ChAdOx1 and MVA based Vaccine Candidates against MERS-CoV Elicit Neutralising Antibodies and Cellular Immune Responses in Mice

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Abstract

The Middle East respiratory syndrome coronavirus (MERS-CoV) has infected more than 1900 humans, since 2012. The syndrome ranges from asymptomatic and mild cases to severe pneumonia and death. The virus is believed to be circulating in dromedary camels without notable symptoms since the 1980s. Therefore, dromedary camels are considered the only animal source of infection. Neither antiviral drugs nor vaccines are approved for veterinary or medical use despite active research on this area. Here, we developed four vaccine candidates against MERS-CoV based on ChAdOx1 and MVA viral vectors, two candidates per vector. All vaccines contained the full-length spike gene of MERS-CoV; ChAdOx1 MERS vaccines were produced with or without the leader sequence of the human tissue plasminogen activator gene (tPA) where MVA MERS vaccines were produced with tPA, but either the mH5 or F11 promoter driving expression of the spike gene. All vaccine candidates were evaluated in a mouse model in prime only or prime-boost regimens. ChAdOx1 MERS with tPA induced higher neutralising antibodies than ChAdOx1 MERS without tPA. A single dose of ChAdOx1 MERS with tPA elicited cellular immune responses as well as neutralising antibodies that were boosted to a significantly higher level by MVA MERS. The humoral immunogenicity of a single dose of ChAdOx1 MERS with tPA was equivalent to two doses of MVA MERS (also with tPA). MVA MERS with mH5 or F11 promoter induced similar antibody levels; however, F11 promoter enhanced the cellular immunogenicity of MVA MERS to significantly higher magnitudes. In conclusion, our study showed that MERS-CoV vaccine candidates could be optimised by utilising different viral vectors, various genetic designs of the vectors, or different regimens to increase immunogenicity. ChAdOx1 and MVA vectored vaccines have been safely evaluated in camels and humans and these MERS vaccine candidates should now be tested in camels and in clinical trials.

Introduction

Middle East respiratory syndrome (MERS) is caused by a novel betacoronavirus (MERS-CoV) that was isolated in late 2012 in Saudi Arabia (1). The syndrome (MERS) is described as a viral infection that causes fever, cough, and/or shortness of breath and to a lesser extent gastrointestinal symptoms such as diarrhea (2). Severe disease from MERS-CoV infection can cause respiratory failure and organ failure, and cases can be fatal, especially in patients with co-morbidities such as diabetes and cardiac complications. However, the infection can be asymptomatic or mild in many cases (3-7). MERS-CoV has spread to 27 countries and infected more than 1900 humans with a mortality rate of 40% (2). Dromedary camels, especially juveniles, contract the infection and shed the virus, without notable symptoms of disease; this is now known to have been occurring since the early 1980s (8-13). The mechanism of camel to human transmission is still not clear, but several primary cases have been associated with camel contact, which is considered an important risk factor (14-16). Therefore, camels are being considered an intermediate host and one of the sources of MERS-CoV infection (8-13). Other livestock animals such as sheep, goats, cows, chicken, and horses have proved seronegative in many studies (17-20). Further, these animals did not productively contract MERS-CoV when they were inoculated experimentally (21, 22). Therefore, to date, dromedary camels are the only confirmed animal reservoir. There is currently no approved vaccine against MERS-CoV for camels or humans despite active vaccine research and development. A number of vaccine candidates have been developed using various platforms and regimens and have been tested in several animal models (23). Viral vectors are potent platform technologies that have been utilised to develop vaccines against malaria, tuberculosis, influenza, HIV, HCV, Ebola, and many viral pathogens. These vectors include adenoviruses, poxviruses, yellow fever viruses, and alphaviruses (24, 25), and they are preferred for their ability to induce cellular immune responses in addition to humoral immunity. Here, we report development of MERS-CoV vaccine candidates that are based on two different viral vectors: Chimpanzee Adenovirus, Oxford

University #1 (ChAdOx1) (26) and Modified Vaccinia virus Ankara (MVA) (27, 28). Each viral vector was developed by generating two alternative versions, resulting in four vaccine candidates that all encode the same complete MERS-CoV spike gene (S). The two ChAdOx1 based vaccines were produced with or without the signal peptide of the human tissue plasminogen activator gene (tPA) at the N terminus. Previous studies have shown that encoding tPA upstream of recombinant antigens enhanced immunogencity, although results differed depending on the antigens employed. The tPA encoded upstream of influenza A virus nucleoprotein, in a DNA vector, enhanced both cellular and humoral immune responses in mice (29, 30), whereas the same leader sequence resulted in increased humoral sequences but decreased cellular responses to HIV Gag (30). The two MVA based vaccines were produced with either the mH5 or F11 poxviral promoter driving antigen expression, both including the tPA sequence at the N terminus of MERS-CoV Spike protein. Previously, we reported the ability of the strong early F11 promoter to enhance cellular immunogenicity of vaccine antigen candidates for malaria and influenza, as compared to utilising p7.5 or mH5 early/late promoters which resulted in a lower level of gene expression immediately after virus infection of target cells, but higher levels at a later stage (31). Here, we continue to assess the F11 promoter in enhancing cellular immunogenicity, and to investigate its ability to impact on humoral immune responses. The four vaccine candidates were evaluated in a number of different regimens in mouse models that showed a single dose of ChAdOx1 MERS inducing higher cellular and humoral immunogenicity than a single dose of MVA MERS, or equivalent to two doses of MVA MERS. ChAdOx1 based vaccines have been tested in different animal models, including camels (32), and in human clinical trials and proved safe and immunogenic (33). Therefore, based on our data, ChAdOx1 MERS can be readily developed for use as a MERS vaccine in humans. Furthermore, utilising ChAdOx1 MERS for camel vaccination can serve the one-health approach whereby blocking MERS-CoV transmission in camels is expected to prevent human infections.

Materials and methods

Transgene and shuttle vector cloning

The spike (S) gene of MERS-CoV camel isolate (Genbank accession number: KJ650098.1) was synthesised by GeneArt Gene Synthesis (Thermo Fisher Scientific). The S transgene was then cloned into four shuttle plasmid vectors following In-Fusion cloning (Clontech). Two plasmids contained the S transgene within the E1 homologous region of ChAdOx1, driven by the human cytomegalovirus major immediate early promoter (IE CMV) that includes intron A. One of the ChAdOx1 shuttle plasmids was designed to include the tPA signal sequence upstream of the transgene sequence while the second plasmid did not contain the tPA. The ChAdOx1 shuttle plasmids contained the S transgene within Gateway® recombination cassettes. To construct MVA MERS, one of the shuttle plasmids for MVA was designed to have the upstream and downstream (flanks) of the *F11L* ORF as homologous sequence arms. Inserting the S transgene within these arms enabled the utilisation of the endogenous F11 promoter, which is part of the right homologous arm, while deleting the native F11L ORF. This resulted in the shuttle vector for generation of F11-MVA MERS (F11 shuttle vector). The mH5 promoter sequence was subcloned upstream of the S transgene; and this mH5-S transgene was then subcloned into the F11 shuttle vector. This resulted in the shuttle vector for generation of mH5-MVA MERS (F11/mH5 shuttle vector). mH5-MVA MERS contained the mH5 promoter at the *F11L* locus, however, the endogenous F11 promoter is intact and located upstream of the mH5 promoter. The endogenous F11 promoter could not be replaced with the mH5 since it is part of the essential upstream ORF.

Immunostaining for Transgene Expression

The ChAdOx1 shuttle plasmid, described above, was used to validate the expression of MERS-CoV spike protein *in vitro*. An African green monkey kidney cell line (Vero cells) was seeded into 6-well plate to 80% confluence. Then the plasmid DNA was transfected into Vero cells using Lipofectamine® 2000

(Thermo Fisher Scientific) following manufacturer's instruction. Twenty four hours after transfection, cells were fixed, permeabilised, and immunostained using a rabbit polyclonal anti-MERS-CoV spike antibody, following standard protocols. DAPI stain was used to label nuclei.

Construction of recombinant ChAdOx1 and MVA encoding MERS-CoV S antigens

The ChAdOx1 MERS vaccines were prepared by Gateway® recombination between the ChAdOx1 destination DNA BAC vector (described in (26)) and entry plasmids containing the coding sequence for MERS-CoV spike gene (ChAdOx1 shuttle vectors explained above), according to standard protocols. ChAdOx1 MERS genomes were then derived in HEK293A cell lines (Invitrogen, Cat. R705-07), the resultant viruses were purified by CsCl gradient ultracentrifugation as previously described (34). The titres were determined on HEK293A cells using anti-hexon immunostaining assay based on the QuickTiter™ Adenovirus Titer Immunoassay kit (Cell Biolabs Inc). For MVA MERS vaccines chicken embryo fibroblast cells (CEFs) were infected with MVA parental virus that encodes dsRed marker instead of the native F11L ORF and transfected with MVA shuttle plasmids containing MERS-CoV spike gene (explained above) to allow recombination with the MVA genome and deletion of dsRed marker whilst keeping the F11 promoter sequence. Recombinant MVA expressing MERS-CoV S protein was purified by plaque-picking and fluorescent selection using the sorting function of CyCLONE robotic module of a MoFlo Flow cytometer (Dako Cytomation, Denmark) as previously described (31). F11-MVA MERS and mH5-MVA MERS were confirmed to lack the native *F11L* ORF (and the dsRed marker), and contain MERS-CoV S by PCR (identity and purity PCR screening). The sequence of the S transgene amplified from these vaccines was confirmed. The recombinant viruses (vaccines) were amplified in 1500 cm^2 monolayers of CEFs cells, partially purified over sucrose cushions and titrated in CEFs cells according to standard practice, and purity and identity were again verified by PCR.

Mouse immunogenicity

Female BALB/c mice (Harlan, UK) aged 6 to 8 weeks were immunised intramuscularly (i.m.) in the upper leg (total volume 50 µL) with a total of 10^8 IU of ChAdOx1 MERS with or without tPA or with a total of 10^6 pfu of either F11-MVA MERS or mH5-MVA MERS. For induction of short-term anaesthesia, animals were anaesthetised using vaporised IsoFloH. In prime only regimens, mice were vaccinated with ChAdOx1 with blood samples taken at 14 days post immunisation (d.p.i) or 28 d.p.i. for serum isolation; and spleens were collected at 28 d.p.i. In heterologous prime-boost regimens, mice were vaccinated with ChAdOx1 MERS and boosted with MVA MERS at 28 d.p.i; mice were bled at 28 d.p.i. (post-prime) or 42 d.p.i (14 days post-boost) for serum isolation, and spleens were collected at 42 d.p.i. In homologous regimens, mice were vaccinated with MVA MERS and boosted with MVA MERS at 21 d.p.i; mice were bled on 21 d.p.i. (post-prime) or 42 d.p.i (post-boost) for serum isolation and spleens were collected at 42 d.p.i.

ELISpot, ICS, and flow cytometry

Splenocytes were harvested for analysis by IFN-γ ELISpot or intracellular cytokine staining (ICS) and flow cytometry as previously described (35, 36), using re-stimulation with 2 µg/mL S291 MERS-CoV S-specific peptide (VYDTIKYYSIIPHSI); for vaccine cellular immunogenicity (37)); or 1 µg/mL E3 and F2(G) MVA vector-specific peptides (38) (for anti-MVA immune responses). In the absence of peptide re-stimulation, the frequency of IFN-γ^+ cells, which was typically 0.1% by flow cytometry or less than 50 SFC by ELISpot, was subtracted from tested re-stimulated samples.

ELISA

2 µg/ml with capturing antigen (S1 recombinant protein from MyBioSource, CA, USA) were used to coat ELISA plates, and standard endpoint ELISA protocol was followed, as previously described (39). Sera were prepared in a 10-fold serial dilution in PBS/T and then 50 µl were plated in duplicate wells. Serum from a naïve BALB/c mouse was included as a negative control. Goat anti-mouse total IgG conjugated to

alkaline phosphatase (Sigma) and PNPP tablet (20 mg p-nitrophenylphosphate, SIGMA) substrate were used in the assay.

MERSpp Neutralisation assay

MERS pseudotyped viral particles (MERSpp) were produced and titrated using Huh7.5 cell line as described previously (40). For the MERSpp neutralization assay, serum samples were serially diluted in 96-well white plates (Nunc). A standard concentration of the MERSpp were added to the wells and plates were incubated for 1 h at 37 °C. After incubation, Huh7.5 cells (10,000 cells per well) were added to the plate in duplicates. Following 48 h incubation, cells were lysed and luciferase activity was measured. IC90 neutralisation titres were calculated for each mouse serum sample using GraphPad Prism.

Virus neutralisation assay

Induction of virus-neutralising antibodies was confirmed according to previously published protocols (37, 41). Briefly, mouse serum samples were tested for their capacity to neutralise MERS-CoV (EMC isolate) infections *in vitro* with 100 50% tissue culture infective doses ($TCID_{50}$) in Huh-7 cells. Sera of non-immunised mice served as negative control.

Statistical analysis

GraphPad Prism (GraphPad software) was used for statistical analysis and to plot data.

Ethics statement

All animal procedures were performed in accordance with the terms of the UK Animals (Scientific Procedures) Act (ASPA) for the project licenses 30/2414 or 30/2889 and were approved by the University of Oxford Animal Care and Ethical Review Committee. All mice were housed for at least 7 days for settlement prior to any procedure in the University animal facility, Oxford, UK under Specific Pathogen Free (SPF) conditions.

Results

Construction and antigen expression of MERS-CoV vaccine candidates

The spike gene from a camel isolate (Camel/Qatar_2_2014 MERS-CoV isolate, GenBank accession number KJ650098.1) was cloned into four shuttle vectors that facilitate homologous recombination with the genome of ChAdOx1 or MVA. Four recombinant viral vectors, two ChAdOx1 and two MVA, were derived as described in the materials and methods. ChAdOx1 based vaccine candidates were generated with or without the signal peptide of the human tissue plasminogen activator gene (tPA). The spike transgene expression in ChAdOx1 MERS vaccine candidates is under the control of the human cytomegalovirus major immediate early promoter (CMV IE) that includes intron A. In MVA MERS vaccine candidates, the tPA was also inserted upstream of the spike transgene, which was under the control of either the ectopic mH5 promoter or the endogenous F11 promoter (Figure 1A). All of our MERS-CoV vaccine candidates contain the same codon-optimized spike transgene.The expression of the newly synthesized transgene was first tested by transfection of an African green monkey kidney cell line (Vero cells) with the adenovirus shuttle vector, and immunofluorescence staining of the transfected cells (Figure 1B and 1C). This was performed to confirm the expression of the codon optimized spike transgene in mammalian cells. The level of transgene expression from the four vaccine candidates was not evaluated *in vitro*. We have previously reported that differences in MVA promoter activity detectable *in vitro* does not correlate with *in vivo* immunogenicity (31), and that only *in vivo* expression correlates with the *in vivo* immunogenicity.

Humoral Immunogenicity of ChAdOx1 based MERS-CoV vaccine candidates

To evaluate humoral immune responses to ChAdOx1 MERS with or without tPA, BALB/c mice were vaccinated with $1x10^8$ IU of ChAdOx1 intramuscularly. Serum samples from 14 and 28 d.p.i. were collected and evaluated by ELISA. Both vaccine candidates induced a high level of S1-specific antibodies (mean endpoint titre (Log_{10}) = 4.8 with tPA, 4.7 without tPA) , unlike the control vaccine, ChAdOx1 encoding enhanced green fluorescent protein (ChAdOx1-eGFP, mean endpoint titre (Log_{10}) = 1). These antibody levels were similar between the two candidates (with or without tPA) at day 14. However, at 28 d.p.i. ChAdOx1 MERS with tPA induced significantly higher S1-specific antibodies than ChAdOx1 MERS without tPA (mean endpoint titre (Log_{10}) = 5.13 with tPA, 4.6 without tPA, Figure 2A). Serum samples from day 28 were selected for MERSpp neutralisation assay. Serum antibodies induced by ChAdOx1 MERS with tPA showed significantly higher neutralisation activity than without tPA (mean titre IC_{90} (Log_{10}) = 2.8 with tPA, 2.2 without tPA; Figure 2B). In order to confirm that the psuedotyped virus neutralisation assay was producing biologically relevant results, serum samples from mice immunised with ChAdOx1 MERS with tPA were also tested in a neutralisation assay utilising wildtype MERS virus. This assay confirmed the neutralisation activity of mouse antibodies (nAb) with a median of 360 VNT (Virus Neutralization Test antibody titre; Figure 2C). We therefore continued to evaluate ChAdOx1 MERS with tPA in addition to generating MVA MERS vaccine candidates with tPA.

Cellular Immunogenicity of ChAdOx1 based MERS-CoV vaccine candidates

Having established the utility of tPA in ChAdOx1 MERS vaccines (referred to as ChAdOx1 MERS in the rest of this report) at increasing humoral responses, spleens were collected at 28 d.p.i. from immunised BALB/c mice. Splenocytes were processed to evaluate cellular immune responses to ChAdOx1 MERS in ELISpot and Intracellular cytokine staining (ICS). Peptide S291, described by others (37), was used to re-stimulate the cells in both assays and ELISpot data showed a high level of IFN-γ secreting splenocytes

(Median = 1300 SFU/10^6 splenocytes; Figure 3A). ICS data confirmed the IFN-γ secreting $CD8^+$ splenocytes also secreted TNF-α and IL-17 (Figure 3B).

Immunogenicity of Heterologous ChAdOx1 and MVA vaccination against MERS-CoV

To evaluate humoral immune responses to heterologous prime-boost vaccination, BALB/c mice were immunised with ChAdOx1 MERS vaccine and boosted with one of two different MVA MERS vaccine candidates four weeks later. The MVA based candidates differ in the promoters that controls the transgene expression: F11-MVA MERS utilises the endogenous strong early F11 promoter and mH5-MVA MERS utilises the ectopic early/late mH5 promoter. Serum samples from 28 d.p.i. (post-prime) or 42 d.p.i. (post-boost) were collected and evaluated by ELISA and MERSpp neutralisation assay. At 28 d.p.i. ChAdOx1 MERS induced similar levels of S1-specific antibodies and nAb as observed previously (Figure 4A and B). At 42 d.p.i. S1-specific antibodies were boosted to a higher level (mean endpoint titre (Log_{10}) = 5 by ChAdOx1 MERS boosted to 5.8 by mH5-MVA MERS or 5.9 by F11-MVA MERS); Figure 4A) with nAb also enhanced to a statistically significant level (mean titre IC_{90} (Log_{10}) = 2.87 by ChAdOx1 MERS boosted to 3.3 by mH5-MVA MERS or 3.5 by F11-MVA MERS; Figure 4B). There was no difference in antibody levels induced using either the F11 or mH5 promoter in the MVA.

At 42 d.p.i. splenocytes were also processed to evaluate cellular immune responses to ChAdOx1 MERS MVA MERS prime-boost vaccination in ELISpot and ICS as shown in Figure 3. The T cell responses to MERS S were boosted by the MVA vaccinations; in the ICS experiments, F11-MVA and mH5-MVA boosted the percentage of IFN-γ^+ splenic $CD8^+$ T cells to 7.3 and 5.2% respectively (Figure 4D) whereas the percentage was 2.5% after ChAdOx1 MERS prime in Figure 3B. The percentage of TNF-α^+ splenic $CD8^+$ T cells were also increased by MVA boost (comparing Figure 3B and 4D). Utilising the F11 promoter resulted in a trend towards greater cell-mediated immunogenicity (Figure 4C and D). Splenocytes were also re-stimulated with MVA backbone-specific E3 and F(G)2 peptides and evaluated in ICS. Both MVA based vaccines induced similar responses to E3 or to F(G)2 peptides, 2 weeks after MVA vaccination (Figure 4E and F). This similarity confirmed the efficiency of vaccine titration, vaccination, and sample processing because responses to each of those peptides are not expected to be different unless there is variation in the doses administered or sample preparation. Overall, MVA MERS vaccines were able to boost the humoral and cellular immune responses to ChAdOx1 MERS prime vaccination. There was no difference between the F11 and mH5 promoter in the resulting antibody titres after ChAdOx1 prime/MVA boost, but there was a trend towards increased cellular immunogenicity when the F11 promoter was used.

Immunogenicity of Homologous MVA vaccination against MERS-CoV

To evaluate humoral immune responses to a homologous MVA MERS prime-boost vaccination, two groups of BALB/c mice were immunised with F11-MVA MERS or mH5-MVA MERS and boosted with the same vaccine after three weeks. Serum samples from 21 d.p.i. (post-prime) or 42 d.p.i. (post-boost) were collected and evaluated in ELISA and MERSpp neutralisation assays. At 21 d.p.i. F11-MVA MERS and mH5-MVA induced similar levels of S1-specific antibodies (mean endpoint titre (Log_{10}) = 3.2 and 2.8 respectively; Figure 5A). At 42 d.p.i S1-specific antibody levels had increased to 4.7 and 4.8 respectively (Figure 5A). The titres of nAb (MERS pp assay) were also similar for both vaccines (mean titre IC_{90} (Log_{10}) = 2.71 (F11-MVA MERS) and 2.76 respectively; Figure 5B). Utilising different promoters in MVA vectors did not result in differences in the induced antibody levels. However, at 42 d.p.i. IFN-γ secreting splenocytes induced by F11-MVA MERS were statistically significantly higher than those of mH5-MVA MERS ((Median = 525 and 249 SFU/10^6 splenocytes, respectively, Figure 5C). Both MVA vaccines induced similar vector-specific immune responses as expected (Figure 5D and E).

Discussion

Vaccines against MERS-CoV have been developed and tested in a number of animal models (including non-human primates (42-44) and camels (45)) as well as in human clinical trials (46). All vaccine candidates focused on the spike antigen because it contains the receptor-binding domain used for cell entry by the virus, against which neutralising antibodies may be induced, and it is conserved. Therefore, the improvement of MERS-CoV vaccines focuses on platform and vaccination regimens rather than antigen selection and optimisation. Here, we focused on using the same antigen (transgene) to develop a vaccine against MERS-CoV, and to assess different vectors, different versions of each vector, and different vaccination regimens. We generated a number of MERS-CoV vaccine candidates based on the same codon optimized spike transgene and ensured its expression *in vitro* before we evaluated the humoral and cellular immunogenicity in a pre-clinical BALB/c mouse model. ChAdOx1 based vaccine candidates were produced with or without tPA. The tPA signal peptide was predicted to enhance the humoral immunogenicity of encoded vaccine antigens, based on previous reports (29). Our data supported this hypothesis and showed a significant increase in the S1-specific antibody levels at 28 d.p.i. The level of neutralising antibodies was also increased when tPA was utilised. However, ChAdOx1 MERS without tPA was still a potent vaccine candidate, inducing a high level of both S1-specific binding antibodies and MERS-CoV neutralising antibodies. Neutralisation activity of mouse serum antibodies was assayed by using MERS-CoV pseudotyped viral particles (MERSpp), an approach used by a number of researchers for other human pathogens such as HIV, Influenza, and HCV to overcome the necessity of handling BSL-3 viruses (40). Additionally, we confirmed the ability of serum samples from vaccinated mice to neutralise live MERS virus. We therefore selected ChAdOx1 MERS with tPA (simply referred to ChAdOx1 MERS) for further evaluation.

ChAdOx1 MERS also induced cellular responses for MERS S, with polyfunctional $CD8^+$ T cells detected in the spleen of immunized mice. This supports the potency of the ChAdOx1 viral vector in inducing T cellular immunity, observed previously in animal models (26, 32, 47) as well as in humans (33). Following ChAdOx1 prime/MVA boost, MVA significantly boosted the neutralizing antibody titres to higher levels. No difference in humoral immunity was found when either the F11 or mH5 promoter was used. Regarding the promoter effect on MVA cellular immunogenicity, we have previously reported that utilising the F11 promoter enhanced malaria and influenza antigens in MVA (31). Here, we again report that F11-MVA MERS induced higher T cell responses than mH5-MVA MERS in a homologous prime-boost MVA MERS vaccination.

All of our vaccine candidates induced humoral (with nAb) and cellular immune (with polyfunctional $CD8^+$ T cell) responses against MERS-CoV spike antigen. Modest effects on immunogenicity of different versions of the vaccines were noted, with the use of the tPA leader sequence in ChAdOx1, and the use of the F11 promoter in MVA producing small increases in immunogenicity compared to no leader sequence, or the mH5 promoter. The protective level of either antibodies or cellular immunity required to counter MERS-CoV infection in humans or in animal models is not yet defined, despite some efforts (48-51). The ideal vaccine would provide rapid onset of immunity and complete protective efficacy after a single dose, with a long duration of immunity. Complete protective efficacy of one dose of ChAdOx1 expressing the external glycoprotein of Rift Valley Fever Virus has been demonstrated in multiple species and it is already known that ChAdOx1 RVF is highly immunogenic in camels (32). To date, the only vaccine against MERS to be tested in camels is an MVA vectored vaccine (41) which was protective in hDPP4 transgenic mice immunized with a homologous prime/boost regimen (37) but in camels required two doses given both intranasally and intramuscularly to provide partial protection and reduction of virus shedding (45). Here we find that a single dose of ChAdOx1 MERS is as immunogenic as two doses of MVA MERS, suggesting that this regimen should be tested for protective efficacy in camels.

However if this is not completely protective, administration of MVA MERS as a heterologous boost should be considered next. In our hands one dose of MVA resulted in an endpoint titre of 3 logs, two doses of MVA produced 4.7 logs, one dose of ChAdOx1 produced 5 logs, and ChAdOx1/MVA prime boost produced 5.9 logs. If a single dose of ChAdOx1 MERs is not protective and a two dose regimen is required, ChAdOx1/MVA would be more likely to provide complete protection than MVA/MVA.

ChAdOx1 MERS should now be evaluated for immunogenicity and efficacy in larger animal species, including both camels and humans.

Figure legends

Figure 1: Construction of MERS-CoV vaccine candidates

A: schematic representation of ChAdOx1 and MVA based vaccines, each encodes the same MERS-CoV spike gene (MERS-CoV S). The S gene was inserted into the E1 region of ChAdOx1 genome or into the *F11L* locus of MVA genome. tPA: Human tissue plasminogen activator (tPA) signal peptide sequence. IE CMV: The human cytomegalovirus major immediate early promoter. mH5 and F11: Poxviral promoters. LHA: left homology arm sequence. RHA: right homology arm sequence. **B:** The expression of spike transgene, cloned into a plasmid vector, was validated by transfection into an African green monkey kidney cell line (Vero cells) and confirmed by immunostaining. C: Untransfected cells control. Green colour represents detection of the spike protein. Blue colour represents nuclei by staining nucleic acid with DAPI.

Figure 2: Antibody responses to ChAdOx1 MERS vaccine candidates.

BALB/c mice (n = 6) were immunised with a single injection of ChAdOx1 MERS that either encodes or lacks tPA signal peptide, intramuscularly at 1x10^8 IU. A control group of mice were immunised with ChAdOx1 expressing eGFP instead of MERS-CoV S gene. Serum samples were collected at 14 and 28 days post immunisation (d.p.i.). S1-binding antibodies were detected at both time points by ELISA (A) and neutralisation activity of the antibodies were confirmed by MERS-CoV pseudotyped viral particles (MERSpp) neutralisation assay (B) or neutralisation assay (C). Individual data points are shown with line as the median. Data are representative of two independent experiments. Statistical significance by Kruskal–Wallis test is shown.

Figure 3: Cellular immune responses to ChAdOx1 MERS vaccine candidate.

BALB/c mice (n = 6) were immunised with a single injection of ChAdOx1 MERS that encodes tPA signal peptide intramuscularly at 1x10^8 IU. Twenty eight days post-immunisation, IFN-γ *ex vivo* ELISpot (A) or Intracellular Cytokine Staining (ICS (B)), were performed to determine the percentage of splenic IFN-γ secreting $CD4^+$ and $CD8^+$ after *in vitro* re-stimulation with a MERS-CoV S-specific peptide. Individual data points are shown with line as the median (A) or error bars as the SD (B). Data are representative of two independent experiments.

Figure 4: Humoral and cellular immunogenicity of heterologous ChAdOx1 MERS and MVA MERS vaccination.

BALB/c mice (n = 6) were immunised with ChAdOx1 MERS that encodes tPA signal peptide, intramuscularly at 1x10^8 IU. At 28 d.p.i. mice were boosted with MVA MERS at 1x10^6 pfu. MVA MERS candidates either contain mH5 or F11 promoter for transgene expression. Serum samples were collected at 28 (post-prime) and 42 (post-boost) d.p.i. S1-binding antibodies were detected at both time points by ELISA (A) and neutralisation activity of serum antibodies at 42 d.p.i. were confirmed by MERSpp neutralisation assay (B). At 42 d.p.i, IFN-γ *ex vivo* ELISpot (C) or Intracellular Cytokine Staining (ICS (D)) were performed to determine the percentage of $CD8^+$ IFN-γ^+ splenocytes after *in vitro* re-stimulation with a MERS-CoV S-specific peptide. ICS of splenocytes re-stimulated with MVA-specific peptides (F(G)2 and E3) was also performed (E and F). Individual data points are shown with line as the median. Data are representative of two independent experiments. Statistical significance by Kruskal–Wallis test is shown. Symbols are closed squares (▪) for ChAdOx1 prime responses, open circles (○) for mH5-MVA boost responses, and closed circles (●) for F11-MVA boost responses.

Figure 5: Humoral and cellular immunogenicity of homologous MVA MERS vaccination.

BALB/c mice (n = 6) were immunised with MVA MERS at 1x10^6 pfu, intramuscularly, in a homologous prime-boost vaccination with three-weeks interval. MVA MERS candidates either contain mH5 or F11 promoter for transgene expression. Serum samples were collected at 21 (post-prime) and 42 (post-boost) d.p.i. S1-binding antibodies were detected at both time points by ELISA (A) and neutralisation activity of serum antibodies at 42 d.p.i. were confirmed by MERSpp neutralisation assay (B). At 42 d.p.i splenocytes were processed and re-stimulated with a MERS-CoV S-specific peptide ($CD8^+$ T cell specific) for IFN-γ *ex vivo* ELISpot (C). ICS of splenocytes re-stimulated with MVA-specific peptides (F(G)2 and E3) was also performed (D and E) as was performed in figure 4. Individual data points are shown with line as the median. Data are representative of two independent experiments. Statistical significance by Kruskal–Wallis test is shown. Symbols are open circles (○) for mH5-MVA and closed circles (●) for F11-MVA.

Conflict of interest

SCG is a co-founder of, consultant to and shareholder in Vaccitech plc which is developing vectored influenza and MERS vaccines.

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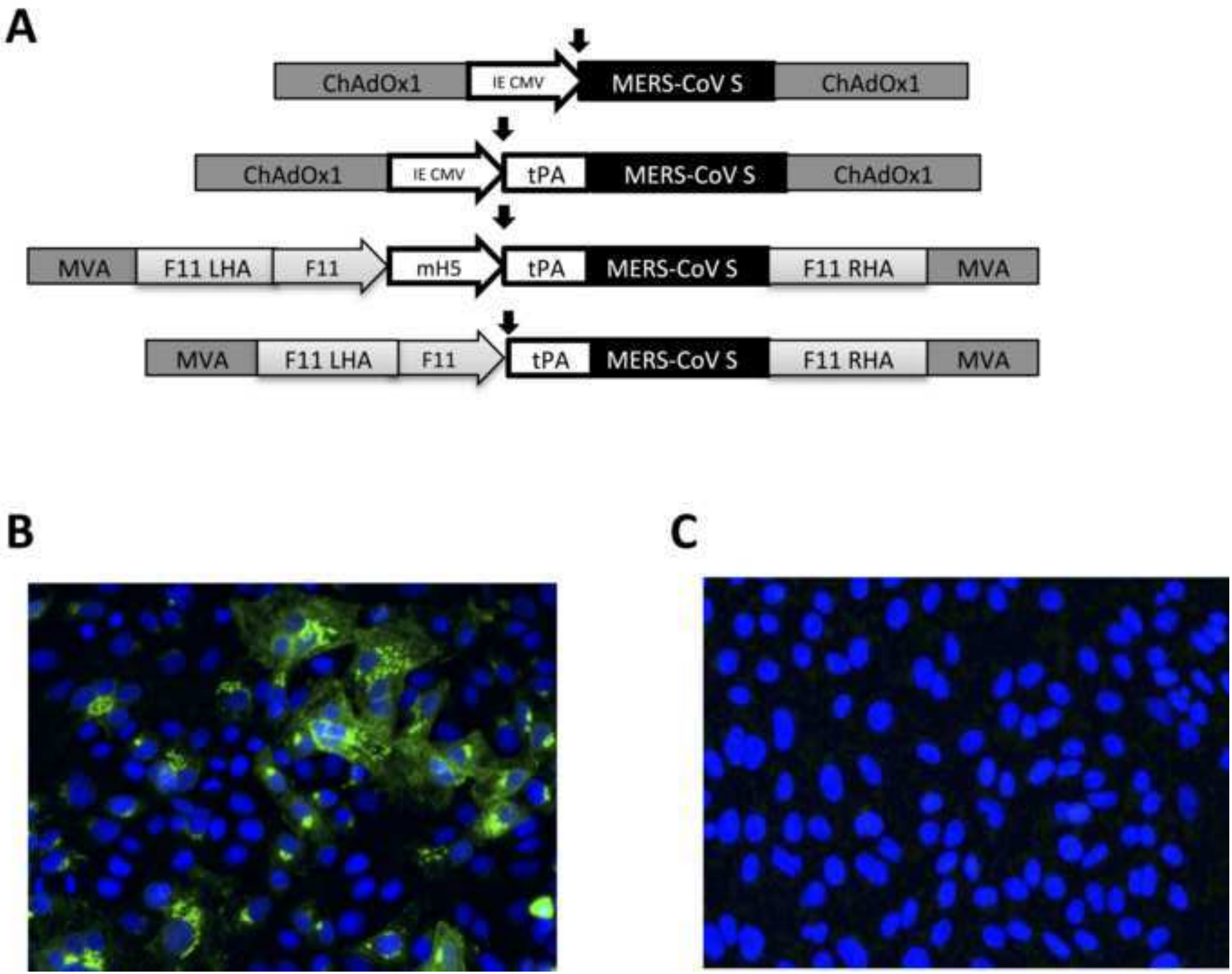

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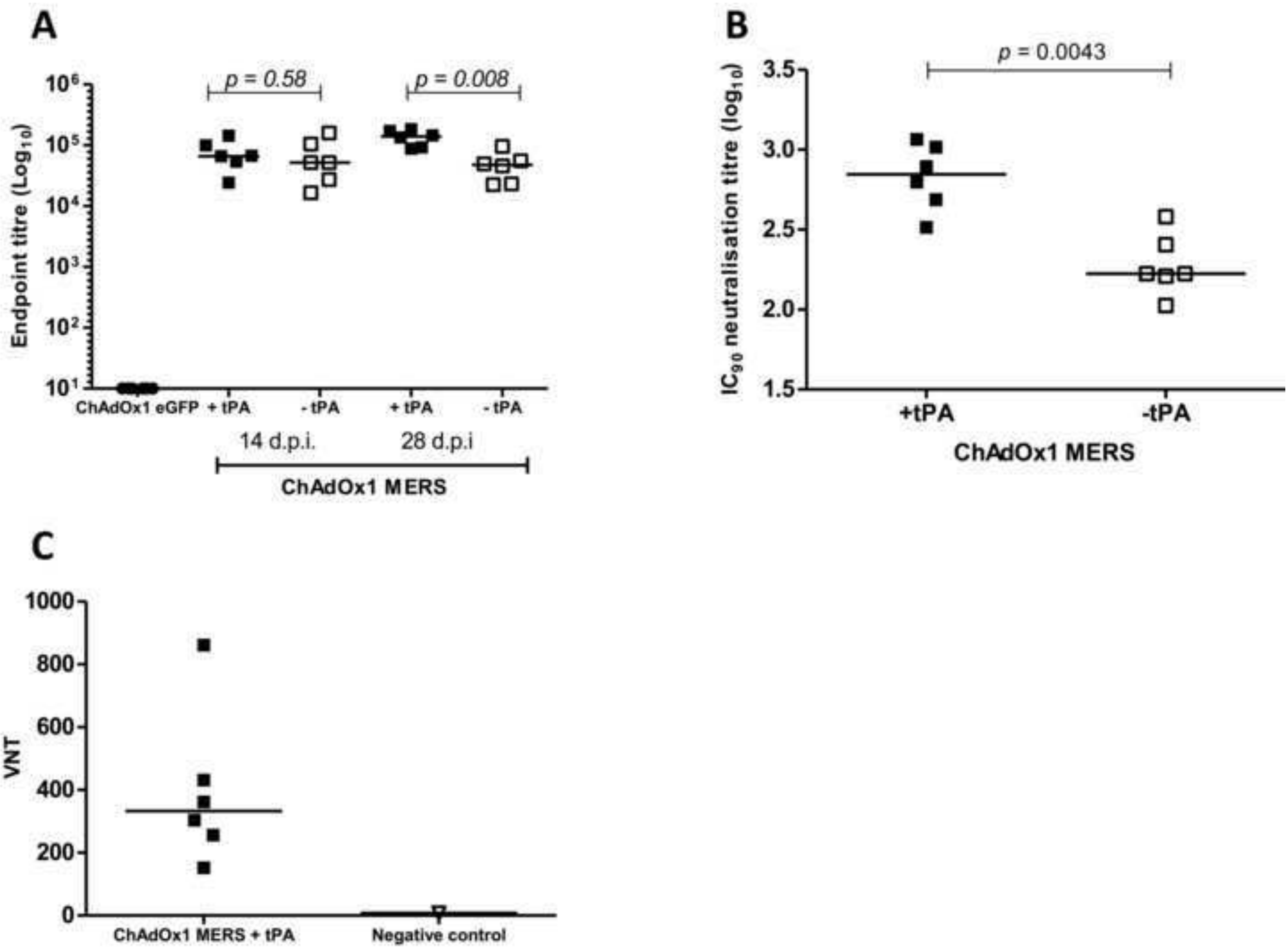

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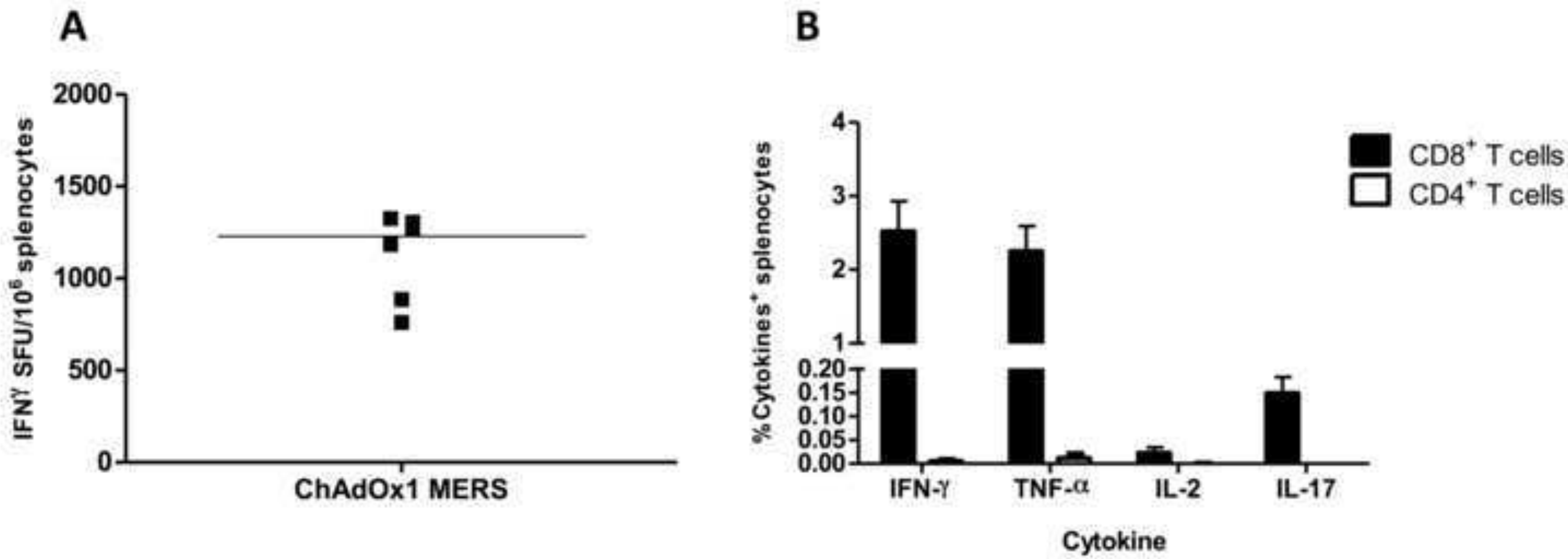

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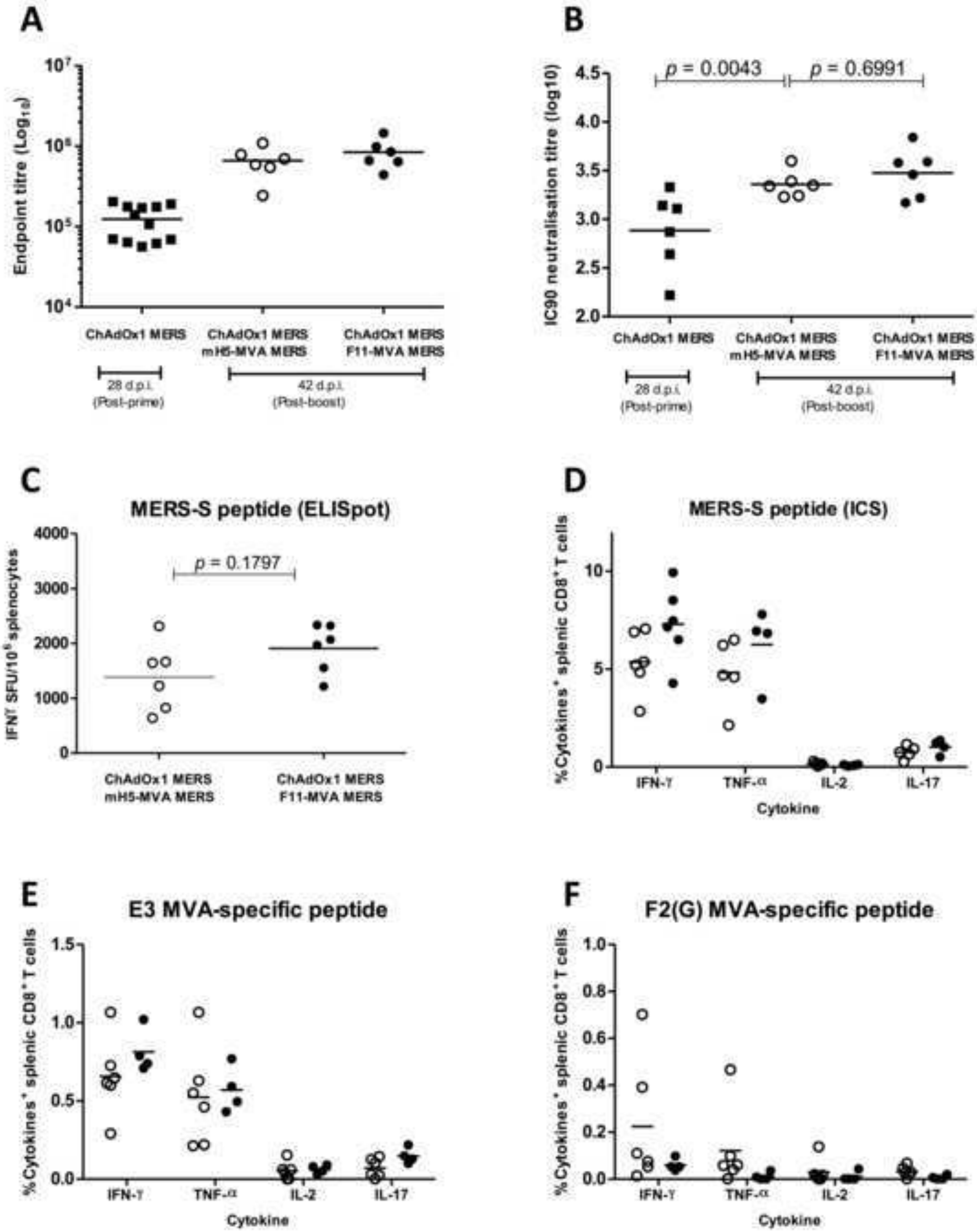

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