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# Distribution and absence of generalized lesions in mice following single dose intramuscular inoculation of the vaccine candidate MVA-MERS-S

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ABSTRACT

Modified Vaccinia Virus Ankara (MVA) is a highly attenuated and replication-deficient virus serving as vaccine against infectious diseases. Here, we assessed the *in vivo* distribution of a recombinant MVA candidate vaccine against the Middle Eastern Respiratory Syndrome (MVA-MERS-S) in mice. Intramuscularly inoculated mice were necropsied at different time points and examined by histology, immunohistochemistry and real-time PCR. We detected inflammation and myonecrosis at the parenteral site and hyperplasia of the draining lymph nodes. MVA-MERS-S did not result in detectable lesions in tissues peripheral to the parenteral site and draining lymph nodes. Real-time PCR analysis of > 240 tissue samples detected MVA-DNA predominantly at the injection site and in the draining lymph nodes, and suggested continuous clearance of the candidate vaccine during the observation period. Levels of parenteral site inflammation and hyperplasia of draining lymph nodes were considered in line with immunological responses to vaccine inoculation.

## 1. Introduction

Modified Vaccinia Virus Ankara (MVA) is a highly attenuated vaccinia virus serving as a well-established viral vector system used for developing vaccines against infectious diseases [1,2]. MVA is largely replication-deficient in mammalian cells, but grows well in chicken embryo fibroblasts [3,4]. Non-replicating MVA vaccines have an excellent safety profile in preclinical models using irradiated rabbits, immunosuppressed macaques, SCID and immunocompetent mice, and in clinical testing in humans using different inoculation routes [5–9].

A recombinant MVA vaccine expressing the full length spike protein of Middle Eastern Respiratory Syndrome Coronavirus (MVA-MERS-S) proved to be suitable for production at an industrial scale, immunogenic, and protective against MERS-CoV infections in mice and dromedaries [10,11]. Here we wished to generate additional preclinical data on the *in vivo* distribution of MVA-MERS-S in order to prepare for the evaluation of the MVA-MERS-S vector vaccine in a first-in-man phase I clinical trial. This 'biodistribution' of a candidate vaccine is important to estimate risks potentially associated with an *in vivo* application of the virus. Relevant aspects include monitoring for signs of virus replication and for potential side effects of the vaccination in the selected animal model. For instance, a dispersion of virus from the parenteral site of inoculation to excretory organs could be indicative of possible viral shedding and spillover into the general environment. Although MVA has been investigated as a safe vector vaccine candidate for years, there are not many studies regarding distribution in sites peripheral to the administration site.

In this study, we assessed the distribution of MVA-MERS-S after intramuscular application in the mouse model using histology, immunohistochemistry, and PCR to detect virus-associated lesions, viral and recombinant antigen *in situ,* and viral DNA.

## 2. Materials & methods

### 2.1. Animals

C57BL/6N mice (6–10 weeks old) were maintained under specified pathogen-free conditions. Animals were allowed to adjust to the facilities (one week) before vaccination experiments were performed and had free access to food and water. Experiments were in compliance with the German regulations for animal experimentation (Animal Welfare Acts).

Mice were inoculated by intramuscular injection with $10^7$ (PCR-study) or $10^8$ plaque-forming units (pfu, histology-study) of MVA-MERS-S or a control recombinant MVA-GFP-mCherry [12] or PBS into the thigh. Mice were monitored daily for signs of disease and were

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**Table 1**
Distribution studies of MVA-MERS-S using qPCR and histology.

**A** C57BL/6N mice

| Group | Treatment | Animals | | Necropsy, animals per time point post inoculation | | | | | | | | Organs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Single i.m. inoculation | ♀ | ♂ | 16 h | 24 h | 2 d | 3 d | 4 d | 6 d | 7 d | 21 d | |
| qPCR | MVA-MERS-S | 10 | 17 | – | 6 | – | 6 | – | – | 6 | 9 | See Part B |
| | PBS | 10 | 3 | – | 4 | – | 3 | – | – | 3 | 3 | |
| Histology | MVA-MERS-S | 1 | 5 | 1 | 1 | 1 | 1 | 1 | 1 | – | – | See Fig. 1 |
| | MVA-GFP-mCherry | 1 | 5 | 1 | 1 | 1 | 1 | 1 | 1 | – | – | |
| | PBS | 4 | – | – | 1 | – | 1 | – | 2 | – | – | |

**B** Organ real-time PCR results

| | Time point/Vaccine | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 24 h | | 3 d | | 7 d | | 21 d | |
| Organ/Tissue | MVA-MERS-S | PBS | MVA-MERS-S | PBS | MVA-MERS-S | PBS | MVA-MERS-S | PBS |
| Gonads | 0/6 | 0/4 | 0/6 | 0/3 | 0/6 | 0/3 | 0/9 | 0/3 |
| Kidneys | 0/6 | 0/4 | 0/6 | 0/3 | 0/6 | 0/3 | 0/9 | 0/3 |
| Liver | 0/6 | 0/4 | 1/6[a] | 0/3 | 0/6 | 0/3 | 0/9 | 0/3 |
| Lung | 1/6[a] | 0/4 | 0/6 | 0/3 | 0/6 | 0/3 | 1/9[a] | 0/3 |
| Rectum + Feces | 0/6 | 0/4 | 0/6 | 0/3 | 0/6 | 0/3 | 0/9 | 0/3 |
| Spleen | 0/6 | 0/4 | 0/6 | 0/3 | 0/6 | 0/3 | 0/9 | 0/3 |
| Urinary Bladder | 0/6 | 0/4 | 0/6 | 0/3 | 0/6 | 0/3 | 0/9 | 0/3 |

[a] Cycle threshold (Ct) > 36.

euthanized and necropsied at different time points (Table 1).

### 2.2. Necropsy, qPCR, histology and immunohistochemistry

A full necropsy was performed and organ samples for PCR and histology/immunohistochemistry were taken. An orthopoxvirus-specific TaqMan-PCR-assay was used to detect viral-DNA [13]. Samples (Table 1A) were frozen at −80 °C until DNA was isolated using the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instructions. For real-time PCR (qPCR) the QuantiTect probe PCR kit (Qiagen) was used. A quantification cycle cut-off ≤38 was regarded specific.

For histology, tissues (Fig. 1) were paraformaldehyde-fixed, paraffin-embedded, cut and sections were stained with hemalum-eosin. Skull, rectum and urinary bladder were taken from 48 h on. Skull and whole thigh were decalcified.

Anti-VACV-immunohistochemistry (IHC) was performed on all tissues as previously described [14] with heat-induced epitope-retrieval. Anti-MERS-S-IHC was performed using a polyclonal rabbit serum (1:1000; Sino Biological, 100208 RP-02) without epitope-retrieval. IHC positive and negative controls were included.

## 3. Results

### 3.1. Histological analyses

#### 3.1.1. MVA-MERS-S inoculated animals

Macroscopic examination revealed swelling of the left thigh with muscular pallor, mild edema and hemorrhage, and swelling of draining lymph nodes. Macroscopic lesions in other organs attributable to MVA inoculation were not observed.

Histologically, at all time points, there was absence of lesions attributable to MVA inoculation in any tissue other than the parenteral site and draining lymph nodes (Fig. 1). At the early time points, there was separation of muscle fibers through inoculum and edema, muscle fiber degeneration and necrosis at the parenteral site. Interstitial areas, adjacent adipose tissue and inoculum were predominantly infiltrated by many neutrophils. At the late time points there was evidence of muscular regeneration, characterized by myoblast tubes and internalized nuclei and many predominantly mononuclear infiltrates in the interstitia and surrounding the inoculum. The draining lymph nodes revealed increased paracortical lymphocytic cellularity (hyperplasia).

VACV antigen was detected only at the parenteral site. Other tissues including draining lymph nodes revealed no VACV antigen. VACV antigen was located in the cytoplasm of spindle cells in the interstitium (Fig. 2) and phagocytic cells (macrophages and neutrophils) in the interstitium or surrounding inoculum. MERS-S antigen was detected in MVA-MERS-S inoculated animals only at the parenteral site in the cytoplasm of similar spindle cells with a comparable distribution pattern (Fig. 2). Draining lymph nodes displayed no specific MERS-S antigen-staining.

#### 3.1.2. MVA-GFP-mCherry inoculated animals

The distribution and severity of lesions were remarkably similar to the MVA-MERS-S inoculated animals. Macroscopic and histologic examinations revealed similar findings in the parenteral site and draining lymph nodes. Acute inflammation, characterized by edema and many infiltrating neutrophils with myofiber degeneration and necrosis were prevalent at the early time points. At later time points, the infiltrate became predominantly mononuclear and there was evidence of muscle regeneration. Macroscopic and microscopic lesions in other organs attributable to MVA inoculation were not observed at any time point. VACV-antigen was only detected at the parenteral site within the cytoplasm of phagocytic and spindle cells (Fig. 2). Other tissues including draining lymph nodes revealed no VACV antigen.

#### 3.1.4. Saline inoculated animals

Macroscopic examination revealed no lesions. Histological analysis displayed mild injection-associated lesions at the parenteral site consisting of band-like degeneration of single muscle fibers, regeneration at later time points and focal scarce interstitial leukocytic infiltrates. The subiliac lymph node of one animal revealed mildly increased paracortical cellularity. Histological changes attributable to inoculation were not detected in any other tissue in any animal. A specific reaction product was not observed in either of the two IHCs.