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SCIENCE DIRECT®
Microbes and Infection 7 (2005) 1400–1404
Microbes and Infection
www.elsevier.com/locate/micinf

Forum in immunology

Vaccines based on Nef and on Nef/ΔV2 Env

Volker Erfle [a,b,*], Frank D. Goebel [c], Carlos A. Guzman [d], Roger Le Grand [e]

[a] *Institute of Molecular Virology, GSF-National Research Center for Environment and Health, Ingolstaedter Landstrasse 1a, 85764 Neuherberg, Germany*
[b] *Institute of Virology, Technical University Munich, Rogerstrasse 4b, 81679 Munich, Germany*
[c] *Department of Infectious Diseases, Medical Policlinic, Ludwig Maximilian University Munich, Pettenkoferstrasse 8a, 80336 Munich, Germany*
[d] *Vaccine Research, GBF-German Research Center for Biotechnology, Mascheroder Weg 1, 38124 Braunschweig, Germany*
[e] *CEA, Laboratoire d'Immuno-Pathologie Experimentale, Service de Neurovirologie, CRSSA, EPHE, IPSC, 18, route du panorama, B.P. 6, 92265 Fontenay aux Roses, France*

Available online 15 September 2005

Abstract

Modified vaccinia virus Ankara (MVA) is a potent vaccine vector, which proved its safety, immunogenity and efficacy in preclinical and clinical studies. The rational for the development of a vaccine against HIV based on the regulatory protein Nef delivered by MVA combined with a V2-deleted Env protein is discussed.

Keywords: HIV Env; MVA vaccine vector; HIV Nef

1. Introduction

The modified vaccinia virus Ankara (MVA) can be considered the current vaccinia virus strain of choice for clinical investigation [1]. Historical development and use of MVA as vaccine against smallpox enabled the establishment of an extraordinary safety profile. In fact, MVA can be used under conditions of biosafety level 1 because of its avirulence and its inability to productively grow in human cells [1].

The distinct replication deficiency of MVA has been thoroughly investigated in various cell lines under multiple and single step growth conditions indicating that the primary block is formation of new infectious progeny. This property has been observed also in primary human cells and is considered an important safety feature of MVA-based vaccines. The latter is further supported by the failure to detect spontaneous revertants under non-permissive conditions and results from marker rescue experiments demonstrating that MVA suffers from multiple host range gene defects that have an additive effect on host cell restriction.

In recent years significant progress has been made with regard to the development of MVA vector technologies. A big advantage when generating recombinant poxviruses, including vaccinia virus MVA, is that large amounts of DNA (theoretically up to 50 kb) can be integrated into the viral genome without need to consider packaging limitations experienced with other viral vectors. Another advantage of MVA is that natural deletion sites, which are distributed in clusters over the genome, can serve as insertion sites for foreign genes automatically precluding an impairment of remaining MVA gene functions. The sites of deletions II, III and VI have been preferentially used for insertion of recombinant genes because they are located in more conserved regions of the vaccinia virus genome, which are likely less affected by sequence rearrangements typically found near the genome termini [1–4].

Also the viral thymidine kinase gene, the standard insertion locus for generating replication competent recombinant vaccinia viruses, has been used to obtain recombinant MVA, albeit there have been observations that genetically stable thymidine kinase-negative MVA in chicken embryo fibroblasts may be difficult to generate and to maintain unless thymidine kinase function is supplemented.

Abbreviations: cGMP, current good manufacturing practices; HAART, highly active anti-retroviral therapy; HLA, human leucocyte antigens; HIV, human immunodeficiency virus; ICS, intracellular cytokine staining; IFNγ, interferon-gamma; MVA, modified vaccinia virus Ankara; SHIV, simian-human immunodeficiency virus; SIV, simian immunodeficiency virus.

* Corresponding author. Tel.: +49 89 3187 3004; fax: +49 89 3187 3249.
E-mail address: erfle@gsf.de (V. Erfle).

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doi:10.1016/j.micinf.2005.07.015

Compared to replication competent vaccinia viruses, MVA provides similar levels of recombinant gene expression even in non-permissive cells. In animal models, MVA vaccines have proven immunogenic and protective against various infectious agents, including immunodeficiency viruses [1]. Furthermore, another important property of MVA and MVA-based vaccines is that the vaccine in a lyophilized form can be kept over long periods under non-refrigerated conditions without any loss of activity.

2. Preclinical studies in mice and monkeys

2.1. Preclinical studies with MVA SIV vaccines

Recombinant MVA expressing immunodeficiency virus antigens were among the initial MVA vectors under investigation as candidate vaccine. Important data have been generated from studies in the macaque infection model with simian immunodeficiency virus (SIV) or simian-human immunodeficiency virus (SHIV). A first study testing a trivalent MVA vaccine delivering SIV Gag, Pol, and Env antigens resulted in significant modulation of virus loads after challenge with highly pathogenic, uncloned SIVsmE660, and demonstrated that macaques immunized with recombinant MVA were better able to control SIV viremia than animals immunized with standard recombinant vaccinia virus.

Further immunization experiments demonstrated the value of combining naked plasmid DNA vaccines and recombinant MVA vaccination, and suggested the regulatory immunodeficiency virus proteins Tat, Rev, and Nef as favorable vaccine antigens. The DNA prime-MVA boost strategy proved also successful in eliciting high-level cytotoxic T cell responses in macaques, and DNA/MVA immunization with multiple SIV and HIV antigens elicited memory immune responses that effectively controlled a mucosal challenge with pathogenic SHIV89.6P. Interestingly, this DNA prime/recombinant MVA vaccination required Env antigen-specific responses to achieve full control of SHIV 89.6 viremia and protection against loss of CD4 T cells. Additional work in the macaque model demonstrated that different patterns of immune responses could be found after MVA or DNA/MVA vaccination despite having similar protective capacity against SHIV89.6P. Compared to DNA/MVA immunization, MVA-only vaccination induced less than 1/10 of vaccine-specific T cells but 10-fold higher levels of Env-binding antibodies. Further interest in inducing potent antibody responses in addition to multivalent T cell immunity re-emerged from results of DNA/MVA vaccinations that elicited significant levels of SIV-(Tat)-antigen specific T cells but failed to protect against progression to disease after challenge infection with highly replication competent pathogenic SIVmac239. From the evaluation in such combined immunization protocols, recombinant MVA vectors emerged as strikingly efficient boosting vaccines for the in vivo amplification of immune responses primed by heterologous vaccines based on, e.g. naked DNA or recombinant Semliki Forest virus [5–19].

MVA vaccination can stimulate vaccinia antigen-specific humoral and cellular immune responses which may affect induction of immunity against the inserted gene [20,21]. As still a substantial proportion of the human population is vaccinated against smallpox, this could be discussed as an argument against the use of MVA as a boosting vaccine vector. Interestingly, when tested as recombinant vaccine for delivery of HIV-1 Env antigen, MVA has been found less affected by pre-existing vaccinia-specific immunity than a vector based on replication competent vaccinia virus Western Reserve. Moreover, applications of recombinant MVA vaccines using different routes for priming and boosting or using combined immunization with DNA vaccines have been found to significantly reduce the effects of pre-existing vaccinia virus-specific immunity.

2.2. Preclinical studies with Nef vaccines

To study the effectivity of Nef alone as a vaccine in preclinical models, Nef vaccines have been produced with the help of vaccinia vector MVA-F6 (MVA SIV Nef, MVA HIV-1 Nef) and as a highly purified protein (rec HIV-1 Nef). The efficacy of a MVA SIV Nef vaccine has been tested in rhesus monkeys. The animals were vaccinated three times i.m. with MVA SIV Nef and three times i.m. with MVA in the control animals. Three weeks after the last vaccination all animals were infected with 50 MID_{50} of a highly pathogenic SIV strain. The challenge was provided intravenously as cell-bound virus. All animals became infected, but after an initial virus peak 2 weeks after infection, the MVA SIV Nef vaccinated animals returned to virus load levels in plasma that were generally one log lower than those of the control animals. Furthermore, during an observation period of 32 weeks more animals died in the control group, than in the vaccinated group, indicating a significant influence of the Nef vaccine on virus replication and spread as well as disease progression.

The MVA HIV-1 Nef vaccine, as well as the rec HIV-1 Nef protein, were used in a preclinical mouse model for prophylactic vaccination. The mice have been challenged after vaccination with a HIV-1/MuLV pseudotype virus. The MVA HIV-1 Nef vaccinated mice became all infected, but showed significant reduction of the virus load by 50%. The rec HIV Nef protein vaccinated mice were as well all infected and the infected animals revealed reduced virus loads by 70%. Interestingly, when the mice were vaccinated with rec HIV Nef protein together with immune stimulatory CpG-DNA, 70% of the animals were protected from infection and the virus load in the infected dropped to 20%. Also the outcome of preclinical studies in mice indicated that vaccination with Nef alone already could have a significant influence on the course of the viral infection [22,23].

In conclusion, the results obtained from experiments in the SIV- or SHIV-macaque models strongly support thorough testing of recombinant MVA containing Nef in humans.