

Lastly, we examined expression of PAI-1 in each group by using immunohistochemical analysis. As expected, overall PAI-1 protein expression was significantly lower in the lungs of  $\text{Kit}^{\text{w-sh/w-sh}}$  and  $\text{PAI-1}^{-/-}$  BMCMC  $\rightarrow \text{Kit}^{\text{w-sh/w-sh}}$  mice compared with WT and WT BMCMC  $\rightarrow \text{Kit}^{\text{w-sh/w-sh}}$  mice after OVA challenge (see Fig E3, A, in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). MCs were strongly positive for PAI-1 staining, and monocytes/macrophages were another source of strong PAI-1 expression (see Fig E3, B).

Yu et al<sup>2</sup> demonstrated previously that MCs play a critical role in airway remodeling in asthma using a MC knockout and reconstitution approach, which is similar to our current experimental approach. Emerging evidence from our laboratory and those of others shows that PAI-1 plays an important role in the pathogenesis of asthma and that MCs are a major source of PAI-1.<sup>5-7</sup> Therefore we further investigated the causative role of MC-derived PAI-1 in allergen-induced asthma by using an MC knockout and reconstitution model. This study confirmed that MC-specific PAI-1 promotes airway inflammation, goblet cell hyperplasia, collagen deposition, and AHR.

Activated MCs in the airways of asthmatic patients are important not only in initiating allergic inflammation after allergen challenge but also in maintaining inflammation through interaction with resident cells and migratory inflammatory cells in the airways. Endothelial cells, macrophages, and other inflammatory cells tend to produce both PAI-1 and tPA simultaneously.<sup>7,8</sup> However, MCs release tPA constitutively in their resting state and PAI-1 only on activation.<sup>6</sup> Consequently, activated MC-derived PAI-1 converts a fibrinolytic environment to a fibrogenic environment by overcoming tPA activity, which is dominant during the resting state.<sup>6</sup> Previously, we reported that MC-derived PAI-1 is expressed at high levels in human lung samples from fatal asthmatic patients.<sup>6</sup> Taken together, allergen-induced MC activation results in increased levels of PAI-1, which in turn converts fibrinolytic environment to fibrogenic environment.

It is interesting that  $\text{PAI-1}^{-/-}$  BMCMC  $\rightarrow \text{Kit}^{\text{w-sh/w-sh}}$  mice had reduced eosinophilic inflammation. Our previous study showed that PAI-1 inhibition by tiplaxtinin decreased the degree of eosinophilic airway inflammation and AHR in an OVA challenge model of allergic asthma.<sup>4</sup> Miyamoto et al<sup>5</sup> also reported that intra-airway administration of small interfering RNA against PAI-1 reduced eosinophilic airway inflammation and AHR in a murine model of acute asthma. They suggest that increased levels of hepatocyte growth factor caused by PAI-1 inhibition might be a mechanism of attenuation in airway inflammation and AHR in their model of acute asthma. We also measured hepatocyte growth factor and other growth factors, such as TGF- $\beta$ 1 and vascular endothelial growth factor, but could not find any significant difference between the groups. Interestingly, however, our *in vitro* study showed that PAI-1 enhanced IL-4/double-stranded RNA-induced thymic stromal lymphopoietin (TSLP) production from airway epithelial cells (see Fig E4 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)), suggesting a possible feedback loop of IgE-PAI-1-TSLP-IL-4/IL-5-IgE/eosinophil inflammation. Based on this result, we speculate that deletion of MC-derived PAI-1 can reduce eosinophilic airway inflammation at least in part through attenuation of the PAI-1-TSLP feedback pathway.

In conclusion, our experiments demonstrated that MCs are an important source of PAI-1 and that MC-derived PAI-1 contributes to airway inflammation, goblet cell hyperplasia, collagen deposition, and AHR. These data suggest that MC-derived PAI-1 can be a novel therapeutic target for airway remodeling.

Ara Jo, PhD<sup>a</sup>  
Sun H. Lee, PhD<sup>b</sup>  
Dong-Young Kim, MD, PhD<sup>b</sup>  
Seung-Jae Hong, MD, PhD<sup>c</sup>  
Michael N. Teng, PhD<sup>a</sup>  
Narasaiah Kolliputi, PhD<sup>a</sup>  
Richard F. Lockey, MD<sup>a</sup>  
Robert P. Schleimer, PhD<sup>b</sup>  
Seong H. Cho, MD<sup>a,b,c,d</sup>

From <sup>a</sup>the Division of Allergy-Immunology, Department of Internal Medicine, Morsani College of Medicine, University of South Florida, Tampa, Fla; <sup>b</sup>the Division of Allergy-Immunology, Department of Medicine, Feinberg School of Medicine, Chicago, Ill; <sup>c</sup>the Division of Allergy-Immunology, James A. Haley Veterans' Hospital, Tampa, Fla; and <sup>d</sup>the Division of Rheumatology, Department of Internal Medicine, School of Medicine, Kyung Hee University, Seoul, Korea. E-mail: [scho2@health.usf.edu](mailto:scho2@health.usf.edu).

Supported by American Heart Association grant 11SDG7590063 and National Institutes of Health grant 1K23AI110731. This material is the result of work supported with resources and the use of facilities at the James A. Haley Veterans' Hospital. The contents do not represent the views of the Department of Veterans Affairs or the United States Government.

Disclosure of potential conflict of interest: R. P. Schleimer has received grants from the National Institutes of Health (NIH); has consultant arrangements with Intersect ENT, GlaxoSmithKline, Allakos, Aurasense, Merck, BioMarck, Sanofi, AstraZeneca/MedImmune, Genentech, Exicure, and Otsuka; and has stock/stock options in Allakos, Aurasense, BioMarck, and Exicure. S. H. Cho has received grants from the American Heart Association, the NIH, and Sanofi and has received personal fees from Sanofi. The rest of the authors declare that they have no relevant conflicts of interest.

## REFERENCES

- Orihara K, Dil N, Anaparti V, Moqbel R. What's new in asthma pathophysiology and immunopathology? *Expert Rev Respir Med* 2010;4:605-29.
- Yu M, Tsai M, Tam SY, Jones C, Zehnder J, Galli SJ. Mast cells can promote the development of multiple features of chronic asthma in mice. *J Clin Invest* 2006; 116:1633-41.
- Oh CK, Ariue B, Alban RF, Shaw B, Cho SH. PAI-1 promotes extracellular matrix deposition in the airways of a murine asthma model. *Biochem Biophys Res Commun* 2002;294:1155-60.
- Lee SH, Eren M, Vaughan DE, Schleimer RP, Cho SH. A plasminogen activator inhibitor-1 inhibitor reduces airway remodeling in a murine model of chronic asthma. *Am J Respir Cell Mol Biol* 2012;46:842-6.
- Miyamoto S, Hattori N, Senoo T, Onari Y, Iwamoto H, Kanehara M, et al. Intra-airway administration of small interfering RNA targeting plasminogen activator inhibitor-1 attenuates allergic asthma in mice. *Am J Physiol Lung Cell Mol Physiol* 2011;301:L908-16.
- Cho SH, Tam SW, Demissie-Sanders S, Filler SA, Oh CK. Production of plasminogen activator inhibitor-1 by human mast cells and its possible role in asthma. *J Immunol* 2000;165:3154-61.
- Cho SH, Lee SH, Kato A, Takabayashi T, Kulka M, Shin SC, et al. Cross-talk between human mast cells and bronchial epithelial cells in plasminogen activator inhibitor-1 production via transforming growth factor- $\beta$ 1. *Am J Respir Cell Mol Biol* 2015;52:88-95.
- Van De Craen B, Declercq PJ, Gils A. The biochemistry, physiology and pathological roles of PAI-1 and the requirements for PAI-1 inhibition *in vivo*. *Thromb Res* 2012;130:576-85.

Available online March 2, 2018.  
<https://doi.org/10.1016/j.jaci.2018.01.040>

## Recurrent wheezing in children following human metapneumovirus infection



### To the Editor:

Human metapneumovirus (HMPV) is a paramyxovirus responsible for a significant portion (5%-10%) of respiratory tract infections.<sup>1</sup> HMPV causes infection ranging from self-limiting acute upper respiratory tract infection to severe lower respiratory

**TABLE I.** Study participant characteristics at time of enrollment

| Characteristic                                                          | HMPV LRTI (n = 38) | Control (n = 30) | P value |
|-------------------------------------------------------------------------|--------------------|------------------|---------|
| Age at enrollment (y)*                                                  | 0.7 (0.1-5.2)      | 1.2 (0.1-3.8)    | .02     |
| Sex: female, n (%)                                                      | 16 (42)            | 11 (37)          | .65     |
| Black, n (%)                                                            | 13 (34)            | 9 (30)           | .71     |
| Birth length (cm)                                                       | 51.3 ± 3.3         | 50.3 ± 3.3       | .29     |
| Birth weight (g)                                                        | 3285 ± 562         | 3306 ± 563       | .96     |
| Gestational age (wk)                                                    | 39.2 ± 1.5         | 39.3 ± 1.2       | .91     |
| Household income <\$20,000, n (%)                                       | 12 (38)            | 8 (33)           | .75     |
| Peripheral blood eosinophils (%)†                                       | 0.9 ± 1.2          | 2.1 ± 1.6        | .003    |
| Total serum IgE (IU/mL)                                                 | 32.7 ± 42.1        | 37.5 ± 70.1      | .41     |
| History of eczema, n (%)                                                | 4 (11)             | 6 (21)           | .31     |
| History of breast-feeding, n (%)                                        | 22 (59)            | 15 (56)          | .75     |
| History of daycare, n (%)                                               | 11 (29)            | 13 (43)          | .22     |
| Exposed to cat, n (%)                                                   | 5 (14)             | 9 (30)           | .10     |
| Exposed to dog, n (%)                                                   | 11 (30)            | 12 (40)          | .38     |
| Mother smoked during pregnancy, n (%)                                   | 13 (35)            | 4 (13)           | .04     |
| Mother smoked at time of enrollment, n (%)                              | 14 (38)            | 8 (27)           | .33     |
| Smoke exposure from other household member at time of enrollment, n (%) | 17 (46)            | 9 (30)           | .18     |
| Maternal history of asthma, n (%)                                       | 5 (14)             | 6 (21)           | .44     |
| Hospitalized, n (%)                                                     | 34 (89)            | NA               |         |
| Hyperinflation on chest x-ray, n (%)                                    | 6 of 34 (18%)      | NA               |         |
| Chest x-ray infiltrates, n (%)                                          | 22 of 34 (65%)     | NA               |         |
| Duration of hospitalization (d)                                         | 2.8 ± 1.8          | NA               |         |
| Lowest percent oxygen saturation (n = 28)                               | 91 ± 5             | NA               |         |

Results presented as means ± SD unless otherwise specified.

\*Median (range).

†n = 25 for each group.

tract infection (LRTI), and is a frequent cause of bronchiolitis in young children.<sup>2</sup> Wheezing from viral infections in early life is associated with an increased likelihood of subsequent recurrent wheezing and asthma.<sup>3-5</sup> We investigated the risk for persistent wheezing and asthma later in childhood in children who experienced severe LRTI with HMPV.

We conducted a prospective cohort study of children with HMPV LRTI and control participants from December 2007 to October 2014 at a tertiary medical center in St Louis, Missouri. Children with lower respiratory tract symptoms had nasopharyngeal swabs performed for clinical purposes at the discretion of the emergency department or inpatient physician. HMPV was detected in nasopharyngeal samples using a direct immunofluorescence assay with the D3 DFA Reagent from Diagnostic Hybrids (Athens, Ohio) at the hospital virology laboratory.

Those patients with evidence of HMPV infection were screened for eligibility. In brief, children met inclusion criteria for the HMPV group if they were 5 years or younger without a history of asthma or chronic lung disease, had detectable HMPV by nasal swab, had wheezing and symptoms of LRTI, and visited the emergency department or were hospitalized. Participants in the control group were screened during surgical outpatient clinic visits and were otherwise healthy children 5 years or younger undergoing minor surgical procedures, such as eye surgery or tonsillectomy. Children who had evidence of any virus other than HMPV, or who had comorbid lung or systemic disease, were also excluded (for inclusion and exclusion criteria, see Table E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). The study was approved by the Washington University School of Medicine Institutional Review Board. Informed consent was obtained from the parents or guardians of the participants.

At time of enrollment, details on the child's history and exposures were collected (Table I) and the outcomes (wheezing and physician-diagnosed asthma) were assessed every 3 to 6 months for the first 3 years of the study, either at clinic visits or by phone interviews, focused on the child's respiratory symptoms and diagnoses. The presence of a wheezing episode with or without colds was defined by an answer of "yes" by the caregiver to the question "Has your child had wheezing with colds?" or "Has your child had wheezing without colds?" at any follow-up encounter. The presence of a physician diagnosis of asthma was defined by a positive answer to "Has your child been diagnosed with asthma by a physician?"<sup>6</sup> The diagnosis of asthma was confirmed by reviewing the caring physician's medical records. A final follow-up phone call was made 6 years after the start of the study, which reviewed respiratory symptoms and diagnoses.

Time to events (wheezing or asthma diagnosis) was calculated from the date of enrollment to the date of the event or the last follow-up date, whichever came first. Kaplan-Meier nonparametric methods were used to determine the freedom from any episode of wheezing or asthma. Cox proportional hazard models were used to compare the risk of events between the HMPV and control groups. Age, mother's smoking history during pregnancy, and peripheral blood eosinophil percent were included in the Cox model, because they were significantly different between groups at enrollment (Table I). Two-sided *P* values of less than .05 were considered significant.

More than 1000 children were screened for eligibility (see Fig E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). We enrolled 38 children who were hospitalized or visited an emergency room with HMPV LRTI and 30 controls. Average age at enrollment was less for the HMPV group at  $1.1 \pm 1.1$  years compared with  $1.6 \pm 1.0$  for controls (*P* = .02) (Table I). In addition, the peripheral eosinophil percentage at enrollment was lower

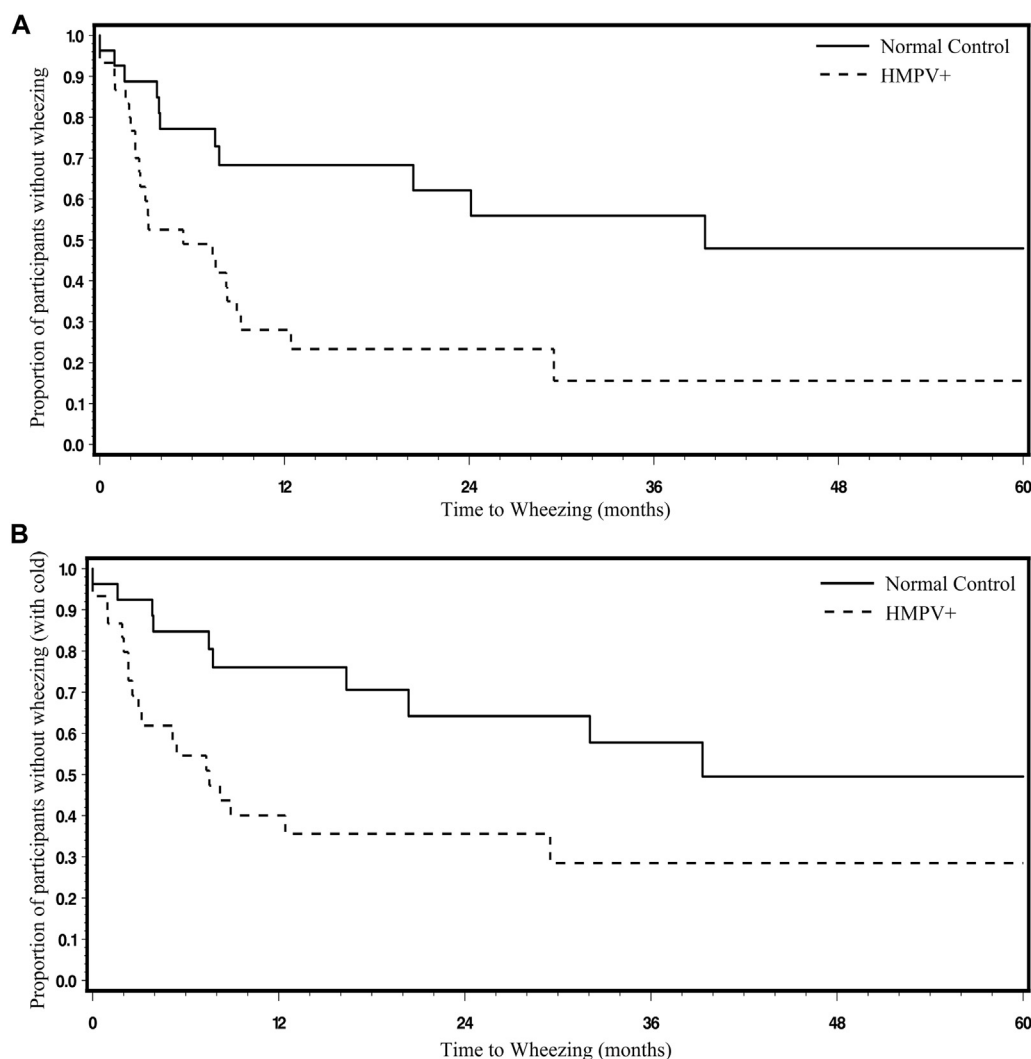


**FIG 1.** Survival analysis for wheezing and asthma. Kaplan-Meier estimates of freedom from any wheezing episodes ( $P = .004$ ) (**A**), wheezing episodes with colds ( $P = .005$ ) (**B**), wheezing episodes without colds ( $P = .003$ ) (**C**), and physician-diagnosed asthma ( $P = .14$ ) (**D**). HMPV LRTI subjects had wheezing earlier in follow-up compared with controls subjects but no significant difference in the time to asthma diagnosis.

in the HMPV group ( $0.9\% \pm 1.2\%$  vs  $2.1\% \pm 1.6\%$ ;  $P = .003$ ), while frequency of maternal smoking during pregnancy was higher in the HMPV group compared with controls (35% vs 13%;  $P = .04$ ).

We obtained follow-up data in 29 children in the HMPV group and 27 controls. Median follow-up duration was 34.9 months (interquartile range, 16.9-53.9 months) for the HMPV group and 31.8 months (interquartile range, 15.6-43.8 months) for the control group ( $P = .6$ ). A significantly higher proportion of HMPV children with follow-up data (23 of 29 [79%]) experienced any wheezing in follow-up compared with controls (12 of 27 [44%];  $P = .007$ ). The HMPV group experienced a higher rate of wheezing episodes compared with controls ( $1.2 \pm 1.4$  vs  $0.6 \pm 1.1$  events/y;  $P = .02$ ). Asthma was diagnosed in a numerically higher proportion of children in the HMPV group (9 of 29 [31%]) in follow-up compared with controls (4 of 27 [15%]); however, this was not statistically significant ( $P = .15$ ).

Survival analysis (Fig 1) indicates that the HMPV group had a shorter duration of time to a wheezing episode after

enrollment ( $P = .004$ ; Fig 1, A) and wheezing with ( $P = .005$ ; Fig 1, B) or without colds ( $P = .003$ ; Fig 1, C), but did not differ significantly in time to asthma diagnosis ( $P = .14$ ; Fig 1, D). In addition, Cox proportional hazard models indicate that HMPV LRTI was associated with a significantly greater likelihood of wheezing after enrollment (hazard ratio [HR], 2.8; 95% CI, 1.4-5.8;  $P = .005$ ), wheezing with a cold (HR, 2.5; 95% CI, 1.1-5.2;  $P = .02$ ), and wheezing apart from a cold (HR, 4.6; 95% CI, 1.7-12.4;  $P = .003$ ) during follow-up compared with controls. We did not find a statistically significant increase in the likelihood for the diagnosis of asthma following HMPV (HR, 2.5; 95% CI, 0.8-8.1;  $P = .12$ ). The risks for asthma and wheeze did not change when adjusted for age at enrollment, baseline eosinophil percentage, or maternal smoking status. A test of confounders (income, daycare status, exposure to cats or dogs, history of eczema, maternal smoking during pregnancy and at time of enrollment, smoke exposure at time of enrollment by others) and their interaction with maternal asthma status did not significantly alter the findings.

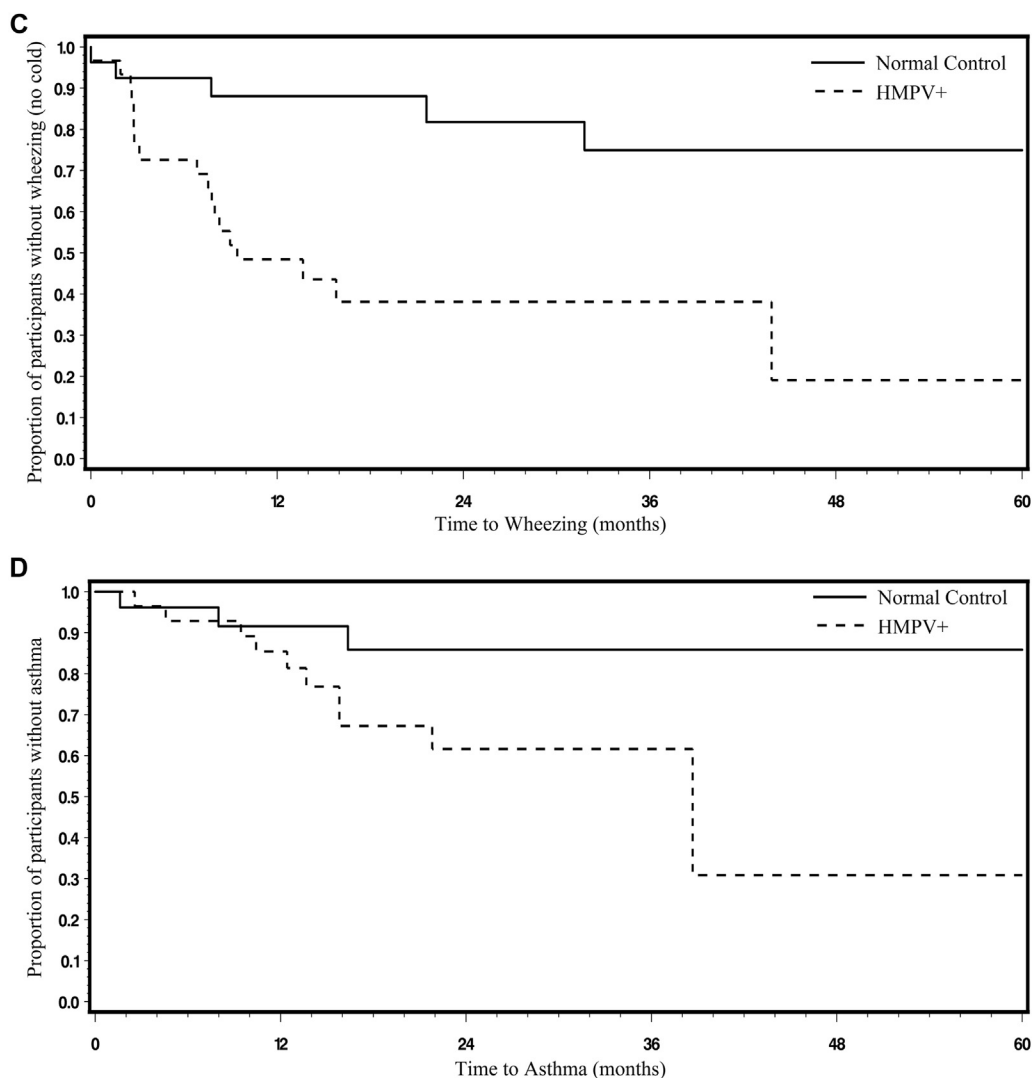


FIG 1. (continued).

We have shown that HMPV LRTI requiring hospital-based care in early life is associated with an increased risk of subsequent wheezing episodes later in childhood. Children with HMPV LRTI early in life had more episodes of wheezing following their initial illness, with increased risk of wheezing both with and without colds. We did not find a statistically significant increased risk of asthma following HMPV LRTI in early life in this study, although the number of children who were diagnosed with asthma was low in both groups. To our knowledge, this study is the first that prospectively assessed the risk of development of recurrent wheezing and asthma in children with HMPV LRTI. HMPV LRTI acts similarly to respiratory syncytial virus (RSV) bronchiolitis as well as other viral LRTIs with lower respiratory symptoms of wheeze and cough.<sup>1-4,7</sup> Many studies have reported the association of HMPV with acute wheezing illnesses in early childhood, but provide limited data on the long-term consequences after the acute infection,<sup>1,2,7,8</sup> unlike the well-established association of the more common RSV bronchiolitis with recurrent wheezing and asthma.<sup>3,5,9</sup>

We acknowledge the limitations of our sample size in evaluating the risk of asthma development as well as our inability to comment on the role of other respiratory viruses in subsequent

wheezing episodes. The outcome of asthma diagnosis was clinically assigned by the participant's physician, as has been done in previous studies,<sup>9</sup> rather than by the study team using a defined algorithm. Wheezing episodes were identified through parental report and may be limited by recall or misclassification bias. Longer follow-up, as well as additional objective measures with spirometry and bronchodilator reversibility, would likely have enhanced the association of metapneumovirus (MPV) with a later asthma diagnosis.<sup>3,5</sup> In addition, we used direct immunofluorescence assay to detect MPV in our clinical viral laboratory, which may have caused underdetection of MPV cases and coinfection with other respiratory viruses compared with using PCR-based methods. Additional larger studies with longer follow-up would be useful to assess the long-term consequences and pathogenesis of HMPV on airway dysfunction, the recurrent wheezing phenotype, and asthma development.

In summary, human metapneumovirus, similar to rhinovirus and RSV, confers an increased risk of recurrent wheezing in children infected early in life.

We acknowledge Jie Zheng, MS, for her work in the initial data analysis, data collection, and power analysis for the study.

Andrea M. Coverstone, MD  
Brad Wilson, MA  
Dana Burgdorf, RN  
Kenneth B. Schechtman, PhD  
Gregory A. Storch, MD  
Michael J. Holtzman, MD  
Mario Castro, MD, MPH  
Leonard B. Bacharier, MD  
Kaharu Sumino, MD, MPH

From the Washington University School of Medicine in St Louis, St Louis, Mo. E-mail: [ksumino@dom.wustl.edu](mailto:ksumino@dom.wustl.edu).

This work was supported by the National Institutes of Health (grant no. 1KMIC156708-1 to K.S.), the National Institute of Allergy and Infectious Diseases (grant no. U19 AI070489), and the National Center for Advancing Translational Sciences (grant no. 5UL1 TR000448-07).

Disclosure of potential conflict of interest: A. M. Coverstone reports grants from the National Institutes of HNIH to Kaharu Sumino/Washington University in St Louis. M. Castro reports grants from the NIH, American Lung Association during the conduct of the study; personal fees from Aviragen, Boehringer Ingelheim, Boston Scientific, Elsevier, Genentech, GSK, Holaira, and Teva; and grants from Amgen, Boehringer Ingelheim, Genentech, Gilead, GlaxoSmithKline, Invivo, Medimmune, Sanofi-Aventis, and Vectura. L. B. Bacharier reports grants from the NIH; personal fees from Aerocrine, GlaxoSmithKline, Genentech/Novartis, Merck, Cephalon, DBV Technologies, Teva, Boehringer Ingelheim, AstraZeneca, WebMD/Medscape, Sanofi/Regeneron, Vectura, and Circassia. The rest of the authors declare that they have no relevant conflicts of interest.

## REFERENCES

- Jartti T, van den Hoogen B, Garofalo RP, Osterhaus AD, Ruuskanen O. Metapneumovirus and acute wheezing in children. *Lancet* 2002;360:1393-4.
- Williams JV, Harris PA, Tollefson SJ, Halburnt-Rush LL, Pingsterhaus JM, Edwards KM, et al. Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. *N Engl J Med* 2004;350:443-50.
- Sigurs N, Gustafsson PM, Bjarnason R, Lundberg F, Schmidt S, Sigurbergsson F, et al. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. *Am J Respir Crit Care Med* 2005;171:137-41.
- Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Pappas TE, et al. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J Respir Crit Care Med* 2008;178:667-72.
- Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. *Am J Respir Crit Care Med* 2000;161:1501-7.
- Castro M, Schweiger T, Yin-Declue H, Ramkumar TP, Christie C, Zheng J, et al. Cytokine response after severe respiratory syncytial virus bronchiolitis in early life. *J Allergy Clin Immunol* 2008;122:726-33.e3.
- Garcia-Garcia ML, Calvo C, Rey C, Diaz B, Molinero MD, Pozo F, et al. Human metapneumovirus infections in hospitalized children and comparison with other respiratory viruses: 2005-2014 prospective study. *PLoS One* 2017;12:e0173504.
- Garcia-Garcia ML, Calvo C, Casas I, Bracamonte T, Rellán A, Gozalo F, et al. Human metapneumovirus bronchiolitis in infancy is an important risk factor for asthma at age 5. *Pediatr Pulmonol* 2007;42:458-64.
- Bacharier LB, Cohen R, Schweiger T, Yin-Declue H, Christie C, Zheng J, et al. Determinants of asthma after severe respiratory syncytial virus bronchiolitis. *J Allergy Clin Immunol* 2012;130:91-100.e3.

Available online March 2, 2018.  
<https://doi.org/10.1016/j.jaci.2018.02.008>

## A critical role for IL-18 in transformation and maturation of naive eosinophils to pathogenic eosinophils



### To the Editor:

Eosinophils have very distinct roles in maintaining innate immunity in healthy subjects and are also involved in the pathogenesis of allergic diseases, autoimmune diseases, and

allograft rejection.<sup>1,2</sup> IL-5 is a well-established differentiation, growth, and survival factor for eosinophils, and earlier reports indicate that IL-5 and eotaxins regulate baseline resident eosinophils.<sup>1,3</sup> Most recently, we and others reported a distinguishable subset of CD101-expressing<sup>4</sup> and CD274-expressing<sup>5</sup> eosinophils, similar to the other immune cell populations that exhibit differences in their phenotype and function.

CD101 expressing eosinophils have been described as IL-5-dependent lung-specific inflammatory eosinophils in a house dust mite (HDM)-induced mouse model of asthma, which is different from the IL-5-independent residential eosinophils detected in healthy mouse lungs.<sup>4</sup> Previously, we also reported CD101- and CD274-expressing eosinophils that are induced in the blood of patients with eosinophilic esophagitis (EoE), and their numbers increase with disease severity and decrease after treatment.<sup>5</sup>

Here we validate our previous findings of CD101- and CD274-expressing eosinophils in patients with EoE and further report that both these subsets are also present in the blood of patients with other allergic diseases, such as asthma (Fig 1, A-D).

Interestingly, we observed that most CD101-expressing blood eosinophils are not CD274-expressing eosinophils (Fig 1, E and F) and that their percentages and absolute numbers increase in the blood of allergic patients (Fig 1, G and H). This observation indicates that both of these eosinophil subsets are either not similar or in a different developmental stage. Most importantly, we report that the tissue-accumulated eosinophils in the nasal lavage fluid of asthmatic patients and esophageal biopsy specimens of patients with EoE are CD101 and CD274 double-positive (CD101<sup>+</sup>CD274<sup>+</sup>) eosinophils (Fig 1, I-N). These observations confirm that CD101- and CD274-expressing eosinophils present in the blood are indeed intermediate-stage eosinophil that mature into CD101<sup>+</sup>CD274<sup>+</sup> double-positive pathogenic eosinophils after accumulation in tissue in patients with allergic diseases. CD101-expressing eosinophils have been shown to be pathogenic in adoptive transfer experiments in a mouse model of asthma.<sup>4</sup> Detailed clinical characteristics of patients are provided in Table E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org).

Mechanistically, to understand eosinophil subset development, we first analyzed IL-5-responsive eosinophils of CD2-IL-5 transgenic mice. This analysis showed that in both spleen (Fig 2, A-F) and blood (Fig 2, G-J) most eosinophils do not express CD101 or CD274 in CD2-IL-5 transgenic mice. These observations are inconsistent with recent reports of CD101-expressing eosinophils as IL-5 dependent,<sup>4</sup> and therefore we investigated the factors that promote CD101 and CD274 expression on eosinophils. Several cytokines (IL-5, IL-13, IL-15, IL-18, IL-21, and IL-33) have been implicated in eosinophil-associated allergic disease.<sup>6,7</sup> Thus we investigated the role of IL-13, IL-15, IL-18, IL-21, and IL-33 in the eosinophil differentiation and transformation of both the CD101 and CD274 eosinophil subsets. We show that CD2-IL-5 transgenic mouse eosinophils become CD101- and CD274-expressing eosinophils kinetically only after *ex vivo* exposure to IL-18. No other cytokine was able to transform IL-5-generated eosinophils into CD101- or CD274-expressing eosinophils (data not shown), indicating that IL-18 is critical in this transformation (Fig 2, K-R). Notably, IL-18 is reported in almost all allergic

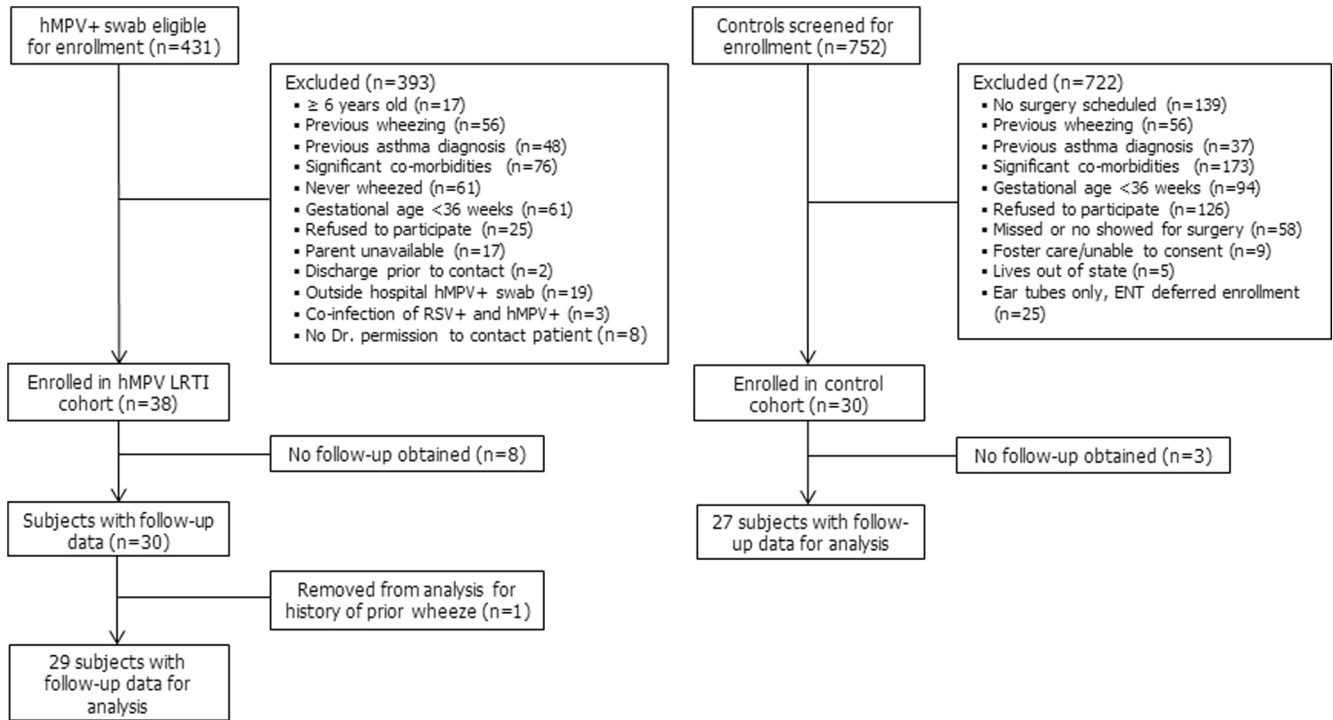
**FIG E1.** Flow diagram of study recruitment, eligibility, and participation.

**TABLE E1.** Inclusion and exclusion criteria

**Inclusion criteria for the HMPV LRTI group**

1. Age 0-5 y with their first episode of wheezing illness (reported by the parents/caregivers) requiring hospitalization or emergency room visit
2. Positive direct immunofluorescence assay for HMPV of nasopharyngeal swab during index hospitalization or emergency room visit
3. Lower viral respiratory tract infection defined as a coryzal illness, with or without fever, with physical examination findings of rapid shallow respirations and audible wheezing, or chest radiographic changes
4. Wheezing episode will be defined as “documented” if
  - A. wheezing was noted by one of the study investigators or by a physician taking care of the child
  - B. the child received bronchodilator therapy as determined by review of the medical record

**Inclusion criteria for control group**

1. Otherwise healthy children of ages 0-5 y
2. Hospitalization for hernia repair or other minimally invasive general surgery or urological procedures, orthopedic procedures, eye surgery, or ENT procedures at the study site children’s hospital

**Exclusion criteria for both groups**

1. Prior episodes of wheezing or a diagnosis of asthma before the index admission
2. Prior use of asthma medication (inhaled corticosteroids, leukotriene modifiers, theophylline, oral corticosteroids)\*
3. Major congenital anomalies of the chest or lung
4. Cystic fibrosis
5. Bronchopulmonary dysplasia
6. Chronic lung injury
7. Significant or life-threatening underlying illness
8. For the control group: symptomatic acute respiratory illness at the time of or within 4 wk before enrollment

ENT, Ear nose throat.

\*Except for limited use in 0-3-year-olds with HMPV if less than 4-month duration (resulted in 2 additional children enrolled).