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Vaccinia-based vaccines to biothreat and emerging viruses

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ABSTRACT

The past few years have seen a rash of emerging viral diseases, including the Ebola crisis in West Africa, the pandemic spread of chikungunya, and the recent explosion of Zika in South America. Vaccination is the most reliable and cost-effective method of control of infectious diseases, however, there is often a long delay in production and approval in getting new vaccines to market. Vaccinia was the first vaccine developed for the successful eradication of smallpox and has properties that make it attractive as a universal vaccine vector. Vaccinia can cause severe complications, particularly in immune suppressed recipients that would limit its utility, but nonreplicating and attenuated strains have been developed. Modified vaccinia Ankara is nonreplicating in human cells and can be safely given to immune suppressed individuals. Vaccinia has recently been modified for use as an oncolytic treatment for cancer therapy. These new vaccinia vectors are replicating; but have been attenuated and could prove useful as a universal vaccine carrier as many of these are in clinical trials for cancer therapy. This article reviews the development of a universal vaccinia vaccine platform for emerging diseases or biothreat agents, based on nonreplicating or live attenuated vaccinia viruses.

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Introduction

The rapid emergence or spread of pathogens such as Ebola, Zika and chikungunya viruses (CHIKV) over the past several years has been both sudden and unexpected. This and the potential use of viruses such as variola, filoviruses and the encephalitic alphaviruses as agents of bioterrorism is an overhanging threat to humanity. Vaccines have proven to be one of the most effective means of providing protection against or gaining control over the spread of a virus, but very few vaccines have been produced for emerging virus infections or to mitigate the

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use of a biological threat virus. In both cases, the cost of developing a vaccine are prohibitive for a pharmaceutical company to produce a fully licensed product, with the over arching risk of having a very limited or unsustainable market for the product. Expression of the antigen of concern in an approved vaccine delivery system may shorten the time taken to develop a vaccine for a current outbreak. The choice of a vaccine platform is limitless, with vaccines under development based on DNA delivery, virus-like particles (VLPs) and a multitude of virus-vectored vaccines (e.g. adenovirus, measles virus, herpes virus and vesicular stomatitis virus) (Ramsauer & Tangy, 2016; Lauer, Borrow, & Blanchard, 2017). However, for the purposes of this update, we will limit our discussions to vaccinia-derived vectors being developed as universal vaccine platforms.

Vaccinia as a universal vaccine carrier

An ideal vaccine platform for emerging infectious diseases and biological threat viruses would have properties such as: (1) a proven safety record in humans; (2) single dose efficacy; (3) multiple routes of administration; (4) a large coding capacity for multiple agents and co-stimulatory molecules; (5) be able to elicit both TH1 and TH2 responses; (6) have a reduced anti-vector response in the host; (7) thermostability of freeze-dried preparations; and (8) be amenable to scale-up manufacturing. Vaccinia virus (VACV) strains used to eliminate the spread of smallpox were effective as vaccines, but have serious adverse effects that would limit their utility as a universal vaccine platform. Adverse effects include progressive vaccinia (at site of inoculation), eczema vaccinatum, postvaccinial encephalitis and generalized vaccinia. These are rare, but serious events that require careful screening of smallpox vaccine recipients for immunodeficiency or skin conditions (Vellozzi et al., 2005). Both non-replicating and attenuated VACV strains are becoming increasingly popular as vaccine vectors for emerging and biothreat viruses.

Modified vaccinia Ankara

A leading candidate is modified vaccinia Ankara (MVA), an attenuated VACV (Mayr, Stickl, Muller, Danner, & Singer, 1978) that was adapted for growth in chicken embryo fibroblasts and is replication-deficient in humans and in other mammals (Tree et al., 2016). MVA-Bavarian Nordic (MVA-BN®) is a well-characterized strain that is approved as a smallpox vaccine in Canada and in the EU (under the trade names Imvamune® and Imvanex®, respectively). Some of the features that make MVA-BN® an excellent vaccine platform include its outstanding safety profile in humans, which was demonstrated in several clinical trials (Vollmar et al., 2006; von Krempelhuber et al., 2010; Frey et al., 2014; Greenberg et al., 2015), and its intrinsic adjuvant capacities to induce both humoral and cellular immune responses (Sutter & Staib, 2003; Gómez, Najera, Krupa, Perdiguero, & Esteban, 2011). Finally, the impact of pre-existing vector immunity to MVA

is limited, unlike other viral vectors such as adenovirus-based vaccines (Harrop, John, & Carroll, 2006), allowing multiple doses of the vaccine to be given. MVA has been used as a vector for many different vaccines ranging from infectious diseases to various cancers, and many of these have reached clinical trials. Table 1 lists some emerging and biothreat virus vaccines being researched using MVA and other VACV strains as a vector for comparative purposes; a short summary of diseases targeted using MVA follows.

MVA-vectored vaccines for emerging infectious diseases

Chikungunya

CHIKV is an alphavirus causing large outbreaks of an acute febrile disease with severe and long lasting polyarthralgia. Currently, no licensed vaccine is available, but it has been the subject of extensive research and development (reviewed in Smalley, Erasmus, Chesson, & Beasley, 2016; Ramsauer & Tangy, 2016). Four groups have developed CHIKV vaccines using MVA as a vector. A study by Garcia-Arriaza et al. (2014) used the entire CHIKV 26S structural polyprotein (C-E3-E2-6K-E1) expressed in an MVA vector. A strong innate immune response was induced in human macrophages or dendritic cells, along with production of interferon (IFN)-β, TNF-α, MIP-1, IP-10 and RANTES. After immunization of C57BL/6 mice with one or two doses, high levels of neutralizing antibodies were induced, as well as a strong CHIKV-specific CD8 + T-cell response. The mice were fully protected, even after a single dose of MVA-CHIKV, when challenged with 10^6 plaque forming units (PFUs) of CHIKV in a model measuring CHIKV-induced swelling of the hind limb (Garcia-Arriaza et al., 2014). van den Doel et al. (2014) compared three different constructs of MVA-vectored CHIKV, using E3-E2-6K-E1, E3-E2 or 6K-E1 constructs. AG129 mice (IFN-α/β/γ deficient) were immunized intramuscular (i.m.) with two doses, 3 weeks apart, and then challenged intraperitoneal (i.p) 6 week later with 10^3 tissue culture infectious dose 50% ($TCID_{50}$) in a lethal model. Both E3-E2 and E3-E2-6K-E1 fully protected the mice, and 6K-E1 provided 75% protection. Only animals vaccinated with the vector expressing the full envelope construct (E3-E2-6K-E1) demonstrated significant titres of neutralizing antibodies. In a third study, Weger-Lucarelli, Chu, Aliota, Partidos, and Osorio (2014) used a E3-E2 construct to express the E2 protein using MVA. When they immunized A129 mice (IFN-α/β deficient) high levels of antibody were obtained, but similar to the previous study (van den Doel et al., 2014), only low levels of neutralizing antibodies were elicited. Subsequently challenged mice were protected against mortality, footpad swelling and viremia. However, passive transfer of induced antibodies did not protect naive mice. Depletion of $CD4^+$ T cells, but not $CD8^+$ T cells, resulted in 100% mortality of immunized and challenged mice, suggesting $CD4^+$ T cells are an important component in protection. Lastly, MVA expressing surface epitopes of the E2 protein (domain A fused to domain B) did produce neutralizing antibody, but this was not sufficient

Table 1. Vaccinia-based vaccines to biothreat and emerging viruses.

Vacv	Antigen	Dose		Notes	Ref
		No.	TCID		
MVA	WEEV E3-E2-6K-E1 EEEV E3-E2-6K-E1 VEEV E3-E2-6K-E1	2 s.c.	1×10^8	Mixture of 3 vaccines protective in mouse model (100% WEEV, 100% VEEV, 60% EEEV)	Hu et al. (2017)
MVA	CHIKV E3-E2-6K-E1, E3-E2, 6 K-E1	2	5×10^6	Protective in AG129 model with MVA E2	van den Doel et al. (2014)
MVA	CHIKV E3-E2	2 s.c.	1×10^7	Protective in BALB/c A129, $CD4^+$ important	Weger-Lucarelli et al. (2014)
MVA	CHIKV C-E3-E2-6K-E1	1–2 i.p.	$1–2 \times 10^7$	Protective in C57BL6 model with a single dose	Garcia-Arriaza et al. (2014)
MVA	CHIKV E2 sAB+	4	1×10^8	No protection in transient infection BALB/c	Weber et al. (2015)
MVA	YFV PreME	1 i.m.	1×10^5	100% protection in a i.c. challenge with YFV 17D	Schäfer et al. (2011)
ΔD4R	YFV PreME				
Ad/26	ZEBOV	1 + 1	10^{11}	Protective in NHP model, using prime boost	Milligan et al. (2016)
MVA	Filo		10^8		
MVA	3 Influenza A/H5N1 HA	2 i.m.	7×10^7	100% protection in BALB/c, Day 0, 28	Pabakaran et al. (2014)
MVA deletion	CHIKV C-E3-E2-6K-E1 ΔC6LΔK7RΔA46R	1–2 i.p.	$1–2 \times 10^7$	100% protection with a single dose	Garcia-Arriaza et al. (2014)
NYVAC	JEV	3	5.8×10^6	NYVAC-JEV protective in NHP (0, 28, 273)	Raengsakulrach et al. (1999)
ALVAC		s.c.	3.1×10^6	ALVAC-JEV not protective in NHP (0, 28, 273)	
SCV ΔD13L	CHIKV C-E3-E2-6K-E1	1 i.p.	10^7	Non-replicating in human cells. Protective in C57BL/6 model with single dose	Eldi et al. (2017)
Wyeth	H5N1 HA, NA, NP, M1, M2, Il-15	2 s.c.	10^7	Two doses 3 weeks apart, challenged 3 weeks later. Protection against H5N1, H1N1, H3N2 and H7N7	Valkenburg et al. (2014)
WR ΔA23T7	H1N1 HA	2 i.m.	10^4, 10^5	2 Doses 4 weeks apart in BALB/c, challenged i.n. with 100 LD_{50} influ A/PR8 – 100% protection with MVA-HA or ΔA23T7-HA	Wyatt et al. (2017)

to protect mice after four immunizations (Weber, Bücher, & Schnierle, 2015). Taken together, MVA-vectored vaccines expressing the E3-E2-6K-E1 or C-E3-E2-6K-E1 constructs induced protective levels of neutralizing antibodies, a strong CHIKV-specific T cell response and induce innate immunity as potential CHIKV vaccine candidates. While the two candidates are not directly comparable as they were tested in different models of infectivity, it would be interesting to see if VLPs are formed (Akahata et al., 2010), or what effect the capsid has in protection in a direct comparison.

Yellow fever

The current live attenuated 17D yellow fever (YF) vaccine has been very effective in providing long-term protection against this severe viscerotropic disease (reviewed in Monath & Vasconcelos, 2015). The vaccine is able to stimulate protective neutralizing antibodies and a robust CD4 + response; however, there have been concerns raised regarding the safety of the vaccine, particularly in recipients over 60 years in age. The incident of YF 17D-associated viscerotropic disease is increased in the elderly and those who are immune compromised (Jonker, Visser, & Roukens, 2013; Monath & Vasconcelos, 2015). To fill this gap, it has been suggested that replacement vaccines may be needed.

Schäfer et al. (2011) inserted the precursor membrane and envelope (PreME) genome fragment of the YFV-17D vaccine strain into MVA (MVA-YF) and into a second non-replicating VACV ΔD4R (defective vaccinia virus – dVV). Single dose i.m. immunization was performed using different concentrations of MVA-YF or dVV-YF in BALB/c mice followed by intracerebral (i.c.) challenge with YFV-17D (10^5 TCID) after 21 days. Despite an inability to detect neutralizing antibody production complete protection was achieved using 10^5 TCID of either MVA-YF or dVV-YF. However, both vaccines were able to induce anti-specific $CD8^+$ T cells and antigen-specific IFN-γ producing $CD\text{-}4^+$ T cells. Overall, the non-replicating VACV vectors induced a broad immune response after a single dose, and were protective even in the presence of pre-vector immunity (Schäfer et al., 2011).

Influenza H5N1

Avian influenza H5N1 is a highly lethal and potential pandemic threat. A trivalent hemagglutinin (HA) vaccine for influenza H5N1 was developed using a single MVA vector expressing three H5N1 HA proteins to broaden the genetic variation with the rapid evolution of new sublineages of H5N1. BALB/c mice received two doses i.m. (8×10^7 $TCID_{50}$) 28 days apart and were challenged intranasal (i.n.) three week after the second immunization. The vector induced strong cross-neutralizing immunity to diverse H5N1 clades and was able to completely protect against 10 minimal lethal dose 50% (MLD_{50}) of related or divergent clades, although loss of weight of up to 10% was observed with the mice challenged with the divergent clade (Pabakaran et al., 2014).

Ebola

A combination vaccine, where an Adenovirus Type 26 Ebola Mayinga glycoprotein (Ad26.ZEBOV) is used as a priming dose and a MVA-BN® booster expressing Ebola Mayinga glycoprotein (gp), Sudan Gula gp, Marburg Musoke gp and Tai Forest nucleoprotein (np) (MVA-BN® -Filo) yielded 100% protection in nonhuman primates (NHPs – Milligan et al., 2016). In a further study, the combined expression of Ebola VP40 matrix protein (mp) with above mentioned gps and Tai Forest np resulted in the production of secreted VLPs which resemble authentic EBOV particles. However, the level of EBOV gp produced was similar to the construct lacking the VP40 mp (which does not produce VLPs), and both induced a similar levels of neutralizing antibodies and a EBOV gp-specific $CD8^+$ T cell response, indicating that generation of VLPs may not confer additional protection (Schweneker et al., 2017).

MVA-vectored vaccines for biothreat viruses

In addition to potential use as vectors for vaccines against emerging diseases, vaccinia-based vectors could be potentially used to protect against biothreats. The encephalitic alphaviruses due to their potential to infect humans through aerosol exposure are a concern as a biological threat agent (for a review of alphaviruses, see Griffin, 2013). Recombinant MVA-BN® -vectored encephalitic alphavirus vaccines were designed to express the E3-E2-6K-E1 polyprotein coding sequences for Venezuelan, western and eastern equine encephalitis viruses (VEEV, WEEV or EEEV), respectively (Hu et al., 2017). The codon usage was adapted for optimal expression in humans and strong VACV promoters were selected for the expression of antigens early after infection of cells (Baur et al., 2010; Wennier et al., 2013). Female BALB/c mice were immunized subcutaneously (s.c.) with two doses of 10^8 TCID of single monovalent MVA-BN encephalitic alphavirus vaccine or with a mixture of three monovalent vaccines at a four-week interval. Fourteen days after the second dose, the mice were then instilled i.n. with 5×10^3 to 1×10^4 PFUs of VEEV, WEEV or EEEV in a lethal challenge. The MVA-BN-VEEV, MVA-BN-WEEV or MVA-BN-EEEV-immunized mice fully survived the corresponding virus challenge without any signs of infection or weight loss. The mixture of three monovalent vaccines could also provide 100% protection to the mice against WEEV and VEEV challenges, but dropped to 60% protection against EEEV challenge. These data suggest that monovalent MVA-BN-EEEV, MVA-BN-WEEV and MVA-BN-VEEV are potential vaccine candidates against encephalitic viruses and the three monovalent vaccines can be given in a mixture without significantly reducing efficacy (Hu et al., 2017).

Other non-replicating vaccinia

Researchers have been looking to improve on VACV as a vector through the specific deletion of gene products required for the replication and immune evasion of VACV in human cell lines. The NYVAC is a highly attenuated VACV which is non-replicating in most human cells, but can be grown in chicken embryo fibroblasts. It was generated by the deletion of 18 virulence associated genes of a VACV Copenhagen isolate (Tartaglia et al., 1992). A study by Raengsakulrach et al. (1999) constructed NYVAC-Japanese encephalitis virus (JEV) and ALVAC-JEV (non-replicating canarypox) vaccine candidates for testing in a NHP model. Animals were immunized s.c. on Day 0, 28 and 273, and challenged i.n. with a 90% effective dose of JEV 60 days after the booster. Three of 4 NYVAC-JEV animals survived challenge, while only 1/4 ALVAC-JEV immunized animals survived challenge. Similarly, a NYVAC vector was shown to develop a better human immunodeficiency virus (HIV)-specific cellular and humoral immune response in NHPs when compared to a ALVAC expressing identical HIV antigens in a 2 plus 2 prime/boost immunization strategy (García-Arriaza et al., 2015). Improved versions of NYVAC were made by reintroduction of genes *K1L* and *C7L* (host range genes) and the deletion of B19R (antagonist of type 1 IFN) in the development of a HIV vaccine (Kibler et al., 2011). Additional combinations of deletion of viral inhibitory genes were shown to increase the immunogenicity of both MVA *ΔC6LΔK7R* (García-Arriaza, Arnáez, Gómez, Sorzano, & Esteban, 2013) and NYVAC *ΔA46L*, *ΔB8R/ΔB19R* vectors (reviewed in García-Arriaza & Esteban, 2014).

Enhancing the immunogenicity of poxvirus vectors

VACV has a wide repertoire of genes which evade the immune response and the deletion of these genes, as described for NYVAC, has been used as an approach to enhance the host immune response and/or attenuate the vector (reviewed in Jackson et al., 2005; Smith et al., 2013; García-Arriaza & Esteban, 2014). For example, it had been previously demonstrated that deletion of the *A41L* gene (immunomodulator) in VACV western reserve (WR) or MVA caused an enhanced VACV-specific IFNγ-producing $CD8^+$ T cell and increased cytotoxic T-cell responses in the spleen. Improved protection was observed against poxviruses using MVA-Δ*A41L* against a LD_{50} 300 dose of VACV WR in a mouse model (Clark, Kenyon, Bartlett, Tschake, & Smith, 2006). García-Arriaza et al. (2013) were able to improve the adaptive and memory immune responses of a MVA HIV vaccine through deletion of two VACV genes, *C6L* and *K7R*, whose products inhibit interferon signalling pathways. Using a DNA-prime and MVA-boost $CD8^+$ T-cells were enhanced in magnitude and duration along with innate immunity up-regulation of IFN-β and other cytokines (García-Arriaza et al., 2013). Similarly, a MVA vector with deletions in *C6L*, *K7R* and A*46*R, and expressing the CHIK 26S

structural genes was constructed and used to immunize C57BL/6 mice using 1 or 2 doses given i.p. (1×10^7, 2×10^7 PFU) two weeks apart. Seven weeks later, mice were challenged with 10^6 PFU of CHIKV in a hind limb swelling model. The mice developed a strong innate response (IFN-β, proinflammatory cytokines, chemokines), broad and long-lasting CHIKV E1 and E2-specific $CD8^+$ T cell response and neutralizing antibodies with a single dose that was completely protective (no swelling or viremia) (Garcia-Arriaza et al., 2014).

In a second approach, the expression of co-stimulatory molecules such as interleukin (IL), interferon and other cytokines alone or in combination have been shown to enhanced the immune response (reviewed in Gómez, Perdiguero, García-Arriaza, & Esteban, 2013; García-Arriaza & Esteban, 2014). An example is the use of IL-15, which was co-expressed with H5N1 antigens (HA, NA, NP, MA1 and MA2) in a replicating VACV Wyeth strain using 2 doses 3 weeks apart. The effect was to broaden the level of heterospecific protection to other subtypes of influenza A (including H1N1, H3N2 and H7N7 strains) with protection ranging from 80–100% (Valkenburg et al., 2014).

Replication competent vaccinia viruses based on oncolytic vectors

While MVA-vectored and other non-replicating VACV have excellent safety profiles, there is evidence to suggest that they may offer less robust and lasting immunity when compared to that of replicating strains (Ferrier-Rembert, Drillien, Tournier, Garin, & Crance, 2008; Russell & Tscharke, 2014). Furthermore, there is evidence that in order to confer protective immunity MVA works best when used as a boost following delivery of priming agents such as DNA or protein (reviewed in Cottingham & Carroll, 2013). With the development of oncolytic virus technologies, there has been a renewed interest in replicating strains, and many oncolytic VACV strains have been engineered to favour growth in tumour cells, while leaving normal cells intact. This can be accomplished in different ways, for example by deleting VACV growth factor (McCart et al., 2001). However, deletions in the VACV nucleotide biosynthetic pathways have been most widely used for targeting replicating vaccinia strains to rapidly dividing and thus dNTP-replete cancer cells (reviewed in Irwin, Hitt, & Evans, 2017). A very common strategy has been to mutate the thymidine kinase locus (*J2R*) and in fact most oncolytic VACV encode *J2R* deletions. More recently, it has been shown that VACV bearing mutations in the small subunit of the ribonucleotide reductase gene (*F4L*) are even more highly attenuated *in vivo* while also still replicating selectively in solid tumours (Potts et al., 2017). The *J2R* locus in Δ*F4L* strains can still then be used as a target for inserting transgene(s) and the double mutant viruses (Δ*J2R* Δ*F4L*) also exhibit a further degree of safety and selectivity. We have used a VACV Δ*F4L* Δ*J2R* Dryvax strain to express CHIKV and YFV antigens at levels that lead to production of VLPs (Nagata et al., 2017). Many other VACV strains are currently being tested for oncolytic activity in clinical trials and some have been used to deliver transgenic

products including immunomodulators like GMCSF. The interested reader can find these studies reviewed in Haddad (2017) and Irwin et al. (2017).

Replicating VACV vectors have also be modified to delete some of the numerous virus-encoded immunomodulatory genes. This can be done either to attenuate the virus and thus improve the safety profile, and/or to increase the immunogenicity of the vector and any encoded transgenic protein (reviewed in Jackson et al., 2005; Smith et al., 2013; Sánchez-Sampedro et al., 2015; Veyer, Carrara, Maluquer de Motes, & Smith, 2017). These kinds of studies can be challenging since a balance is often required between attenuation and immunogenicity when employing replicating VACV vectors. A recent study examined the effects of deleting the N1, C6 and K7 immunomodulators, alone or in combination. These virus-encoded proteins target the NFκB, interferon and NFκB pathways, respectively. The more attenuated viruses bearing multiple gene deletions were less immunogenic in terms of protection afforded against wild type VACV (Sumner, Ren, Ferguson, & Smith, 2016).

Engineering the next generation(s) of vaccinia vectors

Deletion of essential genes required for replication of vaccinia and supplying the key function in trans in a cell line used to propagate the virus has been a novel way of developing non-replicating vaccinia vector systems. Recently, a Sementis Copenhagen Vector (SCV) was derived by deletion of the essential *D13L* gene of the Copenhagen strain of VACV. The vector was non-replicating in human cells, and can only be grown in an engineered Chinese hamster ovary cell line which supplied the D13 protein and a VACV host range factor (CP77) in trans. SCV-CHIK vaccine was engineered by insertion of the CHIKV C-E3-E2-6K-E1 polyprotein into the *A39R* gene locus. A single dose 10^7 $TCID_{50}$ given i.p. provided complete protection against a challenge of 10^4 $TCID_{50}$ CHIKV in footpad swelling of C57BL/6 mice (Eldi et al., 2017).

Previously, a T7 polymerase and promoter system had been engineered into a VACV to produce heterologous proteins (Fuerst, Earl, & Moss, 1987). Control of expression was accomplished by insertion of the lac repressor/operator into VACV (Fuerst, Fernandez, & Moss, 1989). In a recent study, Wyatt, Xiao, Americo, Earl, & Moss (2017) were able to refine expression by deletion of an essential *A23R* (intermediate transcription factor) gene of VACV WR and supplying this function in trans, making the VACV non-replicating in non-complementing cells. The heterologous gene was expressed under a T7 promoter, controlled by the lac repressor/operator system, and the T7 polymerase was expressed under a VACV early promoter. The net effect of the modifications were to increase the heterologous gene expression and reduce VACV expression in the host in non-complementing cells, but maximize virus production and reduce heterologous expression (lac repressor under an intermediate VACV promoter) during VACV production in complementing cells expressing *A23R*. The VACV Δ*A23T7* vector was able to

synthesize the heterologous gene to substantially higher levels than a comparable MVA construct *in vitro* and at a level similar to a replicating WR construct. But there were no differences in the level of protection induced by identical amounts of either the MVA-HA or the Δ*A23T7*-HA vaccine after challenge in an influenza H1N1/PR8 infection model in mice. The authors estimated that the immunogenicity of the Δ*A23T7* vector can be greatly improved upon through selective deletion of VACV immunomodulatory genes (Wyatt et al., 2017).

Recently, Noyce and Evans have a method for assembling a replication competent horsepox virus starting from synthetic DNA fragments (Noyce, Lederman & Evans, 2018) and have re-established interest in the molecular links between VACV and a putative horsepox-like ancestor (Tulman et al., 2006). Whether horsepox virus might offer an alternative to VACV as a smallpox vaccine or novel vector remains to be established. The work also illustrates a novel approach to engineering VACV-like vectors that extends earlier advances in poxvirus reactivation technologies (Yao & Evans, 2003). Synthetic biology now offers an extraordinarily powerful future tool for deleting virus genes, fine-tuning the levels of gene expression, editing immunodominant vector epitopes and rapidly manufacturing new viruses expressing novel antigens.

Summary

Non-replicating strains of vaccinia such as MVA and NYVAC are a very safe and reproducible vaccine platform, which normally require two doses of vaccine for an effective immune response. The immunogenicity of these vectors is being refined using deletions or through the expression of key immunomodulatory genes. New non-replicating vector systems are being developed which use complementing cell lines to allow production of virus particles, which are being optimized for expression using strong promoter systems such as the bacteriophage T7 promoter/polymerase and control of expression optimized using lac promoter/inhibitor and VACV early or late promoters (Wyatt et al., 2017). These produce levels of expression similar to the replicating vaccinia which are also being attenuated and refined through deletion/expression of key gene products. A major difference between MVA and replicating VACV is that MVA does not secrete immunomodulating peptides and is able to induce an IFN-α response in mice. Removal of these genes from VACV vectors (VACV Δ*A23T7*) may give an even better adaptive immune response (Wyatt et al., 2017). The development of oncolytics for safer attenuated viruses, coupled with deletion of immunomodulating genes could lead to very safe and effective replicating VACV vectors. The numerous advantages of vaccinia including proven safety, stability, production, single dose immunization and multiple antigen production lends support to its tremendous potential as universal vaccine carrier for the biothreat and emerging viruses.

Disclosure statement

No potential conflict of interest was reported by the authors.

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