Enhanced airway hyperresponsiveness in a mouse model of asthma with A(H1N1)pdm09 infection

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August 10, 2020

Abstract

Background: Severe asthma exacerbation is an important comorbidity of the 2009 HIN1 pandemic [A(H1N1)pdm09] in asthmatic patients. However, the mechanisms underlying severe asthma exacerbation remain unknown. Using a mouse model of asthma, we evaluated airway hyperresponsiveness (AHR) in mice with A(H1N1)pdm09 infection and those with seasonal influenza for comparison. We also measured AHR in paediatric participants infected with A(H1N1)pdm09. **Methods:** BALB/c mice aged 6-8 weeks were sensitized and challenged with ovalbumin. Either mouse-adapted A(H1N1)pdm09, seasonal H1N1 virus (1×10^5 pfu/20 μL), or mock treatment as a control was administered intranasally. At 3, 7, and 10 days after infection, each group of mice was evaluated for AHR by methacholine challenge using an animal ventilator, flexiVent®. Lung samples were resected and observed using light microscopy to assess the degree of airway inflammation. AHRs in paediatric participants were defined as the provocative acetylcholine concentration causing a 20% reduction in $FEV_{1.0}$ (PC_{20}). **Results:** Airway resistance was significantly enhanced in A(H1N1)pdm09-infected asthmatic mice compared to that in seasonal H1N1-infected mice ($p<0.001$), peaking at 7 days post-infection and then becoming similar to control levels by 10 days post-infection. Histopathological examination of lung tissues showed more intense infiltration of inflammatory cells and severe tissue destruction in A(H1N1)pdm09-infected mice at 7 days post-infection than at 10 days post-infection. AHRs in the paediatric participants were temporarily increased, and alleviated by 3 months after discharge. **Conclusions:** Our results suggest that enhanced AHR could contribute to severe exacerbation in human asthmatic patients with A(H1N1)pdm09 infection.

Introduction

Asthma exacerbation is a major cause of disease morbidity that increases health care costs, and in some patients, progressive loss of lung function[1]. Exposure to aeroallergens and environmental factors, such as smoking, PM2.5, or diesel exhaust particles, triggers asthma exacerbation[2,3]. Respiratory viral infection is also associated with the pathophysiology of asthma exacerbation, particularly in childhood[4]. The most prominent pathogens involved in asthma exacerbation include human rhinovirus, respiratory syncytial virus, enterovirus, influenza virus, and human metapneumovirus[5,6].

Many severe and fatal cases of the 2009 HIN1 pandemic [A(H1N1)pdm09] infection have been reported, both in patients with underlying diseases as well as in healthy children and young adults[7-9]. Asthma is

among the most common underlying conditions in patients hospitalized with A(H1N1)pdm09 infection and asthmatic children show greater susceptibility to A(H1N1)pdm09 viral infection[10], suggesting that severe asthma exacerbation is an important comorbidity of influenza infection in patients with asthma[7-9].

We previously reported that A(H1N1)pdm09 infection, but not seasonal H1N1 infection, induces severe pulmonary inflammation with elevated cytokine levels in a mouse model of asthma[11,12]. Moreover, asthmatic model mice with A(H1N1)pdm09 infection are prone to an earlier onset of severe pulmonary inflammation compared to those with seasonal H1N1 infection[13], suggesting that a hyper-cytokine condition is involved in severe pneumonia and atelectasis. Another report showed that A(H1N1)pdm09 induces AHR in a non-asthmatic mouse model[14]. However, no reports have evaluated the effect of A(H1N1)pdm09 on airway constriction in patients with asthma and, to date, the mechanisms of severe asthma exacerbation due to A(H1N1)pdm09 infection remain unclear.

In this study, we investigated airway hyperresponsiveness (AHR) induced by A(H1N1)pdm09 infection in comparison to that caused by seasonal H1N1 influenza. We show that changes in AHR are greater in asthmatic mice with A(H1N1)pdm09 infection than in those with seasonal H1N1 influenza. Enhanced AHR becomes normalized by 10 days post-infection in the mouse model, a trend that supports post infection observations of AHR in paediatric patients with A(H1N1)pdm09 infection. These findings indicate that enhanced AHR contributes to the severe asthma exacerbation phenotype triggered by A(H1N1)pdm09 infection.

Methods

Sensitization of mice and allergen challenge

BALB/c mice aged 6–8 weeks were obtained from Chiyoda Kaihatsu Co. (Tokyo, Japan) and sensitized and challenged with grade II ovalbumin (Sigma–Aldrich., St. Louis, MO, USA) as previously described[11-13]. All animal experimentation procedures were approved by the Institutional Animal Care and Use Committee of Yamaguchi University (No. 29-S01), and all methods were conducted in accordance with approved guidelines. Additionally, animal experiments conformed to the revised Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council "Guide for the Care and Use of Laboratory Animals" published by the National Academy Press, Washington, D.C. 1996.

Virus infection

Mouse-adapted A(H1N1)pdm09 (strain: A/Narita/1/09) or seasonal H1N1 (strain: A/Puerto Rico) viruses were provided by the National Institute of Infectious Diseases (Tokyo, Japan). On day 31, influenza virus (concentration: 1×10^5 pfu/20 μL) or vehicle (mock-infection) was administered intranasally to the mice. The number of mice in each group ranged from 4 to 7.

Measurement of AHR

Mice were anesthetized by intraperitoneal injection of xylazine (12 mg/kg) and pentobarbital (70 mg/kg) at 3, 7, and 10 days post-infection. After anesthetization, mice were cannulated and connected to an animal ventilator (flexiVent®, SCIREQ, Montreal, QC, Canada). The mice inhaled aerosolized phosphate-buffered saline or 3, 6, 12, 24, or 48 mg/mL methacholine in phosphate-buffered saline. We measured the following parameters: resistance of the respiratory system (R_{rs}) and tissue damping (G); R_{rs} and G reflect the resistance of the central and peripheral airways, respectively[15,16]. These parameters were measured at frequent intervals of 10–15 seconds 12 times following activation of the nebulizer.

Histological examination of the lungs

After the methacholine challenge, the lung was resected from mice that were euthanized with high dose pentobarbital (200 mg/kg). Lung tissues were fixed in 10% buffered formalin for 24 h at room temperature and then embedded in paraffin. Serial sections (3-μm-thick) were cut and stained with haematoxylin and eosin (Muto Pure Chemicals Co., Tokyo, Japan).

Measurement of AHR in children with A(H1N1)pdm09 infection

Studies on paediatric patients were carried out in accord with the Declaration of Helsinki. This study enrolled paediatric participants with A(H1N1)pdm09 infection and hypoxia (SpO_2[?]90%) within the first day of fever admitted to Fukuokahigashi Medical Center between September 2009 and December 2010. Bronchial asthma was diagnosed according to the Japanese Pediatric Guidelines for the Treatment and Management of Bronchial Asthma 2008 (JPGL 2008)[17]. The participants were diagnosed with A(H1N1)pdm09 infection by polymerase chain reaction (PCR) at Fukuoka Institute of Health and Environmental Sciences. AHRs were measured at 1 and 3 months after discharge. Anti-inflammatory drugs and bronchodilators were prohibited 1 month after discharge. Increasing concentrations of acetylcholine (39, 78, 156, 312, 625, 1,250, 2,500, 5,000, 10,000, and 20,000 μg/mL) were inhaled by a nebulizer until the forced expiratory volume in 1 second ($FEV_{1.0}$) was reduced by 20% from a post-nebulized saline value. $FEV_{1.0}$ was measured using a spirometer (HI-801, CHEST M.I., Inc., Tokyo, Japan). AHR was defined as the provocative concentration causing a 20% fall in $FEV_{1.0}$(PC_{20}). This study was approved by the institutional review board of Fukuokahigashi Medical Center (2020-rin-8).

Statistical analysis

For mouse data, differences between two groups and between three groups were analysed using the Mann–Whitney *U* test and Steel–Dwass test, respectively. For human data, differences between groups were analysed using the Wilcoxon signed rank test. When p-values less than 0.05, differences between means were considered to be statistically significant. All analyses and calculations were performed using JMP® Pro version 13.0.0 software (SAS Institute, Inc., Cary, NC, USA).

Results

Enhanced AHR in asthmatic mice with A(H1N1)pdm09 infection

The central airway resistance revealed by the R_{rs} value was significantly increased in the A(H1N1)pdm09 mouse group compared to that in the seasonal H1N1 mouse or control group (**Fig. 1**); this was particularly prominent after treatment with 48 mg/ml methacholine at 3 and 7 days after infection [3 days post-infection A(H1N1)pdm09*vs.* seasonal; 4.40 *vs.* 3.29 s/ml, $p < 0.001$,*vs.* control; *vs.* 3.11 s/ml, $p < 0.001$; 7 days post-infection A(H1N1)pdm09 vs. seasonal; 8.60 *vs.* 3.24 s/ml, $p < 0.001$, *vs.* control; *vs.* 3.00 s/ml, $p < 0.001$]. However, there was no significant difference among the three groups at 10 days post-infection. In contrast, there were no significant differences in the R_{rs} between the seasonal H1N1 and mock groups at 3, 7, or 10 days post-infection.

Next, peripheral airway resistance as reflected by G was compared among the three groups (**Fig. 2**). The peripheral airway resistance was also significantly increased in the A(H1N1)pdm09 group compared to that in the seasonal H1N1 and control groups; this difference was particularly prominent after treatment with 48 mg/ml methacholine at 3 and 7 days post-infection [3 days post-infection A(H1N1)pdm09 vs. seasonal; 25.2 *vs.* 16.5 s/ml, $p < 0.001$, *vs.*control; *vs.* 15.5 s/ml, $p < 0.001$; 7 days post-infection A(H1N1)pdm09 vs. seasonal; 50.0 *vs.* 16.7 s/ml, $p < 0.001$, *vs.* control; *vs.* 15.6 s/ml, $p < 0.001$]. However, these differences in airway resistance were not significantly different among the three groups at 10 days post-infection.

We investigated the changes in AHR of non-asthmatic mice with A(H1N1)pdm09 infection (**Figs. 1, 2**). Although AHR was enhanced with A(H1N1)pdm09 infection in non-asthmatic mice, the changes in AHR were slight compared to the changes observed in asthmatic mice, suggesting that A(H1N1)pdm09 infection more robustly enhances AHR in asthmatic animals.

We further evaluated the alternations of AHR in asthmatic mice during the post-infection period of A(H1N1)pdm09 infection (**Fig. 3**). Airway resistance was significantly enhanced at 7 days post-infection compared to at 3- or 10-days post-infection ($p < 0.001$), whereas there were no differences in airway resistance between 3 and 10 days post-infection. When the body weight of mice was compared among the three groups to evaluate the systemic damage caused by A(H1N1)pdm09 infection at 3, 7, or 10 days post-infection, no significant differences between days were detected (data not shown).

Histopathological findings in the lungs

Haematoxylin and eosin staining of lung tissues from mice at 3, 7, and 10 days post-infection is shown in **Fig. 4** . Inflammatory cell infiltration was prominently observed in A(H1N1)pdm09-infected mice, whereas this was rarely observed in mice infected with seasonal H1N1 at 3 days post-infection (**Fig. 4A**). The lung inflammation was clearly observed in A(H1N1)pdm09- and seasonal H1N1-infected mice at 7 days post-infection, but was more severe in A(H1N1)pdm09-infected mice (**Fig. 4B**). In contrast, the magnitude of inflammation was attenuated by 10 days post-infection (**Fig. 4C**).

Measurement of AHR in children with A(H1N1)pdm09 infection

To elucidate whether AHR was enhanced in patients with A(H1N1)pdm09 infection, we measured AHR of 12 paediatric participants infected with A(H1N1)pdm09 at 1- and 3-months post-infection (**Table 1**). PC_{20} significantly increased at 3 months after discharge compared to that at 1 month after discharge, suggesting that AHR was enhanced in the acute phase (1 month post discharge) of these patients**(Fig. 5** ; 1 month after discharge vs. 3 months after discharge; 1,036 vs. 1,597 μg/dl, p = 0.009).

Discussion

In this study, we comparatively evaluated AHR in a mouse model of bronchial asthma with either A(H1N1)pdm09 infection or seasonal H1N1 infection. Enhanced AHR was observed in asthmatic mice with A(H1N1)pdm09 infection, which peaked at 7 days post-infection and subsequently diminished at 10 days post-infection. Histopathological analysis showed that the onset of lung inflammation in asthmatic mice with A(H1N1)pdm09 infection occurred earlier and was more prominent compared to that in mice with seasonal H1N1 infection; these effects peaked 7 days post-infection and diminished by 10 days post-infection, which was consistent with the observed changes in AHR. These data suggest that A(H1N1)pdm09 induces enhanced AHR complication as a severe phenotype of pneumonia in mice with asthma.

The severity of AHR reflects the inflammatory state of the airways[18]. Several studies have reported that AHR can be enhanced by inflammatory and Th2 cytokines, such as tumour necrosis factor (TNF)-α, interleukin (IL)-6, and IL-13[19-21]. TNF-α secreted from airway macrophages or airway epithelial cells after respiratory virus infection increases levels of adhesion molecules, such as intercellular adhesion molecule-1 on epithelial cells, thereby inducing the recruitment of eosinophils and contributing to epithelial damage and AHR[22-26]. TNF-α is associated with wheezing in human infants[19]. IL-6 is secreted from epithelial cells in respiratory virus infection and induces airway inflammation and bronchospasms in patients with asthma and upper respiratory tract infections[20, 23]. Our previous study showed that IL-6 and TNF-α levels in the bronchoalveolar lavage fluid of A(H1N1)pdm09-infected mice were significantly higher than those in seasonal H1N1-infected mice within 3 days after infection[13]. Therefore, A(H1N1)pdm09 infection may enhance AHR by inducing the production of high levels of inflammatory cytokines during lung inflammation in asthmatic mice, which may also occur in human cases.

Notably, the enhanced AHR in A(H1N1)pdm09-infected mice at 3 and 7 days post-infection decreased to the same level as in control mice at 10 days post-infection, demonstrating that AHR and airway constriction induced by A(H1N1)pdm09 infection is temporary and limited to the acute phase of infection. This finding is supported by our data in A(H1N1)pdm09-infected paediatric participants showing that AHR was alleviated by 3 months after discharge compared to findings at 1 month after discharge. Bozanich et al[34] reported that increased AHR observed in seasonal H3N1 influenza-infected non-asthmatic wild-type mice at 4 days post-infection returned to control levels at 20 days post-infection, which is consistent with our findings. Together, these data indicate that it is pivotal to treat patients with severe asthma exacerbation in the acute phase of post-A(H1N1)pdm09 infection. Established treatments for rescuing acute severe asthma exacerbation complicated with severe pneumonia resulting from A(H1N1)pdm09 infection have not yet been developed. We are currently investigating approaches for treating acute severe asthma exacerbation occurring with A(H1N1)pdm09 infection.

There were some limitations to this study. First, we did not evaluate AHR, inflammatory or Th2 cytokines, or virus titres in the bronchoalveolar lavage fluid at the same time. Lung tissues were collected and used for pathological analysis after AHR evaluation, as we previously reported the cytokine profiles in the bron-

choalveolar lavage fluid of A(H1N1)pdm09 mice[12]. Second, we could not evaluate AHRs of the paediatric participants before or during A(H1N1)pdm09 infection for ethical reasons to verify whether or not enhanced AHR during the infection were alleviated in the post-infection phase. Instead, we measured AHR at 1 and 3 months after discharge.

In conclusion, AHR was significantly enhanced in asthmatic mice with A(H1N1)pdm09 infection compared to that occurring in asthmatic mice with seasonal influenza infection. Furthermore, A(H1N1)pdm09-infected asthma model mice showed more severe pulmonary inflammation in the acute phase post-infection. Enhanced AHR subsequently returned to normal levels with the amelioration of lung inflammation, suggesting that appropriate treatment during the acute phase after A(H1N1)pdm09 infection is essential for avoiding severe respiratory conditions.

References

1. Chipps BE, Szefler SJ, Simons FE, Haselkorn T, Mink DR, Deniz Y, Lee JH; TENOR Study Group. Demographic and clinical characteristics of children and adolescents with severe or difficult-to-treat asthma. J Allergy Clin Immunol 2007;119:1156-1163.
2. Sheehan WJ, Phipatanakul W. Indoor allergen exposure and asthma outcomes. Curr Opin Pediatr 2016;28:772-777.
3. Guarnieri M, Balmes JR. Outdoor air pollution and asthma. Lancet 2014;383:1581-1592.
4. Jartti T, Gern JE. Role of viral infections in the development and exacerbation of asthma in children. J Allergy Clin Immunol 2017;140:895-906.
5. Zheng XY, Xu YJ, Guan WJ, Lin LF. Regional, age and respiratory-secretion-specific prevalence of respiratory viruses associated with asthma exacerbation: a literature review. Arch Virol 2018;163:845-853.
6. Wark PAB, Ramsahai JM, Pathinayake P, Malik B, Bartlett NW. Respiratory viruses and asthma. Semin Respir Crit Care Med 2018;39:45-55.
7. Hasegawa S, Hirano R, Hashimoto K, Haneda Y, Shirabe K, Ichiyama T. Characteristics of atopic children with pandemic H1N1 influenza viral infection: pandemic H1N1 influenza reveals 'occult' asthma of childhood. Pediatr Allergy Immunol 2011;22:e119-e123.
8. Athanasiou M, Lytras T, Spala G, Triantafyllou E, Gkolfinopoulou K, Theocharopoulos G, Patrinos S, Danis K, Detsis M, Tsiodras S, Bonovas S, Panagiotopolous T. Fatal cases associated with pandemic influenza A (H1N1) reported in Greece. PLoS Curr 2010;2:RRN1194.
9. Reichert T, Chowell G, Nishiura H, Christensen RA, McCullers JA. Does glycosylation as a modifier of original antigenic sin explain the case age distribution and unusual toxicity in pandemic novel H1N1 influenza? BMC Infect Dis 2010;10:5.
10. Jain S, Kamimoto L, Bramley AM, Schmitz AM, Benoit SR, Louie J, Sugerman DE, Druckenmiller JK, Ritger KA, Chugh R, Jasuja S, Deutscher M, Chen S, Walker JD, Duchin JS, Lett S, Soliva S, Wells EV, Swerdlow D, Uyeki TM, Fiore AE, Olsen SJ, Fry AM, Bridges CB, Finelli L. 2009 Pandemic Influenza A (H1N1) Virus Hospitalization Investigation Team. Hospitalized patients with 2009 H1N1 influenza in the United States, April-June 2009. N Engl J Med 2009;61:1935-1944.
11. Okada S, Hasegawa S, Hasegawa H, Ainai A, Atsuta R, Ikemoto K, Sasaki K, Toda S, Shirabe K, Takahara M, Harada S, Morishima T, Ichiyama T. Analysis of bronchoalveolar lavage fluid in a mouse model of bronchial asthma and H1N1 2009 infection. Cytokine 2013;63:194-200.
12. Hasegawa S, Wakiguchi H, Okada S, Gui Kang Y, Fujii N, Hasegawa M, Hasegawa H, Ainai A, Atsuta R, Shirabe K, Toda S, Wakabayashi-Takahara M, Morishima T, Ichiyama T. Cytokine profile of bronchoalveolar lavage fluid from a mouse model of bronchial asthma during seasonal H1N1 infection. Cytokine 2014;69:206-210.
13. Fujimoto Y, Hasegawa S, Matsushige T, Wakiguchi H, Nakamura T, Hasegawa H, Nakajima N, Ainai A, Oga A, Itoh H, Shirabe K, Toda S, Atsuta R, Morishima T, Ohga S. Pulmonary inflammation and cytokine dynamics of bronchoalveolar lavage fluid from a mouse model of bronchial asthma during A(H1N1)pdm09 influenza infection. Sci Rep 2017;7:9128.
14. Shim DH. Park YA, Kim MJ, Hong JY, Baek JY, Kim KW, Byun YH, Seong BL, Ryu S, Song

MK, Hong KJ, Na W, Song D, Park JH, Sohn MH, Lee JM. Pandemic influenza virus, pH1N1, induces asthmatic symptoms via activation of innate lymphoid cells. Pediatr Allergy Immunol 2015;26:780-788.
15. McGovern TK, Robichaud A, Fereydoonzad L, Schuessler TF, Martin JG. Evaluation of respiratory system mechanics in mice using the forced oscillation technique. J Vis Exp 2013;75:e50172.
16. Siddiqui S, Tsuchiya K, Risse PA, Bullimore SR, Benedetti A, Martin JG. Site of allergic airway narrowing and the influence of exogenous surfactant in the Brown Norway rat. PLoS One 2012;7:e29381.
17. Kondo N, Nishimuta T, Nishima S, Morikawa A, Aihara Y, Akasaka T, Akasawa A, Adachi Y, Arakawa H, Ikarashi T, Ikebe T, Inoue T, Iwata T, Urisu A, Ebisawa M, Ohya Y, Okada K, Odajima H, Katsunuma T, Kameda M, Kurihara K, Kohno Y, Sakamoto T, Shimojo N, Suehiro Y, Tokuyama K, Nambu M, Hamasaki Y, Fujisawa T, Matsui T, Matsubara T, Mayumi M, Mukoyama T, Mochizuki H, Yamaguchi K, Yoshihara S. Japanese pediatric guidelines for the treatment and management of bronchial asthma 2008. Pediatr Int. 2010;52:319-26.
18. Postma DS, Kerstjens HA. Characteristics of airway hyperresponsiveness in asthma and chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1998;158:S187-S192.
19. Balfour-Lynn IM, Valman HB, Wellings R, Webster AD, Taylor GW, Silverman M. Tumour necrosis factor-alpha and leukotriene E4 production in wheezy infants. Clin Exp Allergy 1994;24:121-126.
20. Yasuda H, Suzuki T, Zayasu K, Ishizuka S, Kubo H, Sasaki T, Nishimura H, Sekizawa K, Yamaya M. Inflammatory and bronchospastic factors in asthma exacerbations caused by upper respiratory tract infections. Tohoku J Exp Med 2005;207:109-118.
21. Risse PA, Jo T, Suarez F, Hirota N, TOlloczko B, Ferraro P, Grutter P, Martin JG. Interleukin-13 inhibits proliferation and enhances contractility of human airway smooth muscle cells without change in contractile phenotype. Am J Physiol Lung Cell Mol Physiol 2011;300:L958-L966.
22. Gern JE, Dick EC, Lee WM, Murray S, Meyer K, Handzel ZT, Busse WW. Rhinovirus enters but does not replicate inside monocytes and airway macrophages. J Immunol 1996;156:621-627.
23. Yamaya M. Virus infection-induced bronchial asthma exacerbation. Pulm Med 2012;2012:834826.
24. Rothlein R, Czajkowski M, O'Neill MM, Marlin SD, Mainolfi E, Merluzzi VJ. Induction of intercellular adhesion molecule 1 on primary and continuous cell lines by pro-inflammatory cytokines. Regulation by pharmacologic agents and neutralizing antibodies. J Immunol 1988;141:1665-1669.
25. Wegner CD, Gundel RH, Reilly P, Haynes N, Letts LG, Rothlein R. Intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. Science 1990;247:456-459.
26. Malaviya R, Laskin JD, Laskin DL. Anti-TNFα therapy in inflammatory lung diseases. Pharmacol Ther. 2017;180:90-98.
27. Anticevich SZ, Hughes JM, Black JL, Armour CL. Induction of human airway hyperresponsiveness by tumour necrosis factor-α. Eur J Pharmacol 1995;284:221-225.
28. Choi IW, Sun K, Kim YS, Ko HM, Im SY, Kim JH, You HJ, Lee YC, Lee JH, Park YM, Lee HK. TNF-α induces the late-phase airway hyperresponsiveness and airway inflammation through cytosolic phospholipase A2 activation. J Allergy Clin Immunol 2005;116:537-543.
29. Li BQ, Sun HC, Nie SN, Shao DB, Liu HM, Qian XM. Effect of penehyclidine hydrochloride on patients with acute lung injury and its mechanisms. Chin J Traumatol 2010;13:329-335.
30. Reper P, Heijmans W. High-frequency percussive ventilation and initial biomarker levels of lung injury in patients with minor burns after smoke inhalation injury. Burns 2015;41:65-70.
31. Singh S, Grover V, Christie L, Charles P, Kelleher P, Shah PL. A comparative study of bronchoscopic microsample probe versus bronchoalveolar lavage in patients with burns-related inhalational injury, acute lung injury and chronic stable lung disease. Respiration 2015;89:19-26.
32. Li T, Luo N, Du L, Zhou J, Zhang J, Gong L, Jiang N. Tumor necrosis factor-α plays an initiating role in extracorporeal circulation-induced acute lung injury. Lung 2013;191:207-214.
33. Neveu W, Allard JB, Dienz O, Wargo MJ, Ciliberto G, Whittaker LA, Rincon M. IL-6 is required for airway mucus production induced by inhaled fungal allergens. J Immunol 2009;183:1732-8.
34. Bozanich E, Gualano RC, Zosky GR, Larcombe AN, Turner DJ, Hantos Z, Sly PD. Acute Influenza A infection induces bronchial hyper-responsiveness in mice. Respir Physiol Neurobiol 2008;162:190-196.

Table 1. Clinical characteristics of patients infected with A(H1N1)pdm09 at 1 month after discharge. FVC,

forced vital capacity; $FEV_{1.0}$, forced expiratory volume in 1 s; V_{50}, maximal flow at 50% vital capacity.

n=12	
Age (years)	7.7 (4.9–11.8)
Sex (M/F)	9/3
IgE (IU/ml)	932 (124–2,680)
%FVC	90.6 (70.0–119)
$\%FEV_{1.0}$	96.4 (75.8–111)
$\%V_{50}$	97.2 (55.5–160)
Previous asthma diagnosis	7

Figure legends

Figure 1. Resistance of respiratory system (Rrs) to methacholine challenge in asthma model mice infected with vehicle (mock), A(H1N1)pdm09, or seasonal H1N1 (A/Puerto Rico) at 3 (A), 7 (B), and 10 (C) days post-infection. Data are presented as means ± standard error of the mean (SEM); *: asthma – mock; : asthma - A(H1N1)pdm09; $^{\cdot}asthma - -seasonalH1N1; * : non - asthma - -A(H1N1)pmd09.Differencesbetweenmeansarepresentedasfollows : asthma - mockvs.asthma - seasonalH1N1, *p<0.05; asthma - seasonalH1N1vs.asthma - A(H1N1)pdm09, +p<0.05; asthma - A(H1N1)pdm09vs.asthma - -mock, + + p<0.05; andasthma - -A(H1N1)pdm09vs.non - asthma - -A(H1N1)pdm09, SSp<0.05$.

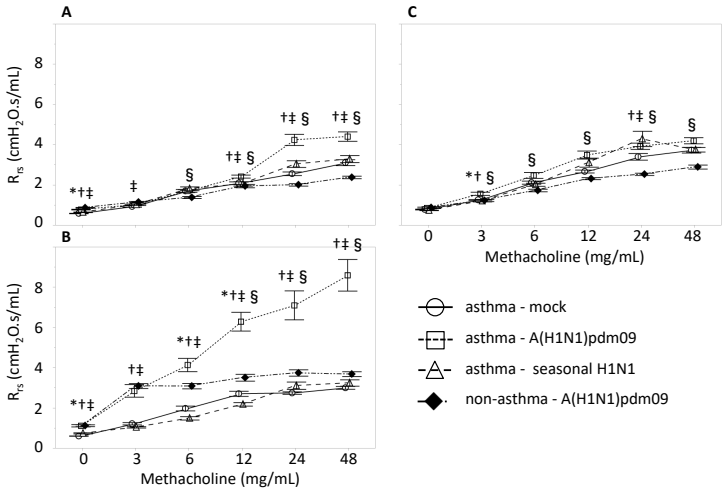
Figure 2. Tissue damping (G) of methacholine challenge in asthma model mice infected with vehicle (mock), A(H1N1)pdm09, or seasonal H1N1 (A/Puerto Rico) at 3 (A), 7 (B), and 10 (C) days post-infection. Data are presented as means +- standard error of the mean (SEM); *: asthma – mock; : asthma - A(H1N1)pdm09; $^{\cdot}asthma - -seasonalH1N1; * : non - asthma - -A(H1N1)pmd09.Differencesbetweenmeansarepresentedasfollows : asthma - mockvs.asthma - seasonalH1N1, *p<0.05; asthma - seasonalH1N1vs.asthma - A(H1N1)pdm09, +p<0.05; asthma - A(H1N1)pdm09vs.asthma - mock, + + p<0.05; andasthma - -A(H1N1)pdm09vs.non - asthma - -A(H1N1)pdm09, SSp<0.05$.

Figure 3. Changes in airway hyperresponsiveness of A(H1N1)pdm09-infected mice at 3, 7, and 10 days post-infection. (A) Resistance of respiratory system (R_{rs}) to methacholine challenge. (B) Tissue damping (G) of methacholine challenge. Data are presented as means +- standard error of the mean (SEM); *: day 3, : day 7, $^{\cdot}day10.Differencesbetweenmeansarepresentedasfollows : day3vs.day7, *p<0.05; day3vs.day10, +p<0.05; andday7vs.day10, + + p<0.05$.

Figure 4. Histopathological findings after influenza infection. Photomicrographs of haematoxylin and eosin-stained lung tissue at 3 (A), 7 (B), and 10 (C) days post-infection with mock, seasonal H1N1 (A/Puerto Rico), or A(H1N1)pdm09 influenza virus. Representative data are shown for tissues from one of four to six independent mice per group.

Figure 5. The provocative concentration causing a 20% reduction in the forced expiratory volume in 1 second (PC_{20}) at 1 and 3 months after discharge.

Figure 1



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