Vaccine 31 (2013) 4241–4246

Contents lists available at SciVerse ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Clinical development of Modified Vaccinia virus Ankara vaccines

Sarah C. Gilbert*

Jenner Institute, University of Oxford, ORCRB, OX3 7DQ, UK

ARTICLE INFO

Article history:
Received 5 March 2013
Accepted 11 March 2013
Available online 21 March 2013

Keywords:
Poxvirus
Vaccine
Clinical safety
Immunogenicity
Efficacy

ABSTRACT

The smallpox vaccine Vaccinia was successfully used to eradicate smallpox, but although very effective, it was a very reactogenic vaccine and responsible for the deaths of one or two people per million vaccinated. Modified Vaccinia virus Ankara (MVA) is a replication-deficient and attenuated derivative, also used in the smallpox eradication campaign and now being developed as a recombinant viral vector to produce vaccines against infectious diseases and cancer. Many clinical trials of these new vaccines have been conducted, and the findings of these trials are reviewed here. The safety of MVA is now well documented, immunogenicity is influenced by the dose and vaccination regimen, and information on the efficacy of MVA-vectored vaccines is now beginning to accumulate.

1. Introduction

In May 1980 the World Health Organisation declared that smallpox had been eradicated [1]. Deliberate cutaneous or intranasal infection with a small quantity of variola virus, the causative agent of smallpox was known as variolation and had been practiced in India and China for many centuries before the practice was introduced to the west during the early 18th century. In a healthy individual, this resulted in a mild case of smallpox, recovery and lifelong immunity. At the time Edward Jenner inoculated his gardener's son with material taken from a lesion on the hand of a milkmaid with a cowpox infection, smallpox claimed the lives of one in three children and was the major cause of blindness in the population, but the prophylactic practice of variolation itself had a fatality rate of 1 in 50. Jenner was not the first person to employ cowpox to protect against smallpox, but he publicized his findings extensively and promoted 'vaccination' as it was now known, as a safer alternative to variolation.

The Vaccinia virus used to vaccinate against smallpox during the 20th century is not cowpox, and its precise origins will never be known [2], following a long history of person to person transfer and production of vaccines on the skin of calves or other large animals. Notwithstanding the important role of Vaccinia virus in the history of vaccination and the eradication of smallpox, it is one of the most reactogenic vaccines that has ever been licensed for use. Side effects typical of viral infection (fever, headache, malaise, muscle ache) were common, and for every million vaccinations there were one or two deaths and hundreds of cases severe enough to require hospitalization [3]. A need for safer alternatives, particularly for use in partially immunocompromised individuals was recognized. Studies only performed after the eradication of smallpox finally clarified the role of humoral and T cell responses to Vaccinia. It is the long-lived humoral responses that persist after exposure to Vaccinia that are responsible for protection against smallpox infection, but the T cell responses against Vaccinia antigens are required to curtail the spread of the Vaccinia virus itself [4]. The practice of pre-vaccination with an attenuated version of Vaccinia was used particularly in Germany [5]. With hindsight, it is clear that the induction of T cell responses against Vaccinia virus antigens provided protection against disseminated Vaccinia virus infection following revaccination with Vaccinia, and the replication-deficient modified Vaccinia virus Ankara (MVA) was used to vaccinate more than 120,000 people in this way, with an excellent safety record.

MVA was produced from the replication-competent Vaccinia virus Ankara following over 570 passages in chick embryo fibroblast cells. During serial passage approximately 12% of the genome was lost [6,7], including genes that interfere with the host immune response to Vaccinia virus such as receptors for interferon-γ, interferon-α/β and CC chemokines [8]. MVA fails to replicate in almost all mammalian cells [9], although this highly restricted host range is determined by other mutations in addition to the six major deletions [10] and in consequence is incapable of causing disseminated infection even in severely immunocompromised animals [11], although is still capable of inducing strong humoral responses and is now stockpiled by the US government as the vaccine to be employed against bioterrorist use of smallpox.

* Tel.: +44 1865 617605.
E-mail address: Sarah.gilbert@ndm.ox.ac.uk

0264-410X/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.vaccine.2013.03.020

2. Use of recombinant MVA-vectored vaccines in malaria vaccine development

Following the demonstration of high levels of recombinant protein production from recombinant MVA [12] it was proposed that recombinant MVA could be employed as a safe but highly immunogenic vaccine in humans, and initial pre-clinical development showed promise [13]. The block in viral replication in human cells occurs after DNA synthesis, and in the majority of mammalian cells both early and late gene expression takes place, such that recombinant antigen is expressed inside the infected cells.

The majority of licensed vaccines are designed to induce humoral rather than cellular immunity, but since poxviruses infect mammalian cells and the recombinant antigen is produced intracellularly, this allows the antigen to be processed by the proteasome and present on MHC molecules at the cell surface of antigen presenting cells which may then engage T cells and initiate a cellular response to the antigen. MVA was not included in a pre-clinical comparison of vaccine delivery systems to determine which were most successful at inducing CD8+ T cell responses to a protective epitope, known as pb9, from *Plasmodium berghei*, [14]. From that study the most immunogenic delivery systems were found to be lipopeptides and yeast-derived virus like particles (VLPs). However the disadvantage of both of these systems is that they each expressed only the nine amino acid epitope sequence, and in order to immunize humans, and provide epitopes that can be recognized by multiple HLA types, a large number of different peptides or VLPs would be required.

The yeast VLPs are in fact capable of carrying more than one T cell epitope, and a version carrying 14 defined T cell epitopes taken from liver stage antigens of *Plasmodium falciparum* in addition to pb9 was then produced [15]. The particles carrying the epitope string were immunogenic in mice, but failed to provide protection against a *P. berghei* infectious challenge. However, when the T cell response that was primed by a single dose of VLPs was boosted by a single dose of recombinant MVA expressing the *P. berghei* antigen circumsporozoite protein (CSP, from which pb9 is derived), or the epitope string including pb9, between 85 and 100% of mice were protected against *P. berghei* challenge [16]. Both the order of immunisations and route of delivery were important for protection. Although MVA expressing CSP or pb9 could prime a T cell response to pb9 in mice, it was not boosted by VLPs carrying pb9, whereas in the reverse order of VLP priming MVA boosting, the T cell response primed by the VLPs was boosted by the MVA to protective levels. The MVA was administered intravenously, which is not a route suitable for prophylactic vaccination of humans.

In a separate study, a DNA vaccine expressing *P. berghei* CSP (whole antigen rather than the defined epitope only) was used in combination with MVA expressing the same antigen, and again, protection against infectious challenge was induced by DNA priming, MVA boosting, but not the reverse order [17,18]. MVA boosting was administered by three different routes which resulted in different levels of protection against infectious challenge with *P. berghei*; intravenous (100%), intradermal (80%) and intramuscular (50%). Administration of the DNA vaccine via gene gun allowed the same level of immunogenicity to be attained with 10-fold less DNA [19]. Intramuscular priming with DNA followed by intramuscular boosting with MVA was shown to be strongly immunogenic in chimpanzees [20].

Following these studies the ability of MVA to boost T cell responses that had been primed by another method had been established. Heterologous prime boost regimes are substantially more immunogenic than homologous priming and boosting with a recombinant MVA [21]. When recombinant MVA is used to boost a pre-existing T cell response to the recombinant antigen, the anamnestic response results in a large amplification of that response and a reduced induction of novel T cell responses to the antigens of the viral vector itself. This is an important concept in achieving strong and protective T cell responses by vaccination, but also in allowing the viral vector to be re-used, since anti-MVA immunity is reduced.

DNA, VLPs and MVA are all vaccine delivery systems that are suitable for use in humans, and the next stage was to initiate clinical trials to test safety, immunogenicity and efficacy of recombinant MVA vectored vaccines for the first time in humans. The antigen that was chosen for these studies was a fusion between the string of multiple defined T cell epitopes originally developed for the VLPs (known as ME), and a complete *P. falciparum* liver stage antigen, thrombospondin relative adhesive protein (TRAP), forming an antigen designated METRAP.

3. First clinical testing of recombinant MVA-vectored vaccines

For any clinical testing of novel vaccines, the first consideration is the safety of the vaccine. All 'adverse events' occurring in the follow up after vaccination must be reported. These may be 'a serious adverse event' (SAE), which is defined as life-threatening, disabling, incapacitating or requiring hospitalisation, or non-serious, in which case the severity can be further divided into mild, moderate or severe. For adverse events which are expected following vaccination, the severity categorization is predefined; for example fever of 37.6–38.0 °C would be recorded as mild, 38.1–39.0 °C as moderate, and >39.0 °C as severe. In addition to considering severity, the relationship to the vaccination is also considered, and defined as definitely, probably, possibly or not related to vaccination. So the death of a vaccinee who was a passenger in an aeroplane that crashed would be recorded as an SAE not related to vaccination, whereas a headache the evening following vaccination might be mild (no impact on daily living), moderate (some impact on daily living) or severe (requiring bed rest) and related to vaccination.

Safety data from the first clinical trials of recombinant MVA reported that no serious or severe adverse events were recorded [22]. MVA-METRAP was administered to 26 healthy volunteers aged between 18 and 55 years by intradermal injection at a dose of 3×10^7 pfu, in some cases following priming with a DNA vaccine also expressing METRAP administered either intramuscularly or by gene gun. Most adverse events were mild, with 10 moderate adverse events occurring; mild flu-like illness, nausea, lethargy, or lymphadenopathy. In general the vaccinations were well tolerated. Swabs were taken from the surface of the skin following injection and from a fluid-filled blister at one injection site and assessed for the presence of MVA-METRAP by PCR which would have detected both live and dead virus, but none was found.

Immunogenicity of the different vaccination regimes was then assessed, using interferon-γ ELISpot assays to enumerate the METRAP-specific T cells induced by vaccination in malaria-naïve volunteers [23]. As predicted by the pre-clinical studies, heterologous prime boost regimes were more immunogenic than multiple doses of either vaccine used alone. Increasing the dose of MVA to 1.5×10^8 pfu resulted in stronger immune responses, but a second dose of MVA, even following initial priming with DNA, did not increase responses. Higher doses of the vaccine were also well tolerated and immune responses persisted for many months following vaccination. Protective efficacy was also assessed following infectious *P. falciparum* challenge. None of the volunteers were completely protected against infection but the group with the highest immune responses (gene gun DNA priming, MVA boosting) exhibited a delay in the time to patent parasitaemia, indicating partial protective efficacy of this regime. Safety and immunogenicity