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Prophylactic and therapeutic benefits of a monoclonal antibody against the fusion protein of human metapneumovirus in a mouse model

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ABSTRACT

Human metapneumovirus (HMPV) is a paramyxovirus causing acute respiratory tract infections in humans. The effects of a monoclonal antibody (MAb 338, MedImmune, Inc.) directed against the HMPV fusion protein were assessed *in vivo*. Different groups of BALB/c mice received an intraperitoneal injection of 25 or 50 mg/kg of MAb 338 either 24 h before or 48 h after viral infection. Lung samples were collected on days 5 and 42 after infection for determination of viral titers and histopathological changes. Pulmonary functions were also evaluated by plethysmography. On day 5 post-infection, lung viral titers were significantly decreased in mice treated with 25 or 50 mg/kg before or after viral infection compared to HMPV-infected control mice. Similarly, HMPV copy numbers on day 42 were decreased for all prophylactic and therapeutic interventions. Histopathological changes were also less severe in all treated groups of mice on days 5 and 42 post-infection, correlating with decreased airways obstruction. Finally, on day 42, all treated groups had a significant decrease in airways hyperresponsiveness following treatment with MAb 338. Both prophylactic and, to a lesser extent, therapeutic administration of MAb 338 improved acute and late consequences of HMPV infection in a relevant mouse model.

1. Introduction

The human metapneumovirus (HMPV) is a member of the *Metapneumovirus* genus within the *Pneumovirinae* subfamily of the *Paramyxoviridae* family (van den Hoogen et al., 2001). Since its initial description by Dutch researchers in 2001, HMPV has been detected in most parts of the world and initially associated with respiratory tract infections in children (Hamelin et al., 2004). Seroprevalence studies have indicated that virtually all children are infected by HMPV before 5–10 years of age (Ebihara et al., 2003; Leung et al., 2005; van den Hoogen et al., 2001; Wolf et al., 2003). Many clinical studies have indicated that HMPV is a leading cause of bronchiolitis and is responsible for 5–10% of hospitalizations for acute respiratory tract infection (ARTI) in young children (Boivin et al., 2002, 2003; Esper et al., 2003; Freymouth et al., 2003). The virus has also been associated with 12–15% of consultations for lower respiratory tract infections (LRTI) and upper respiratory tract infections (URTI) in outpatient children (Williams et al., 2004). Within this population, the clinical features associated with HMPV infections are very similar to those induced by human respiratory syncytial virus (HRSV), another paramyxovirus and the leading cause of bronchiolitis (Boivin et al., 2003; Freymouth et al., 2003; Jartti et al., 2002; Viazov et al., 2003). More recent studies have shown that clinical reinfections with HMPV occurred in children (Ebihara et al., 2004; Pavlin et al., 2008) and are indeed associated with pneumonia and exacerbations of chronic obstructive pulmonary disease in older adults as well (Hamelin et al., 2005a). Severe HMPV pneumonia have also been described in immunocompromised patients (Pelletier et al., 2002).

The HMPV genome consists of a single negative strand of RNA of approximately 13 kb containing 8 genes which code presumably for 9 different proteins (Biacchesi et al., 2003; van den Hoogen et al., 2002). Among the three HMPV surface glycoproteins [i.e. fusion (F), attachment (G) and small hydrophobic (SH) proteins], the F protein is the major antigen that induces a protective immune response in two animal models of HMPV infection, hamsters and African green monkeys (AGMs) (Skiadopoulos et al., 2006; Tang et al., 2005).

Until now, no prophylactic or therapeutic modality has been approved for severe HMPV infection. Palivizumab (Synagis®, MedImmune, Inc., Gaitherburg, MD), a humanized monoclonal antibody (MAb) against the HRSV fusion protein, is very effective at neutralizing this virus and is currently used as a prophylactic agent in high-risk infants during the HRSV season. This preventive strategy

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has dramatically reduced the number of hospitalizations due to HRSV in premature children and in those with underlying cardiopulmonary conditions (Feltes et al., 2003; The IMpact-RSV Study Group, 1998). Although palivizumab is inactive against HMPV (Wyde et al., 2003), similarities in the F proteins of the two viruses suggest that a similar strategy would be feasible and effective for HMPV.

Ulbrandt et al. (2006) generated a series of neutralizing MAbs against the HMPV F protein. One of these MAbs (MAb 338) had the ability to neutralize strains from the 4 subgroups of HMPV and also significantly decreased lung viral titers in hamsters when administered 24 h before intranasal infection. Recently, MAb 338 escape mutants have been characterized and it was reported that the F protein epitope recognized by this MAb was similar to the one recognized on the HRSV F protein by palivizumab (Ulbrandt et al., 2008). Our group previously evaluated MAb 338 in a BALB/c mouse model and observed that an intramuscular administration of 5 or 10 mg/kg 24 h before viral infection significantly reduced lung viral titers, pulmonary inflammation and airways obstruction compared to control BALB/c mice (Hamelin et al., 2008). In contrast, previous pre-clinical work with palivizumab used the intraperitoneal route for immunization and higher MAb concentrations to characterize its effect in mice (Mejías et al., 2004, 2005). Thus, we sought to evaluate both prophylactic and therapeutic administrations of MAb 338 using higher doses (25–50 mg/kg) and a different route of administration (intraperitoneal) to compare its effect with previous palivizumab studies realized in the same animal model.

2. Materials and methods

2.1. Cell line, virus and MAb

LLC-MK2 cells were maintained in minimum essential medium (MEM) (Gibco/BRL, Bethesda, MD) supplemented with 10% fetal bovine serum (FBS). HMPV C-85473 is a clinical strain (group A as are CAN97-83 and NL/00-1), received from the CHUQ-CHUL hospital (Quebec), passaged eight times in LLC-MK2 cells using the Opti-MEM medium (Gibco) supplemented with 0.0002% trypsin (Sigma, St. Louis, MO) and 0.2% gentamicin (Gibco) (HMPV infection medium). The HMPV MAb 338, which neutralizes the F protein, has been described previously (Ulbrandt et al., 2006) and was provided by MedImmune, Inc. Negative control MAb 1A7, which is against the *Escherichia coli* pilus protein FimH, was also provided by MedImmune. The latter did not have any neutralizing activity against hMPV *in vitro*.

2.2. Experimental animal protocol

Groups of eighteen 4–6-week-old BALB/c mice (Charles Rivers Laboratories, Wilmington, MA) received one intraperitoneal injection of either 25 mg/kg (groups A and D) or 50 mg/kg (groups B and E) of MAb 338 or negative control MAb 1A7 (groups C and F). Groups A, B and C received their injection 24 h before infection whereas groups D, E and F received it 48 h after viral challenge. For the challenge, mice were infected intranasally with 1×10^6 $TCID_{50}$ of HMPV strain C-85473 in 80 μl of HMPV infection medium while anesthetized under isoflurane. A sham-infected (infection medium only) group (group G) was also included. On day 5 post-infection (i.e. at the peak of viral replication, Hamelin et al., 2005b), some mice were sacrificed with euthanyl then lungs were collected for viral load determination by cell culture (6 mice per groups) or for histopathological studies (6 mice per groups). The remaining 6 mice per group were used to evaluate pulmonary functions (mainly airways obstruction) using a whole body unrestrained plethysmograph. On day 42 post-infection, which corresponds to late phase of HMPV infection with no detectable infectious virus (Hamelin et al., 2006), the same studies were repeated except that viral load was assessed by real-time PCR instead of cell culture and airways hyperresponsiveness was also determined.

2.3. Virus titration in lungs

On day 5 post-infection, animals were sacrificed and their lungs were removed and quickly frozen in liquid nitrogen. For viral titration, lungs were weighed, homogenized in 1 ml of HMPV infection medium, centrifuged for 10 min at 1200 RPM and then 100 μl of the homogenates was used to determine viral titers by inoculating tenfold serial dilutions of virus in 24-well plates containing LLC-MK2 cells. Before infection, cells were washed twice with PBS to remove residual serum proteins that could inhibit trypsin activity. Infected plates were incubated at 37 °C with 5% CO_2 and replenished with 1 μl of fresh trypsin (0.0002%) every other day. Virus titers were reported as $\log_{10}$ 50% tissue culture infectious dose ($\log_{10}$ $TCID_{50}$) per gram of lung. The lower limit of detection of this assay is 10^2 $TCID_{50}$/g.

2.4. Real-time PCR studies

On day 42 post-infection, animals were sacrificed and their lungs were processed as described above. Viral RNA was extracted from 200 μl of lung homogenates using the QIAamp viral RNA mini kit (Qiagen, Mississauga, Ontario, Canada) according to the Manufacturer's protocol with elution in a final volume of 40 μl. Complementary DNA was synthesized using 10 μl of RNA eluate, random hexamer primers (Amersham Pharmacia Biotech, Baie d'Urfé, Québec, Canada), and the Omniscript reverse transcriptase kit (Qiagen) according to the Manufacturer's instructions. Two μl of cDNA was amplified by a real-time PCR protocol for the L gene as previously reported (Deffrasnes et al., 2005). The limit of detection for this RT-PCR assay is 100 copies per reaction.

2.5. Pulmonary histopathology

On days 5 and 42 post-infection, lungs were removed and fixed with 10% buffered formalin. Fixed lungs were embedded in paraffin, sectioned in slices of 4 μm and stained with hematoxylin-eosin. The histopathological scores (HPS) were determined by a pathologist with experience in pulmonary pathology who was unaware of the infection/treatment status of the animals. A semi-quantitative scale was used to score peribronchial, perivascular, interstitial and intra-alveolar inflammation as previously described (Hamelin et al., 2005b).

2.6. Lung function studies

Whole body flow-through plethysmography (EMKA Technologies, inc., Falls Church, VA) was used to monitor airways obstruction of unrestrained mice on days 5 and 42, as well as airways hyperresponsiveness after methacholine exposure on day 42. The recorded variable included the Penh (enhanced pause) value which consists of the pause parameter ([expiration time (T_E) – relaxation time (T_R)]/T_R) multiplied by the ratio of peak expiratory flow to peak inspiratory flow. This parameter has already been used to characterize airways obstruction of HMPV-infected mice (Darniot et al., 2005; Hamelin et al., 2006).

The system was calibrated by injecting a known volume of air (1 ml) into the chamber with a glass syringe. Fresh air was delivered into the experimental chamber at a constant rate with a bias flow regulator (EMKA Technologies, Falls Church, VA). Mice were first allowed to acclimatize to the plethysmography chamber for 30 min before recordings. Then, respiratory activity was monitored for

5 min to establish airways obstruction parameters. On day 42 post-infection only, mice were subsequently exposed to aerosolized methacholine (acetyl-β-methylcholine chloride; Sigma, St. Louis, MO) 50 mg/ml previously dissolved in PBS 1× for 3 min and plethysmography readings were recorded for another 8 min (Hamelin et al., 2006; Mejías et al., 2004, 2005). Airways hyperresponsiveness was defined by the delta Penh value i.e. the difference between the maximum Penh value post-methacholine challenge and the baseline Penh value.

2.7. Statistical analysis

All data with the exception of histopathological scores are expressed as mean ± SEM. Groups of treated mice were compared for the different variables using the Student's *t* test or, for data not normally distributed, the Mann–Whitney rank sum test.

3. Results

3.1. Lung viral titers in mice

Five days following HMPV infection, a significant decrease in lung viral titers was observed in BALB/c mice that received 25 or 50 mg/kg of MAb 338 either 24 h before or 48 h after the infection compared to HMPV-infected control mice ($3.40 \times 10^3 \pm 4.29$, $1.60 \times 10^3 \pm 1.38$, $1.82 \times 10^4 \pm 1.41$, $6.35 \times 10^3 \pm 2.56$ versus $1.61 \times 10^5 \pm 1.28$ $TCID_{50}$/g, respectively) ($P < 0.05$ for all groups compared to control) (see Fig. 1A for viral titers and Fig. 1B for viral RNA load). Of note, no unexpected change in the viral F gene sequence was observed from the infected lung tissue. Similarly, on day 42 post-infection (at a time when no infectious virus could be recovered; Hamelin et al., 2005b), the number of viral RNA copies in the lungs were significantly decreased in mice treated with 25 or 50 mg/kg of MAb 338 24 h before or 48 h after viral infection compared to infected controls ($6.25 \times 10^2 \pm 7.15$, $2.46 \times 10^2 \pm 1.53$, $8.54 \times 10^2 \pm 4.26$, $7.46 \times 10^2 \pm 1.79$ versus $1.65 \times 10^3 \pm 5.97$ copies per 0.01 g of lungs, respectively) ($P < 0.05$ for all groups compared to control except for 25 mg/kg at 48 h after infection) (Fig. 1C). There was no significant difference between treated groups at either time points. No infectious viruses or viral RNA were found in sham-infected mice at either time point.

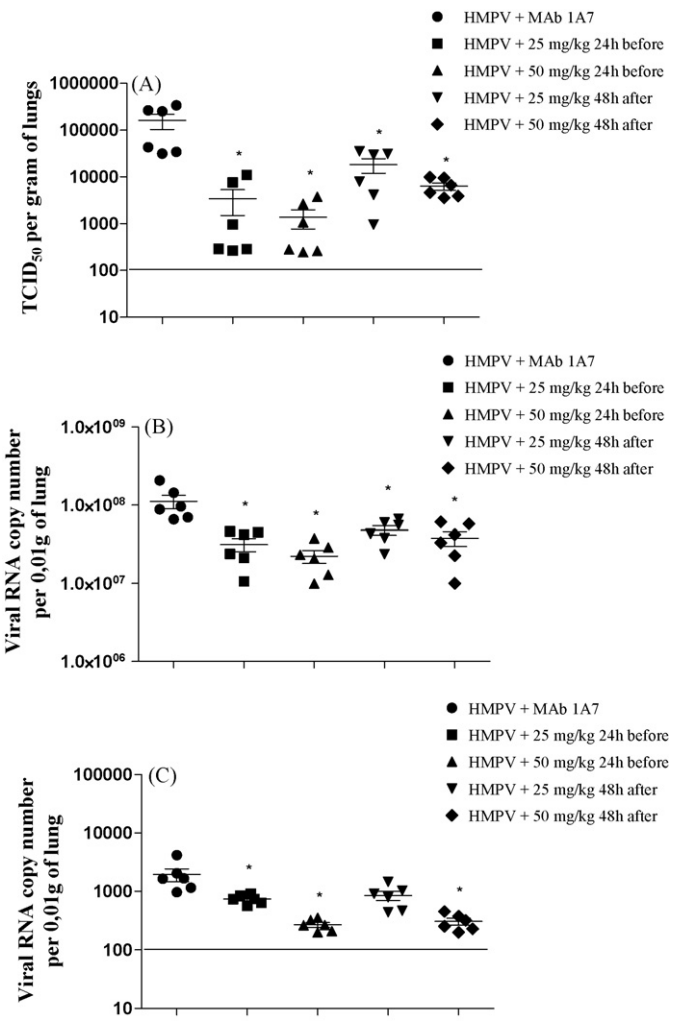

Fig. 1. Lung viral titers in mice treated or not with HMPV MAb 338. Six mice per group were sacrificed on days 5 and 42 post-infection and their lungs were removed. Lung homogenates were serially diluted and inoculated on LLC-MK2 cells for viral titration on day 5 (A) or used to determine HMPV copy numbers with a TaqMan probe on day 5 (B) or 42 (C). Neither viruses nor viral RNA were found in sham-infected mice at both times. The mean values ± SEM are indicated. *Statistically significant differences ($P < 0.05$) were observed between treated groups and HMPV-infected control mice. The line indicates the limit of detection.

3.2. Histopathological changes in lungs of BALB/c mice

On day 5 after HMPV infection, the lung inflammation was mostly characterized by perivascular, alveolar and interstitial infiltrates in HMPV-infected control mice, which was decreased in mice treated with 25 or 50 mg/kg of MAb 338 either before or after viral challenge (Fig. 2A). Overall, total HPS were 9, 7.83, 9.17, 8.83 and 11.33 for mice pre-treated with 25 or 50 mg/kg of MAb, post-treated with 25 or 50 mg/kg of MAb and for the HMPV-infected control group, respectively (Fig. 3A). In mice examined 42 days post-HMPV infection, the lung inflammation was mostly perivascular and peribronchiolar with some remaining alveolar and interstitial inflammation (Fig. 2B). Total HPS were 3, 2.83, 3.33, 2.83 and 3.83 for mice pre-treated with 25 or 50 mg/kg of MAb, post-treated with 25 or 50 mg/kg of MAb and for the HMPV-infected control group, respectively (Fig. 3B). A few infiltrating cells were found around bronchioles of uninfected mice immunized with MAb 338. The latter does not seem to be responsible for these basal responses as this phenomenon has been previously observed in sham-infected BALB/c mice (Hamelin et al., 2006).

3.3. Airways obstruction in mice

On day 5 following HMPV infection, infected BALB/c mice that had received the control MAb presented a significant increase in Penh value compared to shams, suggesting airways obstruction. Mice that had received 25 or 50 mg/kg of MAb 338 before or after viral infection also presented significant increased Penh values compared to sham-infected mice, although their mean values were significantly lower than that of HMPV-infected control mice (Fig. 4A) ($P < 0.05$ for all groups compared to controls). On day 42 post-infection, infected HMPV control mice still had a significant increase in the Penh value compared to sham-infected mice indicating that airways obstruction was still present. Mean Penh values of the different treated groups were significantly higher than that of sham-infected mice, but significantly lower than that of infected controls (Fig. 4B) ($P < 0.05$ for all groups compared to controls except for 25 mg/kg 48 h after infection). There was no significant difference between treated groups at either time point.

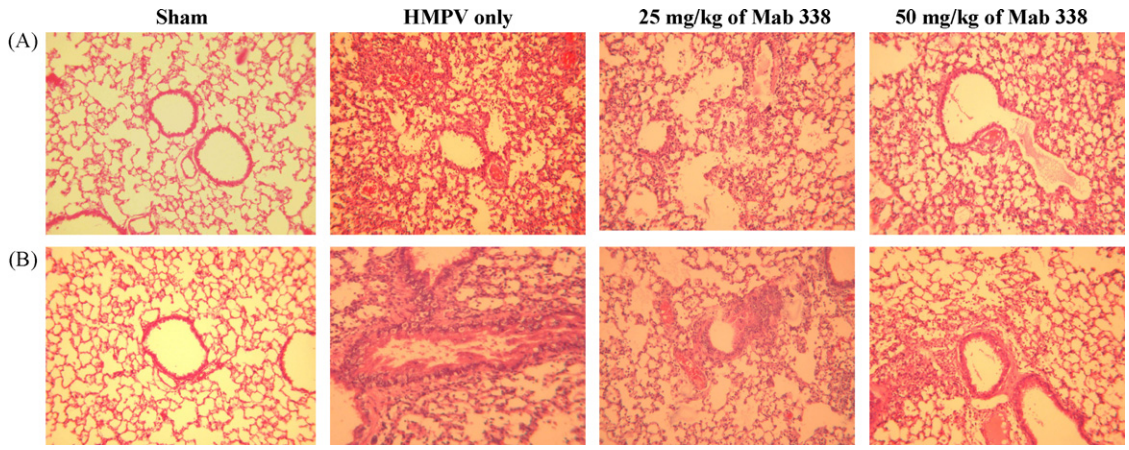

Fig. 2. Lung staining in mice treated or not with HMPV MAb 338. Mice that had received MAb 338 24 h prior to infection were euthanized on days 5 and 42 post-infection and their lungs were removed and fixed with 10% formalin. Four μm sections of paraffin-embedded lung tissues were cut and stained with hematoxylin and eosin. A representative section is shown for the different groups of mice on days 5 (A) and 42 (B) post-infection.

3.4. Development of airways hyperresponsiveness

Nebulization of methacholine has been reported to further increase the Penh value in HMPV-infected mice, illustrating the development of airways hyperresponsiveness (Hamelin et al., 2006). Data in Fig. 5 are shown as delta Penh, which is obtained by comparing post- and pre-methacholine values. Methacholine challenge on day 42 induced changes in respiratory function parameters in all groups of mice. HMPV-infected mice that received the control MAb were extremely sensitive to methacholine challenge, and these animals died within 2 min after methacholine nebulization. In contrast, none of the mice that received MAb 338 either before or after HMPV infection died following the methacholine challenge indicating a significant decrease in airways hyperresponsiveness. No significant changes in delta Penh values were noted between sham-infected mice and treated mice, as well as, between different treatment groups.

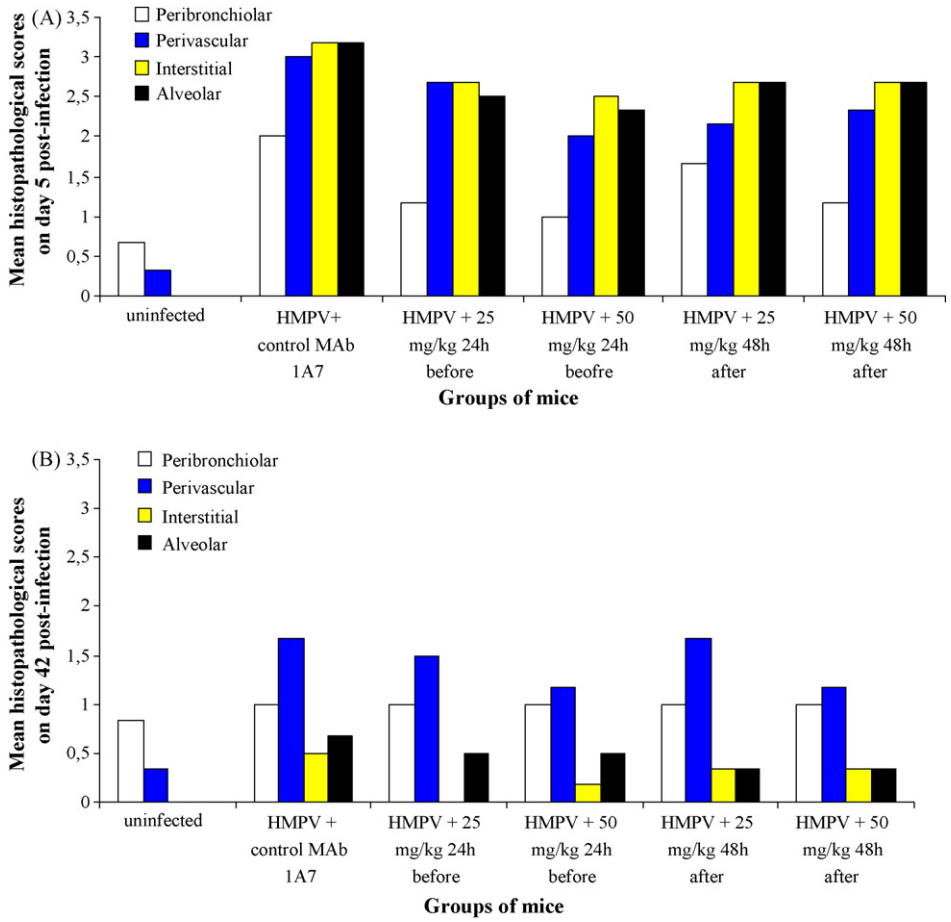

Fig. 3. Lung histopathological scores in mice treated or not with HMPV MAb 338. The degree of lung inflammation (mean histopathological score) was evaluated for peribronchial, perivascular, interstitial and intra-alveolar inflammation on days 5 (A) and 42 (B) post-infection, as previously described (Hamelin et al., 2005b).

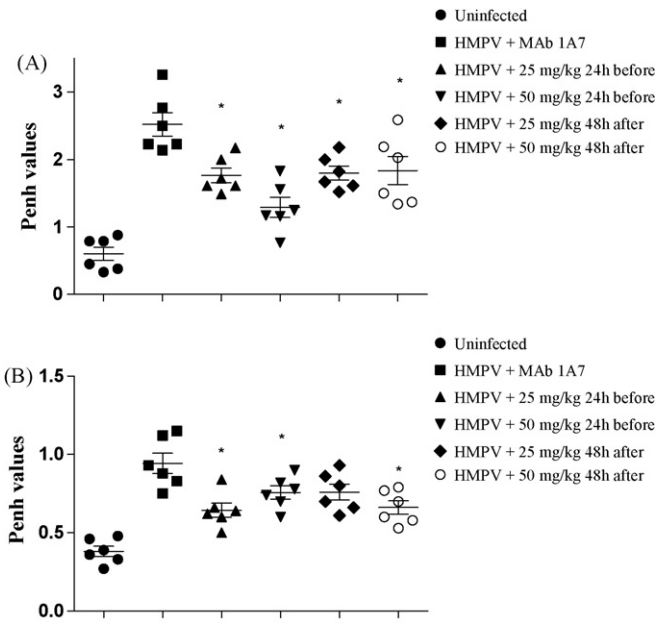

Fig. 4. Airways obstruction in mice treated or not with HMPV MAb 338. Six mice per group were evaluated on days 5 (A) and 42 (B) post-infection. Airways obstruction, as defined by the Penh value, was determined using whole body unrestrained plethysmography. The mean values ± SEM are indicated. *Statistically significant differences ($P < 0.05$) were observed between treated groups and HMPV-infected control mice.

4. Discussion

We characterized the prophylactic and therapeutic effects of MAb 338 directed against the fusion (F) protein of HMPV on the acute and late phases of viral infection using a BALB/c mouse model. On day 5 post-infection, lung viral titers were significantly decreased in mice treated with 25 or 50 mg/kg of MAb 338 either 24 h before or 48 h after viral infection. Pulmonary inflammation and airways obstruction were also decreased in all groups treated with MAb 338. Similarly, on day 42 post-infection, the functional consequences of HMPV infection were less pronounced in the treated groups. In particular, airways hyperresponsiveness was less severe in all treated groups compared to HMPV-infected control mice. Overall, our data suggest that there is a possible role for MAb 338 not only in prophylaxis as previously reported by our group (Hamelin et al., 2008) but also as an early treatment to attenuate acute and late consequences of HMPV infection.

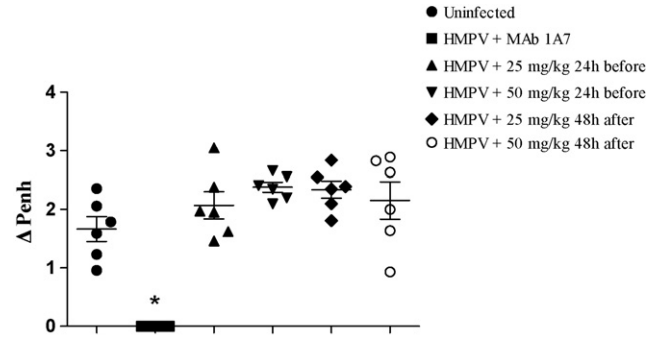

Fig. 5. Airways hyperresponsiveness in mice treated or not with HMPV MAb 338. Six mice per group received nebulized methacholine (50 mg/ml) on day 42 post-infection. Lung functions were determined using whole body unrestrained plethysmography. Airways hyperresponsiveness, represented by the delta Penh value, corresponds to the difference between the maximum values recorded post-methacholine challenge and those from baseline. The mean values ± SEM are indicated. *No values are shown due to deaths of all mice following methacholine challenge.

HMPV is responsible for a significant proportion of bronchiolitis in young children (Boivin et al., 2003), although no intervention is currently available to alter the course of this viral infection. Some animal studies in hamsters and monkeys have indicated that the intranasal administration of one HMPV strain protects (although for a limited period of time) against subsequent challenge with homologous and heterologous strains (MacPhail et al., 2004; Skiadopoulos et al., 2004). Pre-clinical immunization studies have shown that the HMPV F protein is the major immunodominant protein. Indeed, recombinant HMPV viruses lacking the SH and/or G proteins administered intranasally conferred complete protection against lung replication in hamsters following challenge with wild-type HMPV (Biacchesi et al., 2004). In another approach, the HMPV F protein was inserted in a bovine parainfluenza type 3 (PIV-3) recombinant virus. The administration of this recombinant virus to hamsters and African green monkeys also conferred protection against both groups of HMPV and hPIV-3 (Tang et al., 2003, 2005).

The latter studies suggest that administration of MAbs targeting the F protein might effectively protect against HMPV infection. Recently, Ulbrandt et al. (2006) have reported the protective efficacy of a series of MAbs in an animal model (hamsters) of acute HMPV infection. When administered intramuscularly to hamsters 24 h before infection at a dose of 3 mg/kg, lung viral titers were decreased by approximately 2–3 $\log_{10}$ on day 4 post-infection. We also tested one of these MAbs (338) in a BALB/c mouse model and observed that a single intramuscular administration of 5 or 10 mg/kg of MAb 338 24 h before infection decreased lung viral titers by the same magnitude as in hamsters and also reduced pulmonary inflammation (Hamelin et al., 2008). However, to date, there has been no report on the activity of MAb 338 when administered after infection, which is potentially clinically relevant in immunocompromised patients and high-risk premature infants. Therapeutic effect of MAb 338 was characterized 48 h following viral infection, as performed for previous HRSV studies.

The prophylactic administration of MAb 338 in our BALB/c mouse model significantly reduced HMPV replication by approximately 2 $\log_{10}$ $TCID_{50}$/g of lung on day 5 post-infection (Fig. 1A), which is a reduction similar to what we reported in our previous study (Hamelin et al., 2008) despite the use of different doses and mode of administration (intraperitoneal instead of intramuscular) for MAb 338 and a higher HMPV inoculum in this study which was needed to produce more significant inflammation. We acknowledge that the 2 experiments were not performed at the same time and the viral inoculum was slightly higher in the latter. Observing similar results with those different conditions might reinforce the effect of the treatment. The therapeutic administration of MAb 338 was evaluated for the first time with significant benefits shown when the dose was administered 48 h after infection especially for the highest concentration of 50 mg/kg. Although the therapeutic benefits were lower when the MAb was administered post-infection compared to pre-infection, we report significant decreases in lung titers, pulmonary inflammation, airways obstruction and hyperresponsiveness in mice treated 48 h after HMPV infection compared to untreated animals. Of note, airway responses that we observed following HMPV infection are likely due to the presence of the virus and not LLC-MK2 cells as we already reported that uninfected cells (a control that was not used in this study) do not produce this kind of response (Hamelin et al., 2006).

Although we observed a reduction in lung inflammation (mainly perivascular, interstitial and alveolar on day 5 compared to perivascular and peribronchiolar on day 42) with MAb 338 treatment, such reduction was modest compared to the pronounced decrease in viral titers. This could suggest that the host inflammatory response is an important contributor to the pathogenesis of HMPV disease

at least in this animal model. A similar dichotomy between lung inflammation and viral titers has been reported in mice receiving palivizumab and then infected with HRSV (Mejías et al., 2004, 2005). In cotton rats, only the addition of systemic steroids to palivizumab was able to reduce the severity of pulmonary inflammation induced by HRSV (Prince et al., 2000); such combination might be worth evaluating for HMPV.

The Penh value determined by plethysmographic evaluation is a parameter that correlates well with pulmonary airflow resistance or obstruction. It also increases in parallel with the severity of small airways disease, thus remaining a useful tool to evaluate the consequences of microorganisms on airways functions (Hamelmann et al., 1997). In correlation with the pulmonary inflammation noted, HMPV-infected mice that received the control MAb had significant airways obstruction (Fig. 2A) as illustrated by the high Penh value on day 5 post-infection as previously described (Darniot et al., 2005; Hamelin et al., 2006). Therapeutic administration of MAb 338 also improved respiratory functions in our mouse model during both the acute and the late phases of infection (Fig. 4), although only the highest concentration of MAb (i.e. 50 mg/kg) was effective at reducing airways obstruction on day 42.

Peribronchiolitis, as noted in the late phase of HMPV infection, is usually the type of inflammation associated with asthma and the histopathological changes observed during this animal study support clinical findings indicating that HMPV may trigger asthma exacerbations in both children and adults (García-García et al., 2007; Jartti et al., 2002; Williams et al., 2005). In addition, a study indicated that HMPV infection in infancy is an important risk factor for asthma at age 5 (García-García et al., 2007). Prevention or attenuation of severe paramyxovirus infections occurring in young infants (particularly in premature babies) could then become an important goal to achieve in order to prevent the development of those important consequences. Indeed, preliminary results from a prospective case-cohort multicenter study have suggested that infants who had received palivizumab during their first HRSV season had a lower risk of subsequent recurrent wheezing at 12 months (Simoes et al., 2007). In the present study, we observed that mice treated with MAb 338 tolerated methacholine challenge on day 42 post-infection and did not exhibit significantly more airways hyperresponsiveness than sham (uninfected) mice.

In conclusion, the present study demonstrates the additional benefit of MAb 338 when administered 48 h after HMPV challenge on the acute and late complications of this viral infection. The results obtained so far in various animal models with MAb 338 are very similar to those published with palivizumab (Mejías et al., 2005), which support further pharmacokinetics studies and possible clinical development of this molecule. A potential indication for this MAb could include the prevention of severe HMPV infections in high-risk infants in combination with HRSV-related MAb since clinical presentation and risk factors appear similar for both paramyxoviruses (Boivin et al., 2003). In addition, such a molecule could be used for the treatment of severe HMPV infections in immunocompromised individuals. Lastly, evaluation of MAb 338 in combination with immunomodulatory compounds might also be warranted.

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G.B. holds a Canada Research Chair on Emerging Viruses and Antiviral Resistance.

References

Biacchesi, S., Skiadopoulos, M.H., Boivin, G., Hanson, C.T., Murphy, B.R., Collins, P.L., Buchholz, U.J., 2003. Genetic diversity between human metapneumovirus subgroups. Virology 315, 1–9.
Biacchesi, S., Skiadopoulos, M.H., Yang, L., Lamirande, E.W., Tran, K.C., Murphy, B.R., Collins, P.L., Buchholz, U.J., 2004. Recombinant human Metapneumovirus lacking the small hydrophobic SH and/or attachment G glycoprotein: deletion of G yields a promising vaccine candidate. J. Virol. 78, 12877–12887.
Boivin, G., Abed, Y., Pelletier, G., Ruel, L., Moisan, D., Côté, S., Peret, T.C., Erdman, D.D., Anderson, L.J., 2002. Virological features and clinical manifestations associated with human metapneumovirus: a new paramyxovirus responsible for acute respiratory-tract infections in all age groups. J. Infect. Dis. 186, 1330–1334.
Boivin, G., De Serres, G., Côté, S., Gilca, R., Abed, Y., Rochette, L., Bergeron, M.G., Déry, P., 2003. Human metapneumovirus infections in hospitalized children. Emerg. Infect. Dis. 9, 634–640.
Darniot, M., Petrella, T., Aho, S., Pothier, P., Manoha, C., 2005. Immune response and alteration of pulmonary function after primary human metapneumovirus (hMPV) infection of BALB/c mice. Vaccine 23, 4473–4480.
Deffrasnes, C., Côté, S., Boivin, G., 2005. Analysis of replication kinetics of the human metapneumovirus in different cell lines by real-time PCR. J. Clin. Microbiol. 43, 488–490.
Ebihara, T., Endo, R., Kikuta, H., Ishiguro, N., Yoshioka, M., Ma, X., Kobayashi, K., 2003. Seroprevalence of human metapneumovirus in Japan. J. Med. Virol. 70, 281–283.
Ebihara, T., Endo, R., Ishiguro, N., Nakayama, T., Sawada, H., Kikuta, H., 2004. Early reinfection with human metapneumovirus in an infant. J. Clin. Microbiol. 42, 5944–5946.
Esper, F., Boucher, D., Weibel, C., Martinello, R.A., Kahn, J.S., 2003. Human metapneumovirus infection in the United States: clinical manifestations associated with a newly emerging respiratory infection in children. Pediatrics 111, 1407–1410.
Feltes, T.F., Cabalka, A.K., Meissner, H.C., Piazza, F.M., Carlin, D.A., Top Jr., F.H., Connor, E.M., Sondheimer, H.M., 2003. Palivizumab prophylaxis reduces hospitalization due to respiratory syncytial virus in young children with hemodynamically significant congenital heart disease. J. Pediatr. 143, 532–540.
Freymouth, F., Vabret, A., Legrand, L., Eterradossi, N., Lafay-Delaire, F., Brouard, J., Guillois, B., 2003. Presence of the new human metapneumovirus in French children with bronchiolitis. Pediatr. Infect. Dis. J. 22, 92–94.
García-García, M.L., Calvo, C., Casas, I., Bracamonte, T., Rellan, A., Gozalo, F., Tenorio, T., Pérez-Brena, P., 2007. Human metapneumovirus bronchiolitis in infancy is an important risk factor for asthma at age 5. Pediatr. Pulmonol. 42, 458–464.
Hamelin, M.E., Abed, Y., Boivin, G., 2004. Human metapneumovirus: a new player among respiratory viruses. Clin. Infect. Dis. 38, 983–990.
Hamelin, M.E., Côté, S., Laforge, J., Lampron, N., Bourbeau, J., Weiss, K., Gilca, R., DeSerres, G., Boivin, G., 2005a. Human metapneumovirus infection in adults with community-acquired pneumonia and exacerbation of chronic obstructive pulmonary disease. Clin. Infect. Dis. 41, 498–502.
Hamelin, M.E., Yim, K., Kuhn, K.H., Cragin, R.P., Boukhvalova, M., Blanco, J.C., Prince, G.A., Boivin, G., 2005b. Pathogenesis of human metapneumovirus lung infection in BALB/c mice and cotton rats. J. Virol. 79, 8894–8903.
Hamelin, M.E., Prince, G.A., Gomez, A.M., Kinkead, R., Boivin, G., 2006. Human metapneumovirus infection induces long-term pulmonary inflammation associated with airway obstruction and hyperresponsiveness in mice. J. Infect. Dis. 193, 1634–1642.
Hamelin, M.E., Couture, C., Sackett, M., Kiener, P., Suzich, J., Ulbrandt, N., Boivin, G., 2008. The prophylactic administration of a monoclonal antibody against human metapneumovirus attenuates viral disease and airways hyperresponsiveness in mice. Antivir. Ther. 13, 39–46.
Hamelmann, E., Schwarze, J., Takeda, K., Oshiba, A., Larsen, G.L., Irvin, C.G., Gelfand, E.W., 1997. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. Am. J. Respir. Crit. Care Med. 156, 766–775.
Jartti, T., van den Hoogen, B., Garofalo, R.P., Osterhaus, A.D., Ruuskanen, O., 2002. Metapneumovirus and acute wheezing in children. Lancet 360, 1393–1394.
Leung, J., Esper, F., Weibel, C., Kahn, J.S., 2005. Seroepidemiology of human metapneumovirus (hMPV) on the basis of a novel enzyme-linked immunosorbent assay utilizing hMPV fusion protein expressed in recombinant vesicular stomatitis virus. J. Clin. Microbiol. 43, 1213–1219.
MacPhail, M., Schickli, J.H., Tang, R.S., Kaur, J., Robinson, C., Fouchier, R.A., Osterhaus, A.D., Spaete, R.R., Haller, A.A., 2004. Identification of small-animal and primate models for evaluation of vaccine candidates for human metapneumovirus (hMPV) and implications for hMPV vaccine design. J. Gen. Virol. 85, 1655–1663.
Mejías, A., Chávez-Bueno, S., Ríos, A.M., Saavedra-Lozano, J., Fonseca Aten, M., Hatfield, J., Kapur, P., Gómez, A.M., Jafri, H.S., Ramilo, O., 2004. Anti-respiratory syncytial virus (RSV) neutralizing antibody decreases lung inflammation, airway obstruction, and airway hyperresponsiveness in a murine RSV model. Antimicrob. Agents Chemother. 48, 1811–1822.
Mejías, A., Chávez-Bueno, S., Ríos, A.M., Aten, M.F., Raynor, B., Peromingo, E., Soni, P., Olsen, K.D., Kiener, P.A., Gómez, A.M., Jafri, H.S., Ramilo, O., 2005. Comparative effects of two neutralizing anti-respiratory syncytial virus (RSV) monoclonal antibodies in the RSV murine model: time versus potency. Antimicrob. Agents Chemother. 49, 4700–4707.
Pavlin, J.A., Hickey, A.C., Ulbrandt, N., Chan, Y.P., Endy, T.P., Boukhvalova, M.S., Chunsuttiwat, S., Nisalak, A., Libraty, D.H., Green, S., Rothman, A.L., Ennis, F.A., Jarman, R., Gibbons, R.V., Broder, C.C., 2008. Human metapneumovirus reinfection among children in Thailand determined by ELISA using purified soluble fusion protein. J. Infect. Dis. 198, 836–842.

Pelletier, G., Déry, P., Abed, Y., Boivin, G., 2002. Respiratory tract reinfections by the new human Metapneumovirus in an immunocompromised child. Emerg. Infect. Dis. 8, 976–978.

Prince, G.A., Mathews, A., Curtis, S.J., Porter, D.D., 2000. Treatment of respiratory syncytial virus bronchiolitis and pneumonia in a cotton rat model with systemically administered monoclonal antibody (Palivizumab) and glucocorticosteroid. J. Infect. Dis. 182, 1326–1330.

Simoes, E.A., Groothuis, J.R., Carbonell-Estrany, X., Rieger, C.H., Mitchell, I., Fredrick, L.M., Kimpen, J.L., 2007. Palivizumab prophylaxis, respiratory syncytial virus, and subsequent recurrent wheezing. J. Pediatr. 151, 34–42.

Skiadopoulos, M.H., Biacchesi, S., Buchholz, U.J., Riggs, J.M., Surman, S.R., Amaro-Carambot, E., McAuliffe, J.M., Elkins, W.R., St Claire, M., Collins, P.L., Murphy, B.R., 2004. The two major human metapneumovirus genetic lineages are highly related antigenically, and the fusion (F) protein is a major contributor to this antigenic relatedness. J. Virol. 78, 6927–6937.

Skiadopoulos, M.H., Biacchesi, S., Buchholz, U.J., Amaro-Carambot, E., Surman, S.R., Collins, P.L., Murphy, B.R., 2006. Individual contributions of the human metapneumovirus F, G, and SH surface glycoproteins to the induction of neutralizing antibodies and protective immunity. Virology 345, 492–501.

Tang, R.S., Schickli, J.H., MacPhail, M., Fernandes, F., Bicha, L., Spaete, J., Fouchier, R.A., Osterhaus, A.D., Spaete, R., Haller, A.A., 2003. Effects of human metapneumovirus and respiratory syncytial virus antigen insertion in two 3′ proximal genome positions of bovine/human parainfluenza virus type 3 on virus replication and immunogenicity. J. Virol. 77, 10819–10828.

Tang, R.S., Mahmood, K., Macphail, M., Guzzetta, J.M., Haller, A.A., Liu, H., Kaur, J., Lawlor, H.A., Stillman, E.A., Schickli, J.H., Fouchier, R.A., Osterhaus, A.D., Spaete, R.R., 2005. A host-range restricted parainfluenza virus type 3 (PIV3) expressing the human metapneumovirus (hMPV) fusion protein elicits protective immunity in African green monkeys. Vaccine 23, 1657–1667.

The IMpact-RSV Study Group, 1998. Palivizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. Pediatrics 102, 531–537.

Ulbrandt, N.D., Ji, H., Patel, N.K., Riggs, J.M., Brewah, Y.A., Ready, S., Donacki, N.E., Folliot, K., Barnes, A.S., Senthil, K., Wilson, S., Chen, M., Clarke, L., Macphail, M., Li, J., Woods, R.M., Coelingh, K., Reed, J.L., McCarthy, M.P., Pfarr, D.S., Osterhaus, A.D., Fouchier, R.A., Kiener, P.A., Suzich, J.A., 2006. Isolation and characterization of monoclonal antibodies which neutralize human metapneumovirus in vitro and in vivo. J. Virol. 80, 7799–7806.

Ulbrandt, N.D., Ji, H., Patel, N.K., Barnes, A.S., Wilson, S., Kiener, P.A., Suzich, J., McCarthy, M.P., 2008. Identification of antibody neutralization epitopes on the fusion protein of human metapneumovirus. J. Gen. Virol. 89, 3113–3118.

van den Hoogen, B.G., de Jong, J.C., Groen, J., Kuiken, T., de Groot, R., Fouchier, R.A., Osterhaus, A.D., 2001. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat. Med. 7, 719–724.

van den Hoogen, B.G., Bestebroer, T.M., Osterhaus, A.D., Fouchier, R.A., 2002. Analysis of the genomic sequence of a human metapneumovirus. Virology 295, 119–132.

Viazov, S., Ratjen, F., Scheidhauer, R., Fiedler, M., Roggendorf, M., 2003. High prevalence of human metapneumovirus infection in young children and genetic heterogeneity of the viral isolates. J. Clin. Microbiol. 41, 3043–3045.

Williams, J.V., Harris, P.A., Tollefson, S.J., Halburnt-Rush, L.L., Pingsterhaus, J.M., Edwards, K.M., Wright, P.F., Crowe Jr., J.E., 2004. Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. N. Engl. J. Med. 350, 443–450.

Williams, J.V., Crowe Jr., J.E., Enriquez, R., Minton, P., Peebles Jr., R.S., Hamilton, R.G., Higgins, S., Griffin, M., Hartert, T.V., 2005. Human metapneumovirus infection plays an etiologic role in acute asthma exacerbations requiring hospitalization in adults. J. Infect. Dis. 192, 1149–1153.

Wolf, D.G., Zakay-Rones, Z., Fadeela, A., Greenberg, D., Dagan, R., 2003. High seroprevalence of human metapneumovirus among young children in Israel. J. Infect. Dis. 188, 1865–1867.

Wyde, P.R., Chetty, S.N., Jewell, A.M., Boivin, G., Piedra, P.A., 2003. Comparison of the inhibition of human metapneumovirus and respiratory syncytial virus by ribavirin and immune serum globulin in vitro. Antivir. Res. 60, 51–59.