Protective efficacy of recombinant Modified Vaccinia virus Ankara (MVA) delivering Middle East Respiratory Syndrome coronavirus spike glycoprotein

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ABSTRACT

Middle East Respiratory Syndrome coronavirus (MERS-CoV) causes severe respiratory disease in humans. We tested a recombinant MVA vaccine expressing full-length MERS-CoV spike glycoprotein (S) by immunizing BALB/c mice using either intramuscular or subcutaneous regimens. In all cases MVA-MERS-S induced MERS-CoV-specific CD8+ T-cells and virus-neutralizing antibodies. Vaccinated mice were protected against MERS-CoV challenge infection after transduction with the human dipeptidyl peptidase 4 receptor. This MERS-CoV infection model demonstrates the safety and efficacy of the candidate vaccine.

In 2012 the MERS-CoV emerged as the causative agent of severe human respiratory disease in Saudi Arabia. Since then the virus continues to circulate and cases of human infections are regularly reported, mostly linked to Middle East countries. The highest incidence of MERS-CoV infection occurs in the elderly or immunocompromised individuals. The virus is suspected to persist in dromedary camels and cause sporadic zoonotic infections, followed by intra-familial or health-care-related transmissions (1-3). MERS-CoV uses a cell surface amino peptidase, dipeptidyl peptidase 4 (DPP4) or CD26, as a functional receptor (4). Expression of human DPP4 in mice using adenovirus transduction or transgenesis permits productive infection of MERS-CoV in mouse model systems (5, 6). Rapid development of MERS-CoV specific vaccines is warranted (3, 7), and several initial candidate vaccines based on the spike glycoprotein have been shown to elicit MERS-CoV neutralizing antibodies (8-13).

Modified Vaccinia virus Ankara (MVA), a safety-tested and replication-deficient vaccinia virus, is an advanced viral vector platform for developing new vaccines against infectious diseases and cancer (14-16). Recently, we constructed a recombinant MVA stably expressing the full-length MERS-CoV spike (S) protein (MVA-MERS-S) (13). Here, we assessed safety, immunogenicity and protective capacity of this MVA-MERS-S candidate vaccine in a
BALB/c mouse/MERS-CoV infection model using dose escalation and two different application routes.

The MVA-MERS-S vaccine was prepared and quality-controlled following standard procedures (17). The recombinant virus MVA-MERS-S proved genetically stable after five repetitive large-scale amplifications in primary chicken embryo fibroblasts (CEF) under serum-free conditions with >95% of the resulting virus population producing the MERS-S target antigen (data not shown).

**Antibody response induced after vaccination with recombinant MVA-MERS-S.** Single subcutaneous (s.c.) immunization with doses of $10^7$ or $10^8$ pfu MVA-MERS-S elicited detectable MERS-CoV neutralizing antibodies (Fig. 1A). S.c. booster immunizations resulted in increased titers of MERS-CoV neutralizing antibodies, and even the low dose of $10^6$ pfu MVA-MERS-S induced measurable neutralizing antibodies. Vaccination doses of $10^7$ and $10^8$ pfu MVA-MERS-S resulted in similar antibody levels.

Intramuscular (i.m.) immunization resulted in MERS-CoV neutralizing antibodies with all dosages of MVA-MERS-S after a single primary immunization (Fig. 1B). Repeated i.m. immunization further increased the levels of MERS-CoV neutralizing antibodies to higher titers than those obtained upon s.c. immunization. However, the peak antibody titers elicited by s.c. or i.m. immunizations did not differ significantly.

**T-cell immune responses after immunization with MVA-MERS-S.** To evaluate T-cell responses in BALB/c mice we measured MERS-CoV-specific CD8+T-cells by IFN-γ-ELISPOT. We tested several S antigen-derived peptides for CD8+ T-cell specificities recognizing the MERS-S antigen (6). Primary immunizations with MVA-MERS-S given s.c. or i.m. elicited CD8+T-cells specific for both MERS-S antigen epitopes S291 (KYYSIIPHSI) and S823 (EYGQFCSKI) (data not shown). We chose peptide S291 for *in vitro* stimulations.
since this peptide consistently activated high numbers of S antigen-specific T-cells. Single s.c. immunizations with $10^6$ and $10^7$ pfu MVA-MERS-S induced nearly equivalent levels of S291-specific CD8+T-cells; however immunization with $10^8$ pfu MVA-MERS-S resulted in about three-fold higher responses (Fig. 2A). Booster s.c. immunizations further increased the magnitude of IFN-γ-secreting MERS-S291-specific CD8+T-cells, particularly with the lower dosage of $10^6$ or $10^7$ pfu MVA-MERS-S. Notably, i.m. immunizations resulted in comparable levels of CD8+T-cell responses for all doses of MVA-MERS-S vaccine after single and prime-boost immunizations (Fig. 2B). The i.m. booster increased the level of MERS-S291-specific T-cell responses about three-fold. Moreover, we detected MERS-S291-specific IFN-γ-producing T-cells in splenocytes 56 days following the primary or secondary immunization, demonstrating an antigen-specific memory CD8+ T-cell response (Fig. 2C).

**Protective capacity of MVA-MERS-S upon MERS-CoV challenge.** To model a productive infection of MERS-CoV, we intranasally transduced MVA-MERS-S-vaccinated BALB/C mice with $2.5 \times 10^8$ pfu of an adenoviral vector encoding both the human DPP4 receptor and mCherry (ViraQuest) at 45 days post prime-boost immunization. Five days later the animals were infected with $7 \times 10^4$ TCID$_{50}$ MERS-CoV (strain EMC/2012), and 4 days post challenge the animals were sacrificed and the lungs harvested to measure viral loads and for histopathological analysis. High virus loads, on average >11,000 to >20,000 MERS-CoV genome equivalents/ng of total RNA, were found in both mock-immunized and non-recombinant MVA-immunized control groups. In sharp contrast, the lung tissue of MVA-MERS-S-immunized subjects contained significantly lower levels of MERS-CoV RNA, indicating efficient inhibition of MERS-CoV replication by vaccine-induced immune responses (Fig. 3). Furthermore, adenoviral vector transduction levels were also monitored by real-time RT-PCR analysis for mCherry RNA.
Histopathological examination demonstrated that the total percentage of lung tissue affected by MERS-CoV infection varied greatly between the groups (Fig. 4). Lung tissue of control mice revealed large areas of densely packed inflammatory cells, mainly comprising macrophages, lymphocytes, and to a lesser extent, neutrophils (Fig. 4A,C). Inflamed foci were mainly seen around larger bronchi, and some bronchi were filled with cellular debris and inflammatory cells, while other areas of the lungs remained unaffected. Lungs from control mice showed extensive MERS-CoV-S-specific staining, primarily in areas severely affected by inflammation (Fig. 4E). Tissues from MVA-MERS-S immunized animals showed minimal lesions, mostly mild hyperplasia of the bronchus associated lymphoid tissue and little positive staining of virus infected cells in lung tissues (Fig. 4B,F). Occasionally, small areas of inflammation resembling those prominently seen in tissues from control mice were also noted (Fig. 4D).

Conclusions. Here we report that the MVA-MERS-S vector vaccine is compatible with clinical use and industrial scale production. The vector can be grown in CEF without the need for additional animal-derived components in culture and MVA-MERS-S stably synthesizes S glycoprotein antigen upon serial amplifications at low multiplicity-of-infection.

The immunogenicity data required before initiating clinical trials (18) include evaluating immune responses according to dosage, route of administration and intervals of application, as well as characterizing humoral and cell-mediated immunity. In this study, s.c. and i.m. routes were associated with comparable immune responses, particularly when using the standard dosage of $10^8$ pfu MVA-MERS-S in prime-boost applications. The present results are in good agreement with other data in support of the licensing of MVA as replacement smallpox vaccine demonstrating nearly equivalent immunogenicity of s.c. or i.m. immunization (19-23). Moreover the efficiency of i.m. MVA-MERS-S immunization here in inducing humoral and cell-mediated immune responses is similar to the immunogenicity data.
from other recombinant MVA vaccine studies in clinical testing (15, 24). Interestingly, i.m. immunizations induced nearly equal amounts of MERS-S-specific CD8+ T-cells across all doses used here, and also in prime and prime-boost vaccination schemes. These findings are also in agreement with the previously observed induction of fully protective levels of virus-specific CD8+ T-cells upon low dose MVA immunization (25). S.c. vaccination was somewhat less immunogenic when using lower dosages of virus; only higher dose of 10^8 pfu MVA-MERS-S immunization resulted in high levels of MERS-specific CD8+ T-cells and MERS-CoV neutralizing antibodies after the prime-boost regimen.

An examination of the efficacy of MVA-MERS-S vaccination in a mouse model of MERS-CoV lung infection revealed that all immunized mice exhibited little or no replication of MERS-CoV, irrespective of the route or dose of vaccination. This data confirms that the S glycoprotein of MERS-CoV, like that of SARS-CoV (26), is an important and safe vaccine antigen. Notably we found no evidence of an increased inflammatory response or the potential enhancement of MERS-CoV infection through S-antigen-specific antibody induction, as has been previously speculated for SARS-CoV infections (27-29). Thus, the MVA-MERS-S vector merits further development as candidate vaccine against MERS-CoV for potential human use.

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Figure Legends

**Figure 1.** Antibody response induced by MVA-MERS-S vaccination. Groups of BALB/c mice (n=5) were immunized subcutaneously (A) or intramuscularly (B) with $10^6$, $10^7$, or $10^8$ pfu MVA-MERS-S, $10^8$ pfu non-recombinant MVA (WT) or PBS (Mock). To monitor antibody responses we analyzed the MERS-CoV neutralizing capacity of mouse sera taken at d21 and d40. Serum antibodies against MERS-CoV were measured by virus neutralization assays (VNT) after primary vaccination (prime) and after prime-boost vaccination (prime-boost). Shown is the mean of serum titers (log2) from individual animals. The statistical evaluation was performed with GraphPad Prism for Windows (GraphPad Prism Software, USA). Statistical significance of differences between groups is indicated by * for p-value <0.05, ** for p-value <0.01 and *** for p-value <0.001.

**Figure 2.** Virus-specific CD8+ T-cell responses induced by MVA-MERS-S. BALB/c mice were immunized by single shot and prime-boost vaccinations with $10^6$, $10^7$, or $10^8$ pfu MVA-MERS-S vaccine via the subcutaneous (A) or intramuscular (B) route. Animals inoculated with non-recombinant MVA (WT) or PBS (Mock) were used as controls. Splenocytes were prepared at 8 days after prime or prime-boost vaccination, and S291-specific IFN-γ-producing CD8+ T-cells (IFN-γ-spot forming cells) were measured by ELISPOT. (C) Virus-specific memory CD8+ T-cell responses induced by MVA-MERS-S. Spleen cells were harvested at 56 days after prime or prime-boost vaccination. MERS S-specific CD8+ T-cells were stimulated with peptide S291. Peptide SPYAAGYDL (F2L) served for comparative analysis of MVA-specific CD8+ T-cells (30). MERS-CoV S-specific T cells were quantified by IFN-g ELISPOT (AELVIS, Hannover, Germany). The statistical evaluation by t-test was performed with GraphPad Prism for Windows (GraphPad Prism Software, USA). For statistical significant results the following convention was used: * - p-value < 0.05, ** - p-value < 0.01 and *** - p-value < 0.001.
**Figure 3.** Protective capacity of MVA-MERS-S immunization against challenge with MERS-CoV in human DPP4 transduced BALB/c mice. BALB/c mice were infected with 7x10^4 tissue culture infectious doses 50 (TCID50) MERS-CoV 45 days after immunization with 10^6, 10^7, 10^8 pfu MVA-MERS. MERS-CoV RNA loads in lung tissues were determined by quantitative real-time RT-PCR (31). Viral genome copies/ng RNA are shown for groups of animals (n, number of animals per group) immunized by (A) subcutaneous route with 10^6 (n=5), 10^7 (n=2), 10^8 (n=2) pfu MVA-MERS-S (MVA-S), non-recombinant MVA (WT) (n=1) and PBS (Mock) (n=4) or (B) intramuscular vaccination with 10^6 (n=5), 10^7 (n=5), 10^8 (n=5) pfu MVA-MERS-S (MVA-S), non-recombinant MVA (WT) (n=3) and PBS (Mock) (n=4). The statistical evaluation was performed with GraphPad Prism for Windows (GraphPad Prism Software, USA). Statistical significance of differences between groups is indicated by * for p-value <0.05, ** for p-value <0.01 and *** for p-value <0.001.
Histopathological and immunohistochemical examination of MVA-MERS-S immunized (B, D, F, H), non-recombinant MVA vaccinated (A, C, E) and mock vaccinated (G) mice that had been transduced with a non-replicating adenoviral vector encoding human DPP4 and mCherry. Mice were infected with MERS-CoV (A-H) or mock infected to monitor for inflammation caused by adenoviral vector transduction (I, J). Lungs were collected 4 days post infection (A-H) or 5 days after transduction with control adenoviral vector (I, J); fixed tissue was routinely embedded in paraffin and stained with hematoxylin and eosin (H&E). For immunohistochemical detection of MERS-CoV a rabbit polyclonal antibody against the spike protein S1 (Sino Biological Inc., cat. no. 100208-RP) was used. Since all tested antibodies against the human DPP4 showed partial cross-reactivity with murine DPP4, a mouse monoclonal antibody against mCherry (abcam®, cat no. ab125096) was used to monitor adenoviral transduction. H&E staining (A-D, I), immunohistochemistry for MERS-CoV spike protein (E, F, J) or mCherry (G, H); scale bar: 500 µm (A, B), 200 µm (I, J), 100 µm (C-H).
References


