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BRIEF REPORT

Monkeypox Virus Cross-Neutralizing Antibodies in Clinical Trial Participants Vaccinated With Modified Vaccinia Virus Ankara Encoding Middle East Respiratory Syndrome–Coronavirus Spike Protein

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Modified vaccinia virus Ankara (MVA) is used as a vaccine against monkeypox virus and as a viral vaccine vector. MVA-MERS-S is a vaccine candidate against Middle East respiratory syndrome (MERS)–associated coronavirus. Here, we report that cross-reactive monkeypox virus neutralizing antibodies were detectable in only a single study participant after the first dose of MVA-MERS-S vaccine, in 3 of 10 after the second dose, and in 10 of 10 after the third dose.

Keywords. Middle East respiratory syndrome–related coronavirus; MPXV; MVA; modified vaccinia virus Ankara; monkeypox virus.

Monkeypox virus (MPXV), an orthopoxvirus first recognized to cause a zoonotic infection in 1970 [1], is endemic in Central and West Africa, with sporadic small outbreaks in non-endemic regions introduced by travel. However, since May 2022, numerous cases and local transmission has been identified in Europe and the Americas.

Vaccinia virus (VACV) was used to immunize against smallpox (caused by variola virus), eventually leading to the eradication of this disease in 1980. Modified VACV Ankara (MVA) is a VACV-derived strain, which was used in Germany in the final stages of smallpox eradication. It is highly attenuated, having lost its ability to replicate in most mammalian cell types, making it nonpathogenic and safe for use even in immunocompromised individuals [2]. Compared with ACAM2000, an earlier smallpox vaccine based on replication-competent VACV, MVA was found to have an improved safety profile, while also eliciting neutralizing antibody (nAb) responses and attenuation of major cutaneous reactions, considered to be protective against smallpox [3]. In 2019, the United States Food and Drug Administration granted approval to vaccine manufacturer Bavarian Nordic for the use of MVA-BN vaccine to prevent smallpox and monkeypox; along with ACAM2000, MVA-BN was included in strategic stockpiles maintained in case of an outbreak [4].

Because recombinant MVA expressing genes from other pathogens are relatively easy to make, there is interest in using MVA as a vector for novel vaccines [5]. MVA-MERS-S—MVA expressing the spike (S) protein from Middle East respiratory syndrome–related coronavirus (MERS-CoV)—is a candidate viral vector vaccine currently being investigated in clinical trials. MERS-CoV is an emerging zoonotic pathogen in humans, first identified as a cause of severe pneumonia in 2012 and included by the World Health Organization in its research and development blueprint for action to prevent epidemics [6]. A previously completed phase 1a trial showed favorable safety and the induction of MERS-CoV–specific antibodies and T cells in individuals given 2 injections of a high- or low-dose vaccine with a 1-month interval [7]. However, immunity waned by 180 days after the second dose, prompting a follow-up study that demonstrated robust antibody boosting when an additional dose was administered approximately 1 year after the initial dose in a subgroup of 10 participants from the initial trial. Over a 2-year follow-up period, nAbs were maintained, with titers remaining above the peak value after the second dose in 4 of 5 participants [8, 9].

A bivalent vaccine capable of protecting against both emerging coronaviruses and poxviruses would have clear benefits from an epidemic preparedness perspective. Here, we report cross-nAb responses against MPXV for the MVA-MERS-S phase 1a clinical trial participants and absence of cross-

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reactivity of MERS spike–specific antibodies to other human coronaviruses (HCoVs).

METHODS

This study used serum samples collected from a single-center, open-label phase 1 clinical trial with MVA-MERS-S in healthy individuals (NCT03615911), specifically a subgroup of individuals (n = 10) who agreed to receive an additional booster dose 1 year after the initial dose was administered. Full details of the trial design and results have been published [8]. The study design of the clinical trial was reviewed and approved by the competent national authority (Paul Ehrlich Institute) and the Ethics Committee of the Hamburg Medical Association. The study was performed in accordance with the Declaration of Helsinki in its version of Fortaleza 2013. All study participants provided written informed consent before enrollment in the studies. Participants were aged 18–40 years and had not previously been exposed to VACV. The trial was performed in 2017 and 2018, before the coronavirus disease 2019 pandemic.

Serum samples were analyzed using an in-house orthopoxvirus plaque reduction neutralization test (PRNT). A detailed description of the procedures used in this assay has been described elsewhere [10]. In short, Vero cells were infected with 1500 plaque-forming units (PFUs) of recombinant MVA encoding green fluorescent protein or 400 PFUs of MPXV, strain 2022_NL001 (European Virus Archive reference 010V-04721), which were preincubated with serum diluted 1:20–1:2560 in 2-fold steps. After fixation and immunofluorescent staining of MPXV-infected cells, plates were imaged using an automated confocal imaging microscope (Opera Phenix; Perkin Elmer). The numbers of fluorescence-positive cells per well were quantified using Harmony software (version 4.9; Perkin Elmer). A 50% reduction in the number of infected cells compared with the no-serum control was considered to be neutralizing ($PRNT_{50}$). Nonneutralizing serum samples were given a titer of 10, whereas $PRNT_{50}$ titers were interpolated using a 4-parameter logistic regression curve (GraphPad Prism software, version 9.4.1). An anti–vaccinia immunoglobin reference standard was included as a positive control, with a $PRNT_{50}$ of 349 against MVA and 1005 against MPXV in these experiments.

An in-house indirect VACV enzyme-linked immunosorbent assay (ELISA) was used as described elsewhere [10]. VACV-Elstree–infected cell lysate was coated on 96-well high-binding ELISA plates. Mock-infected cell lysate-coated plates were used to subtract background signal. Serum samples were titrated using 5-fold serial dilutions (range, 1:10–1:7 81 250), and resultant net OD_{450} (optical density at 450 nm) responses were transformed to a minimum-to-maximum OD_{450} S curve based on a positive reference serum pool. Results were expressed as 30% end-point titers relative to this positive control S curve.

An in-house HCoV protein microarray assay was used, as described elsewhere [11]. Array slides contained full spike ectodomain proteins for MERS-CoV, severe acute respiratory syndrome-related coronavirus (SARS-CoV and SARS-CoV-2), and the seasonal HCoVs HKU1, NL63, and OC43.

RESULTS

Serum samples taken during the MVA-MERS-S phase 1a clinical trial and initially used to analyze immune responses against MERS-CoV were reanalyzed to detect binding antibodies against VACV and HCoVs and nAbs against MVA and MPXV. These analyses included study participants who completed the booster extension trial and received a third dose of MVA-MERS-S 1 year after the primary 2-dose series (n = 10). Of these individuals, 3 were primed with a low dose (10^7 PFUs) of the vaccine and 7 with a high dose (10^8 PFUs). The third vaccination was high dose (10^8 PFUs) in all participants. The analysis was performed at 4 time points: at baseline and at 4 weeks (28 days) after each dose of MVA-MERS-S (Figure 1*A*). Baseline results from the VACV ELISA and MVA PRNT confirmed that none of the participants had previously been exposed to VACV- or MVA-based vaccines (Figure 1*B* and 1*C*).

After a single dose of MVA-MERS-S, VACV-reactive binding antibodies and MVA nAbs were detectable in 9 of 10 study participants, with a VACV ELISA geometric mean titer (GMT) of 17 (95% confidence interval [CI], 12–23) and an MVA PRNT GMT of 32 (21–51). After the second dose, all individuals seroconverted, with GMTs of 424 (95% CI, 237–758) for VACV-reactive antibodies and 211 (136–327) for MVA nAbs. After the third dose, the VACV GMT increased further to 11 785 (95% CI, 4425–31 387), and the MVA nAb GMT to 793 (425–1481). In contrast, MPXV nAbs were detectable in only a single participant after the first dose, in 3 of 10 after the second dose, and finally in 10 of 10 after the third dose (Figure 1*D*). MPXV-neutralizing titers after 3 doses were relatively low to moderate, with a GMT of 107 (95% CI 36–323). The 3 participants who were primed using low-dose vaccine did not appear to have different responses compared with their high-dose counterparts, although there was insufficient power to compare the 2 groups statistically.

Robust antibody responses against MERS-CoV S were previously detected using ELISA [7]. These results were further confirmed using an in-house protein microarray assay, capable of detecting binding antibodies against full S antigens of multiple HCoVs in parallel. While MERS-CoV S-binding antibodies were detectable in all study participants 28 days after the second dose and increased further after the third dose, antibody levels against SARS-CoV and SARS-CoV-2 remained undetectable in all participants. Antibody responses against HCoVs were unaffected (Figure 1*E*).

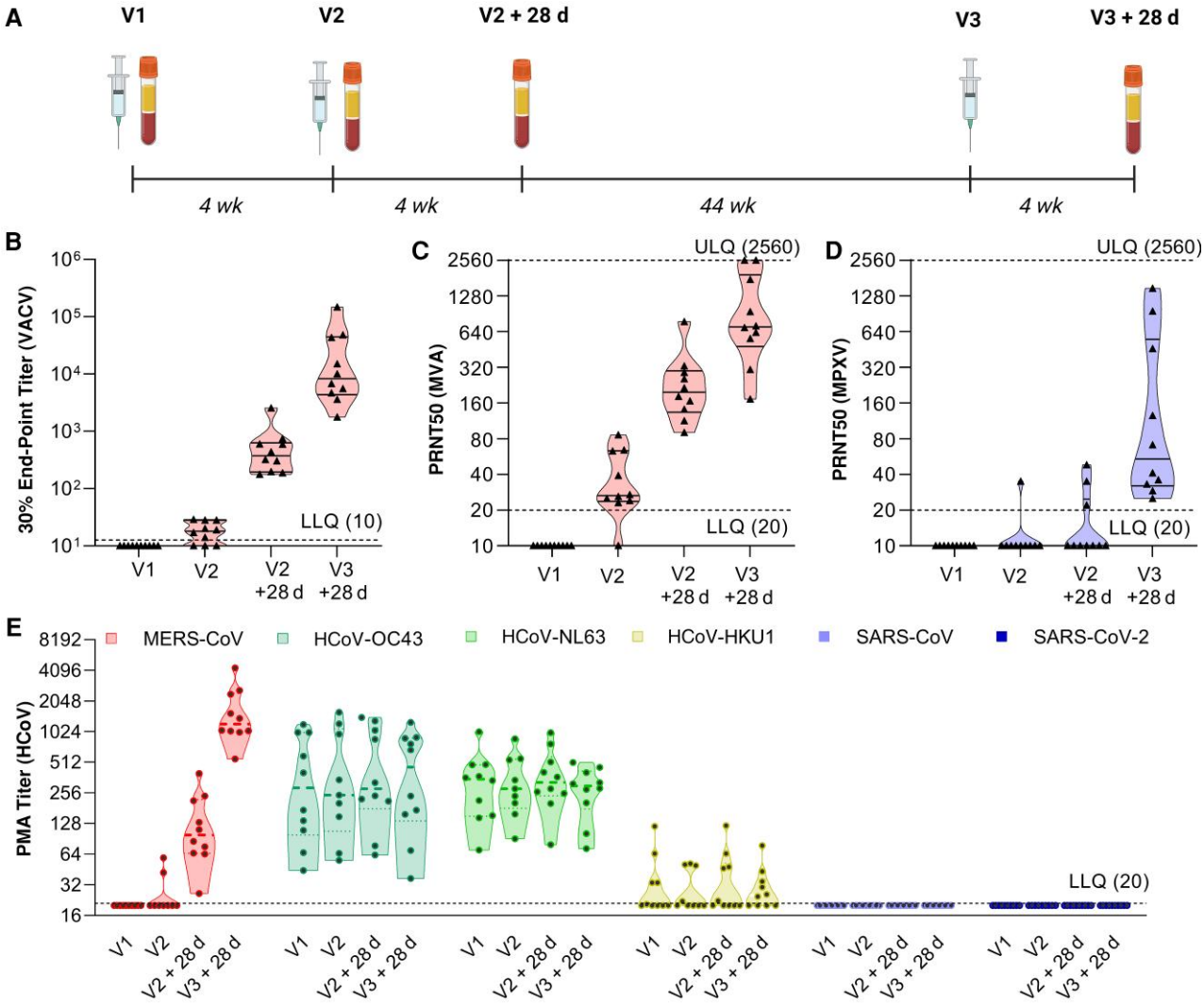

Figure 1. Antibody titers in individuals (n = 10) receiving 3 doses of the MVA-MERS-S vaccine candidate. *A,* Time points are identified by consecutive vaccine dose (vaccine doses 1, 2, and 3 [V1, V2, and V3]), followed by the approximate number of days between vaccination and sampling (0 or 28 days), created with BioRender.com. *B–D,* MVA-MERS-S vaccination elicited binding antibodies against vaccinia virus (VACV) (*B*), which neutralized modified VACV Ankara (MVA) (*C*) and cross-neutralized monkeypox virus (MPXV) after the third dose (*D*). *E,* MVA-MERS-S vaccination did not elicit cross-reactive binding antibodies against other human coronavirus (HCoV). Violin plots show individual titers with median and interquartile range. Abbreviations: LLQ, lower limit of quantification; MERS-CoV, Middle East respiratory syndrome–related coronavirus; PMA, protein microarray; $PRNT_{50}$, 50% plaque reduction neutralization test; SARS-CoV, severe acute respiratory syndrome-related coronavirus; SARS-CoV-2, severe acute respiratory syndrome-related coronavirus 2; ULQ, upper limit of quantification.

DISCUSSION

The suitability of MVA as a vaccine vector, combined with its potential for broad protection against multiple orthopoxvirus species, makes it a promising platform for developing vaccines against emerging pathogens. In this report, we have shown the ability of MVA-MERS-S to elicit a bivalent antibody response to both MERS-CoV and MVA. Cross-neutralization of MPXV was detected in all study participants after 3 doses, though with relatively low titers. We did not detect cross-reactivity of MERS-CoV S–specific antibodies with S proteins of other HCoVs.

No correlate of protection against MPXV in humans has been identified to date. For the approval of MVA as a smallpox vaccine, attenuation of major cutaneous reactions against VACV and the formation of VACV nAbs have been accepted [3]. These serve as surrogates for nAbs against variola virus, the testing of which is challenging given the highly restricted access to the virus. In serum samples from individuals vaccinated with MVA or replication-competent VACV, nAb levels were higher when the neutralization target and the vaccine strain used were the same (homologous), compared with the cross-neutralizing (heterologous) responses against either the other

vaccine strain or variola virus [12]. Similarly, we found that MPXV cross-neutralizing nAb responses were detectable after vaccination but lower compared with homologous MVA nAb titers.

A prior study revealed that MPXV cross-neutralization of serum samples from a similarly designed phase 1/2a clinical trial, using a recombinant MVA-based vaccine candidate against influenza A virus H5N1 (MVA-H5), showed comparable results [10, 13]. Both MVA-H5 and MVA-MERS-S were administered intramuscularly in these trials, whereas MVA-BN, currently used to vaccinate at-risk populations against MPXV, is given subcutaneously. Both routes were shown to be equally immunogenic [14], suggesting that comparative analysis between these different vaccines is possible.

The formation of robust MPXV nAbs only after 3 doses of MVA-MERS-S raises the question whether high-risk individuals currently being vaccinated with MVA-BN would benefit from an additional booster. However, a preliminary analysis of an observational cohort study suggests high effectiveness against MPXV infection of even a single dose of MVA-BN in moderate- to high-risk individuals [15]. It is possible that the nAbs present at the time of virus exposure are not what protects the vaccinated individual. Rather, the memory B and T cells activated on exposure to MPXV could result in a rapid boost in nAbs that block infection, similar to the response to the third dose of MVA-MERS-S in this study. Further studies are required to better understand the immunogenicity and to determine the protective effectiveness of MVA against MPXV infection and the optimal dosing regimens in different populations.

Notes

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