Research

A rat model of picornavirus-induced airway infection and inflammation

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Abstract

Background: Infection of the lower airways by rhinovirus, a member of the picornavirus family, is an important cause of wheezing illnesses in infants, and plays an important role in the pathogenesis of rhinovirus-induced asthma exacerbations. Given the absence of natural rhinovirus infections in rodents, we investigated whether an attenuated form of mengovirus, a picornavirus whose wild-type form causes systemic rather than respiratory infections in its natural rodent hosts, could induce airway infections in rats with inflammatory responses similar to those in human rhinovirus infections.

Results: After inoculation with 10⁷ plaque-forming units of attenuated mengovirus through an inhalation route, infectious mengovirus was consistently recovered on days 1 and 3 postinoculation from left lung homogenates (median Log₁₀ plaque-forming units = 6.0 and 4.8, respectively) and right lung bronchoalveolar lavage fluid (median Log₁₀ plaque-forming units = 5.8 and 4.0, respectively). Insufflation of attenuated mengovirus, but not vehicle or UV-inactivated virus, into the lungs of BN rats caused significant increases (P < 0.05) in lower airway neutrophils and lymphocytes in the bronchoalveolar lavage fluid and patchy peribronchiolar, perivascular, and alveolar cellular infiltrates in lung tissue sections. In addition, infection with attenuated mengovirus significantly increased (P < 0.05) lower airway levels of neutrophil chemoattractant CXCR2 ligands [cytokine-induced neutrophil chemoattractant-1 (CINC-1; CXCL1) and macrophage inflammatory protein-2 (MIP-2; CXCL2)] and monocyte chemoattractant protein-1 (MCP-1; CCL2) in comparison to inoculation with vehicle or UV-inactivated virus.

Conclusion: Attenuated mengovirus caused a respiratory infection in rats with several days of viral shedding accompanied by a lower airway inflammatory response consisting of neutrophils and lymphocytes. These features suggest that mengovirus-induced airway infection in rodents could be a useful model to define mechanisms of rhinovirus-induced airway inflammation in humans.

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Background

Human rhinovirus (HRV) infections are the most frequent cause of common colds and virus-induced asthma exacerbations, and wheezing HRV infections in infancy are associated with an increased risk for the development of childhood asthma [1-3]. A central conundrum with regard to HRV, a member of the picornavirus family, is explaining how a virus that usually causes a self-limiting upper airway infection, a common cold, can induce asthma exacerbations and provoke persistent lower airway sequelae in susceptible children [4,5]. An important clue in addressing this issue is the substantial evidence that HRV can infect the lower airways [6-11]. HRV infection of lower airway epithelial cells induces the secretion of a variety of proinflammatory cytokines, chemokines, and mediators [4].

Neutrophils are the predominant inflammatory cell initially recruited to the airways during HRV infections [12,13], and clinical studies have demonstrated that there is a positive correlation between this inflammatory response and respiratory symptoms and airway dysfunction [14-17]. Although these relationships have been observed in a variety of clinical and experimental infection studies, the nature of this relationship is still enigmatic. It is possible that 1) neutrophilic inflammation causes respiratory symptoms, 2) neutrophils recruited to the airways in response to HRV infection have antiviral effects and contribute to resolution of the infection, or 3) neutrophilic inflammation is an epiphenomenon that does not significantly affect the course of the disease. Finally, perhaps the difference between a relatively uneventful cold and more severe HRV-induced airway sequelae resides in the balance between beneficial and detrimental effects of the neutrophilic inflammatory response.

Progress in understanding the relationship between HRV infection, inflammation, and respiratory symptoms has been significantly hampered by the absence of rodent-specific rhinoviruses. Recently, murine experimental models have been established using either minor group HRV in wild-type mice or major group HRV in mice that are transgenic for human intercellular adhesion molecule-1 (ICAM-1; CD54), the receptor for major group HRV [18,19]. While these models will be useful, a significant drawback to these models is that HRV replication is short-lived (≤ 24 h) in the mouse. In studying the relationship between viral replication, inflammation, and respiratory dysfunction, it would be advantageous to develop a model with viral replication lasting several days, as occurs during clinical or experimental infections with HRV.

Mengovirus is a picornavirus that naturally infects rodents [20], and the native virus causes systemic infections that resemble poliovirus infections, rather than HRV infections, of humans. The poly(C) tract in the distal region of the 5’ untranslated region of the mengovirus genome is a critical virulence determinant that inhibits interferon responses [21-25]. A panel of attenuated mengovirus mutants with varying deletions of the poly(C) tract (wild-type mengovirus has a poly(C) tract length of 44) has been derived, including vMC0, which has no poly(C) tract [21-25]. In contrast to the systemic and often lethal infections caused by wild type mengovirus, intracerebral or intraperitoneal administration of vMC0 induces self-limited infections, and vMC0 also stimulates vigorous type I interferon responses [21-25]. Furthermore, attenuated mengoviruses replicate well in epithelial cells but poorly in macrophage lineage cells [25]. These features are similar to those of HRV infection [4], and led us to hypothesize that inoculation of rats with vMC0 via inhalation could produce infection limited to the respiratory tract, and could serve as a model for HRV infections in humans.

Results

Expression of infectious virus in the lungs after inhalation of attenuated mengovirus

To examine whether attenuated mengovirus could induce lower airway infections in rats, 10^7 plaque-forming units (PFU) of attenuated mengovirus, vMC0, an equivalent amount of UV-inactivated vMC0, or vehicle were instilled into the lungs of adult BN rats. On days 1 and 3 postinoculation, significant levels of infectious mengovirus were recovered from left lung homogenates (median Log_{10} PFU = 6.0 and 4.8, respectively) and right lung bronchoalveolar lavage (BAL) fluid (median Log_{10} PFU = 5.8 and 4.0, respectively) of BN rats inoculated with the attenuated mengovirus, vMC0 (Figure 1; P < 0.005). By day 5 postinoculation, viral titers in the lung homogenates and BAL fluid of vMC0-inoculated rats were either low or undetectable. Infectious mengovirus was not detected in lung homogenates and BAL fluid from BN rats inoculated with either UV-inactivated vMC0 or vehicle. Examination of brain, heart, and spleen homogenates and plasma revealed no evidence of systemic infection with vMC0.

Reduction in body weight gain after inhalation of attenuated mengovirus

A reduction in body weight or in the rate of body weight gain is a sensitive measure of viral respiratory infections in rodents [26]. The percent gain in body weight from the day of the inoculation to day 3 postinoculation was significantly lower in BN rats inoculated with 10^7 PFU of vMC0 (median = 8.8%; n = 10 rats) than in those receiving the vehicle (median = 2.2%; n = 6 rats; P = 0.04). However, there was no significant difference between the percent gain in body weight in rats inoculated with UV-inactivated vMC0 (median = 1.6%; n = 5 rats) and those inoculated with vehicle, indicating the requirement for
replication-competent virus for the observed effects on body weight.

**Development of neutrophilic lower airway inflammation after inhalation of attenuated mengovirus**

Insufflation of vMC$_0$ (10$^7$ PFU) into the lungs of adult BN rats induced the recruitment of neutrophils and lymphocytes into the lower airways. The total number of BAL cells and the numbers of BAL neutrophils and lymphocytes were significantly elevated on days 3 and 5 postinoculation in BN rats inoculated with attenuated mengovirus compared with those inoculated with an equivalent amount of UV-inactivated vMC$_0$ or vehicle (Figure 2; $P < 0.05$). Levels of BAL lymphocytes were also significantly elevated on day 1 postinoculation in vMC$_0$-inoculated BN rats compared with vehicle- or UV-inactivated vMC$_0$-inoculated rats, respectively (Figure 4C; $P < 0.05$). As shown with regard to CXCR2 ligand expression, UV-inactivation of vMC$_0$ abrogated its ability to induce a significant elevation in BAL fluid MCP-1 levels, demonstrating the need for replication-competent virus.

**Expression of MCP-1 in the lower airways after inhalation of attenuated mengovirus**

Because HRV infection induces high levels of MCP-1 expression [28], and MCP-1 indirectly contributes to neutrophil recruitment to the lungs [29-32], we examined the BAL fluid from BN rats that had been inoculated with 10$^7$ PFU of vMC$_0$ for MCP-1 expression. The levels of MCP-1 in BAL fluid were significantly increased on days 1 and 3 postinoculation in vMC$_0$-inoculated rats compared with vehicle- or UV-inactivated vMC$_0$-inoculated rats, respectively (Figure 4C; $P < 0.05$). As shown with regard to CXCR2 ligand expression, UV-inactivation of vMC$_0$ abrogated its ability to induce a significant elevation in BAL fluid MCP-1 levels, demonstrating the need for replication-competent virus.

**Effect of inoculation dose on inflammatory response to inhalation of attenuated mengovirus**

Inoculation with a ten-fold lower dose of vMC$_0$ yielded a similar inflammatory response in the lower airways. Insufflation of 10$^6$ PFU of vMC$_0$ into the lungs of BN rats ($n = 4$) induced a significant increase ($P < 0.05$) in the numbers [10$^6$ cells: median (interquartile range)] of neutrophils [0.19 (0.16, 0.21)] and lymphocytes [0.23 (0.20, 0.30)], but not total cells, eosinophils, or macrophages in the BAL fluid on day 3 postinoculation as compared with the values from vehicle-inoculated rats. In addition, the levels [pg: median (interquartile range)] of CINC-1 [715 (611, 835)], MIP-2 [188 (168, 208)], and MCP-1 [385 (266, 452)] in the BAL fluid were significantly elevated ($P < 0.05$) in these rats as compared with vehicle-inoculated controls. An inoculation dose of 10$^5$ PFU of vMC$_0$ was substantially less effective at generating an inflammatory response in the lower airways of the rats, leading to the recruitment of about 75% fewer BAL neutrophils and 60% fewer BAL lymphocytes on day 3 postinoculation com-
Figure 2
Recruitment of neutrophils and lymphocytes to the lungs after inhalation of attenuated mengovirus. Numbers of (A) total cells, (B) neutrophils, (C) lymphocytes, (D) eosinophils, and (E) macrophages in the BAL fluid harvested on days 1, 3, and 5 postinoculation from the right lungs of BN rats inoculated with 10^7 PFU of vMC0 (n = 4, 10, and 4 rats, respectively) and on day 3 postinoculation from those inoculated with an equivalent amount of UV-inactivated vMC0 (n = 5 rats) or vehicle (n = 7 rats). Data are presented as box plots. * P < 0.05 (mengovirus vs. vehicle); † P < 0.05 (vMC0 vs. UV-inactivated vMC0).
Recruitment of inflammatory cell infiltrates to the lungs after inhalation of attenuated mengovirus.

Giemsa-stained sections of the left lungs from BN rats inoculated with (A) vehicle, (B) \( \text{vMC}_0 \) (10⁷ PFU) or (C) an equivalent amount of UV-inactivated \( \text{vMC}_0 \). Lungs were harvested on day 3 postinoculation. Magnification, 20×.

**Figure 3**

**Figure 4**
In the rat model described here, infectious mengovirus was consistently detected in the lungs at high levels, and persisted for at least 3 days after inoculation. The inoculation dose of $10^6$–$10^7$ PFU of attenuated mengovirus in the rats was similar to the dose of $5 \times 10^6$ TCID$_{50}$ (50% tissue-culture infective dose) administered in the HRV models in mice [18,19], especially considering that the body weight of the rats is about an order of magnitude greater compared to that of mice. Furthermore, inhalation of attenuated mengovirus, but not vehicle or UV-inactivated virus, into the lungs of BN rats resulted in increases in chemokines (CINC-1, MIP-2, and MCP-1) and cellular inflammation (neutrophils, lymphocytes, and total BAL cells). Compared to the HRV mouse models, infection with vMC$_0$ represents a rodent model of picornavirus-induced airway inflammation in which the roles of viral replication and persistence are more prominent.

Mengovirus-induced expression of CXCR2 ligands is consistent with the increased expression of CXCR2 ligands that is observed in response to rhinovirus infection [34-36]. A similar induction of CXCR2 ligands was also observed in the murine HRV infection models [18,19]. We also observed the induction of MCP-1 expression in response to inhalation of attenuated mengovirus, which represents another similarity between this rat model of attenuated mengovirus-induced airway inflammation

Figure 5
Effect of inhalation of attenuated mengovirus on pulmonary physiology. BN rats were inoculated with either vehicle or $10^7$ PFU of vMC$_0$ (n = 5 rats per group), and on day 3 postinoculation, pulmonary physiology measurements were obtained after exposure to aerosols of normal saline followed by escalating concentrations of methacholine. Values for respiratory system resistance (Rrs) are presented as the group means ± the standard error.

Discussion
The establishment of useful small animal models to study HRV pathogenesis has been an important goal to enable mechanistic studies and facilitate the development of new therapeutics. The earliest reported effort to develop a HRV infection model in mice required very large input doses of virus and pretreatment of the mice with actinomycin D [33]. Recently, more robust murine experimental models of HRV infection have been established. These models employ either a murine cell culture-adapted minor group HRV in wild-type mice or a major group HRV in mice that are transgenic for human ICAM-1 [18,19]. Although the development of these novel tools represents a significant advance in the study of HRV-induced airway inflammation, an important limitation is that HRV shedding is limited to ≤ 24 h postinoculation [18].
and human host responses to HRV infection [28]. Therefore, the induction of rat CXC2 ligand and MCP-1 expression in airway fluids in response to inhalation of attenuated mengovirus closely resembles the HRV-induced enhancement of these chemokines.

Another similarity between this rat model and HRV infection in humans is the relative kinetics of the viral infection vs. the lower airway neutrophilic inflammatory response. Mengovirus titers in the lung peak earlier than the neutrophilic inflammatory response in the lower airways. This parallels data from experimental HRV inoculations in human volunteers [11,13]. In addition, the patchiness of the mengovirus-induced airway inflammation in this rat model is consistent with the patchy infection of airway epithelial cells observed in HRV infections in human subjects [11,37-39].

Infection of the lower airways with mengovirus did not result in significant changes in baseline pulmonary physiology measurements or in AHR to methacholine challenge in this rat model. It is important to note that experimentally naïve adult rats without existing airway disease were used in these studies. Similar to this rat model, several studies involving experimental HRV inoculations of healthy, nonasthmatic, nonallergic human subjects have demonstrated no changes in baseline pulmonary function or AHR after HRV infection [10,40-44]. In one study showing a small change in AHR after experimental HRV infection of nonasthmatic, nonallergic subjects, the small difference was only detected by employing a methacholine concentration that was a half-log higher than the highest concentration typically used [45]. In contrast, experimental inoculation with HRV has been shown to increase AHR in individuals with asthma and/or allergic rhinitis in several studies [10,17,44,46,47], although not in others [40,41,43,45,48]. Therefore, the absence of changes in AHR in these healthy adult rats without existing airway disease is consistent with the outcomes of experimental HRV infections in healthy humans who had no underlying airway disease, such as asthma or allergic rhinitis. The absence of viral effects on AHR in this mengovirus model and in the experimental HRV inoculations in humans is consistent with the murine experimental model of HRV infection described by Bartlett et al. in which there was no increase in AHR to methacholine challenge after HRV infection unless the BALB/c mice had also been sensitized and challenged with allergen [18]. However, in the murine experimental HRV infection model described by Newcomb et al., an increase in AHR to methacholine challenge was observed after infection of C57BL/6 mice with HRV [19], which may be related to the use of a different mouse strain. Overall, the lack of significant changes in pulmonary physiology during mengovirus-induced respiratory infection in adult rats without existing airway disease is consistent with previous observations in experimental HRV infections in humans. In future studies, it will be of interest to investigate the effects of mengovirus-induced respiratory infection on rats with existing airway injury related to prior exposures to allergens or other respiratory viruses [49] with the objective of modeling aspects of HRV-induced asthma exacerbations.

A potential limitation of this animal model is the use of mengovirus, which is neurotropic, to serve as a model for HRV, which primarily causes respiratory infections. In this regard, it is important to note that poliovirus, which is closely related to HRV, is also neurotropic. The attenuated mengovirus, vMC0, used in these studies induced a self-limited respiratory infection when administered through an inhalation route. This indicates that there is plasticity in the tissue tropism of vMC0 that makes it suitable for a model of picornavirus-induced airway infection and inflammation. Another consideration is that there are both similarities and differences in CXCR2 and its ligands between rats and humans [50]. Humans express IL-8 and two IL-8 receptors, CXCR1 and CXCR2, whereas rats do not express an IL-8 ortholog and only express CXCR2. However, rats do express relevant CXCR2 ligands, such as CINC-1 and MIP-2, which are functional analogs of IL-8 with regard to neutrophil recruitment and activation. We believe that the rat represents an attractive, relevant, and simplified model for examining the role of CXC chemokines in neutrophil recruitment and activation in response to picornavirus-induced respiratory infection because of the reduced number of chemokines and chemokine receptors to be examined.

**Conclusion**

Overall, our data support the feasibility of using this novel rat model of picornavirus-induced lower airway infection and inflammation to study, among other questions, the role of neutrophilic inflammation in the host response to picornavirus-induced respiratory infections. Although this model does not fully encompass all aspects of HRV infection in humans, it does demonstrate a remarkable number of parallel developments that will provide novel opportunities to study the interactions between picornavirus replication and the host antiviral immune responses in a relevant small animal model.

**Methods**

**Animals**

BN/SsN male rats were purchased from Harlan (Indianapolis, IN) and had a median body weight of 250 g when used for inoculation studies. The rats were housed in HEPA-filtered isolation cubicles (Britz and Co., Wheatland, WY) in an American Association for Accreditation of Laboratory Animal Care-accredited laboratory animal facility at the University of Wisconsin School of Medicine.
and Public Health. All procedures were approved by the University of Wisconsin Animal Care and Use Committee and conformed to the Guide for the Care and Use of Laboratory Animals (1996).

**Virus**

Stock preparations of the attenuated mengovirus, vMC₀ (which has no poly(C) tract) [21-25], were prepared by transfection of HeLa cells with viral RNA transcribed from a plasmid encoding the vMC₀ genome followed by amplification of viral titers via passage in HeLa cell cultures as described [51]. Supernates from uninfected HeLa cell cultures were used as vehicle controls, and UV-inactivated vMC₀ stocks were prepared by exposing vMC₀ to a germicidal UV lamp at a distance of 10 cm for 1 h. Plaque assays using HeLa cells were employed to determine the titer of the active virus preparations and to verify UV-inactivation. Active virus was undetectable (< 10 PFU/ml) in the UV-inactivated preparations.

**Virus inoculation**

Rats were lightly anesthetized by inhalation of 5% isoflurane, and vMC₀, UV-inactivated vMC₀, or vehicle in a total volume of 0.1 ml were insufflated into the lungs via an orotracheal catheter.

**Measurements of pulmonary inflammation**

At various times after inoculation, rats were anesthetized with urethane and euthanized by exsanguination. The chest was opened, and the left mainstem bronchus was clamped to allow BAL of the right lung. The right lung was filled with phosphate buffered saline (PBS) to total lung capacity by gravity and drained 5 times, the BAL fluid was centrifuged, and the cell pellet was resuspended in 1 ml PBS. The total number of BAL leukocytes was determined with an automated cell counter (model Z, Beckman Coulter, Hialeah, FL), and cytospin slides were prepared for a differential leukocyte count based on 200 cells. BAL fluid was concentrated 15× using a centrifugal filter device with a molecular weight cutoff of 5,000 (Millipore, Bedford, MA) and stored at -80°C until analyzed for chemokine expression. Samples of unconcentrated BAL fluid were determined by plaque assay using HeLa cells as described [24,51]. Briefly, HeLa cell monolayers were inoculated with dilutions of the samples, incubated for 24–48 h at 37 °C (until plaques form), formalin fixed, stained with crystal violet, and scored for plaques. Stock vMC₀ preparations served as the positive control.

**Histological assessment of pulmonary inflammation**

Sections (5 μM) were prepared from formalin-fixed, paraffin-embedded left lungs. Giemsa staining was performed on these sections, which were evaluated for inflammation by light microscopy.

**Measurement of chemokine expression**

Chemokine levels in BAL fluid were determined using commercially available rat-specific enzyme-linked immunosorbent assay (ELISA) kits for CINC-1 (R&D Systems, Minneapolis, MN), MIP-2, and MCP-1 (Biosource, Camarillo, CA) with sensitivities of 7.8, 7.8, and 8 pg/ml, respectively, according to the manufacturers’ instructions.

**Statistical analysis**

Analysis of variance (general linear model) was performed on the BAL fluid CINC-1 and MIP-2 ELISA data and on pulmonary physiology data after a log transformation, and Fischer’s least significant difference test was used for planned pairwise comparisons. A residual analysis was employed to test the adequacy of the models. Nonparametric tests were used to analyze all other data. For comparisons between two groups, the Mann-Whitney test was used. The Kruskal-Wallis test was used for comparisons among three or more groups and was followed by planned pairwise comparisons using the Mann-Whitney test. Because infectious virus was undetectable in the lung homogenate and BAL fluid samples from rats inoculated with vehicle or UV-inactivated virus, these groups were combined for statistical analysis of viral titers. Box plots depict the median and the interquartile range between the 25th and 75th percentile, and whiskers show the 10th and 90th percentiles. Analyses were performed using the sta-
tical software package SYSTAT 11.0 (Systat Software, Chicago, IL).

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
LAR co-conceived the study, designed and coordinated the experiments, participated in the animal and immunological studies, performed the data and statistical analysis, analyzed and interpreted the data, and drafted the manuscript. SPA carried out the virology studies and participated in the experimental design and interpretation of the data. RFL carried out the animal, immunological, and histological studies and participated in the interpretation of the data. RFL participated in the interpretation of the data and revision of the manuscript. JEG co-conceived the study and participated in the interpretation of the data and revision of the manuscript. RLS co-conceived the study and participated in the experimental design, the animal and immunological studies, the interpretation of the data, and the revision of the manuscript. All authors read and approved the final manuscript.

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References
1. Lemanske RF Jr, Jackson DJ, Gangnon RE, Evans MD, Li Z, Shult PA, Kirk CJ, Reisdorf E, Roberg KA, Anderson EL, Carlson-Dakes KT, Adler KJ, Gilbertson-White S, Hamilton R, Shult PA, Kirk CJ, Da Silva DF, Sund SA, Kosorok MR, Lemanske RF Jr: Rhinovirus infections during infancy predict subsequent childhood wheezing. J Allergy Clin Immunol 2005, 116:571-577.
2. Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Pappas TE, Printz MC, Lee WM, Shult PA, Reisdorf E, Carlson-Dakes KT, Salazar LP, DaSilva DF, Tisler CJ, Gern JE, Lemanske RF Jr: Wheezing rhinovirus infections in early life predict asthma development in high-risk children. Am J Respir Crit Care Med 2008, 178:667-672.
3. Kusel MM, de Klerk NH, Bodeza T, Vohma V, Holt PG, Johnston SL, Sly PD: Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. J Allergy Clin Immunol 2007, 119:105-110.
4. Kelly JT, Busse WW: Host immune responses to rhinovirus: mechanisms in asthma. J Allergy Clin Immunol 2008, 122:671-682.
5. Singh AM, Moore PE, Gern JE, Lemanske RF Jr, Hartert TV: Bronchiolitis to asthma: a review and call for studies of gene-virus interactions in asthma causation. Am J Respir Crit Care Med 2007, 175:108-119.
6. Gern JE, Galagan DM, Jarjour NN, Dick EC, Busse WW: Detection of rhinovirus RNA in lower airway cells during experimentally induced infection. Am J Respir Crit Care Med 1997, 155:1159-1161.
7. Papadopoulos NG, Bates PJ, Bardin PG, Papi A, Leir SH, Fraenkell DJ, Meyer J, Lackie PM, Sanderson G, Holgate ST, Johnston SL: Rhinoviruses infect the lower airways. J Infect Dis 2000, 181:1875-1884.
8. Mosser AG, Brockman-Schneider R, Aminova S, Burchell L, Sedgwick JB, Busse WW, Gern JE: Similar frequency of rhinovirus-infected cells in upper and lower airway epithelium. J Infect Dis 2002, 185:734-743.
9. Schroth MK, Grimm E, Frindt P, Galagan DM, Konno SI, Love R, Gern JE: Rhinovirus replication causes RANTES production in primary bronchial epithelial cells. Am J Respir Cell Mol Biol 1999, 20:1220-1228.
10. Message SD, Laza-Stanca V, Mallia P, Parker HL, Zhu J, Kebadze T, Concari M, Sanderson G, Kon OM, Papi A, Jeffery PK, Stanciu LA, Johnston SL: Rhinovirus-induced lower respiratory illness is increased in asthma and related to virus load and Th1/2 cytokine and IL-10 production. Proc Natl Acad Sci USA 2008, 105:13562-13567.
11. Mosser AG, Vrtis R, Burchell L, Lee WM, Dick CR, Weisshaar E, Bock D, Swenson CA, Cornell RD, Meyer KC, Jarjour NN, Busse WW, Gern JE: Quantitative and qualitative analysis of rhinovirus infection in bronchial tissues. Am J Respir Crit Care Med 2005, 171:645-651.
12. Gern JE, Vrtis R, Jarjour NN, Gern JE, Swenson CA, Busse WW: The effect of an experimental rhinovirus 16 infection on bronchial lavage neutrophils. J Allergy Clin Immunol 2000, 105:1169-1177.
13. Gern JE, Vrtis R, Grindle KA, Swenson CA, Busse WW: Relationship of upper and lower airway cytokines to outcome of experimental rhinovirus infection. Am J Respir Crit Care Med 2000, 162:2226-2231.
14. Fahy JV, Kim KW, Liu J, Boushey HA: Prominent neutrophilic inflammation in sputum from subjects with asthma exacerbation. J Allergy Clin Immunol 1995, 95:843-852.
15. Wark PA, Johnston SL, Moric I, Simpson JL, Hensley MJ, Gibson PG: Neutrophil degranulation and cell lysis is associated with clinical severity in virus-induced asthma. Eur Respir J 2002, 19:68-75.
16. Gern JE, Martin MS, Anklaam KA, Shen K, Roberg KA, Carlson-Dakes KT, Adler K, Gilbertson-White S, Hamilton R, Shult PA, Kirk CJ, Da Silva DF, Sund SA, Kosorok MR, Lemanske RF Jr: Relationships among specific viral pathogens, virus-induced interleukin-8, and respiratory symptoms in infancy. Pediatr Allergy Immunol 2002, 13:386-392.
17. Grunberg K, Timmers MC, Smits HH, de Klerk EP, Dick EC, Spaan WJ, Hiemstra PS, Sterk PJ: Effect of experimental rhinovirus 16 colds on airway hyperresponsiveness to histamine and interleukin-8 in nasal lavage in asthmatic subjects in vivo. Clin Exp Allergy 1997, 27:36-45.
18. Bartlett NW, Walton RP, Edwards MR, Anisencko J, Caramori G, Zhu J, Glaville N, Choy KJ, Jourard P, Burnet J, Tuthill TJ, Pedrick MS, Hurle MJ, Plumpton C, Sharp NA, Bussell JN, Swallow DM, Schwarze J, Guy B, Almond JW, Jeffery PK, Lloyd CM, Papi A, Killington RA, Rowlands DJ, Blair ED, Clarke NJ, Johnston SL: Mouse models of rhinovirus-induced disease and exacerbation of allergic airway inflammation. Nat Med 2008, 14:199-204.
19. Newcomb DC, Sajan US, Nagarkar DR, Wang Q, Nanua S, Zhou Y, McHenry CL, Henricks K, Tsai WC, Bentley JK, Lukacs NW, Johnston SL, Hershenson MB: Human rhinovirus 1B exposure induces phosphatidylinositol 3-khase-dependent airway inflammation in mice. Am J Respir Crit Care Med 2008, 177:1111-1121.
20. Palmenberg AC, Osorio JE: Cardioviral poly(C) tract syndromes. J Virol 1981, 36:393-406.
21. Duke GM, Osorio JE, Palmenberg AC: Mammalian cardiovirus poly(C) tract length. Nature 1999, 343:474-476.
22. Martin LR, Duke GM, Osorio JE, Hall DJ, Palmenberg AC: Pathogenesis of cardiovirus poly(C) tract length. J Virol 2006, 80:6377-6384.
23. Martin LR, Duke GM, Osorio JE, Hall DJ, Palmenberg AC: Muta-
with interferon-γ treatment in rats. Am J Respir Crit Care Med 1999, 160:705-710.

27. Shibata F, Kishihara K, Kato H, Komorita N, Al-Mokdad M, Fujioka M, Nakagawa H: Recombinant production and biological properties of rat cytokine-induced neutrophil chemotactants, GRO/CINC-2 alpha, CINC-2 beta and CINC-3. Eur J Biochem 1995, 231:306-311.

28. Hall DJ, Baxes ME, Guu L, Cronan M, Korpi N, Bertics PJ: The role of p38 MAPK in rhinovirus-induced monocyte chemotactant protein-1 production by monocytic-lineage cells. J Immunol 2005, 174:8056-8063.

29. Gonzalo JA, Lloyd CM, Wen D, Albar JP, Wells TN, Proudfoot A, Martinez A, Dorf M, Bjerke T, Coyle AJ, Gutierrez-Ramos JC: The coordinated action of CC chemokines in the lung orchestrates allergic inflammation and airway hyperresponsiveness. J Exp Med 1998, 188:157-167.

30. Maus U, von GK, Kuziel WA, Mack M, Miller EJ, Coyle AJ, Gutierrez-Ramos JC: Monocytes are potent facilitators of alveolar neutrophil emigration during lung inflammation: role of the CCL2-CCR2 axis. Am J Respir Crit Care Med 2002, 166:268-273.

31. Maus UA, Waelsch K, Kuziel WA, Delbeck T, Mack M, Blackwell TS, Christman JW, Schindler-FD, Seeger W, Lohmeyer J: Monocytes are potent mediators of alveolar neutrophil emigration during lung inflammation: role of the FFAR2-CXCR2 axis. Am J Respir Cell Mol Biol 2004, 30:55-62.

32. Vozzelli MA, Mason SN, Whorton MH, Auten RL Jr: Antimacrophage chemokine treatment prevents neutrophil and macrophage influx in hyperoxia-exposed newborn rat lung. Am J Physiol Lung Cell Mol Physiol 2004, 286:L488-L493.

33. Yin FH, Lomax NB: Establishment of a mouse model for human rhinovirus infection. J Gen Virol 1986, 67:2335-2340.

34. Zhu Z, Tang W, Gwaltney JM Jr, Wu Y, Elias JA: Rhinovirus stimulation of interleukin-8 in vivo and in vitro: role of NF-kappaB. Am J Physiol 1997, 273:L814-L824.

35. Subauste MC, Jacoby DB, Richards S, Proud D: Infection of a human respiratory epithelial cell line with rhinovirus. Induction of cytokine release and modulation of susceptibility to infection by cytokine exposure. J Clin Invest 1995, 96:549-557.

36. Donninger H, Glashoff R, Hachi HM, Syce JA, Ghildyal R, van RE, Bardin PG: Rhinovirus induction of the CXC chemokine epithelial neutrophil activating peptide-78 in bronchial epithelial cells. J Infect Dis 2003, 187:1809-1817.

37. Arruda E, Boyle TR, Winther B, Pwee DC, Gwaltney JM Jr, Hayden FG: Localization of human rhinovirus replication in the upper respiratory tract by in situ hybridization. J Infect Dis 1995, 171:1329-1333.

38. Pitaro J, Puhakka T, Makela MJ, Ruuskanen O, Carpen O, Vaheri A: Detection of rhinovirus RNA in middle turbinate of asymptomatic rhinovirus carriers. J Med Virol 2003, 70:319-323.

39. Winther B, Gwaltney JM Jr, Mygind N, Turner RB, Hendley JO: Sites of rhinovirus recovery after point inoculation of the upper airway. JAMA 1986, 256:1763-1767.

40. Skoner DP, Doyle WJ, Serokky J, Vandeuse MA, Fireman P: Lower airway responses to rhinovirus 39 in healthy allergic and nonallergic subjects. Eur Respir J 1996, 9:1402-1406.

41. Zambrano JC, Carper HT, Rakes GP, Patrice J, Murphy MD, Platts-Mills TA, Hayden FG, Gwaltney JM Jr, Hatley TK, Owens AM, Heymann TM, Boushey HA: Rhinovirus infection preferentially increases lower airway responsiveness in allergic subjects. Am J Respir Crit Care Med 1997, 155:1872-1876.

42. de Klerk EP, de Koker K, Kato H, Komorita N, Al-Mokdad M, Fujioka M, Nakagawa H: Recombinant production and biological properties of rat cytokine-induced neutrophil chemotactants, GRO/CINC-2 alpha, CINC-2 beta and CINC-3. Eur J Biochem 1995, 231:306-311.

43. Angelini B, Van Deusen MA, Doyle WJ: Rhinovirus infection preferentially increases lower airway responsiveness in allergic subjects. Am J Respir Crit Care Med 1999, 160:100-108.

44. Lemanske RF Jr, Dick EC, Swenson CA, Vrtis RF, Busse WW: Rhinovirus upper respiratory infection increases airway hyperactivity and late asthmatic reactions. J Clin Invest 1989, 83:1-10.

45. de Gouw HW, Grunberg K, Schot R, Kroes AC, Dick EC, Sterk PJ: Relationship between exhaled nitric oxide and airway hyperresponsiveness following experimental rhinovirus infection in asthmatic subjects. Eur Respir J 1998, 11:126-132.

46. Avila PC, Abisheganaden J, Wong H, Liu J, Yagi S, Schnurr D, Kishiyama YL, Boushey HA: Effects of allergic inflammation of the nasal mucosa on the severity of rhinovirus 16 cold. J Allergy Clin Immunol 2000, 105:923-932.

47. Sir Paul Nurse, Cancer Research UK