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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 intrafamilial 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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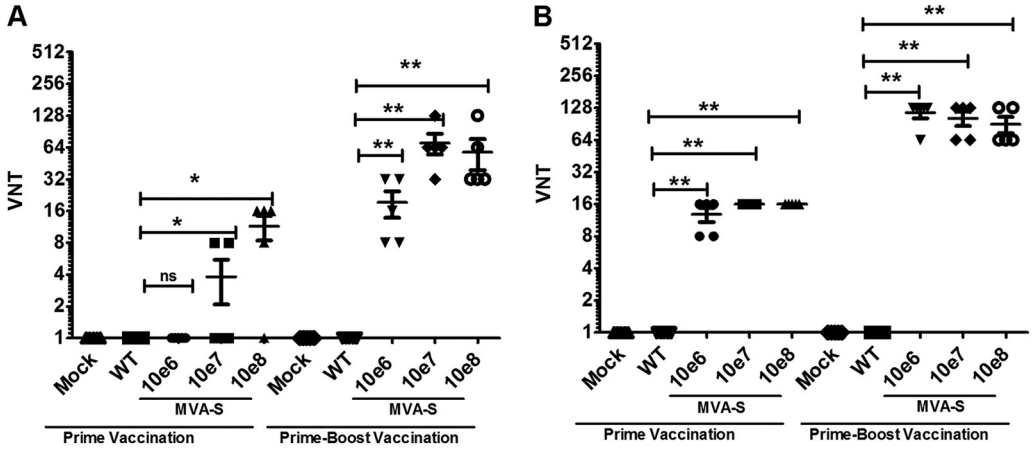


FIG 1 Antibody response induced by MVA-MERS-S vaccination. Groups of BALB/c mice ($n = 5$) were immunized s.c. (A) or i.m. (B) with 10^6, 10^7, or 10^8 PFU of MVA-MERS-S, 10^8 PFU of nonrecombinant MVA (WT), or phosphate-buffered saline (Mock). To monitor antibody responses, we analyzed the MERS-CoV-neutralizing 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.

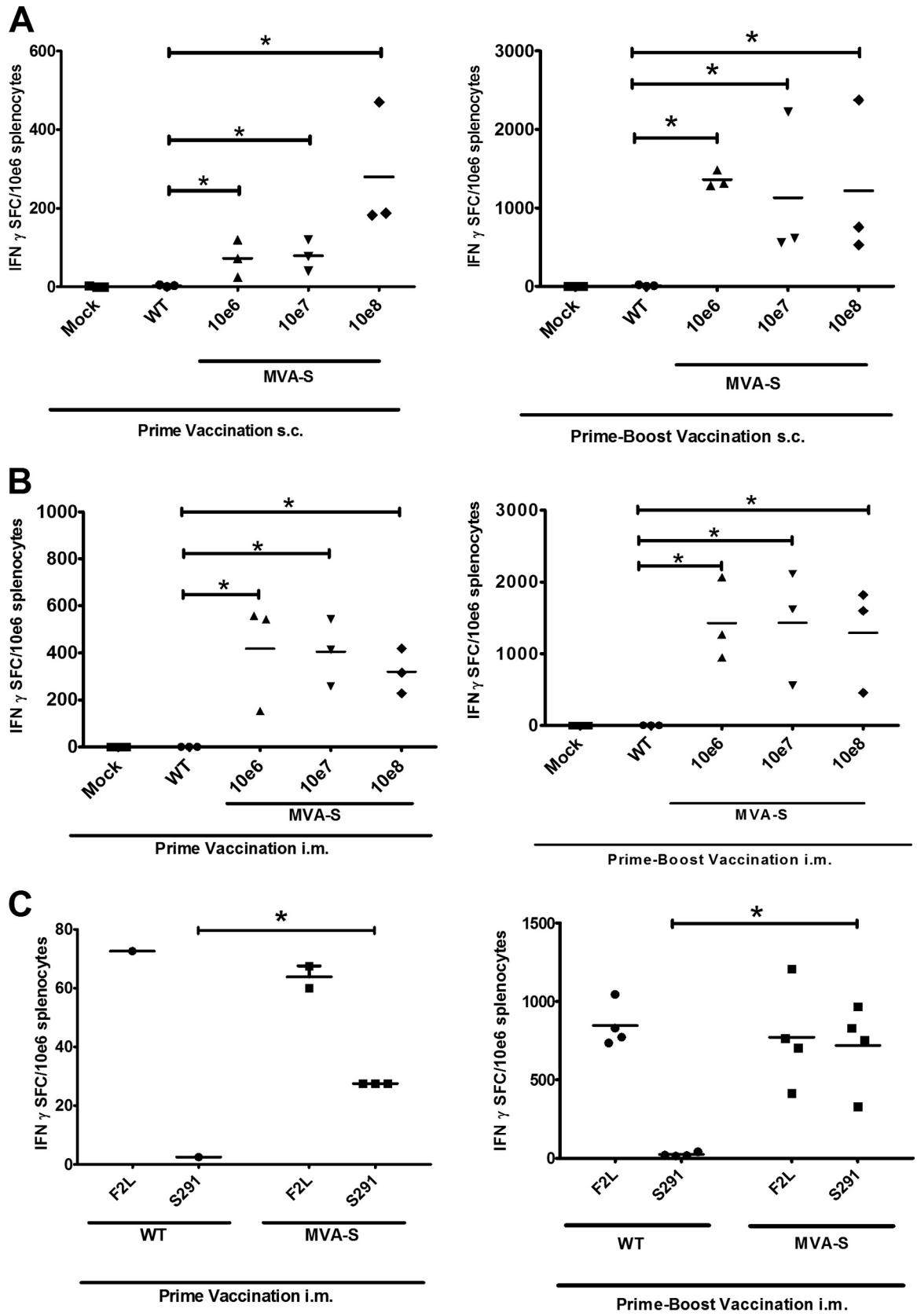


FIG 2 Virus-specific $CD8^+$ T-cell responses induced by MVA-MERS-S. BALB/c mice were immunized by single-shot and prime-boost vaccinations with 10^6, 10^7, or 10^8 PFU of MVA-MERS-S vaccine via the s.c. (A) or i.m. (B) route. Animals inoculated with nonrecombinant MVA (WT) or phosphate-buffered saline (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.EL.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-specifc 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).

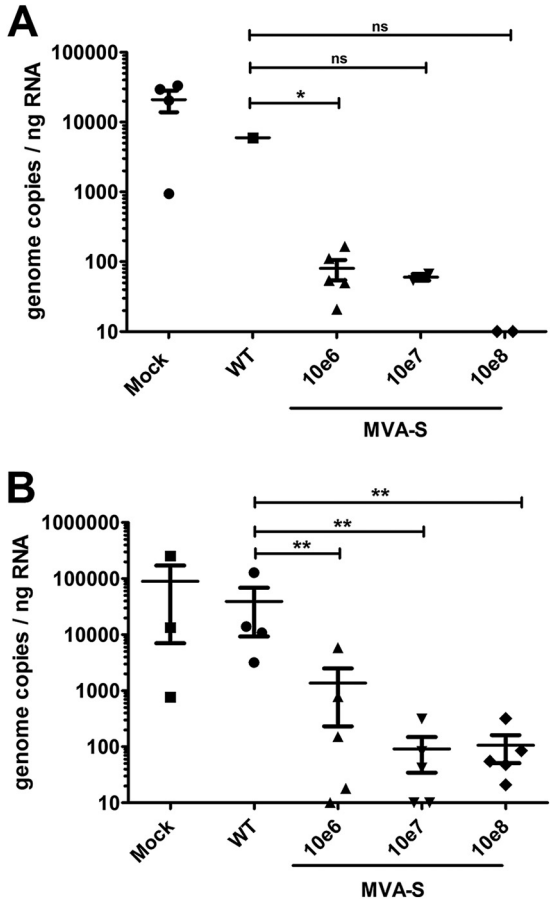
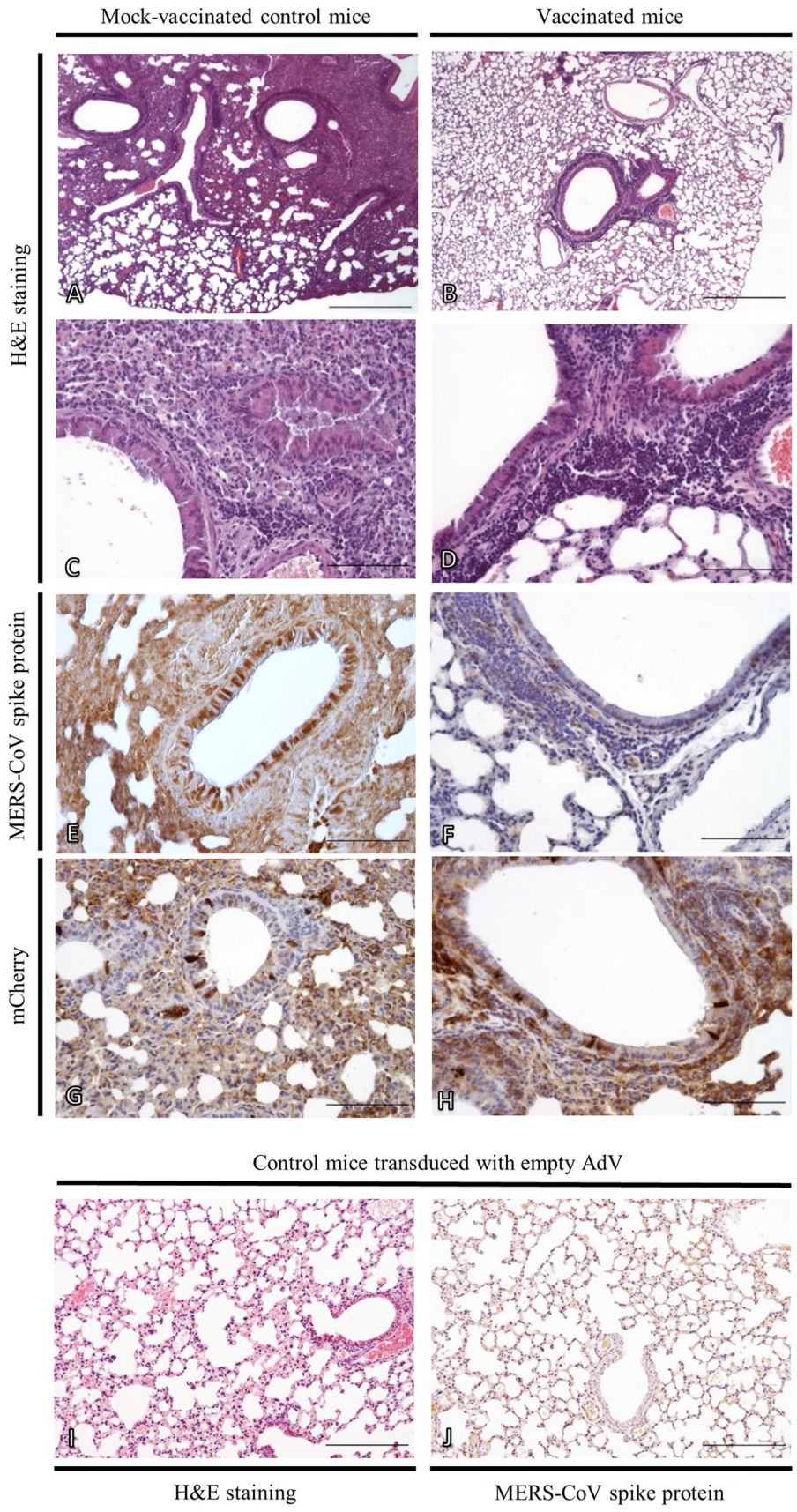


FIG 3 Protective capacity of MVA-MERS-S immunization against challenge with MERS-CoV in human DPP4-transduced BALB/c mice. BALB/c mice were infected with 7×10^4 $TICD_{50}$ of MERS-CoV 45 days after immunization with 10^6, 10^7, or 10^8 PFU of MVA-MERS. MERS-CoV RNA loads in lung tissues were determined 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.

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 μm (A, B), 200 μm (I, J), and 100 μm (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 ($TICD_{50}$) 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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REFERENCES

1. **Al-Tawfiq JA, Memish ZA.** 2014. Middle East respiratory syndrome coronavirus: epidemiology and disease control measures. Infect Drug Resist **7:**281–287.
2. **Al-Tawfiq JA, Memish ZA.** 2014. Middle East respiratory syndrome coronavirus: transmission and phylogenetic evolution. Trends Microbiol **22:** 573–579. http://dx.doi.org/10.1016/j.tim.2014.08.001.
3. **Coleman CM, Frieman MB.** 2014. Coronaviruses: important emerging human pathogens. J Virol **88:**5209–5212. http://dx.doi.org/10.1128/JVI.03488-13.
4. **Raj VS, Mou H, Smits SL, Dekkers DHW, Muller MA, Dijkman R, Muth D, Demmers JAA, Zaki A, Fouchier RAM, Thiel V, Drosten C, Rottier PJM, Osterhaus ADME, Bosch BJ, Haagmans BL.** 2013. Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. Nature **495:**251–254. http://dx.doi.org/10.1038/nature12005.
5. **Agrawal AS, Garron T, Tao X, Peng B-H, Wakamiya M, Chan T-S, Couch RB, Tseng C-TK.** 2015. Generation of transgenic mouse model of Middle East respiratory syndrome coronavirus infection and disease. J Virol **89:**3659–3670. http://dx.doi.org/10.1128/JVI.03427-14.
6. **Zhao J, Li K, Wohlford-Lenane C, Agnihothram SS, Fett C, Zhao J, Gale MJ, Baric RS, Enjuanes L, Gallagher T, McCray PB, Perlman S.** 2014. Rapid generation of a mouse model for Middle East respiratory syndrome. Proc Natl Acad Sci U S A **111:**4970–4975. http://dx.doi.org/10.1073/pnas.1323279111.
7. **Hotez PJ, Bottazzi ME, Tseng C-TK, Zhan B, Lustigman S, Du L, Jiang S.** 2014. Calling for rapid development of a safe and effective MERS vaccine. Microbes Infect **16:**529–531. http://dx.doi.org/10.1016/j.micinf.2014.05.002.
8. **Coleman CM, Liu YV, Mu H, Taylor JK, Massare M, Flyer DC, Glenn GM, Smith GE, Frieman MB.** 2014. Purified coronavirus spike protein nanoparticles induce coronavirus neutralizing antibodies in mice. Vaccine **32:**3169–3174. http://dx.doi.org/10.1016/j.vaccine.2014.04.016.
9. **Du L, Zhao G, Kou Z, Ma C, Sun S, Poon VKM, Lu L, Wang L, Debnath AK, Zheng B-J, Zhou Y, Jiang S.** 2013. Identification of a receptor-binding domain in the S protein of the novel human coronavirus Middle East respiratory syndrome coronavirus as an essential target for vaccine development. J Virol **87:**9939–9942. http://dx.doi.org/10.1128/JVI.01048-13.
10. **Kim E, Okada K, Kenniston T, Raj VS, AlHajri MM, Farag EABA, Al Hajri F, Osterhaus ADME, Haagmans BL, Gambotto A.** 2014. Immunogenicity of an adenoviral-based Middle East respiratory syndrome coronavirus vaccine in BALB/c mice. Vaccine **32:**5975–5982. http://dx.doi.org/10.1016/j.vaccine.2014.08.058.

11. **Ma C, Wang L, Tao X, Zhang N, Yang Y, Tseng C-TK, Li F, Zhou Y, Jiang S, Du L.** 2014. Searching for an ideal vaccine candidate among different MERS coronavirus receptor-binding fragments—the importance of immunofocusing in subunit vaccine design. Vaccine **32:**6170–6176. http://dx.doi.org/10.1016/j.vaccine.2014.08.086.
12. **Mou H, Raj VS, van Kuppeveld FJM, Rottier PJM, Haagmans BL, Bosch BJ.** 2013. The receptor binding domain of the new Middle East respiratory syndrome coronavirus maps to a 231-residue region in the spike protein that efficiently elicits neutralizing antibodies. J Virol **87:**9379–9383. http://dx.doi.org/10.1128/JVI.01277-13.
13. **Song F, Fux R, Provacia LB, Volz A, Eickmann M, Becker S, Osterhaus ADME, Haagmans BL, Sutter G.** 2013. Middle East respiratory syndrome coronavirus spike protein delivered by modified vaccinia virus Ankara efficiently induces virus-neutralizing antibodies. J Virol **87:**11950–11954. http://dx.doi.org/10.1128/JVI.01672-13.
14. **Altenburg AF, Kreijtz JHCM, de Vries RD, Song F, Fux R, Rimmelzwaan GF, Sutter G, Volz A.** 2014. Modified vaccinia virus Ankara (MVA) as production platform for vaccines against influenza and other viral respiratory diseases. Viruses **6:**2735–2761. http://dx.doi.org/10.3390/v6072735.
15. **Gilbert SC.** 2013. Clinical development of modified vaccinia virus Ankara vaccines. Vaccine **31:**4241–4246. http://dx.doi.org/10.1016/j.vaccine.2013.03.020.
16. **Gómez CE, Perdiguero B, García-Arriaza J, Esteban M.** 2013. Clinical applications of attenuated MVA poxvirus strain. Expert Rev Vaccines **12:**1395–1416. http://dx.doi.org/10.1586/14760584.2013.845531.
17. **Kremer M, Volz A, Kreijtz JCM, Fux R, Lehmann M, Sutter G.** 2012. Easy and efficient protocols for working with recombinant vaccinia virus MVA, p 59–92. *In* Isaacs SN (ed), Vaccinia virus and poxvirology, vol 890. Humana Press, Totowa, NJ.
18. **European Medicines Agency.** 2010. Guideline on quality, non-clinical and clinical aspects of live recombinant viral vectored vaccines. EMA/CHMP/VWP/141697/2009. European Medicines Agency, London, United Kingdom.
19. **Frey SE, Newman FK, Kennedy JS, Sobek V, Ennis FA, Hill H, Yan LK, Chaplin P, Vollmar J, Chaitman BR, Belshe RB.** 2007. Clinical and immunologic responses to multiple doses of IMVAMUNE® (modified vaccinia Ankara) followed by Dryvax challenge. Vaccine **25:**8562–8573. http://dx.doi.org/10.1016/j.vaccine.2007.10.017.
20. **Frey SE, Winokur PL, Hill H, Goll JB, Chaplin P, Belshe RB.** 2014. Phase II randomized, double-blinded comparison of a single high dose (5×10(8) TCID50) of modified vaccinia Ankara compared to a standard dose (1×10(8) TCID50) in healthy vaccinia-naïve individuals. Vaccine **32:**2732–2739. http://dx.doi.org/10.1016/j.vaccine.2014.02.043.
21. **Greenberg RN, Overton ET, Haas DW, Frank I, Goldman M, von Krempelhuber A, Virgin G, Bädeker N, Vollmar J, Chaplin P.** 2013. Safety, immunogenicity, and surrogate markers of clinical efficacy for modified vaccinia Ankara as a smallpox vaccine in HIV-infected subjects. J Infect Dis **207:**749–758. http://dx.doi.org/10.1093/infdis/jis753.
22. **Walsh SR, Wilck MB, Dominguez DJ, Zablowsky E, Bajimaya S, Gagne LS, Verrill KA, Kleinjan JA, Patel A, Zhang Y, Hill H, Acharyya A, Fisher DC, Antin JH, Seaman MS, Dolin R, Baden LR.** 2013. Safety and immunogenicity of modified vaccinia Ankara in hematopoietic stem cell transplant recipients: a randomized, controlled trial. J Infect Dis **207:**1888–1897. http://dx.doi.org/10.1093/infdis/jit105.
23. **European Medicines Agency.** 2013. Imvamex: modifiziertes Vacciniavirus Ankara lebend. EMA/490157/2013. European Medicines Agency, London, United Kingdom.
24. **Kreijtz JHCM, Goeijenbier M, Moesker FM, van den Dries L, Goeijenbier S, De Gruyter HLM, Lehmann MH, de Mutsert G, van de Vijver DAMC, Volz A, Fouchier RAM, van Gorp ECM, Rimmelzwaan GF, Sutter G, Osterhaus ADME.** 2014. Safety and immunogenicity of a modified-vaccinia-virus-Ankara-based influenza A H5N1 vaccine: a randomised, double-blind phase 1/2a clinical trial. Lancet Infect Dis **14:**1196–1207. http://dx.doi.org/10.1016/S1473-3099(14)70963-6.
25. **Volz A, Langenmayer M, Jany S, Kalinke U, Sutter G.** 2014. Rapid expansion of $CD8^+$ T cells in wild-type and type I interferon receptor-deficient mice correlates with protection after low-dose emergency immunization with modified vaccinia virus Ankara. J Virol **88:**10946–10957. http://dx.doi.org/10.1128/JVI.00945-14.
26. **Bisht H, Roberts A, Vogel L, Bukreyev A, Collins PL, Murphy BR, Subbarao K, Moss B.** 2004. Severe acute respiratory syndrome coronavirus spike protein expressed by attenuated vaccinia virus protectively immunizes mice. Proc Natl Acad Sci U S A **101:**6641–6646. http://dx.doi.org/10.1073/pnas.0401939101.
27. **Jaume M, Yip MS, Cheung CY, Leung HL, Li PH, Kien F, Dutry I, Callendret B, Escriou N, Altmeyer R, Nal B, Daëron M, Bruzzone R, Peiris JSM.** 2011. Anti-severe acute respiratory syndrome coronavirus spike antibodies trigger infection of human immune cells via a pH- and cysteine protease-independent FcγR pathway. J Virol **85:**10582–10597. http://dx.doi.org/10.1128/JVI.00671-11.
28. **Jaume MYM, Kam YW, Cheung CY, Kien F, Roberts A, Li PH, Dutry I, Escriou N, Daeron M, Bruzzone R, Subbarao K, Peiris JS, Nal B, Altmeyer R.** 2012. SARS CoV subunit vaccine: antibody-mediated neutralisation and enhancement. Hong Kong Med J **18**(Suppl 2):31–36.
29. **Roberts A, Lamirande EW, Vogel L, Jackson JP, Paddock CD, Guarner J, Zaki SR, Sheahan T, Baric R, Subbarao K.** 2008. Animal models and vaccines for SARS-CoV infection. Virus Res **133:**20–32. http://dx.doi.org/10.1016/j.virusres.2007.03.025.
30. **Tscharke DC, Karupiah G, Zhou J, Palmore T, Irvine KR, Haeryfar SMM, Williams S, Sidney J, Sette A, Bennink JR, Yewdell JW.** 2005. Identification of poxvirus $CD8^+$ T cell determinants to enable rational design and characterization of smallpox vaccines. J Exp Med **201:**95–104. http://dx.doi.org/10.1084/jem.20041912.
31. **Corman VMEI, Bleicker T, Zaki A, Landt O, Eschbach-Bludau M, van Boheemen S, Gopal R, Ballhause M, Bestebroer TM, Muth D, Müller MA, Drexler JF, Zambon M, Osterhaus AD, Fouchier RM, Drosten C.** 2012. Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Euro Surveill **17:**pii=20285. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20285.