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Deletion of the vaccinia virus F13L gene results in a highly attenuated virus that mounts a protective immune response against subsequent vaccinia virus challenge

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ABSTRACT

Vaccinia virus F13L encodes the envelope protein p37, which is the target of the anti-pox virus drug ST-246 (Yang et al., 2005) and that is required for production of extracellular vaccinia virus. The F13L (p37)-deleted (and ST-246 resistant) vaccinia virus recombinant (Vac-ΔF13L) produced smaller plaques than the wild-type vaccinia (Western Reserve vaccinia). In addition, Vac-ΔF13L proved, when inoculated either intravenously or intracutaneously in both immunocompetent and immunodeficient (athymic nude or SCID) mice, to be severely attenuated. Intravenous or intracutaneous inoculation of immunocompetent mice with the ΔF13L virus efficiently protected against a subsequent intravenous, intracutaneous or intranasal challenge with vaccinia WR (Western Reserve). This was corroborated by the observation that Vac-ΔF13L induced a humoral immune response against vaccinia following either intravenous or intracutaneous challenge. In conclusion, F13L-deleted vaccinia virus may have the potential to be developed as a smallpox vaccine.

1. Introduction

In 1980, successful global immunization campaigns with the vaccinia virus led by the World Health Organization, resulted in the eradication of smallpox and vaccination programs were consequently suspended (Geddes, 2006). The etiological agent of smallpox, variola virus, like vaccinia virus, belongs to the family of the orthopoxviridae (Moss, 2001). Use of vaccinia virus may be associated with serious adverse events and rare fatal reactions, particularly in immunodeficient patients and patients with atopic dermatitis (Bray, 2003; Lane and Goldstein, 2003). In light of the potential use of the variola virus as a bioweapon it is important to have a safer vaccine at hand.

Several vaccinia viruses with attenuated properties that mount a protective immunity against smallpox have been reported (Gurt et al., 2006; Jentarra et al., 2008; Vijaysri et al., 2008). Vaccinia mutants with deletions in the E3 interferon resistance gene (Jentarra et al., 2008) have been proven to be highly attenuated in both immunocompetent and immunodeficient mice following either intranasal or intracranial infection and induced a protective immune response following intranasal vaccination (Jentarra et al., 2008). Furthermore, vaccination by scarification resulted in a potent cell mediated and humoral immunity. Gurt et al. (2006) isolated two mutants of the Western Reserve (WR) strain of vaccinia virus, that carried mutations in A33R or B5R in genes coding for two proteins of the outer membrane of the extracellular enveloped virus (EEV) (Katz et al., 2002, 2003). Whereas a high degree of attenuation in mice was achieved with the A33R mutant, the B5R mutant remained somewhat pathogenic. Mice intranasally infected with one of both mutant viruses were all protected against a subsequent challenge of wild-type virus (Gurt et al., 2006).

We here report that vaccinia virus, from which the F13L has been deleted may have the potential to be further developed as a potential poxvirus vaccine. The F13L gene encodes a 37-kDa palmitylated peripheral membrane protein required for extracellular virus particle formation (Grosenbach and Hruby, 1998; Husain and Moss, 2001, 2002) and p37 is the target of the anti-pox virus drug ST-246 (Yang et al., 2005; Duraffour et al., 2008).

2. Materials and methods

2.1. Cells and viruses

BSC-40 (CRL-2761) cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA). The origin of vaccinia virus strain Western Reserve (WR) has been described

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before (Neyts et al., 2004; Yang et al., 2005). The vaccinia virus recombinant was derived from strain WR and expressed green fluorescent protein (GFP) from the F13L locus (Vac-ΔF13L-GFP).

Vac-ΔF13L-GFP: To construct Vac-ΔF13L-GFP, vaccinia virus F13L was amplified by PCR using primers Vac39831 (5′-CAT CCA TCC AAA TAA CCC TAG-3′) and Vac42787 (5′-AGA TAC TCC TAG ATA CAT ACC ATC-3′). Primers were designed based upon the published sequence information for vaccinia virus strain WR (GenBank Accession No. AY243312). The resulting PCR product (2956 bp) was cloned into pCR2.1 (Invitrogen, CA), to generate plasmid pF13L-KO1. Plasmid pF13L-KO1 was digested with Hind III and Bam HI restriction enzymes and the overhanging nucleotides were removed with Klenow fragment. The construct was religated to generate plasmid pF13L-KO1-ΔKpnI which resulted in the removal of sequences in the vector that included a KpnI site. The EGFP2 gene was amplified by PCR from plasmid pEGFP2 using primers EGFP2_kpn1 (5′-GGT ACC GAG TAA AGG AGA AG-3′) and EGFP2_BglII (5′-AGA TCT TTA TTT GTA TAG TTC ATC C-3′) and cloned into pCR2.1 to generate pEGFP2-KO2. This plasmid was digested with KpnI and EcoRV to release the EGFP2 gene that was subsequently cloned into the KpnI-PmlI site of pF13L-KO1-ΔKpnI where all but the first six amino acids of F13L were deleted to generate pEGFP2-ΔF13L-KO4. This plasmid contained the EGFP2 gene fused in frame to the first six amino terminal amino acids of F13L. The EGFP2-ΔF13L gene was amplified by PCR using primers Vac39831 and Vac42787, and the PCR product was gel purified using QIAquick purification kit (Qiagen, MA). The PCR product was transferred into the wild-type vaccinia virus strain WR genome by marker transfer (Yao and Evans, 2003). Virus recombinants were identified by fluorescence microscopy of infected BSC40 cell monolayers, and virus from small plaques that expressed GFP was isolated. The recombinant virus was plaque-purified three times prior to large scale stock preparation. The genotype of the recombinant was verified by DNA sequencing of a PCR product amplified from purified Vac-ΔF13L-GFP DNA.

Virus stocks were propagated in BSC40 cells. BSC40 cell cultures were grown at 37 °C in a humid incubator containing 5% CO_2 in Dulbecco's modified minimal essential medium containing (D-MEM), 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 5% fetal bovine serum (Invitrogen). Vaccinia WR, and Vac-ΔF13L-GFP stocks were cultured using standard methods. Briefly, BSC40 cells were seeded at a density of 1×10^7 cells per 15 cm diameter dish. The cultures were infected at a multiplicity of infection (MOI) of 0.01 PFU vaccinia WR/cell or 0.1 PFU Vac-ΔF13L-GFP/cell for efficient virus propagation. The cultures were incubated with the viral inoculum for 1 h at 37 °C in 5 ml medium with constant gentle rocking. The inoculum was removed and fresh culture medium was added to the dishes. The cultures were incubated 2–3 days or until extensive CPE was observed. The cultures were harvested and cell associated virus was released by sonication at 40% power in a Misonex Sonicator® 3000 (Farmingdale, NY) using a cup horn attachment. The cell debris was removed by centrifugation 1000*g* for 10 min at 4 °C and the crude virus suspension was stored at −80 °C. Subsequently virus stocks were purified by velocity sedimentation on a sucrose gradient (Hruby et al., 1979). Vaccinia titers were determined by plaque assay on BSC40 cell monolayers.

2.2. Mice and inoculation strategies

SCID (Severe Combined Immune Deficient) mice (C.B.-14 *scid/scid* inbred strain) were bred at the Rega Institute under germ-free conditions and were housed under specific-pathogen free conditions during the experiments. Female immunocompetent outbred NMRI (RjHan:NMRI, Naval Medical Research Institute) mice were obtained from the Animal Production Centre of the University of Leuven. Hairless (FVB/NRj-hr^{rh}) outbred mice were bred in house by backcrossing and intercrossing of the homozygous parents. Both mouse strains were housed under conventional conditions during the experiments. Female athymic outbred nude mice (Rj:NMRI-nu) were obtained from Elevage Janvier (Le Genest Saint Isle, France). Athymic nude mice (in which the development and differentiation of hair is severely impaired) lack functional T-cell immunity. Animals were placed in group housing and did not receive antibiotics.

NMRI, hairless, SCID and athymic nude mice were infected intravenously with 2×10^5 PFU (Table 1) or 2×10^4 PFU of virus (Tables 2 and 3). An inoculum of 2×10^5 PFU for intravenous inoculation of mice with WR was selected since this inoculum resulted in a countable number of tail lesions in NMRI mice but did otherwise not result in morbidity and mortality. For challenge (vaccination) studies whereby mice were inoculated twice, a 10-fold lower inoculum (2×10^4 PFU) was chosen.

In some experiments hairless mice, SCID and athymic nude mice were inoculated intracutaneously by means of scarification at the lumbosacral area (5×10^5 PFU). This inoculum was selected because it resulted in a reproducible induction of cutaneous lesions. Prior to scarification, animals received light ether anesthesia and were immobilized manually by one person; scarifications were made by a second person. A 50-μl droplet of the viral inoculum was placed at the lumbosacral area. A sterile stainless steel blood lancet (Maersk Medical, Sheffield, United Kingdom) was used to

Table 1
Effect of intravenous or intracutaneous inoculation of immunocompetent and immunodeficient mice with vaccinia WR or Vac-ΔF13L-GFP on virus-induced lesion formation and mortality.

Mouse strains	Virus	Inoculation [a]	# (death/total)	# Mice with lesions	Mean lesion number ± SD[b]	MTD ± SD[c]
NMRI	WR	i.v.	0/4	4/4	59 ± 11	>50
	Vac-ΔF13L-GFP	i.v.	0/4	0/4	0*	>50
Hairless	WR	i.cut.	0/13	13/13		>50
	Vac-ΔF13L-GFP	i.cut.	0/15	0/15		>50
Athymic nude	WR	i.v.	4/4	4/4	37 ± 7	9 ± 0.5
	Vac-ΔF13L-GFP	i.v.	4/4	0/4	0*	22 ± 1*
	WR	i.cut.	5/5	5/5		11 ± 1
	Vac-ΔF13L-GFP	i.cut.	0/15	0/15		>50
SCID	WR	i.v.	4/4	4/4	20 ± 8	11 ± 1
	Vac-ΔF13L-GFP	i.v.	0/4	0/4	0*	>50
	WR	i.cut.	5/5	5/5		18 ± 2
	Vac-ΔF13L-GFP	i.cut.	0/5	5/5		>50

[a] i.cut.: intracutaneous infection with 5×10^5 PFU – lesions on the back, i.v.: intravenous infection with 2×10^5 PFU – lesions on the tail.
[b] Lesion score as determined on day 7 post-WR infection.
[c] MTD: mean time to death.
* $p < 0.001$.