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# Immune response and alteration of pulmonary function after primary human metapneumovirus (hMPV) infection of BALB/c mice☆

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**Abstract**

Human metapneumovirus (hMPV), a recently identified virus, causes upper and lower respiratory tract diseases. In this study, we show that BALB/c mice inoculated with hMPV exhibited significant morbidity on 1–2 days post-infection, when airway obstruction was found. Increased airway hyper-responsiveness to metacholine was found on day 4 concurrent with lung viral replication. Both IgG1 and IgG2a hMPV-specific antibodies were found in sera, while interferon-gamma (IFN-γ) and interleukin-4 (IL-4) were found in bronchoalveolar lavage. Lung histology showed parenchymal pneumonia and increased lymphocytic infiltration. We present here an animal model that may be helpful in studying hMPV pathogenesis and evaluating the effects of vaccines.


*Keywords:* Human metapneumovirus; BALB/c mice; Animal model

## 1. Introduction

Human metapneumovirus (hMPV) is a human respiratory pathogen of the *Paramyxoviridae* family in the *Metapneumovirus* genus. First isolated in the Netherlands in 2001 from nasopharyngeal aspirates of young children suffering from respiratory tract diseases [1], hMPV has since been reported in many parts of the world and identified in both young children and elderly adults [2]. The genomic organization of hMPV resembles that of the avian pneumovirus (APV), with strains that cluster in two main genotypes (A and B). To date, most studies on hMPV have been based on the evaluation of its prevalence and the identification of its clinical features. HMPV is associated with upper and lower respiratory tract infections. Clinical symptoms, characterized by cough, bronchiolitis and pneumonia, are indistinguishable from those associated with respiratory syncytial infection (RSV) infection. Some authors have suggested an association between hMPV infection and asthma exacerbation [3–6]. However, two other reports suggest that this association is less strong than that of asthma and rhinovirus infection [7,8].

It is important to establish an animal model to understand immunologic events following hMPV infection. This is primordial for the development of treatment and effective vaccines. Attempts to replicate hMPV have been performed in several animal species (poultry, ferrets, monkeys and rodents) [1,9–12]. We report here on a BALB/c mouse model of hMPV infection, where administration of a moderate-titer inoculum resulted in clinical illness, lung replication and pulmonary pathology. This murine model has enabled us to evaluate the pathology and immune response to hMPV infection.

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## 2. Materials and methods

### 2.1. Mice

Six–eight-week-old female, pathogen-free BALB/c mice were purchased from Charles River (France). Throughout the study they were allowed access to food and water ad libitum.

### 2.2. Cells and virus

Vero cells were maintained in Eagles' minimal essential medium (EMEM) supplemented with 2 mM L-glutamine, 2.08 g/ml sodium bicarbonate, $10^4$ U of penicillin per liter, 50 mg of streptomycin per liter and 5% fetal calf serum. The hMPV strain (NL-001) was kindly provided by Dr. Ron Fouchier (Dept of Virology, Erasmus MC, Rotterdam, Netherlands). HMPV was cultured on Vero cells in EMEM containing 0.3% BSA and 0.025% trypsin (infection medium). At full cytopathic effect, the cell monolayer was disrupted with sterile glass beads and the resulting cell suspension divided into aliquots, snap-frozen, and stored at −80 °C until required. Uninfected Vero cell lysate was prepared similarly to the virus preparation.

### 2.3. Experimental model

At the time of infection, the viral suspension was rapidly thawed at room temperature and immediately inoculated into the mice. The mice were divided into two main groups: (1) hMPV mice, where intranasal challenge with $3.3\,10^5$ pfu/mouse of hMPV was performed on day 0; (2) mock mice, inoculated with a control without virus which consisted of uninfected cell preparation prepared as described above. Before inoculation, both infected and mock mice were anesthesized by i.p. injection of ketamine–xylazine (ketamine 10%; xylazine 2%, 16 μl in 100 μl of PBS/mouse). A third group of naïve mice, with no treatment or inoculation was used as control for weight measurement.

The mice were weighed daily from day 0 to 8, they were also observed for signs of illness; they were killed by pentothal injection. The lungs of the hMPV mice were harvested on day 1–8 post-challenge to assess the peak of viral replication, or from day 1 to 14 post-challenge to measure cytokine levels. Bronchoalveolar lavages (BAL) were collected from day 1 to 14. Airway reactivity was measured daily. Histopathologic scores were determined at days 2 and 6 post-infection.

### 2.4. Virus infectivity assay

Vero cells were seeded into 24-well plates 24 h prior to titration. Immediately before titration, the medium was aspirated from the cells, which were washed with PBS before inoculation with serial 10-fold dilutions of the virus in infection medium. The plates were incubated at 37 °C for 2 h. The inoculum was subsequently removed and the cells were covered with infection medium containing 0.5% agarose. The virus infection was left to propagate for 3 days. Detection of hMPV cytopathic plaques by immunostaining was as follows: the agarose was removed and the cells were fixed in cold methanol containing 10% acetone (between each of the steps the cells were washed with PBS). A human anti-hMPV serum diluted to 1:100 in PBS was added to the cells, and the plates were incubated at 37 °C for 30 min. The cells were then incubated with an HRP-labeled anti-human IgG (Southern Biotechnology Associates, Birmingham) at 37 °C for 30 min before the addition of an AEC substrate for peroxidase (Vector laboratories, Burlingame, CA, USA). They were then left for 10 min at room temperature. The plates were finally rinsed with water. CPE were counted after AEC staining. To determine the peak of viral replication, the lungs were individually homogenized with 1 mm glass beads in a Mini-BeadBeater homogenizer (Biospec products). The suspension was centrifuged at $10{,}000 \times g$ for 1 min at 4 °C and the resulting supernatant was titrated as described above. Virus titer was expressed as $\log_{10}$ pfu/g of lung.

### 2.5. Detection of hMPV-specific IgG, IgG1, IgG2a and IgA antibodies in serum by ELISA

Wells of Maxisorp plates (Nunc, Denmark) were coated with hMPV-infected or uninfected Vero cells. The plates were blocked with PBS-containing 10% milk. Serial dilutions of sera (starting dilution of 1:100) in PBS with 5% BSA were incubated at 37 °C for 1 h, and then added to the wells. Subclasses of IgG and IgA were detected by the subsequent addition of biotinylated goat anti-mouse antibody (Southern Biotechnology Associates, Birmingham) followed by neutralite avidin–horseradish peroxidase (Southern Biotechnology Associates). Titers in ELISA were expressed as the $\log_{10}$ of the reciprocal of the final dilution with an optical density of 0.2, which was at least twice that of the negative control.

### 2.6. IgE antibodies in serum

Total IgE antibodies in serum were determined by ELISA using a rat anti-mouse IgE monoclonal antibody as the coating antibody (Pharmingen). IgE was detected by using biotinylated rat anti-mouse IgE antibody (Southern biotechnology). Concentrations of IgE were calculated by interpolation from a standard titration curve with known concentrations of purified mouse IgE standard (Pharmingen). The detection limit of the total IgE assay was 8 ng/ml.

### 2.7. Bronchoalveolar lavage (BAL)

Lavage of the airways was performed twice via a trachea cannula with 1 ml of PBS. The resulting fluid was immediately centrifuged (at $500 \times g$ for 5 min) [13]. Supernatants were removed and stored at −80 °C for cytokine quantification, and pellets of BAL cells were resuspended in 500 μl of RPMI medium.