Protective Efficacy of Recombinant Modified Vaccinia Virus Ankara Delivering Middle East Respiratory Syndrome Coronavirus Spike Glycoprotein

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Middle East respiratory syndrome coronavirus (MERS-CoV) causes severe respiratory disease in humans. We tested a recombinant modified vaccinia virus Ankara (MVA) vaccine expressing full-length MERS-CoV spike (S) glycoprotein by immunizing BALB/c mice with 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, Middle East respiratory syndrome coronavirus (MERS-CoV) emerged as the causative agent of severe human respiratory disease in Saudi Arabia. Since then, the virus has continued to circulate and cases of human infections are regularly reported, mostly linked to Middle Eastern countries. The highest incidence of MERS-CoV infection occurs in 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 transmission (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 by 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 the development of new vaccines against infectious diseases and cancer (14–16). Recently, we constructed a recombi-

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FIG 1 Antibody response induced by MVA-MERS-S vaccination. Groups of BALB/c mice ($n = 5$) were immunized s.c. (A) or i.m. (B) with 10^6 , 10^7 , or 10^8 PFU of MVA-MERS-S, 10^8 PFU of nonrecombinant MVA (WT), or phosphate-buffered saline (Mock). To monitor antibody responses, we analyzed the MERS-CoV-neutralizing capacity of mouse serum samples taken at days 21 and 40. Serum antibodies against MERS-CoV were measured by virus neutralization assay (VNT) after primary vaccination and after prime-boost vaccination. Shown are the mean serum antibody titers ($\log_2$) of individual animals. The statistical evaluation was performed with GraphPad Prism for Windows (GraphPad Software, La Jolla, CA). Statistical significance of differences between groups is indicated as follows: *, $P < 0.05$; **, $P < 0.01$; ns, no statistically significant difference.

FIG 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 10⁶, 10⁷, or 10⁸ PFU of MVA-MERS-S vaccine via the s.c. (A) or i.m. (B) route. Animals inoculated with nonrecombinant MVA (WT) or phosphate-buffered saline (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 assay. (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- γ ELISPOT assay (A.E.L.VIS, Hannover, Germany). Statistical evaluation by *t* test was performed with GraphPad Prism for Windows (GraphPad Software, La Jolla, CA). For statistically significant differences between results, the following convention is used: *, *P* < 0.05.

nant MVA stably expressing the full-length MERS-CoV spike (S) protein (MVA-MERS-S) (13). Here, we assessed the safety, immunogenicity, and protective capacity of this MVA-MERS-S candidate vaccine in a BALB/c mouse MERS-CoV infection model by using dose escalation and two different application routes.

The MVA-MERS-S vaccine was prepared and quality controlled in accordance with standard procedures (17). The MVA-MERS-S recombinant virus 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. A single subcutaneous (s.c.) immunization with a dose of 10^7 or 10^8 PFU of MVA-MERS-S elicited detectable MERS-CoV-neutralizing antibodies (Fig. 1A). Booster s.c. immunizations resulted in increased titers of MERS-CoV-neutralizing antibodies, and even a low dose of 10^6 PFU of MVA-MERS-S induced measurable neutralizing antibodies. Vaccination doses of 10^7 and 10^8 PFU of MVA-MERS-S resulted in similar antibody levels.

A single primary intramuscular (i.m.) immunization resulted in MERS-CoV-neutralizing antibodies with all of the dosages of MVA-MERS-S used (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. and 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 gamma interferon (IFN- γ) enzyme-linked immunospot (ELISPOT) assay. We tested several S antigen-derived peptides for CD8⁺ T-cell specificity for 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 (EYGQF CSKI) (data not shown). We chose peptide S291 for *in vitro* stimulation, as this peptide consistently activated high numbers of S antigen-specific T cells. Single s.c. immunizations with 10^6 and 10^7 PFU of MVA-MERS-S induced nearly equivalent levels of S291-specific CD8⁺ T cells; however, immunization with 10^8 PFU of MVA-MERS-S resulted in about 3-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 of 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 3-fold. Moreover, we detected MERS-S291-specific IFN- γ -producing T cells in splenocytes 56 days following the primary or

FIG 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 7×10^4 TICD₅₀ of MERS-CoV 45 days after immunization with 10^6 , 10^7 , or 10^8 PFU of MVA-MERS. MERS-CoV RNA loads in lung tissues were determined by quantitative real-time reverse transcription-PCR (31). Numbers of viral genome copies per nanogram of RNA are shown for groups of animals (*n*, number of animals per group) immunized by the s.c. route with 10^6 (*n* = 5), 10^7 (*n* = 2), or 10^8 (*n* = 2) PFU of MVA-MERS-S (MVA-S), nonrecombinant MVA (WT) (*n* = 1), or phosphate-buffered saline (Mock) (*n* = 4) (A) or vaccinated by the i.m. route with 10^6 (*n* = 5), 10^7 (*n* = 5), or 10^8 (*n* = 5) PFU of MVA-MERS-S (MVA-S), nonrecombinant MVA (WT) (*n* = 3), or phosphate-buffered saline (Mock) (*n* = 4) (B). The statistical evaluation was performed with GraphPad Prism for Windows (GraphPad Software, La Jolla, CA). Statistical significance of differences between groups is indicated as follows: *, *P* < 0.05; **, *P* < 0.01; ns, no statistically significant difference.

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 productive infection with MERS-CoV, we intranasally transduced MVA-MERS-S-vaccinated BALB/c mice with

FIG 4 Histopathological and immunohistochemical examination of MVA-MERS-S-immunized (B, D, F, H), nonrecombinant MVA-vaccinated (A, C, E), and mock-vaccinated (G) mice that had been transduced with a nonreplicating adenoviral vector encoding human DPP4 and mCherry. Mice were infected with MERS-CoV (A to H) or mock infected and monitored for inflammation caused by adenoviral vector transduction (I, J). Lungs were collected 4 days postinfection (A to H) or 5 days after transduction with the 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., catalog no. 100208-RP) was used. Since all of the antibodies tested against human DPP4 showed partial cross-reactivity with murine DPP4, a mouse monoclonal antibody against mCherry (Abcam catalog no. ab125096) was used to monitor adenoviral transduction. Shown are the results of H&E staining (A to D, I) and immunohistochemical analyses for MERS-CoV spike protein (E, F, J) and mCherry (G, H). Scale bars: 500 μ m (A, B), 200 μ m (I, J), and 100 μ m (C to H).

2.5×10^8 PFU of an adenoviral vector encoding both the human DPP4 receptor and mCherry (ViraQuest) at 45 days after prime-boost immunization. Five days later, the animals were infected with 7×10^4 50% tissue culture infective doses (TICD₅₀) of MERS-CoV (strain EMC/2012), and 4 days postchallenge, the animals were sacrificed and their lungs were harvested for measurement of viral loads and for histopathological analysis. Large virus loads, on average, $>11,000$ to $>20,000$ MERS-CoV genome equivalents/ng of total RNA, were found in both mock-immunized and nonrecombinant 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 and C). Inflamed foci were seen mainly around larger bronchi, and some bronchi were filled with cellular debris and inflammatory cells, while other areas of the lungs remained unaffected. Lungs of control mice showed extensive MERS-CoV-S-specific staining, primarily in areas severely affected by inflammation (Fig. 4E). Tissues of 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 and 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 multiplicities of infection.

The immunogenicity data required before initiation of clinical trials (18) include evaluation of immune responses according to dosage, route of administration, and intervals of application, as well as characterization of humoral and cell-mediated immunity. In this study, the s.c. and i.m. routes were associated with comparable immune responses, particularly when using the standard dosage of 10^8 PFU of 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 a replacement smallpox vaccine demonstrating nearly equivalent immunogenicity of s.c. and i.m. immunizations (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 of the 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 at lower virus doses; only immunization with a higher dose of 10^8 PFU of MVA-

MERS-S 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 of the immunized mice exhibited little or no replication of MERS-CoV, irrespective of the route or dose used for vaccination. These data confirm 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 a candidate vaccine against MERS-CoV for potential human use.

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