Published in final edited form as:
Adv Exp Med Biol. 1996 ; 397: 7–13.

HOST RANGE RESTRICTED, NON-REPLICATING VACCINIA VIRUS VECTORS AS VACCINE CANDIDATES

Bernard Moss[1], **Miles W. Carroll**[1], **Linda S. Wyatt**[1], **Jack R. Bennink**[1], **Vanessa M. Hirsch**[2], **Simoy Goldstein**[2], **William R. Elkins**[2], **Thomas R. Fuerst**[3], **Jeffrey D. Lifson**[3], **M Piatak**[3], **Nicholas P. Restifo**[4], **Willem Overwijk**[4], **Ronald Chamberlain**[4], **Steven A. Rosenberg**[4], and **Gerd Sutter**[1]

1 *Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases National Institutes of Health, Bethesda, Maryland 20892*

2 *Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases National Institutes of Health, Rockville, Maryland 20852*

3 *GeneLabs Technologies Redwood City, California 94063*

4 *Surgery Branch, National Cancer Institute National Institutes of Health, Bethesda, Maryland 20892*

1. INTRODUCTION

The use of a recombinant virus containing a heterologous gene of another microorganism as a live vaccine was suggested more than 10 years ago (Mackett et al., 1982; Panicali and Paoletti, 1982). Vaccinia virus was considered for such a purpose because of its success as a smallpox vaccine and ease and economy of production, distribution and administration (Fenner et al., 1988). The extensive experimental use of recombinant vaccinia viruses was facilitated by the construction of plasmid transfer vectors containing a vaccinia virus promoter, one or more convenient restriction endonuclease sites for inserting a foreign gene, flanking DNA sequences for homologous recombination into a non-essential site of the vaccinia virus genome and for selection and/or screening of recombinant viruses (Chakrabarti et al., 1985; Mackett et al., 1984). Humoral and cell mediated immune responses to an expressed foreign protein and protection of experimental animals against challenge with the corresponding pathogen were demonstated in a variety of animal model systems (Cox et al., 1992; Moss, 1991).

Initial testing of recombinant vaccinia viruses in humans has been reported. A first generation recombinant vaccinia virus AIDS vaccine was considered to be immunogenic and safe (Cooney et al., 1991). Nevertheless, the occurrence of rare adverse reactions to smallpox vaccination and the increased susceptibility of immunodeficient individuals has made further attenuation and improved safety a priority for new human vaccines based on vaccinia virus. Attenuation can be achieved by deleting genes that contribute to virulence but are non-essential for replication in tissue culture (Buller et al., 1988; Buller et al., 1985; Tartaglia el al., 1992) or by insertion of lymphokine genes (Flexner et al., 1987; Ramshaw et al., 1987). An alternative approach is to use one of several highly attenuated strains of vaccinia virus that were developed but not extensively used during the smallpox eradication campaign. One of these, known as modified vaccinia virus Ankara (MVA), is avirulent in normal or immunosuppressed animals and elicited no adverse reactions in 120,000 humans, many of whom were at risk for the conventional smallpox vaccine (Hochstein-Mintzel et al., 1972; Mayr and Danner, 1979; Mayr et al., 1975; Mayr et al., 1978; Stickl et al., 1974; Werner et al., 1980).

Edited by S. Cohen and A. Shafferman, Plenum Press, New York, 1996.

MVA was generated by over 500 passages of the parental strain in chicken embryo fibroblasts, during which it became severely host restricted and unable to propagate efficiently in mammalian cells. In this respect, MVA resembles Avipoxviruses which are also being developed as safe vaccines (Taylor et al., 1992). Compared to the parental vaccinia virus, MVA contains six major deletions of genomic DNA resulting in the loss of 30,000 base pairs (bp) or 15% of its genetic information (Meyer et al., 1991). The block in replication of MVA in human and other mammalian cells occurs at a step in virion assembly, allowing unimpaired expression of early and late viral or recombinant genes (Sutter & Moss, 1992). Thus, MVA is an efficient as well as a safe vector system. Here, we review examples of the use of recombinant MVA to protectively immunize against influenza virus, simian immunodeficiency virus (SIV), and neoplastic cells in animal model systems.

2. IMMUNIZATION WITH MVA

2.1. Immunization against Influenza Virus

Influenza virus infection of mice provides an experimental model for vaccination against a respiratory disease. Previous studies demonstrated that recombinant vaccinia viruses expressing the influenza virus hemagglutinin gene (*ha*) induced type-specific humoral and cell mediated immune responses and protectively immunized mice against a lethal influenza virus challenge (Andrew et al., 1986; Bennink et al., 1984). Recombinant vaccinia viruses expressing the influenza virus nucleoprotein gene (*np*) induced a less protective but cross-reactive CTL response (Andrew et al., 1986; Yewdell et al., 1985). To evaluate MVA as a candidate vaccine, both the *ha* and *np* regulated by vaccinia virus synthetic strong early/late promoters were inserted into the MVA genome to form MVA-INF$_{ha/np}$ (Sutter et al., 1994). Preliminary experiments verified that the genes were expressed and that the recombinant virus did not cause a spreading infection or discernible cytopathathology in monolayers of mouse L929 cells. Mice inoculated intramuscularly with MVA-INF$_{ha/np}$ developed humoral and CTL immune responses to influenza virus proteins in a dose-dependent manner. A single vaccination with 10^4 or more infectious units of MVA-INF$_{ha/np}$protected mice against a challenge with 100 times the lethal dose of influenza virus (Table 1; Sutter et al., 1994). Surprisingly, all parameters of immunity including protection were similar or better than those induced by standard intradermal vaccination with equivalent doses of replication competent vaccinia virus strain WR expressing influenza *ha* and *np.* (The lower dose of recombinant virus required for protection than for detectable hemagglutinin inhibition probably reflects different sensitivities of the *in vivo* and *in vitro* assays). Protection was also achieved by nasal vaccination, although higher vaccine doses were required (Table 1).

2.2. Immunization against SIV

SIV and human immunodeficiency virus (HIV) are closely related viruses with similar genome organizations and CD4 lymphocyte/macrophage tropism. Moreover, SIV causes an immunodeficiency disease in macaques that has many of the features of AIDS. For these reasons, SIV has been used as an HIV surrogate for vaccine studies. Good protection has been obtained by vaccination with live attenuated SIV (Daniel et al., 1992). Varying degrees of protection, perhaps partly due to differences in challenge strains, were observed with recombinant vaccinia viruses alone or combined with other immunogens (Giavedoni et al., 1993; Hu et al., 1992; Israel et al., 1994). MVA could provide a safer alternative to conventional vaccinia virus-based vaccines particularly in populations in which AIDS is prevalent.

A recombinant MVA (MVA-SIV$_{env/gag/pol}$)virus containing the complete envelope and gag-polymerase coding regions of HIV-1 regulated by a strong synthetic vaccinia virus early/late promoter and a moderate natural vaccinia virus early/late promoter, respectively, was constructed (Hirsch et al., 1995). For comparative purposes, the same SIV genes were inserted

into the Wyeth (WY) vaccine strain of vaccinia virus to generate WY-SIV$_{env/gag/pol}$. We verified that the SIV genes were expressed by both recombinant viruses in monkey BS-C-1 cells. Twelve juvenile rhesus macaques, divided into four groups, were immunized four times over a period of 28 weeks with MVA-SIV$_{env/gag/pol}$ (n = 4), WY-SIV$_{env/gag/pol}$, (n = 4), MVA control virus (n = 2) or WY control virus (n = 2). After 44 weeks, animals receiving either recombinant virus were also vaccinated with 250 μg of whole SIV, inactivated with psoralen and ultraviolet light (Johnson et al., 1992), in saline. No visible lesions were formed after the intramuscular inoculations of 5×10^8 infectius units of MVA or MVA-SIV$_{env/gag/pol}$, whereas typical cutaneous lesions occured after the first intradermal injection of 10^8 infectious units of WY viruses. MVA-SIV$_{env/gag/pol}$ induced a sustained antibody response to both env and gag proteins whereas WY-SIV$_{env/gag/pol}$ induced detectable antibody only to the envelope protein. *In vitro* neutralizing activity to SIV was transient, peaking after the second recombinant virus administration, and was not enhanced by the inactivated whole SIV.

Four weeks after the final boost, all macaques were challenged by intravenous injection with 50 monkey infectious doses of cell-free, uncloned, homologous SIV (sm/E660) that had been generated in macaque peripheral blood lymphocyte cultures (Goldstein et al., 1994). The results of the SIV challenge are summarized (Table 2; Hirsch et al., 1995). All of the control animals, immunized with non-recombinant MVA or WY strains of vaccinia virus, were infected and three had severe disease requiring them to be sacrificed at 14,22, and 54 weeks post challenge; the fourth appears healthy with a low virus load and a normal CD4 count. In contrast to the control animals, the macaques that had been vaccinated with recombinant viruses all displayed a rapid anamnestic antibody response to SIV. Although the vaccinated macaques were infected with SIV, virus replication was restricted particularly in three of the four that received MVA-SIV$_{env/gag/pol}$ In the latter, plasma viremia was absent, the viral load in peripheral blood mononuclear cells was reduced, lymph nodes contained 1 % or less of the virus from control animals and the architecture was normal, the CD4 counts were maintained, and the animals are still healthy after 62 weeks. The fourth MVA-SIV$_{env/gag/pol}$ immunized macaque has lymphadenopathy and a low CD4 count. The group vaccinated with WY-SIV$_{env/gag/pol}$ was less well protected than the group vaccinated with MVA-SIV$_{env/gag/pol}$ three of the four macaques had to be sacrificed between 51 and 58 weeks because of secondary infections and the fourth has lymphadenopathy and a low CD4 count. At this time, we cannot determine whether inherent genetic differences between the vectors accounted for the better immunity induced by recombinant MVA compared to recombinant WY or whether differences in the dose or route of inoculation were important. Further vaccine trials with MVA-SIV$_{env/gag/pol}$ are in progress.

2.3. Immunization against Neoplastic Cells

Tumor-associated antigens, that are recognized by CD8$^+$ CTL, are potential targets for cancer immunotherapy. To evaluate MVA as a vector for tumor antigens, we used a murine model system that was previously tested with recombinant vaccinia and fowlpox viruses (Bronte et al., 1995; Wang et al., 1995). The BALB/c colon carcinoma cell line CT26.WT was stably transfected with the *Escherichia coli lacZ* gene, which encodes (β-galactosidase, to generate CT26.CL25. BALB/c mice, immunized intramuscularly with MVA expressing the *lacZ* model tumor antigen (MVA-βgal), were protected against intravenous challenge with a lethal number of CT26.CL25 cells (Table 3). Pulmonary metastases and death occurred in control animals vaccinated with MVA-βgal and challenged with CT26.WT or vaccinated with parental MVA and challenged with CT26.CL25 (Table 3). Therefore, protection was specific for virus and cell-lines expressing the model tumor antigen. In treatment experiments using mice bearing 3-day established pulmonary tumors, either prolonged survival or a reduction in the number of metastases was obtained by immunization with MVA-βgal or by adoptive transfer of *in vitro* stimulated splenocytes from normal mice vaccinated with MVA-βgal. Comparative studies

suggested that MVA-βgal might be more effective than WR-βgal (a replication-competent vaccinia virus expressing β-galactosdiase) when used for treatment of established tumors.

3. SUMMARY

Three model sytems were used to demonstrate the immunogenicity of highly attenuated and replication-defective recombinant MVA. (1) Intramuscular inoculation of MVA-IN-$F_{ha/np}$ induced humoral and cell-mediated immune responses in mice and protectively immunized them against a lethal respiratory challenge with influenza virus. Intranasal vaccination was also protective, although higher doses were needed. (2) In rhesus macaques, an immunization scheme involving intramuscular injections of MVA-$SIV_{env/gag/pol}$ greatly reduced the severity of disease caused by an SIV challenge. (3) In a murine cancer model, immunization with MVA-βgal prevented the establishment of tumor metastases and even prolonged life in animals with established tumors. These results, together with previous data on the safety of MVA in humans, suggest the potential usefulness of recombinant MVA for prophylactic vaccination and therapeutic treatment of infectious diseases and cancer.

References

Andrew ME, Coupar BEH, Ada GL, Boyle DB. Cell-mediated immune response to influenza virus antigens expressed by vaccinia virus recombinants. Microb Path 1986;1:443–452.

Bennink JR, Yewdell JW, Smith JW, Moller C, Moss B. Recombinant vaccinia virus primes and stimulates influenza virus HA-specific CTL. Nature 1984;311:578–579. [PubMed: 6332992]

Bronte V, Tsung K, Rao JB, Chen PW, Wang M, Rosenberg SA, Restifo NP. 1L-2 enhances the function of recombinant poxvirus-based vaccines in the treatment of established pulmonary metastases. J Immunol 1995;154:5282–5292. [PubMed: 7730632]

Buller RM, Chakrabarti S, Cooper JA, Twardzik DR, Moss B. Deletion of the vaccinia virus growth factor gene reduces virus virulence. J Virol 1988;62:866–877. [PubMed: 3339716]

Buller RML, Smith GL, Cremer K, Notkins AL, Moss B. Decreased virulence of recombinant vaccinia virus expression vectors is associated with a thymidine kinase-negative phenotype. Nature 1985;317:813–815. [PubMed: 4058585]

Chakrabarti S, Brechling K, Moss B. Vaccinia virus expression vector: Coexpression of β-galactosidase provides visual screening of recombinant virus plaques. Mol Cell Biol 1985;5:3403–3409. [PubMed: 3939316]

Cooney EL, Collier AC, Greenberg PD, Coombs RW, Zarling J, Arditti DE, Hoffman MC, Hu SL, Corey L. Safety of and immunological response to a recombinant vaccinia virus vaccine expressing HIV envelope glycoprotein. Lancet 1991;337:567–572. [PubMed: 1671940]

Cox, WI.; Tartaglia, J.; Paoletti, E. Poxvirus recombinants as live vaccines. In: Binns, MM.; Smith, GL., editors. Recombinant poxviruses. CRC Press; Boca Raton: 1992. p. 123-162.

Daniel MD, Kirchhoff F, Czajak SC, Sehgal PK, Desrosiers RC. Protective effects of a live attenuated SIV vaccine with a deietion in the nef gene. Science 1992;258:1938–1941. [PubMed: 1470917]

Fenner, F.; Henderson, DA.; Arita, I.; Jezek, Z.; Ladnyi, ID. Smallpox and its eradication. World Health Organization; Geneva: 1988.

Flexner C, Hugin A, Moss B. Prevention of vaccinia virus infection in immunodeficient nude mice by vector-directed IL-2 expression. Nature 1987;330:259–262. [PubMed: 3118219]

Giavedoni LD, Planelles V, Haigwood NL, Ahmad S, Kluge JD, Marthas ML, Gardner MB, Luciw PA, Yilma TD. Immune response of rhesus macaques to recombinant simian immunodeficiency virus-gp130 does not protect from challenge infection. J Virol 1993;67:577–583. [PubMed: 8416384]

Goldstein S, Elkins WR, London WT, Hahn A, Goeken R, Martin JE, Hirsch VM. Immunization with whole inactivated vaccine protects from infection by SIV grown in human but not macaque cells. J Med Primatol 1994;23:75–82. [PubMed: 7966237]

Hirsch VM, Goldstein S, Chanock R, Elkins WR, Sutter G, Moss B, Sisler J, Lifson J, Fuerst T. Limited virus replication following SIV challenge of macaques immunized with attenuated MVA vaccinia expressing SIVsm *env* and *gag-pol*. Vaccines 1995;95:195–200.

Hochstein-Mintzel V, Huber HC, Stickl H. Virulenz und immunogenitat eines modiftzierten vaccinia-virus (Stamm MVA). Z Immun-Forsch 1972;144:140–145.

Hu SL, Abrams K, Barber GN, Moran P, Zarling JM, Langlois AJ, Kuller L, Morton WR, Beneviste RE. Protection of macaques against SIV infection by subunit vaccines of SIV envelope glycoprotein gp160. Science 1992;255:456–459. [PubMed: 1531159]

Israel ZR, Edmonson PF, Maul DH, O'Neill SP, Mossman SP, Thiriart C, Fabry L, Van Opstal O, Bruck C, Bex F, Burny A, Fultz PN, Mullins JI, Hoover EA. Incomplete protection, but suppression of virus burden, elicited by subunit simian immunodeficiency virus vaccines. J Virol 1994;68:1843–1853. [PubMed: 8107246]

Johnson PR, Montefiori DC, Goldstein S, Hamm TE, Zhou JY, Kitov S, Haigwood NL, Misher L, London WT, Gerin JL, Allison A, Purcell RH, Chanock RM, Hirsch VM. Inactivated whole SIV vaccine in Macaques - evaluation of protective efficacy against challenge with cell-free virus or infected cells. AIDS Res Human Retroviruses 1992;8:1501–1505. [PubMed: 1466990]

Mackett M, Smith GL, Moss B. Vaccinia virus: a selectable eukaryotic cloning and expression vector. Proc Natl Acad Sci, USA 1982;79:7415–7419. [PubMed: 6296831]

Mackett M, Smith GL, Moss B. General method for production and selection of infectious vaccinia virus recombinants expressing foreign genes. J Virol 1984;49:857–864. [PubMed: 6321770]

Mayr A, Danner K. Bedeutung von tierpocken für den menschen nach aufhebung der pflichtimpfung gegen pocken. Berl Münch Tierärzil Wochenschr 1979;92:251–256.

Mayr A, Hochstein-Mintzel V, Stickl H. Abstammung, eigenschaften und verwendung des attenuierten vaccinia-stammes MVA. Infection 1975;3:6–14.

Mayr A, Stickl H, Müller HK, Danner K, Singer H. Pockenimpfstamm MVA: marker. genetische struktur, erfahrungen mit der parenteralen schutzimpfung und verhalten im abwehrgesch- wächten organismus. Zbl Bakt Hyg I Abt Orig B 1978;167:375–390.

Meyer H, Sutter G, Mayr A. Mapping of deletions in the genome of the highly attenuated vaccinia virus MVA and their influence on virulence. J Gen Virol 1991;72:1031–1038. [PubMed: 2033387]

Moss B. Vaccinia virus: a tool for research and vaccine development. Science 1991;252:1662–1667. [PubMed: 2047875]

Panicali D, Paoletti E. Construction of poxviruses as cloning vectors: insertion of the thymidine kinase gene from herpes simplex virus into the DNA of infectious vaccinia virus. Proc Natl Acad Sci USA 1982;79:4927–4931. [PubMed: 6289324]

Ramshaw A, Andrew ME, Phillips SM, Boyle DB, Coupar BEH. Recovery of immunodeficient mice from a vaccinia virus/IL-2 recombinant infection. Nature 1987;329:545–546. [PubMed: 3498904]

Stickl H, Hochstcin-Mintzel V, Mayr A, Huber HC, Schäfer H, Holzncr A. MVA- stufenimpfung gegen pocken. Kleinische erprobung des attenuierten pocken-lebendimpfstoffes stamm MVA. Dtsch Med Wschr 1974;99:2386–2392. [PubMed: 4426258]

Sutter G, Moss B. Nonreplicating vaccinia vector efficiently expresses recombinant genes. Proc Natl Acad Sci USA 1992;89:10847–10851. [PubMed: 1438287]

Sutter G, Wyatt LS, Foley PL, Bennink JR, Moss B. A recombinant vector derived from the host range-restricted and highly attenuated MVA strain of vaccinia virus stimulates protective immunity in mice to influenza virus. Vaccine 1994;12:1032–1040. [PubMed: 7975844]

Tartaglia J, Perkus ME, Taylor J, Norton EK, Audonnet JC, Cox WI, Davis SW, Vanderhoeven J, Meignier B, Riviere M, Languet B, Paoletti E. NYVAC - A highly attenuated strain of vaccinia virus. Virology 1992;188:217–232. [PubMed: 1566575]

Taylor J, Wcinberg R, Tartaglia J, Richardson C, Alkhatib G, Breidis D, Appel M, Norton E, Paoletti E. Nonreplicating viral vectors as potential vaccines: recombinant canarypox virus expressing measles virus fusion (F) and hemagglutinin (HA) glycoproteins. Virology 1992;187:321–328. [PubMed: 1736535]

Wang M, Bronte V, Chen PW, Gritz L, Panicali D, Rosenberg SA, Restifo NP. Active immunotherapy of cancer with a non-replicating recombinant fowlpox virus encoding a model tumor-associated antigen. J Immunol 1995;154:4685–4692. [PubMed: 7722321]

Werner GT, Jentsch U, Metzger E, Simon J. Studies on poxvirus infection in irradiated animals. Arch Virol 1980;64:247–256. [PubMed: 6250514]

Yewdell JW, Bennink JR, Smith GL, Moss B. Influenza A virus nucleoprotein is a major target for cross-reactive anti-influenza virus cytotoxic T lymphocytes. Proc Natl Acad Sci USA 1985;82:1785–1789. [PubMed: 3872457]

Table 1

Protection against lethal challenge with influenza virus[1]

Inoculation site	Vaccine	Dose[2]	Increased HI titer[3]	Survivors[4]
Intramuscular	MVA	8	0/8	0/8
	WR	6	0/8	0/8
	WR-INF$_{ha/np}$	4	0/8	5/8
		5	1/8	8/8
		6	4/8	8/8
	MVA-INF$_{ha/np}$	4	0/16	14/16
		5	3/16	16/16
		6	7/8	8/8
Intranasal	MVA	6	0/8	0/8
	MVA-INF$_{ha/np}$	4	0/8	0/8
		5	0/8	0/8
		6	0/8	6/8

[1] data from Sutter et al. (1994) with permission.

[2] log tissue culture infectious dose$_{50}$ or plaque forming unit/animal.

[3] Number of animals with >4 fold increase in hemagglutinin (HI) titer/total animals for each group.

[4] Surviving animals/total animals challenged for each group: 100 LD$_{50}$ Influenza A/PR/S challenge delivered to 10-week-old-mice. 4 weeks post vaccination.

Table 2

Responses of vaccinated macaques to S1V challenge

Parameter	MVA or WY controls	MVA-SIVenv/gag/pol	WY-SIVenv/gag/pol
Anamnestic antibody	No	Yes	Yes
PBMC viremia	Persistent in 3 of 4	Transient in 3 of 4	Persistent in 4 of 4
Acute phase			
Antigenemia	3 of 4	None	1 of 4
Plasma viral RNA	High	Severely reduced	Reduced
PBMC viral copies	High	Reduced	Reduced
Lymph nodes			
Morphology	Hyperplastic	Normal	Hyperplastic
In situ	Numerous positive	Negative (3 of 4)	Positive
Virus load	High	Low	Moderate
CD4 lymphocytes	Low (3 of 4)	Low(l of 4)	Low (4 of 4)
Survivors	1 of 4	4 of 4	1 of 4

Table 3

Protection against neoplastic cells

Vaccination intramuscular	Dose[1]	CT26.WT number of metastases[2]	CT26.CL25 number of metastases[2]
None		411	>500
MVA	10^8	>500	>500
MVA-βgal	10^8	>500	0
MVA-βgal	10^6	>500	2

[1] Infectious units.

[2] Pulmonary nodules per lung, average of 5 animals.