Immunization of Syrian golden hamsters with F subunit vaccine of human metapneumovirus induces protection against challenge with homologous or heterologous strains

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Human metapneumovirus (hMPV), a newly discovered paramyxovirus, is associated with acute respiratory-tract illness, primarily in young children, individuals with underlying disease and the elderly. Two genetic lineages of hMPV circulate around the world, and viruses from these two lineages demonstrate antigenic differences. The clinical impact of hMPV warrants the development of vaccines. Recombinant soluble fusion (F) proteins of prototype viruses of the two main lineages of hMPV that can be produced in high yields have been constructed. In this study, the antigenicity, immunogenicity and protective efficacy of these soluble F subunit vaccines were evaluated in Syrian golden hamsters (Mesocricetus auratus). Immunization of hamsters with the soluble F proteins, adjuvanted with Specol or iscom matrix, induced high virus-neutralization titres, with higher titres against the homologous than the heterologous virus. The neutralizing antibodies protected from subsequent infection of the lungs with both homologous and heterologous virus. Upon challenge, viral titres in the nasal turbinates of immunized animals were reduced significantly compared with those of PBS-immunized animals. In conclusion, a soluble F subunit vaccine for hMPV that induces cross-protective immunity for infection of the lower respiratory tract in Syrian golden hamsters has been generated.

INTRODUCTION

Human metapneumovirus (hMPV), a recently described paramyxovirus, was first isolated from respiratory specimens obtained from children in the Netherlands who were hospitalized for acute respiratory-tract illness (van den Hoogen et al., 2001). Clinical manifestations of hMPV infections are similar to those caused by respiratory syncytial virus (RSV), ranging from mild respiratory illness to bronchiolitis and pneumonia (van den Hoogen et al., 2003; Williams et al., 2006). RSV and hMPV are both members of the subfamily Pneumovirinae within the family Paramyxoviridae. However, within this subfamily, RSV is a member of the genus Pneumovirus and hMPV is the first mammalian member of the genus Metapneumovirus, based on differences in genome organization (van den Hoogen et al., 2002). Phylogenetic analysis of fusion (F) and attachment (G) genes of a large number of hMPV isolates revealed the existence of two main genetic virus lineages (A and B), each divided into at least two sublineages (1 and 2). The two main lineages, with prototype viruses NL/1/00 and NL/1/99 for lineages A and B, respectively, were found to be antigenically distinct in virus-neutralization assays with ferret sera (van den Hoogen et al., 2004).

The genome of hMPV encodes three surface glycoproteins: F, G and the small hydrophobic protein (SH). Whereas the G and SH proteins are highly variable, the F protein is highly conserved between the major lineages A and B. Antibodies induced against the F protein are correlates of protection in animal models (Skiadopoulos et al., 2006; Tang et al., 2005). The fusion protein of hMPV demonstrates similarities to those of other paramyxoviruses (van den Hoogen et al., 2002, 2004). The F proteins of paramyxoviruses are class I viral F proteins, which are synthesized as an inactive precursor F0 that must be cleaved by host endoproteases, resulting in two disulfide-linked polypeptides, F1 and F2 (Lamb et al., 2006; Russell et al., 2001).

The clinical impact of hMPV warrants the development of vaccines. For RSV, subunit vaccines based on the F or G proteins have been developed and tested in a variety of rodent and primate models, where they demonstrated
protective efficacy (Hancock et al., 2000; Murphy et al., 1989; Walsh et al., 1987). In addition, RSV F subunit vaccines have been evaluated in clinical trials in healthy adults, children older than 1 year with or without underlying pulmonary disease, the elderly, and pregnant women, and have been found to be safe and effective (Falsey & Walsh, 1996; Munoz et al., 2003; Piedra et al., 2003; Tristram et al., 1993). Based on the genetic relationship of RSV and hMPV, we hypothesized that a similar subunit vaccine for hMPV would induce protective immunity against hMPV infection. Soluble F proteins of prototype viruses representing the two main lineages of hMPV were produced to high yields in stable NSO murine myeloma cell lines (Ulbrandt et al., 2006). We evaluated the antigenicity, immunogenicity and cross-protective efficacy of these soluble hMPV F proteins in Syrian golden hamsters (Mesocricetus auratus). As adjuvants are known to enhance the immune response, the immunogenicity of the soluble F proteins was tested in combination with different adjuvants: Alum, Specol and iscom matrix (Stills, 2005). Alum induces low antibody titres when used with subunit vaccines, but it was chosen because of its long history of use in humans. Iscom matrix is known to induce high antibody titres and is currently being evaluated in humans. Specol was chosen to function as positive control, as it induces high antibody titres in rodents. This study demonstrates that soluble hMPV F proteins adjuvanted with Specol or iscom matrix are promising candidate subunit vaccines for the induction of protective antibody levels against homologous and heterologous hMPV infections.

METHODS

Cells and viruses. Subclone 118 of Vero-WHO cells (Vero-118 cells) (Kuiken et al., 2004) were grown in Iscove's modified Dulbecco's medium (IMDM; BioWhittaker) supplemented with 10% fetal calf serum (Greiner Bio-One), 100 IU penicillin ml⁻¹, 100 μg streptomycin ml⁻¹ and 2 mM glutamine. The construction of recombinant hMPV NL/1/00 (prototype virus for lineage A) and NL/1/99 (prototype virus for lineage B) has been described previously (Herfst et al., 2004). High-titre virus stocks were produced in Vero-118 cells in infection medium: IMDM supplemented with 4% BSA fraction V (Invitrogen), 100 IU penicillin ml⁻¹, 100 μg streptomycin ml⁻¹, 2 mM glutamine and 3.75 μg trypsin ml⁻¹. After 7 days, the cultures were harvested and stored in 25% sucrose at −70 °C.

Construction of soluble F proteins. Constructs expressing a truncated version of the hMPV F protein, lacking the transmembrane domain, were generated as described previously (Ulbrandt et al., 2006). In brief, full-length hMPV cDNA plasmids were used as templates to generate F gene PCR products by using the following oligonucleotides for both the NL/1/00 and NL/1/99 sequences: 5’- AATCAAGGTCGCCCACTGTGGAAGTG-3’ and 5’- TATATGATTGGGTTGGGTGGTGACTGACGCGTTTCCCTTCTGTC-3’. The PCR products were digested with restriction endonucleases Rsfl and EcoRl and ligated to the pEEl5.1 vector (Lonza) digested with the same restriction endonucleases. Stable NSO murine myeloma cell lines expressing NL/1/00 or NL/1/99 F proteins were generated as described by Bebbington et al. (1992). Soluble F protein (Fsol) was purified by affinity chromatography using an F-specific mAb, 1017, which binds to both NL/1/00 and NL/1/99 F proteins (Ulbrandt et al., 2006), coupled to cyanogen bromide-activated Sepharose and eluted with 0.1 M glycine, pH 2.8. The eluate was neutralized with 0.1 vol. 1 M Tris/HCl, pH 8.0, and was dialysed against PBS.

Vaccine preparations. For the iscom matrix-adjuvanted Fsol preparation, N-decanoyl-N-methylglucamine (MEGA-10; Sigma-Aldrich) at a final concentration of 2% was supplemented with cholesterol [1 mg (mg protein)⁻¹; Sigma], phosphatidylethanolamine [1 mg (mg protein)⁻¹; Sigma] and Quil-laja glucosides [5 mg ISCOMREP 703 (mg protein)⁻¹; Isotec] and dialysed against PBS. The iscom matrix preparation was analysed by negative-contrast electron microscopy, revealing the typical iscom matrix particles with a diameter of approximately 40 nm. The iscom matrix preparation was added to the F protein immediately before immunization, resulting in preparations containing 10 μg Fsol in 100 μl.

Concerning the correct choice of an aluminium-containing adjuvant, the charge of adjuvants and antigens is characterized by the point of zero charge (PZC) and isoelectric point (pI), respectively (al-Shakhshir et al., 1994). Maximum adsorption of protein by aluminium adjuvants is reached at pH conditions under which the antigen and adjuvant have opposite charges. Based on the approximate pI of 5.9 of the Fsol proteins (http://au.expasy.org/cgi-bin/pi-tool), Alhydrogel with PZC value 11.1 was chosen as aluminium-containing adjuvant (a gift from Dr Erik B. Lindblad, Brenttag Biosector, Frederikssund, Denmark). A vaccine dose was prepared by mixing 50 μl Fsol (200 μg protein ml⁻¹ in PBS) with 50 μl 2% Alhydrogel (10.3 mg Al³⁺ ml⁻¹), resulting in preparations containing 10 μg Fsol and 0.52 mg Al³⁺.

Doses of Specol vaccine (Stimune; Cedi Diagnostics) were prepared by mixing 4 vols water phase containing 10 μg Fsol with 5 vols Specol while mixing vigorously.

To obtain inactivated whole-virus vaccines, hMPV strains NL/1/00 and NL/1/99 were grown to 70–90% cytopathic effect on Vero-118 cells. After one freeze–thaw cycle, cell-free supernatants were purified and concentrated by using a 30–60% (w/w) sucrose gradient. Virus was inactivated with β-propiolactone (Sigma-Aldrich) and complete inactivation was confirmed by titration on Vero-118 cells. SDS-PAGE analysis and Western blotting with an F monoclonal antibody (mAb) indicated that 100 μg inactivated virus contained approximately 10 μg F protein (data not shown); therefore, a vaccine dose of 100 μg inactivated virus (in 100 μl PBS) was used.

Animal experiments. All intramuscular immunizations, intranasal inoculations, orbital punctures and euthanasia were performed under anaesthesia with inhaled isoflurane. All animal studies were approved in accordance with animal experimentation guidelines.

Immunization and challenge. Five- to seven-week-old female Syrian golden hamsters (Harlan Sprague–Dawley) were immunized twice intramuscularly at a 3 week interval with a dose of 100 μl vaccine containing 10 μg Fsol or 100 μg inactivated virus in PBS. Three weeks after the second immunization in the immunization-optimization experiment, or 4 days post-infection in the challenge experiment, blood samples were collected by orbital puncture. Blood samples were stored overnight at room temperature and centrifuged for 15 min at 1200 g; serum was collected and stored at −20 °C.

Animals were challenged intranasally with 100 μl NL/1/00 virus, diluted in PBS in order to obtain the required dose [10⁴, 10⁵ or...
RESULTS

Virus titrations. Tissues from the inoculated hamsters were homogenized by using a Polytron homogenizer (Kinematica AG) in infection medium. After removal of tissue debris by centrifugation, supernatants were used for virus titration in Vero-118 cells. Titrations were performed with tenfold serial dilutions in 96-well plates (Greiner Bio-One). Confluent monolayers of Vero-118 cells were spin-inoculated (15 min, 2000 g) with 100 μl monolayer serial dilutions of each sample and incubated at 37 °C for 2 h. Two hours after the spin inoculation, infected wells were identified by raising in guinea pigs, as described previously (van den Hoogen et al. 2001). Titres, expressed as TCID50, were calculated as described by Reed & Muench (1938). Titres, expressed as the dilution resulting in 50% reduction of the number of plaques, calculated according to the method of Reed & Muench (1938). Per assay, each serum was tested in duplicate against hMPV NL/1/00 and NL/1/99.

Immunogenicity of Fsol

Soluble F proteins of the two prototypes of hMPV (NL/1/00 and NL/1/99) were produced: Fsol/1/00 and Fsol/1/99. NSO murine myeloma cells were chosen for their capacity to produce high yields of protein, as shown by Ulbrandt et al. (2006). By using these stable NSO murine myeloma cell lines, we were able to affinity-purify up to 20 mg soluble hMPV F protein ml−1. Analyses of these proteins raised in guinea pigs, as described previously (van den Hoogen et al., 2001). Titres, expressed as TCID50, were calculated as described by Reed & Muench (1938). Titres were calculated (g tissue)−1, with a detection limit of 10−4 and 10−2 TCID50 (g tissue)−1 for nasal turbinates and lungs, respectively.

Challenge model

We first set up a challenge model by inoculating hamsters with different virus doses and euthanasia of the animals at different days post-inoculation (2, 4 and 6 days p.i.). Three groups of 12 hamsters were inoculated intranasally with 10⁶, 10⁵ or 10⁴ TCID50 NL/1/00. From each group, four animals were euthanized at 2, 4 or 6 days p.i. In general, both in nasal turbinates and in lungs, virus shedding was higher at 2 and 4 days p.i. than at 6 days p.i. (Fig. 1), except for viral shedding from the nose at 4 days p.i. at the lowest inoculation dose (Fig. 1a). At 2 and 4 days p.i., animals inoculated with 10⁶ TCID50 harboured more virus in their nose and lungs than did those inoculated with lower virus doses. Only at 4 days p.i., and only with the two highest doses (10⁵ and 10⁶), was virus detected in the lungs of all animals (Fig. 1b). Based on these results, challenge experiments were conducted with 10⁶ TCID50 with subsequent euthanasia at 4 days p.i.
The immunogenicity of these two proteins was tested in combination with three different adjuvants and compared with that of inactivated viruses containing the native form of the F protein.

Six Syrian golden hamsters per group were immunized twice intramuscularly with 10 mg Fsol alone, Fsol with adjuvant (iscom matrix, alum or Specol), 100 mg inactivated virus or PBS. Three weeks later, sera were subjected to F-specific ELISA at a 1:1600 dilution. All animals, except the PBS-immunized animals, demonstrated the presence of F-specific antibodies (Fig. 3). Animals immunized with an NL/1/00 F protein preparation displayed higher ELISA antibody titres than those immunized with NL/1/99 preparations, although a mixture of antigens from both prototype viruses was used to coat the ELISA plates. The non-adjuvanted Fsol proteins induced slightly lower antibody titres compared with the whole virus preparation. Addition of adjuvants increased the immunogenicity of the Fsol proteins, with the highest titres induced by iscom matrix- and Specol-adjuvanted vaccines (Fig. 3). High ELISA antibody titres correlated with high VN antibody titres (data not shown). Based on the high antibody titres induced by immunization, the protective efficacy of the iscom matrix- and Specol-adjuvanted F subunit vaccines was tested in immunization/challenge experiments.

**Immunization/challenge experiment**

Nine groups of eight hamsters (six hamsters for the adjuvant-control groups) were immunized twice with 10 µg Fsol alone, Fsol with adjuvant (iscom matrix or Specol), adjuvants alone or PBS. Three weeks after the second immunization, all hamsters were inoculated intranasally with 10⁶ TCID₅₀ NL/1/00.

Immunization of control animals with PBS, iscom matrix or Specol (without antigen) did not induce detectable virus-specific antibodies (Fig. 4), which upon challenge resulted in virus shedding in the nose of all animals (Fig. 5a). Unfortunately, in contrast to the animals in the challenge-optimization experiment (Fig. 1), some of these control animals (e.g. three of eight, two of six and one of six for the PBS, iscom matrix and Specol groups, respectively) did not secrete virus from the lower respiratory tract (LRT) (Fig. 5b). Similarly, immunization of hamsters with Fsol alone induced low to undetectable levels of antibodies (Fig. 4). Upon challenge, virus was detected in the upper respiratory tracts (URTs) of all these animals and in the LRTs of five and six of eight animals (Fsol/1/99 and Fsol/1/00, respectively) (Fig. 5).

Despite these uninfected-control animals, significant protective efficacies were observed for animals immunized with Fsol adjuvanted with iscom matrix or Specol. Immunization with these vaccines induced high virus-specific antibody titres (Fig. 4). Upon challenge, these antibody titres partly protected from infection of the URT, with viral titres in the nose being statistically significantly lower than those observed in the nasal turbinates of the PBS-immunized animals (Mann–Whitney test, $P<0.05$). Complete (cross-) protection of the LRT was induced by immunization with these adjuvanted Fsol proteins. None of the animals demonstrated the presence of infectious virus (Fig. 5b) or viral genomes in their lung samples (data not shown). Immunization with adjuvanted Fsol vaccines induced significantly higher protective efficacy for LRT.
than did immunization with PBS (Mann–Whitney test, 
P<0.038). Immunization with adjuvanted Fsol/1/00 as well as Fsol/1/99 (cross-) protected the LRT against challenge infection with hMPV/NL/1/00.

All sera were tested in PRVN assays against NL/1/00 (Fig. 4a) and NL/1/99 (Fig. 4b). Sera from all animals from the control groups (PBS, iscom matrix or Specol) were negative for neutralizing antibodies. Animals immunized with adjuvanted vaccines had higher antibody titres than those immunized with Fsol alone. In general, homologous VN antibody titres were higher than heterologous VN antibody titres, with the highest homologous titres observed in the NL/1/00-immunized animals. Mean homologous titres induced by the adjuvanted vaccines were 20–30-fold and 2.5–3.4-fold higher than the heterologous titres for NL/1/99 and NL/1/00, respectively (Table 1). The difference in homologous and heterologous titres was not reflected in differences in protective efficacy, as both homologously and heterologously challenged animals were protected from LRT infection and displayed an equal reduction of viral titres in nasal turbinates.

**DISCUSSION**

The clinical impact of hMPV warrants the development of vaccines, and subunit vaccines are promising candidate vaccines for boosting pre-existing antibody titres, as described for RSV subunit vaccines (Falsey & Walsh, 1996; Munoz et al., 2003; Piedra et al., 2003; Tristram et al., 1993). We have produced recombinant soluble F proteins (Fsol proteins) for the prototypes of the two main lineages of hMPV (NL/1/00, lineage A; NL/1/99, lineage B) in a system that allows production of high yields of recombinant proteins. The use of these proteins as subunit vaccines was evaluated in Syrian golden hamsters.

The F proteins of paramyxoviruses are class I viral F proteins, which are synthesized as an inactive precursor, F0. This immature F protein is subsequently modified by
the addition of N-linked carbohydrate and then assembled into homo-oligomers in the rough endoplasmic reticulum. F0 is subsequently cleaved endoproteolytically, resulting in two polypeptides, F1 and F2, that are linked covalently to each other by disulfide bonds (Lamb et al., 2006; Russell et al., 2001). Thus, the F protein is present during infection in immature and mature forms (Sakurai et al., 1999).

The F glycoprotein is one of the major targets of RSV- or hMPV-neutralizing antibodies (Skiadopoulos et al., 2006; Tang et al., 2005) and is therefore an ideal candidate to use as subunit vaccine. Upon vaccination and/or natural infection with RSV, both neutralizing and non-neutralizing antibodies can be detected (Tsui et al., 1996). Several studies have suggested that non-neutralizing antibodies are directed against the uncleaved form of the F protein, whilst neutralizing antibodies are directed against the cleaved form of the protein (Sakurai et al., 1999; Tsui et al., 1996).

Both Fsol proteins used in this study were produced from viruses containing a serine at position 101 in the RQSR motif at the putative cleavage site of the F protein. It has previously been demonstrated that hMPV expressing an F protein with 101S is incapable of initiating multicycle growth without the addition of trypsin, as the F protein was not efficiently cleaved proteolytically (Schickli et al., 2005). In the present study, the production of the Fsol proteins of hMPV occurred in the presence of serum and in the absence of trypsin, so most of the Fsol proteins were not cleaved proteolytically. This was also confirmed by performing Western blot analysis on the Fsol proteins after incubation with or without trypsin. After the addition of trypsin, a smaller band was revealed, corresponding to the predicted size of cleavage fragment hMPV F1, by using F-specific mAb 1017 (data not shown).

Although most of the proteins used for the vaccine preparation were uncleaved, we have demonstrated that these truncated Fsol proteins were as immunogenic as the proteins in the native form. Immunization with the soluble proteins induced neutralizing-antibody titres similar to those induced by immunization with whole inactivated virus. However, antibody titres in animals immunized with Fsol were low. Therefore, several adjuvants were tested for their potential to induce higher neutralizing-antibody titres.

Alum was chosen because of its long history of use in humans, although it is known to induce low antibody titres when used with subunit vaccines (Gupta, 1998). Specol and iscom matrix were chosen because they both generate long-lasting, functional antibody responses, but neither adjuvant is licensed for human use (Beck et al., 2003; Leenaars et al., 1994; Sanders et al., 2005). However, several studies in human volunteers have shown that iscom-based vaccines are highly immunogenic, as well as safe and well-tolerated (Davis et al., 2004; Frazer et al., 2004). The addition of Alum to the Fsol proteins resulted in antibody levels similar to those induced by the non-adjuvanted Fsol. This weak response might be the result of a low degree of adsorption of Fsol to Alhydrogel, which is considered to be an important parameter for the function of aluminium adjuvants (Lindblad, 2004).

Addition of both Specol and iscom matrix to the Fsol protein enhanced the hMPV-specific antibody response. This enhanced effect was also seen in cotton rats immunized with 25 μg soluble hMPV F protein adjuvanted with Titermax Gold (Cseke et al., 2007). Although Titermax Gold is known to induce high antibody titres, the adjuvant induced sterile abscesses in non-human primates and is not licensed for human use (Deng et al., 2002).

The iscom matrix- and Specol-adjuvanted F-subunit vaccines were tested for protective efficacy against homologous and heterologous infection in Syrian golden hamsters. For this purpose, we developed a challenge model for NL/1/00 infections in Syrian golden hamsters. An optimum inoculation dose of 10^6 TCID50, followed by collection of lungs and nasal turbinates at 4 days p.i., resulted in virus detection in samples collected from all animals.

Using this challenge model in an immunization/challenge experiment, none of the vaccines induced complete protection against URT infection, although viral titres in the nose of adjuvanted Fsol-immunized animals were significantly lower than those in the PBS-immunized animals. However, the primary goal of immunization against respiratory viruses is prevention of serious LRT illnesses. The control vaccines (iscom matrix or Specol without antigen, PBS or non-adjuvanted Fsol) did not prevent LRT infections, whilst immunization with adjuvanted Fsol induced complete protection against LRT infection. Even after optimization of the challenge model, subsequent challenges of the control animals in the immunization/challenge experiment resulted in <100 % infection. Although intranasal inoculation of Syrian golden hamsters has been used successfully as the infection procedure in other studies, this route of infection might.

### Table 1. HMPV NL/1/00- and NL/1/99-specific PRVN antibody titres in sera collected from Syrian golden hamsters immunized twice with Fsol NL/1/99 (B) or NL/1/00 (A) adjuvanted with Specol or iscom matrix

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<tr>
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<td>1044</td>
<td>4816</td>
<td>963</td>
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<td>Ratio A : B</td>
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Mean PRVN antibody titres are depicted for each group. Homologous PRVN antibody titres are shown in bold; ratios are given as homologous PRVN titres divided by heterologous PRVN antibody titres.
be less efficient for the virus strains used in the present study. Perhaps a higher inoculum dose or even intratracheal infection would have resulted in 100% infection. However, intranasal infection, with a risk for a less robust infection, resembles a natural infection more closely. Despite the fact that not all control animals harboured virus in their lungs upon challenge, significant protective efficacy was demonstrated for the adjuvanted Fsol proteins. All animals immunized with Fsol adjuvanted with iscom matrix or Specol were protected from infection of their lungs, compared with three of eight animals in the PBS-immunized group (Mann–Whitney test, \( P = 0.038 \)).

In passive-transfer experiments in which RSV-specific mAbs were used, it was demonstrated that URT protection requires significant higher antibody doses than does protection of the LRT (Siber et al., 1994). This suggests that antibody titres induced by the studied vaccines might be too low to protect against URT infection. A third vaccination could be considered to increase the antibody levels or the use of alternative adjuvants in order to achieve protection of the URT. Alternatively, although the uncleaved Fsol proteins induced neutralizing antibodies, immunization with a cleaved form of Fsol might induce higher neutralizing-antibody titres that protect against URT infection. Both the adjuvanted Fsol/1/00 and Fsol/1/99 proteins induced higher homologous than heterologous VN antibody titres, indicating serological differences between the two main lineages of hMPV, as described previously (van den Hoogen et al., 2004). However, antibodies raised against the NL/1/99 F protein provided cross-protection against heterologous NL/1/00 virus infection of the LRT. Although we demonstrate cross-protection in only one direction, it seems likely that cross-protection will be obtained in both directions. This has been observed previously with the F protein in its native form (Skiadopoulos et al., 2004; Tang et al., 2005).

Vaccine development aimed at protecting naive individuals from infection with RSV or hMPV is hampered by an experience with a formalin-inactivated RSV vaccine. Immunization of naive children with this vaccine induced enhanced disease upon subsequent infection (Kim et al., 1969). Enhanced disease has so far not been observed in employment of F subunit vaccines for RSV in patients with pre-existing immunity and is not to be expected for hMPV subunit vaccines. Therefore, the focus of this study was the protective efficacy of F subunit vaccines. Syrian golden hamsters are not the ideal animal model to study vaccine-induced immune pathology, as immunological tools are scarce. The presented results justify subsequent studies in non-human primates, in which the safety issue can be addressed properly.

Our results demonstrate that immunization of Syrian golden hamsters with adjuvanted hMPV Fsol subunit vaccines induced a strong antibody response that provided complete protection against LRT infection with homologous and heterologous viruses. The availability of this protein in high quantities, in combination with the demonstrated beneficial use of the safe adjuvant iscom matrix, makes this F subunit vaccine an excellent candidate for further exploration to boost antibody titres in humans with pre-existing antibody titres.

ACKNOWLEDGEMENTS

We thank Karim Hussain and Leo Sprong for excellent technical assistance and Rik de Swart and Guus Rimmelzwaan for stimulating discussions.

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