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Attenuation and immunogenicity of host-range extended modified vaccinia virus Ankara recombinants

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ABSTRACT

Modified vaccinia virus Ankara (MVA) is being widely investigated as a safe smallpox vaccine and as an expression vector to produce vaccines against other infectious diseases and cancer. MVA was isolated following more than 500 passages in chick embryo fibroblasts and suffered several major deletions and numerous small mutations resulting in replication defects in human and most other mammalian cells as well as severe attenuation of pathogenicity. Due to the host range restriction, primary chick embryo fibroblasts are routinely used for production of MVA-based vaccines. While a replication defect undoubtedly contributes to safety of MVA, it is worth considering whether host range and attenuation are partially separable properties. Marker rescue transfection experiments resulted in the creation of recombinant MVAs with extended mammalian cell host range. Here, we characterize two host-range extended rMVAs and show that they (i) have acquired the ability to stably replicate in Vero cells, which are frequently used as a cell substrate for vaccine manufacture, (ii) are severely attenuated in immunocompetent and immunodeficient mouse strains following intranasal infection, (iii) are more pathogenic than MVA but less pathogenic than the ACAM2000 vaccine strain at high intracranial doses, (iv) do not form lesions upon tail scratch in mice in contrast to ACAM2000 and (v) induce protective humoral and cell-mediated immune responses similar to MVA. The extended host range of rMVAs may be useful for vaccine production.

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1. Introduction

Modified vaccinia virus Ankara (MVA) is a host-range restricted, highly attenuated vaccine strain that was obtained by passaging chorioallantoic vaccinia virus Ankara (CVA) >500 times in primary chick embryo fibroblasts (CEF) [1–4]. In addition to serving as an attenuated smallpox vaccine, MVA has potential as a safe vector for recombinant vaccines against other microbial pathogens and cancer [5–8]. Despite the current interest in MVA and the availability of the complete genome sequence [9], the basis for the host range restriction is incompletely understood. Although MVA replication is deficient in human and most other mammalian cells, growth can occur in baby hamster kidney 21 cells [10,11] and fruit bat cells [12]. Unlike other vaccinia virus (VACV) host range mutants [13], the replication defect of MVA is manifested by formation of aberrant non-infectious virus particles with no impairment of viral protein synthesis in non-permissive cells [5]. Severe attenuation coupled with robust gene expression has made MVA an extremely useful vector.

There are six major deletions in the MVA genome, comprising about 15% of the total DNA [14,15] as well as numerous smaller mutations [9]. The region of the MVA genome responsible for the host range defect was interrogated by carrying out homologous recombination with a panel of cosmids prepared from a replication-competent VACV strain and assessing plaque formation in African green monkey BS-C-1 cells, which are marginally permissive for MVA [16]. Recombinant MVAs (rMVAs) derived from three overlapping cosmids, each containing approximately 40 kbp of DNA near the left end of the VACV genome, exhibit enhanced MVA replication in monkey, human and rabbit cells. Two of the host-range extended viruses, rMVA 51.1 and rMVA 44/47.1, were derived by recombination with one and two cosmids, respectively, and share some newly acquired DNA [16]. In another approach, DNA sequences corresponding to the six major deletions of MVA were removed from the parental CVA [17]. However, these deletions failed to confer mammalian host range restriction or strong attenuation, indicating that other genetic changes are responsible for the MVA phenotype. On the other hand, introducing a deletion corresponding to one near

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the left end of MVA into the Lister strain of VACV reduced replication in human cells and a combination of multiple MVA deletions provided attenuation [18]. Further work is needed to determine the genetic basis for MVA host restriction and the contribution of this property to attenuation.

While the inability of MVA to replicate in human and most other mammalian cells can account for the absence of pathogenicity, MVA contains mutations of immune evasion and other genes that could contribute to attenuation even in host-range extended rMVAs. Thus, we were interested in determining the degree of attenuation of rMVA 51.1 and rMVA 44/47.1 in mice. In addition, the host range restriction of MVA limits the cell substrates that can be used for vaccine manufacture. Vero cells, which are widely used to make vaccines, would be an attractive alternative to primary CEF for production of MVA-based vaccines. The abilities of rMVA 44/47.1 and rMVA 51.1 to replicate in BS-C-1 cells [16] encouraged us to consider that they would also replicate in Vero cells. Here we demonstrate that rMVA 44/47.1 and rMVA 51.1 can be propagated in Vero cells, are greatly attenuated in immunocompetent and immunodeficient mice, and induce protective humoral and cell mediated immune responses similar to those of MVA.

2. Materials and methods

2.1. Cells and viruses

Primary chick embryo fibroblasts (CEF) and continuous cell lines were maintained at 37 °C with 5% CO_2 in modified Eagle minimal essential medium (Quality Biologicals, Inc., Gaithersburg, MD) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 10 units of penicillin/ml and 10 μg of streptomycin/ml. VACV strains ACAM2000, Western Reserve (WR) and "Ankara" were grown, purified and titrated as described previously [19,20]. ACAM2000 (Lot number: VV04-003-A) was grown and titrated on BS-C-1 cells. VACV Ankara, used to construct the cosmid library for marker rescue and formation of rMVAs was originally understood to be the parent strain of MVA [16]. However, sequence analysis (J. Mendez-Rios, personal communication) indicates that VACV Ankara does not correspond to the sequence of CVA [21], the actual progenitor of MVA, and may be related to the Copenhagen vaccine strain. However, for consistency the name Ankara has been retained for this report. Generation and purification of rMVA 44/47.1 and rMVA 51.1 were described previously [16]. Titers of MVA, rMVAs and Ankara were determined by immunostaining in CEF as described [10,16]. All procedures with viruses were performed in a registered BSL-2 laboratory.

2.2. Virus replication in Vero cells

Vero cells (5×10^5) were infected with 0.1 plaque forming units (pfu)/cell in 12-well plates. After 1 h, the monolayers were washed twice and overlaid with fresh medium. At various times post-infection, cells from triplicate wells were harvested individually in 1 ml of medium and stored at −80 °C. The cells were then lysed and sonicated; cell-associated virus yields were determined by assay on CEF for MVA and rMVAs and on BS-C-1 cells for ACAM2000.

2.3. Mouse pathogenicity studies

Viruses were diluted in phosphate-buffered saline (PBS) containing 2% fetal bovine serum, and the virus concentration of each dilution used in animals was verified by plaque assay on the same day. Groups of 4–10, 5–6 weeks-old BALB/c, C57BL/6 or ICR-SCID female mice (Taconic Biotechnology, Germantown, NY) were inoculated with viruses by intranasal or intracranial routes. For intranasal infections, mice were anesthetized by inhalation of isoflurane and 20 μl (10^6 pfu) of virus was introduced into one nostril. For intracranial infections, mice were anesthetized with a mixture of ketamine (75 mg/kg) and xylazine (7.5 mg/kg) in PBS. A volume of 30 μl (10^4–10^8 pfu) of virus was injected with a syringe connected to a half-inch 27-gauge needle, which was inserted through the parietal bone of the skull at an angle of about 90° above the horizontal. A limiting tubing device was attached allowing the needle to penetrate not more than 2 mm under the bone. Animals were weighed and observed three to seven times per week for up to 47 days post-infection for ICR-SCID mice or 21 days for BALB/c or C57BL/6. Animals that lost 30% or more of their starting weight were euthanized in accordance with NIAID Animal Care and Use protocols. Experiments were performed in an ABSL-2 facility with approval of the NIAID Animal Care and Use Committee.

2.4. Immunogenicity studies

Intramuscular vaccination of 6 weeks-old mice with 10^5–10^8 pfu of virus and tail scratch with 10^4–10^8 pfu were carried out as described [22]. For intramuscular vaccination, the total 100 μl dose was divided and 50 μl was injected into the gastrocnemius muscles of each back leg. Blood collection from the mandibular plexus into serum collection tubes (BD Biosciences, San Jose, CA) was carried out at 3 weeks after vaccination. Serum was isolated from clotted blood samples by centrifugation as directed by the manufacturer. Challenge with 10^7 pfu of VACV Western Reserve (WR) by the intranasal route was performed as described above. Mock-infected control animals were inoculated with an equivalent volume of diluent.

2.5. Intracellular cytokine staining of mouse splenocytes

Procedures for preparation and staining of splenocytes were modified from the method previously described [23] as follows. Mouse P815 mastocytoma target cells, at a concentration of 10^7 cells/ml in RPMI medium were infected with 10 pfu/cell of MVA for 90 min at 37 °C, brought up to 10^6 cells/ml in RPMI medium and incubated 4–5 h at 37 °C. After washing, cells were suspended at 2.5×10^6 cells/ml. Splenocytes were prepared from individual mice one week post-vaccination. For stimulation, 1.5×10^6 splenocytes were mixed with 2.5×10^5 MVA-infected P815 cells. After 4 h at 37 °C, brefeldin A (Sigma, St. Louis, MO) was added to a concentration of 10 μg/ml, and the incubation was continued for 10–15 h. Cells were incubated for 10 min with Fc block (anti-CD16/CD32, clone 2.4G2, a gift from J. Bennink, Laboratory of Viral Diseases), and then stained with peridinin chlorophyll-a protein (PerCP) conjugated anti-CD8 (clone 53.6-7) for 30 min at room temperature. After fixation and permeabilization, the cells were stained with allophycocyanin (APC)-conjugated anti-gamma interferon (IFN-γ) (clone XMG 1.2), fluorescein isothiocyanate (FITC)-conjugated anti-interleukin-2 (IL-2) (clone JES6-5H4) and R-phycoerythrin (PE)-conjugated anti-tumor necrosis factor (TNFα) (clone MP6-XT22) for 1 h. Cells were then washed and suspended in 2% paraformaldehyde. All staining reagents were purchased from BD Biosciences (San Jose, CA). At least 100,000 cells were acquired on a FACSCalibur cytometer using CellQuest software (BD Biosciences) and analyzed with FlowJo software (TreeStar, Cupertino, CA).

2.6. VACV enzyme-linked immunosorbent assay (ELISA)

Microtiter plates (96-well, Thermo Labsystems, Franklin, VA) were coated with 10^7 pfu/ml of sucrose gradient purified MVA in