chromatin immunoprecipitation analysis[28,29] was performed by using a mouse anti–c-Jun antibody (Santa Cruz Biotechnology, Santa Cruz, Calif) or control murine IgG. Immunoprecipitated DNA was amplified by using quantitative real-time RT-PCR with the SYBR Green Supermix (Bio-Rad Laboratories, Hercules, Calif) and primers that cover human IRAK-M gene promoter region containing the putative AP-1/c-Jun binding site. All values were normalized to input DNA and expressed as the fold induction in chromatin enrichment with the c-Jun antibody to indicate c-Jun binding intensity.

### IRAK-M gene knockdown in normal human brushed bronchial epithelial cells

Human IRAK-M short hairpin RNA (shRNA) encoded in pLL3.7 was generated as previously described.[30,31] Briefly, epithelial cells under immersed conditions were transduced with either pLL3.7-shLUC (an irrelevant gene control) or pLL3.7–shIRAK-M once daily for 3 consecutive days. Forty-eight hours after the last transduction, IRAK-M gene knockdown was verified at both the mRNA and protein levels. The remaining cells were used for ALI culture, as described above. On day 10 of ALI culture, cells were treated with BSA or 10 ng/mL rhIL-13 for 6 days. Then cells were treated for 24 hours with BSA or rhIL-13 with or without 100 ng/mL of the TLR2 agonist Pam2CSK4 (InvivoGen, San Diego, Calif). Thereafter, nuclear factor κB (NF-κB) p65 activity, TLR2 protein, and apical hBD2 peptide were examined.

### Generation of a human lung epithelial cell line stably overexpressing human IRAK-M protein

Human IRAK-M cDNA was obtained from Open Biosystems (Huntsville, Ala) and cloned into a mammalian expression plasmid by means of PCR