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ORIGINAL ARTICLE

Defective innate immune responses to respiratory syncytial virus infection in ovalbumin-sensitized mice

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Background/Purpose: Respiratory viral infections have frequently been reported to closely correlate with asthma exacerbations. Distinctive expression of cytokine/chemokine and anomalous responses of innate immunity induced by respiratory viral infections were suggested to play a key role. This study further evaluates the effects of airway sensitization on innate immunity in response to different viruses.
Methods: Murine sensitization was established using an ovalbumin (OVA) sensitization model. Mice were subsequently infected with either respiratory syncytial virus (RSV) or human metapneumovirus (hMPV). Type I interferon (IFN), cytokines, and chemokines were measured in bronchoalveolar lavage (BAL) fluid. Pulmonary tissue samples were collected for the analysis of viral titers and type I IFN signal transcriptors.
Results: Distinct expressions of cytokine/chemokine responses after viral infection were also found in mice with OVA sensitization. A significant increase of virus replication was found in lungs of RSV-infected sensitized mice. The increment of RSV titer was associated with the decreased levels of type I IFN. Although Toll-like receptor 3 (TLR3) expression was significantly increased in the lungs, the key signal transcriptor, IFN regulatory factor 3, was significantly suppressed in the RSV-infected sensitized mice.
Conclusion: A defective antiviral innate response was observed in the murine respiratory allergy model. Suppressed expression of IFN signal transcriptor contributes to decreased production of type I IFN. The defective innate immune response might result in acute viral

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exacerbations of allergic airway diseases.

Introduction

Asthma is an allergic airway disease characterized by a chronic inflammatory reaction and airway hyperresponsiveness (AHR). During episodes of asthma exacerbations, respiratory viral infections can be detected in nearly 80—85% of school-age children and 75—80% in adults.[1,2] Respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) belong to the family of *Paramyxoviridae*. They share similar clinical manifestations and seasonal epidemics in young children.[3] Both are often detected in young children with asthma exacerbation.[4—8]

Whether respiratory virus induces asthma exacerbation, or if asthmatics are more prone to respiratory viral infection still remains an enigma. A report from Wark et al[9] showed an increased viral replication after rhinovirus inoculation in *ex vivo* cultured primary bronchial epithelial cells collected from asthmatic adults. Decreased expression of type I interferons (IFNs) and inhibited apoptosis of infected bronchial epithelial cells were suggested to contribute to the increased viral replication.[9] Another important antiviral immune response is the pattern-recognition receptors (PRRs)-induced type I IFNs production. Among these PRRs, retinoic acid-inducible gene I (RIG-I) and Toll-like receptor 3 (TLR3) seemed to play an important role in mediating the recognition of RSV and hMPV.[10,11] pattern recognition receptor (PPR) domains transmit signals into the nucleus through several transcription factors. The key signal transcriptors, such as IFN regulatory factor 3 (IRF3), IRF7, and nuclear factor-κb (NF-κb), once activated, initiate IFNs reponses.[12]

In this study, we examined how sensitization affects innate immune responses. Ovalbumin (OVA)-sensitized mice were utilized in the infection with RSV or hMPV. The study demonstrated that after RSV infection, sensitization status impaired type I IFN expression, and resulted in enhanced viral replication. Gene and protein analysis further verified that the reduced expression of IRF3 contributed to the decline of type I IFNs.

Materials and methods

Mice and viral stocks

BALB/c female mice (6—8 weeks old) were purchased from the National Laboratory Animal Center (Taipei, Taiwan). All mice were housed in accordance with the guidelines of National Institute of Health for animal health care. The hMPV strain CAN97—83 and RSV A2 were obtained from the University of Texas Medical Branch, Galveston, TX, USA with kind permission from Dr. Roberto P Garofalo (Division of Clinical and Experimental Immunology and Infectious Diseases, University of Texas Medical Branch). hMPV was propagated in LLC-MK2 cells (ATCC CCL-7), and viral titer was determined by a 50% Tissue Culture Infection Dose ($TCID_{50}$) plaque assay as described previously.[13] RSV A2 was grown in HEp2 cells (ATCC CCL-23), and viral titer was determined by a methylcellulose plaque assay, as previously described.[14]

Experimental protocol

The study protocol is outlined as in Fig. 1. The OVA sensitization and viral inoculation are described below. On Day 0, mice were immunized with 50 μg OVA (Sigma-Aldrich, St. Louis, MO, USA) and 2 mg aluminum hydroxide. Mice were subsequently challenged with 1% OVA solution diluted with sterilized Phosphate Buffered Saline (PBS) daily using a small volume nebulizer on Days 14—16. On Day 16, mice were inoculated intranasally with purified hMPV, RSV, or PBS. A total of 1×10^7 plague-forming unit (pfu) hMPV or RSV was used for infecting one mouse. Analytic procedures were performed on Day 17. Lung virus titers were determined on Day 21 (Day 5 postinfection). Mice were divided into Mock, OVA mock, RSV, OVA + RSV, hMPV, and OVA + hMPV groups. The detailed manipulations of each group are shown in Fig. 1.

Sample collection

On protocol Day 17, 1 day after the last OVA challenge, mice of the different groups were sacrificed. Bronchoalveolar lavage (BAL) was performed with 1.0 mL of ice-cold PBS. BAL cells were counted, fixed, and stained using the Protocol Hema3 kit (Fisher Diagnostics, Middletown, VA, USA). Cellular differentials of BAL fluid were determined. Supernatants of BAL fluid were collected for determining the levels of cytokines and chemokines. After lavage, lungs were collected for histopathological exam or stored at −80°C until real-time quantitative polymerase chain reaction (RT-qPCR) analysis. On protocol Day 21, lungs were collected and stored at −80°C until the titration of viral titer.

Viral titration of murine lungs

Harvested murine lungs for viral titration were homogenized in 1 mL Dulbecco's Modified Eagle Medium (DMEM) and serially $\log_{10}$ diluted and applied onto HEp2 cells for RSV titration or onto LLC-MK2 cells for hMPV titration as described above.

RT-qPCR

Harvested lungs for RT-qPCR were homogenized in 1 mL PBS. A total of 100 μL of lung homogenate was treated with 1 mL of TRIzol reagent to extract RNA. RNA was resuspended in 10 μL of diethyl pyrocarbonate-treated water. cDNA synthesis was performed using 1 μL of the RNA suspension and a random hexamer cDNA synthesis kit (Applera, Norwalk, CT, USA). A total of 2/20 μL of cDNA suspension