Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

*These authors contributed equally to this work.
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REFERENCES

1. Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. Science 2008;319:1096-100.
2. Hino S, Miyata H. Torque teno virus (TTV): current status. Rev Med Virol 2007; 17:45-57.
3. Focosi D, Maggi F, Albani M, Macera L, Ricci V, Gragnani S, et al. Torquetenovirus viremia kinetics after autologous stem cell transplantation are predictable and may serve as a surrogate marker of functional immune reconstitution. J Clin Virol 2010;47:189-92.
4. Dupre L, Andolfi G, Tangye SG, Clementi R, Locatelli F, Aricò M, et al. SAP controls the cytolytic activity of $CD8^+$ T cells against EBV-infected cells. Blood 2005;105:4383-9.
5. Xia Z, Liu Q, Berger CT, Keenan BT, Kaliszewska A, Cheney PC, et al. A 17q12 allele is associated with altered NK cell subsets and function. J Immunol 2012;188: 3315-22.
6. Horton MR, Powell JD. Quieting T cells with Slfn2. Nat Immunol 2010;11:281-2.
7. Bustos O, Naik S, Ayers G, Casola C, Perez-Lamigueiro MA, Chippindale PT, et al. Evolution of the Schlafen genes, a gene family associated with embryonic lethality, meiotic drive, immune processes and orthopoxvirus virulence. Gene 2009;447:1-11.
8. Berger M, Krebs P, Crozat K, Li X, Croker BA, Siggs OM, et al. An Slfn2 mutation causes lymphoid and myeloid immunodeficiency due to loss of immune cell quiescence. Nat Immunol 2010;11:335-43.

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Plasminogen activator inhibitor-1 in sputum and nasal lavage fluids increases in asthmatic patients during common colds

To the Editor:

The possibility that recurrent asthma exacerbations associated with common colds promote airway remodeling is suggested by the finding of accelerated lung function decline over time in patients with asthma who have frequent exacerbations.[1] The presumed cause of loss of lung function in asthma is airway remodeling. One of the inflammatory mediators thought to promote airway remodeling is plasminogen activator inhibitor-1 (PAI-1), which inhibits both the fibrinolytic system and the matrix metalloproteinase system.[2] We have previously reported that PAI-1 is highly expressed in patients with fatal asthma[3] and that elevated plasma levels of PAI-1 are associated with diminished forced vital capacity.[4] We here report that common colds are associated with increased PAI-1 production in airways of asthmatic subjects.

TABLE I. Demographic and clinical characteristics

	Asthma (n = 52)	Allergic rhinitis (n = 9)	Healthy control (n = 14)	*P* value
Age (y), median (IQR)	32.5 (16.3)	35.0 (24.5)	29.5 (7.0)	.184*
Sex: female, n (%)	40 (77)	7 (78)	8 (57)	.315†
Atopy, n (%)	**38 (73)**	**9 (100)**	2 (14)	**<.0001**†
Race, n (%)				
White	33 (63)	5 (56)	13 (93)	
Black	6 (12)	1 (11)	0 (0)	
Hispanic	5 (10)	1 (11)	0 (0)	
Others	8 (15)	2 (22)	1 (7)	
Baseline FEV_1 % predicted	**92.2**‡	109.0	104.0	**.0008***
Virus detection, n (%)				
Rhinovirus only	22 (42)	3 (33)	7 (50)	
Coronavirus only	6 (12)	0 (0)	3 (21)	
RSV only	2 (4)	0 (0)	0 (0)	
Enterovirus only	1 (2)	0 (0)	0 (0)	
Influenza virus only	0 (0)	1 (11)	0 (0)	
RSV + coronavirus	1 (2)	0 (0)	0 (0)	
RSV + influenza virus	1 (2)	0 (0)	0 (0)	
Any virus	33 (63)	4 (44)	10 (71)	.177†
Current asthma control meds, n (%)				
ICS only	8 (15)			
Singular only	1 (2)			
ICS + LABA	6 (12)			
ICS + LABA + others	4 (8)			
No medications	33 (63)			
Nasal steroids, n (%)	9 (17)	0 (0)		

ICS, Inhaled corticosteroids; *IQR*, interquartile range; *LABA*, long-acting β-2-adrenergic receptor agonists; *meds*, medications; *RSV*, respiratory syncytial virus.
**P* from the Kruskal-Wallis test comparing all 3 groups.
†*P* from the χ^2 test comparing all 3 groups.
‡Asthma severity based on baseline FEV_1 (spirometry was available in 44 patients out of 52): FEV_1 is 80% or more in 86.4% (n = 38), 60% to 79% in 13.6% (n = 6), and less than 60% in 0% of asthmatic patients.

Fifty-two asthmatic subjects, 9 subjects with allergic rhinitis, and 14 healthy controls were evaluated within 1 to 3 days of cold onset (visit 1), then between day 5 and 7 of cold symptoms (visit 2), and at 6 weeks or longer thereafter to assess baseline status (visit 3). At each visit, induced sputum and nasal lavage fluid (NLF) samples were collected and spirometry performed as described previously.[5] The Virochip[5] was used to detect viruses at visit 1. Allergy skin testing was performed at visit 3 to assess atopy. This study was approved by the Internal Review Boards of the University of California at San Francisco and Northwestern University. PAI-1 concentrations in sputum and NLF were determined by using ELISA (AssayPro, St Charles, Mo). All statistical analyses were performed with the Prism software, version 5 (GraphPad, San Diego, Calif). First, all the 3 groups were compared by using the Kruskal-Wallis test, and then 2-group

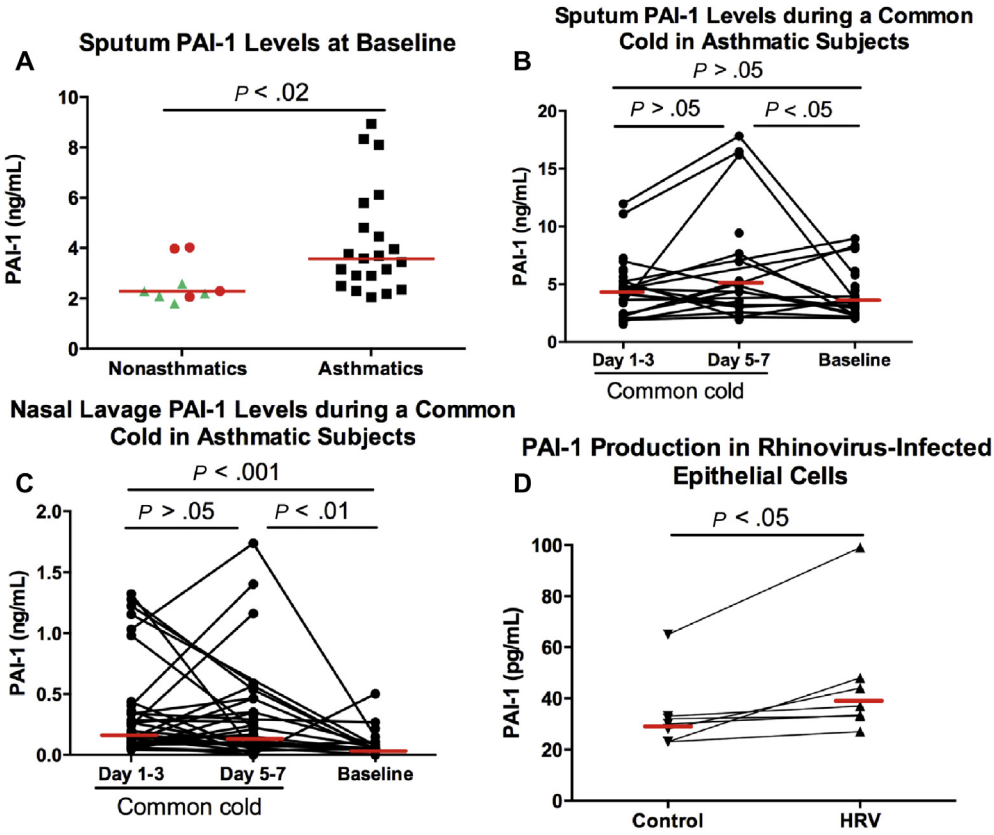

FIG 1. PAI-1 in airway secretions during a common cold. Baseline sputum PAI-1 levels were measured in asthmatic and nonasthmatic subjects **(A**, *red circles*—allergic rhinitis; *green triangles*—healthy controls**)**. Both sputum **(B)** and nasal lavage **(C)** PAI-1 levels were also measured during colds in asthmatic patients. **D,** PAI-1 levels in supernatants of nasal epithelial cells from asthmatic patients 48 hours after human rhinovirus *(HRV)* infection.

comparisons were done with the Mann-Whitney *U* test. Serial measurements were analyzed with the Friedman rank test and paired comparisons by the Wilcoxon paired test. A *P* value of less than .05 was considered statistically significant.

Clinical characteristics of subjects showed expected differences (Table I). A higher proportion of allergic rhinitis and asthmatic subjects were atopic compared with healthy controls, and asthmatic subjects had the lowest FEV_1. There were no significant differences in age and sex among the groups. The proportion of respiratory virus detection was similar between asthmatic and healthy subjects (63.4% vs 71.4%). Among the detected viruses, rhinovirus was the most prevalent in the 3 subject groups. At baseline, sputum PAI-1 levels were significantly higher in asthmatic subjects than in nonasthmatic controls (median ± interquartile range, 3.6 ± 2.6 vs 2.3 ± 2.1 ng/mL; $P < .02$) (Fig 1, *A*). In asthmatic patients, sputum PAI-1 levels increased significantly on day 5 to 7 compared with the baseline levels ($P < .05$; Fig 1, *B*), whereas they did not change significantly in nonasthmatic subjects (see Fig E1 in this article's Online Repository at www.jacionline.org). Sputum PAI-1 levels in asthmatic patients with exacerbation (FEV_1 drop ≥10%, n = 4) were higher than in those without exacerbation (n = 17), although it was not statistically significant (6.6 vs 4.7 ng/mL on days 1-3, $P = .9$; 11.7 vs 4.8 ng/mL on days 5-7, $P = .3$). There was no significant difference in baseline NLF PAI-1 levels between asthmatic and nonasthmatic subjects (0.05 vs 0.08 ng/mL, $P = .2$). PAI-1 levels in NLF samples from asthmatic patients were significantly higher both at days 1 to 3 and at days 5 to 7 than at baseline ($P < .001$ and $P < .01$, respectively; Fig 1, *C*). Interestingly, asthmatic subjects had an early elevation in PAI-1 levels (days 1-3) in NLF samples, which was not observed in NLF samples from nonasthmatic subjects (see Fig E2 in this article's Online Repository at www.jacionline.org). To investigate whether rhinovirus, the most prevalent common cold virus, induces airway epithelial cells from asthmatic subjects to produce PAI-1, we obtained and cultured primary nasal epithelial cells in submerged medium from 7 asthmatic subjects, and treated them with either human rhinovirus (HRV) serotype 16 at multiplicity of infection of 1 or vehicle control for 48 hours. PAI-1 levels in the supernatants of infected cultures from asthmatic patients increased significantly compared with noninfected cultures ($P < .05$; Fig 1, *D*).

Our results show that at baseline, sputum PAI-1 levels are significantly higher in asthmatic patients versus nonasthmatic controls. In addition, the common cold increased PAI-1 levels in upper and lower airways of asthmatic subjects but not in control subjects. Lastly, *in vitro*, HRV induced epithelial production of PAI-1. Our data on increased sputum PAI-1 levels at baseline in asthma are similar to previous reports.[6] Previous studies suggest that PAI-1 may be related to airway obstruction by not only extracellular matrix deposition in the airway wall but also intraluminal fibrin deposition.[7,8] This may explain at least in part the mechanism by which frequent exacerbations may cause progressive

airway obstruction in a subset of patients, and why reduction in FEV_1 is associated with a history of frequent exacerbations in asthmatic patients.[9] A similar study of asthmatic subjects with cold showed that there was a very high level of fibrinogen in induced sputum on day 4.[10] We hypothesize that this highly elevated fibrinogen level in airways of asthmatic subjects can potentiate conversion to fibrin, which is not degraded because of the elevated local PAI-1 level, an occurrence that may lead to the airway obstruction. Although we could not find a negative correlation between sputum PAI-1 levels and lung function due to the small sample size, we found that 2 patients with very high sputum PAI-1 level on days 1 to 3 and days 5 to 7 (Fig 1, *B*) were among 4 patients who had significant asthma exacerbation with FEV_1 drop of 10% or more. It would be interesting to conduct further studies on this observation. A recent study showed that sputum levels of PAI-1 were significantly higher in patients with a longer duration than in those with a shorter duration of asthma.[6] Our results raise the hypothesis that repeated respiratory viral infections may lead to repeated transient increases in airway PAI-1 levels in susceptible asthmatic patients, which over several years could lead to accelerated remodeling and progressive airway obstruction.[6,8]

In summary, this study demonstrates that lower airway PAI-1 levels are higher in asthmatic subjects than in healthy subjects and a common cold further increases upper and lower airway PAI-1 levels in asthmatic subjects. These results may explain the association between recurrent exacerbations and persistent lower airway obstruction. Further studies are needed to understand whether elevated PAI-1 levels lead to the accumulation of fibrin and extracellular matrix in airways of asthmatic patients.

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Seong H. Cho, MD[a,b]
Seung J. Hong, MD, PhD[a,b]
Haimei Chen, PhD[a]
Ali Habib, BA[a]
David Cho[a]
Sun H. Lee, PhD[a]
Joseph Kang, PhD[c]
Theresa Ward, RN[d]
Homer A. Boushey, MD[d]
Robert P. Schleimer, PhD[a]
Pedro C. Avila, MD[a]

From [a]the Division of Allergy-Immunology, Department of Medicine, Northwestern Feinberg School of Medicine, Chicago, Ill; [b]the Division of Rheumatology, Department of Medicine, Kyung Hee University School of Medicine, Seoul, Korea; [c]the Department of Preventive Medicine, Northwestern Feinberg School of Medicine, Chicago, Ill; and [d]the Department of Medicine, Cardiovascular Research Institute, University of California at San Francisco, San Francisco, Calif. E-mail: seong-cho@northwestern.edu.

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REFERENCES

1. McDonald VM, Gibson PG. Exacerbations of severe asthma. Clin Exp Allergy 2012;42:670-7.
2. Ma Z, Paek D, Oh CK. Plasminogen activator inhibitor-1 and asthma: role in the pathogenesis and molecular regulation. Clin Exp Allergy 2009;39:1136-44.
3. 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.
4. Cho S, Kang J, Lyttle C, Harris K, Daley B, Grammer L, et al. Association of elevated plasminogen activator inhibitor 1 levels with diminished lung function in patients with asthma. Ann Allergy Asthma Immunol 2011;106:371-7.
5. Kistler A, Avila PC, Rouskin S, Wang D, Ward T, Yagi S, et al. Pan-viral screening of respiratory tract infections in adults with and without asthma reveals unexpected human coronavirus and human rhinovirus diversity. J Infect Dis 2007;196:817-25.
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.
7. Pampuch A, Kowal K, Bodzenta-Lukaszyk A, Di Castelnuovo A, Chyczewski L, Donati MB, et al. The -675 4G/5G plasminogen activator inhibitor-1 promoter polymorphism in house dust mite-sensitive allergic asthma patients. Allergy 2006;61:234-8.
8. 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.
9. Miller EK. New human rhinovirus species and their significance in asthma exacerbation and airway remodeling. Immunol Allergy Clin North Am 2010;30:541-52.
10. Pizzichini MM, Pizzichini E, Efthimiadis A, Chauhan AJ, Johnston SL, Hussack P, et al. Asthma and natural colds: inflammatory indices in induced sputum: a feasibility study. Am J Respir Crit Care Med 1998;158:1178-84.

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Pulmonary alveolar proteinosis in adenosine deaminase–deficient mice

To the Editor:

Adenosine deaminase (ADA) is a ubiquitous enzyme important for the metabolism of adenosine and deoxyadenosine. Inherited defects in the function of ADA lead to severe immune abnormalities with increased susceptibility to lethal infections. Current treatments for ADA deficiency include allogeneic hematopoietic stem cell transplantation, autologous gene therapy, and enzyme replacement therapy with bovine ADA conjugated to polyethylene glycol (PEG-ADA).

Recently, we identified the accumulation of a surfactant-like substance, suggestive of pulmonary alveolar proteinosis (PAP), in ADA-deficient patients.[1] PAP is a rare lung disorder characterized by impaired pulmonary surfactant homeostasis resulting in progressive respiratory failure.[2] To further establish and understand the role of ADA deficiency in the development of PAP, we studied ADA-deficient (ADA-KO) mice (see the Methods section in this article's Online Repository at www.jacionline.org for additional information), which display many of the metabolic, immune, and systemic abnormalities observed in ADA-deficient patients.[3] Previous reports found increased bronchial airways mucus, inflammatory cells accumulation, and structural abnormalities in the lungs of ADA-KO mice, although PAP was not described.[4,5] All our ADA-KO mice died by age 18 to 21 days, as previously

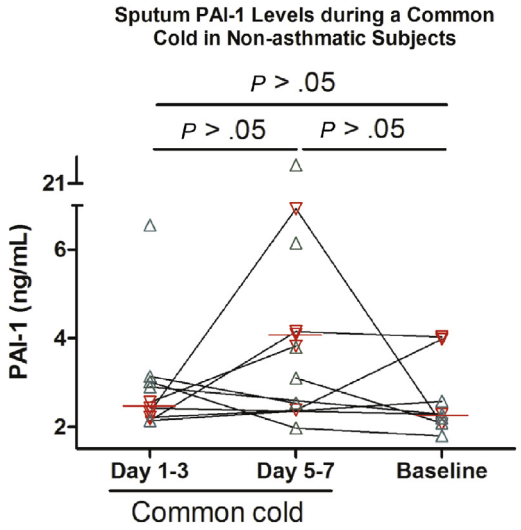

FIG E1. Sputum PAI-1 levels of nonasthmatic subjects (healthy controls, *green upward triangle*; allergic rhinitis, *red downward triangle*) on days 1 to 3 and days 5 to 7 of the common cold onset were compared with those at baseline visit (Wilcoxon paired test, *red lines* indicate median value, $P > .05$).

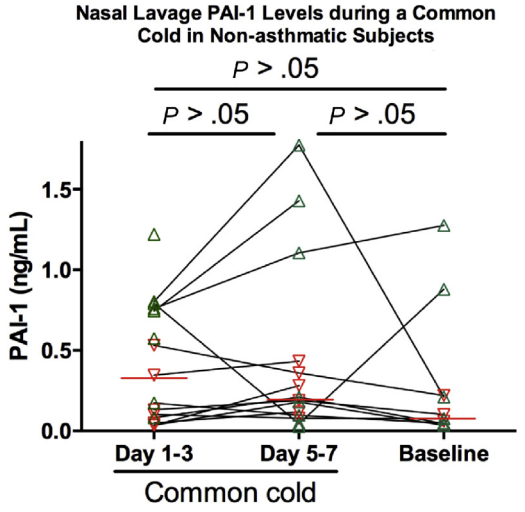

FIG E2. Nasal lavage fluid levels of PAI-1 of nonasthmatic subjects (healthy controls, *green upward triangle*; allergic rhinitis, *red downward triangle*) on days 1 to 3 and days 5 to 7 of the common cold onset were compared with those at baseline visit (Wilcoxon paired test, $P > .05$).