An orthopoxvirus-based vaccine reduces virus excretion after MERS-CoV infection in dromedary camels

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Middle East respiratory syndrome coronavirus (MERS-CoV) infections cause an ongoing outbreak in humans fueled by multiple zoonotic MERS-CoV introductions from dromedary camels. Besides implementing hygiene measures to limit further camel-to-human and human-to-human transmissions, vaccine-mediated reduction of MERS-CoV spread from the animal reservoir may be envisaged. Here, we show that a modified vaccinia virus Ankara (MVA) virus vaccine expressing the MERS-CoV spike protein confers mucosal immunity in dromedary camels. Significant reduction of excreted infectious virus and viral RNA transcripts was observed in vaccinated animals upon MERS-CoV challenge as compared to controls. Protection correlated with the presence of serum neutralizing antibodies to MERS-CoV. Induction of MVA-specific antibodies that cross-neutralize camelpox virus, would also provide protection against camelpox.

Coronaviruses (CoVs) cause common colds in humans, but zoonotic transmissions occasionally introduce more pathogenic viruses into the human population, including the severe acute respiratory syndrome (SARS) outbreak in 2003. In 2012 a previously unknown virus, now named Middle East respiratory syndrome CoV (MERS-CoV), was isolated from the sputum of a 60-year-old man in Saudi Arabia who suffered from acute pneumonia and subsequently died (1, 2). To date, several infection clusters have been reported over the past three years in the Middle East but also in South Korea with approximately 35% of the reported human cases being fatal (3, 4). Dromedary camels (*Camelus dromedarius*) were suspected to be the reservoir host after detecting neutralizing antibodies to MERS-CoV in these animals (5–8). Subsequently, virus detected in nasal swabs of these animals was found to be similar to that in human MERS cases asso-

ciated with those farms where the dromedaries were kept (9, 10). In addition, MERS-CoV from dromedary camels replicates in human lung sections cultured ex vivo (11). More recent studies also provide serological evidence for camel-human transmission (12, 13). Because of its widespread presence in dromedary camels (14–16), continued introduction of MERS-CoV through zoonotic infections in humans will occur. Therefore, strict implementation of quarantine and isolation measures, as well as the development of candidate vaccines and antivirals are urgently needed.

The spike (S) protein is considered a key component for vaccines against coronavirus infections. The identification of dipeptidyl peptidase 4 (DPP4) as the MERS-CoV receptor (17) has facilitated the subsequent characterization of the receptor binding domain in the S1 region of the MERS-CoV spike protein (18, 19). When tested as a vaccine in mice, full-length S protein of MERS-CoV expressed by Modified Vaccinia virus Ankara (MVA-S) induced high levels of circulating antibodies that neutralize MERS-CoV and limited lower respiratory tract replication in animals transduced with the human receptor DPP4 and

inoculated with MERS-CoV (20, 21). MVA, a highly attenuated strain of vaccinia virus, serves as one of the most advanced recombinant poxvirus vectors in preclinical and clinical trials for vaccines against infectious diseases and cancer. As a proof of principle we tested the protective efficacy of a MVA-MERS-CoV candidate vaccine in dromedary camels.

In dromedary camels, MERS-CoV replication is mainly restricted to the upper respiratory tract (22). Therefore, we inoculated four dromedary camels twice with a 4 week interval with 2×10^8 PFU MVA-S via both nostrils using a mucosal atomization device to disperse the vaccine on the nasal epithelium and 10^8 PFU MVA-S intramuscularly in the neck of each animal (23). Similarly, four control animals received non-recombinant wildtype MVA (MVA-wt, $n = 2$) or PBS ($n = 2$). All animals vaccinated with the MVA-S vaccine devel-

oped detectable serum neutralizing MERS-CoV specific antibody titers (Fig. 1A). No MERS-CoV specific antibodies were detected in sera of the PBS- or MVA-wt-immunized control animals. The specificity of the antibody response was confirmed by ELISA using recombinant S1 protein (fig. S1). In addition, low levels of MERS-CoV-neutralizing antibodies (virus neutralization titer of 1:20 to 1:40) were detected at three weeks after the boost immunization in the nasal swabs of three animals (Fig. 1B). Since a MVA-vectored vaccine was used, antibodies neutralizing MVA were also detected (Fig. 1C); these antibodies cross-neutralized camelpox virus (Fig. 1D). Camelpox virus infections occur frequently in dromedaries and cause severe disease that can be prevented by vaccines based on attenuated camelpox viruses (24).

Three weeks post-boost, all dromedary camels were inoculated with a high dose of 10^7 TCID₅₀ of MERS-CoV via the intranasal route using a mucosal atomization device. Upon challenge, the animals only showed mild clinical signs, mainly limited to a relatively small rise in body temperature observed in control-vaccinated animals one day after challenge (fig. S2). In addition, whereas some dry mucus was observed in one of the nostrils of most animals after day 4, from day 8-10 onward all control-vaccinated animals exhibited a runny nose that was not observed in MVA-S vaccinated animals (Fig. 2, A and B). Previous studies have shown that both experimentally and naturally infected dromedary camels may show nasal discharge after MERS-CoV infection (15, 22). We next tested nasal respiratory tract samples for the presence of infectious virus. Whereas MERS-CoV was found at high titers in all four control-vaccinated animals, mean viral titers in the animals that received the MVA-S vaccine were significantly reduced (Fig. 2C). After day 1 pi, an increase in MERS-CoV RNA levels was noted in MVA-S vaccinated animals (Fig. 2D). Low levels of infectious virus was excreted (10^3 TCID₅₀/ml) by one of the vaccinated animals at 6 days pi (Fig. 2C). Sequencing of the spike gene of this virus showed no amino acid changes in the receptor binding domain (fig. S3), suggesting that this virus did not emerge as a result of escape from vaccine-induced antibodies (Fig. 2C). Rather, the observation that this animal had no detectable MERS-CoV antibody response in the nasal swab at time of challenge may indicate that priming with the MVA-S vaccine was less effective in this animal, compared with the other vaccinated animals, for unknown reasons. Antibodies to MERS-CoV rapidly increased 8 days pi in control vaccinated animals (fig. S4), consistent with the absence of infectious virus in the nasal swabs at that time (Fig. 2C). Low levels of viral RNA, but no infectious virus, was detected in rectal swabs after MERS-CoV challenge (fig. S5) but not in any of the sera tested.

To analyze pathological changes and viral replication in organs of the animals, two animals per group were euthanized and necropsies were performed at days 4 and 14 pi. Gross pathology showed no significant changes in the or-

gans of any of the animals. However, at four days pi MERS-CoV RNA transcripts were detected in several organs of the control-vaccinated animals (Fig. 3A), although infectious virus particles were restricted to noses and tracheas (Fig. 3B). Relatively high levels of viral RNA in the absence of infectious MERS-CoV has also been observed in tissues of experimentally infected Rhesus macaques and rabbits (25, 26). By contrast, infectious MERS-CoV particles were found at low levels in the nose of animals that had received MVA-S vaccine (Fig. 3B). At day 14 pi, only viral RNA was detected, mainly in control vaccinated animals (fig. S6).

Differences in viral replication in the upper respiratory tract between vaccinated groups were confirmed by MERS-CoV in situ hybridization (ISH) and immunohistochemistry (IHC). In the nose of MVA-S vaccinated dromedaries at 4 days pi, a few cells stained for MERS-CoV RNA by ISH compared with control camels (Fig. 4, A and B). Viral replication in the control-vaccinated animals was consistent with histopathological analyses showing multifocal moderate rhinitis with multifocal epithelial necrosis, lymphocytic and neutrophilic exocytosis (Fig. 4C). In the nasal submucosa, edema and infiltrates with lymphocytes, neutrophils, plasma cells and macrophages were observed. In the trachea and bronchi there was a multifocal mild tracheitis and bronchitis with epithelial necrosis and lymphocytic and neutrophilic exocytosis and infiltration in the lamina propria. In the lymph nodes and the tonsil there was follicular hyperplasia. Associated with the nasal lesions there was marked MERS-CoV antigen expression in the nasal epithelium (Fig. 4C). By ISH, MERS-CoV RNA was confirmed in the nasal cavity in similar cells as those that scored positive by IHC (Fig. 4C). Furthermore, a few epithelial cells in trachea, bronchus and those covering the palatum molle, as well as large stellate cells (consistent with dendritic cells) in lymphoid tissue of the palatum molle, tonsil, tracheal and cervical lymph nodes were found positive for viral antigen by IHC (fig. S7). In contrast, in MVA-S vaccinated animals the rhinitis was accompanied by less submucosal edema with antigen expression in some cells in the nose (Fig. 4C). Eosinophilic granulocytes were not observed in the lungs of MVA-S vaccinated animals that were challenged with MERS-CoV. In one MVA-S vaccinated animal, viral antigen expression was found in a few dendritic-like cells in lymphoid tissue of the palatum molle, tonsil, tracheal and cervical lymph nodes and in the gut-associated lymphoid tissue of the duodenum (table S1). At day 14 pi, there was multifocal mild rhinitis, tracheitis and bronchitis, and follicular hyperplasia in the lymphoid tissue in the control-vaccinated and MVA-S-vaccinated animals. In the lungs of almost all animals there was multifocal mild infiltration of neutrophils, histiocytes and lymphocytes that was not associated with viral antigen expression. In other extra-respiratory tissues examined, no significant morphological changes or viral antigen expression were found. Overall these results indicate that vaccination of dromedary camels with MVA-S induces protective immunity resulting

in reduction of excreted infectious MERS-CoV, without evidence for antibody-dependent enhancement of viral replication as seen in feline coronavirus infection (27). Given the potential transient nature of mucosal immune responses, follow up studies need to determine the longevity of the responses induced by the MVA-S vaccine with respect to protection as well as antibody dependent enhancement of viral replication when antibody levels are waning. In addition, dosing of the vaccine and alternative methods of administration need to be explored in more detail to further develop this candidate vaccine to be useful in the field.

Protective immunity to coronaviruses is orchestrated by antibody and cellular immune responses. Investigations in mice have already provided evidence that vaccination with MERS-CoV spike-protein-based candidate vaccines, monoclonal antibodies directed against the spike protein, or dromedary immune serum, induce protective immunity against lower respiratory tract MERS-CoV infection (28–30). A DNA vaccine encoding the spike protein induced MERS-CoV neutralizing antibody responses in dromedary camels to similar levels as the MVA-S vaccine but no challenge experiments were performed (31). However, also studies in the field indicated that MERS-CoV seropositive dromedaries may carry MERS-CoV viral RNA in their nasal excretions (8, 15, 16). Thus, sterilizing immunity may not be possible to achieve as virus replicates in the upper respiratory tract even in the presence of specific antibodies, similarly to other respiratory viruses. Because dromedary camels do not show severe clinical signs upon MERS-CoV infection, vaccination of dromedaries should primarily aim at reducing virus excretion to prevent virus spreading. Young dromedaries excrete more infectious MERS-CoV than adults (8, 15, 16) thus would be the first target to vaccinate. Our results reveal that MVA-S vaccination of young dromedary camels may significantly reduce infectious MERS-CoV excreted from the nose, when analyzing two groups of each four animals challenged with a high dose of MERS-CoV. Two major advantages of the orthopoxvirus-based vector used in our study include its capacity to induce protective immunity in the presence of pre-existing (e.g., maternal) antibodies (32) and the observation that MVA-specific antibodies cross-neutralize camelpox virus, revealing the potential dual use of this candidate MERS-CoV vaccine in dromedaries. Dromedary camels vaccinated with conventional vaccinia virus showed no clinical signs upon challenge with camelpox virus while control animals developed typical symptoms of generalized camelpox (33). The MVA-S vectored vaccine may also be tested for protection of humans at risk, such as healthcare workers and people with camel contacts.

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SUPPLEMENTARY MATERIALS

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Materials and Methods

Figs. S1 to S7

Table S1

References (34, 35)

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Fig. 1. Virus neutralizing antibody responses to MERS-CoV, MVA and camelpox virus in vaccinated dromedary camels. (A to D) Individual virus neutralization titers (VNT) from dromedary camels vaccinated either with PBS, MVA-wt or MVA-S, against MERS-CoV [(A) and (B)], MVA (C) and camelpox virus (D) as determined in sera [(A), (C), and (D)] and nasal swabs (B). Different symbols indicate time points after immunization sera were analyzed; week 0 (black circles), week 4 (blue triangles) and week 7 (red squares).

Fig. 2. Clinical signs and MERS-CoV excretion in nasal swabs of dromedary camels vaccinated with MVA-S vaccine. (A and B) Two MVA-S vaccinated (A) and two control vaccinated dromedary camels (B) were analyzed for the presence of mucus excretion 8-10 days after MERS-CoV challenge. **(C and D)** Detection of infectious MERS-CoV (C) and MERS-CoV RNA (D) at different time points after challenge in nasal swabs of dromedary camels vaccinated with MVA-S (open bars) or MVA-wt/PBS (closed bars). The dashed line depicts the detection limit of the assays. Bars represent mean values $\pm$ SEM, * $P < 0.05$; $n = 4$ per group.

Fig. 3. Detection of MERS-CoV in tissues of vaccinated dromedary camels. (A and B) MERS-CoV viral RNA (A) and infectious virus (B) were determined in tissue homogenates from dromedary camels vaccinated with MVA-S (green and black bars) or control vaccinated (red and blue bars) at 4 days after challenge.

Fig. 4. Histopathology, expression of viral antigen and viral RNA in nasal respiratory epithelium of MVA-S vaccinated and control vaccinated dromedaries at 4 days post challenge with MERS-CoV. (A to C) Detection of MERS-CoV viral RNA by in situ hybridization in the nose of dromedary camels vaccinated with MVA-S (A) or control vaccinated (B). Nasal respiratory tissue of a representative MVA-S vaccinated dromedary demonstrated no significant lesions (C), revealed by hematoxylin and eosin (HE) staining, a few viral antigen positive cells visualized by immunohistochemistry (IHC) or viral RNA visualized by in situ hybridization (ISH). Nasal respiratory tissue of a control (ctrl) vaccinated dromedary demonstrated multifocal necrosis of epithelial cells and infiltration of neutrophils, lymphocytes and a few macrophages in the epithelium and lamina propria with abundant viral antigen and viral RNA present at the same location (C).