

Attenuated poxvirus vectors MVA and NYVAC as promising vaccine candidates against HIV/AIDS

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As yet, the only human infectious disease eradicated from our planet is smallpox, caused by variola virus a member of the poxvirus family. The vaccination success, with the declaration by WHO in 1980 of a worldwide free of smallpox, was largely due to the availability of a quite effective and stable live vaccine, as well as the restricted human host for virus infection. Variola was considered one of the most devastating diseases of human mankind. With the sudden appearance of the HIV/AIDS in 1981, an infection which spread rapidly to become a pandemic in a short time, causing up to date more than 22 million deaths, about 40 million people infected and a current incidence of about 3 million deaths per year, this dreadful pandemic has become one of the most severe diseases in the World, specially in poor countries. While different antiviral drugs have been developed that block virus replication at various stages of infection, however the rapid virus escape that follows during the drug therapy due to mutations, makes the development of vaccines the most secure option to control and eradicate the disease. Numerous vaccines have been developed, but to date the clinical trials have failed to show any efficacy against HIV infection. Due to the proven success of vaccinia virus in the control of smallpox as well as of poxvirus recombinants against veterinary diseases, a major effort has been directed to document the advantages of poxvirus vectors as vaccines against multiple diseases. Two of the most promising poxvirus vectors are the highly attenuated modified vaccinia virus Ankara (MVA) and the

modified Copenhagen strain NYVAC. In this commentary I describe the biological characteristics of the attenuated poxvirus vectors, MVA and NYVAC, with emphasis in their application in HIV preclinical and clinical trials, and considerations as future HIV vaccines.

Characteristics of the Attenuated Poxvirus Vectors MVA and NYVAC

The demonstration in the early 1980's that the genome of vaccinia virus could be manipulated and foreign genes inserted, immediately suggested that poxvirus vectors could be useful as potential vaccines against different diseases.¹⁻⁴ The success came with the application in the wild of a vaccinia virus recombinant to control rabies in animals.⁵ However for human use a higher level of attenuation of vaccinia virus was demanded as a vaccine. This was due to the complications found during the smallpox vaccination campaign, particularly in immunocompromised individuals. In an effort to search for attenuated vaccinia virus mutants several approaches were followed that include, the continuous passage of the virus in tissue culture cells and removal of selected genes in the viral genome.

Mayr and colleagues used primary chicken embryo fibroblast cells to grow chorioallantoid vaccinia virus Ankara (CVA), a Turkish smallpox vaccine, and isolated after more than 570 passages attenuated mutants. In this way they obtained what we now know as MVA.⁶ This virus was sequenced and shown to have lost about 30 kb, particularly at both ends of the viral genome, with multiple genes deleted that

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counteracted host immune defense mechanisms.⁷ An important characteristic of MVA is a highly attenuated phenotype as shown in several animal models and in humans, and high levels of expression of heterologous antigens. In fact, MVA was used during the eradication campaign in Germany in over 120,000 individuals without adverse effects.^{6,8} Using *in vivo* live imaging we have shown that MVA when inoculated in mice by various routes remains active in terms of viral gene expression at least during 24 h⁹ and does not reach the brain. This has also been shown in monkeys after MVA delivery by aerosol.¹⁰ In human cells MVA replicates efficiently but is unable to form infectious virus particles, thus restricting its capacity to express viral genes exclusively in the initially infected cell.¹¹⁻¹³ The ability of MVA to produce viral progeny is limited to few cell lines, like CEF and BHK-21.¹⁴ This is why MVA is grown in CEF for industrial purposes. The restricted MVA phenotype is probably related to the cellular signals triggered during virus infection. Through the use of microarray analyses of MVA infected immature human dendritic cells we have shown that MVA infection triggered the induction of cellular genes involved in innate immune responses, especially interferon- β , TNF- α , RIG-I, MDA-5, TLRs.¹⁵ A detailed analysis of immune sensing of MVA in human macrophages and mouse bone-derived macrophages (BMDMs) revealed that the TLR2-TLR6-MyD88, MDA-5-IPS-1 and NALP3 inflammasome pathways play specific and coordinated roles in regulating cytokine, chemokine and interferon response to MVA infection.¹⁶ Consistent with the fact that several pattern recognition receptors are engaged in the sensing of MVA by the innate immune system, multiple signaling pathways, like NF κ B, ERK1/2, JNK, IRF3, IRF7 and STAT1, are activated in macrophages infected with MVA.¹⁶ Interferon-dependent and independent mechanisms are induced by MVA infection.^{16,17} Thus, MVA is an effective vector to activate innate immune responses that might be important to enhance the vector efficacy when administered as a vaccine.

Tartaglia and colleagues approached the generation of attenuated vaccinia virus mutants by selectively deleting specific

regions in the virus genome. Using the Copenhagen vaccine strain of vaccinia virus and gene targeting techniques, these investigators produced a virus, referred as NYVAC, with 18 viral genes inactivated.¹⁸ This virus is highly attenuated, expresses efficiently foreign genes and it grows in limited number of cells for industrial purpose like CEF and Vero. In human cells virus replication is restricted, some late structural proteins are not synthesized and virus morphogenesis is blocked at the level of immature virus (IV) formation.¹⁹ A reduction in viral proteins that elicit antibodies with neutralizing capacity has also been observed in sera from individuals vaccinated with NYVAC,²⁰ a property of interest since fewer neutralizing antibodies to the vector will be produced in vaccinees with NYVAC. The innate immune sensing triggered by NYVAC in dendritic cells and macrophages was lower compared to MVA,¹⁶ probably due to the number of virus immune modulators that still remain active in the NYVAC genome. Recombinants based on NYVAC have been demonstrated to be safe and immunogenic in animal and human vaccine studies.² Due to the biological and immunological characteristics of NYVAC, this vector is considered a promising vector against HIV, as discussed later.

Esteban and colleagues developed a persistent vaccinia virus infection cell culture system with the WR strain that generated highly attenuated virus mutants with large deletions at both ends of the viral genome and mutations in some structural genes.^{21,22} Recombinants based on these WR mutants and expressing parasite antigens for malaria and leishmania were shown in prime/boost combination (influenza or DNA as priming and vaccinia virus vectors as booster) to elicit protection after challenge with the parasites.^{23,24} This led to the first demonstration in vaccination of a heterologous prime/boost approach with different vectors to activate CD8⁺ T cell responses that were protective after challenge with a pathogen.^{23,25} Moreover, these studies establish that a vaccinia virus vector was most effective when inoculated as a booster. Whether these WR mutant vectors exhibit immunogenic characteristics different from MVA and NYVAC is under investigation.

Preclinical Studies of MVA and NYVAC Recombinants as HIV Vaccines

Soon after the demonstration that MVA and NYVAC vectors could be used as potential vaccines due to their highly attenuated phenotype and capacity to express efficiently foreign genes,^{2,11} these vectors have been tested in a number of preclinical trials in animal models, particularly in mice and monkeys.²⁶ We have shown, as part of the EuroVacc program, that MVA and NYVAC vectors expressing Env/Gag-Pol-Nef antigens of HIV-1 from clades B and C (referred as MVA-B and MVA-C; NYVAC-B and NYVAC-C), triggered in mice specific immune responses to the HIV antigens.^{27,28} These responses are enhanced when heterologous prime/boost combination is used, like priming with a DNA vector expressing the same four HIV antigens followed by a booster with MVA or NYVAC expressing the same antigens. While the DNA/MVA or DNA/NYVAC combination favors Env responses, the combination of NYVAC/MVA gave broader antigen responses. All immune responses were predominantly Th1 type.^{27,28} When similar recombinants were tested in macaques, but now with DNA, MVA or NYVAC vectors expressing Env of HIV-1 89.6p and Gag-Pol-Nef of SIVmac239, it was found that the immune response elicited by the combination DNA/NYVAC elicited predominantly a CD4⁺T cell response compared to DNA/MVA protocol that was more biased to a CD8⁺T cell response. Significantly, after challenge the vaccinated macaques with SHIV89.6p, all animals (n = 7) were able to control the infection and survived, similarly for both the DNA/MVA or DNA/NYVAC combinations.²⁹ This is consistent with the findings by other groups showing protection in macaques after immunization with MVA vectors expressing SIV antigens.³⁰⁻³³ Protection in macaques immunized with DNA/MVA vectors against SIV infection has been correlated with effective CD8⁺T cell responses. The degree of protection achieved in macaques has been variable and this is probably related to the prime/boost vector combination used. In fact, reduced protection was observed in macaques immunized with three doses

of MVA expressing Gag and Tat,³¹ while other studies with DNA/MVA expressing Env/Gag-Pol showed better protection after challenge with SIVmac251.³⁰ That an immune control of an SIV challenge by a T-cell based vaccine in macaques is possible has been shown in a prime/boost combination with two different adenovirus vectors expressing Gag.³⁴ While there is consensus that for vaccine efficacy in macaques the virus challenge has to be done with heterologous and highly pathogenic SIV, the studies thus far revealed that activation of T cell responses are important but not sufficient in the control of SIV/AIDS disease. Protection from HIV/AIDS by vaccines will likely require induction of rapid and potent HIV specific B and T cell responses in mucosal tissue as well as systemic, leading to reduction in virus load and in virus dissemination.^{35,36}

Clinical Studies with MVA and NYVAC as HIV Vaccines

A limited number of prophylactic and therapeutic HIV clinical studies have been performed with MVA and NYVAC vectors.²⁶ These studies confirmed the safety of both vectors and ability to induce specific immune responses to foreign expressed antigens. A therapeutic study carried out in young adults infected with HIV and on antiretroviral therapy (HAART), using the prime/boost combination of MVA and fowlpox vectors expressing Env, Gag, Tat, Rev and Nef-RT fusion antigens, revealed that the immunization protocol elicited increased frequencies of HIV-1 specific CD4⁺ breath of HIV-1 specific CD8⁺ T cell responses.³⁷ However, plasma HIV-1 specific antibody levels and neutralizing activity were unchallenged following vaccination.³⁷ A series of prophylactic studies have been performed with MVA vectors expressing various HIV antigens and administered either alone or in prime/boost combination with DNA.²⁶ While there were variations in the number of responders between studies (from 15–50%),^{38,39} a recent phase I clinical trial proved by the standardized ELISPOT assay using fresh PBMCs that prime/boost with DNA and MVA expressing a variety of HIV antigens from different clades induced specific HIV responses in about 90% of

the volunteers.⁴⁰ Considering that several factors were suboptimal in this study the demonstration of strong immunogenicity in this trial in spite of pre-existing immunity to vaccinia virus⁴¹ is encouraging. As part of the EuroVacc program, two phase I clinical trials have been performed (EVO1 and EVO2;^{42,43}) with two arms, either NYVAC alone or the combination DNA/NYVAC. The DNA and NYVAC vectors express Env (as gp120) and the fusion protein Gag-Pol-Nef of 160 kDa, as indicated in the above section.⁴⁴ These studies established that the administration of two doses of NYVAC induced immune responses specific to HIV-1 in about 40% of the volunteers, while when DNA was given as a priming followed by a booster with NYVAC recombinant, the number of responders increased to over 90%. Significantly, the response to HIV antigens was predominantly directed by CD4⁺ T cells, it was broad with immunodominance for Env, polyfunctional and durable with maintenance of memory T cells for a year.⁴⁴ Thus, the recent clinical studies provided evidence that MVA and NYVAC vectors are efficient activators of T cell responses, with MVA favoring preferentially specific CD8⁺ T cells while NYVAC triggers more CD4⁺ T cells. These observations are consistent with the difference in behavior observed between the two vectors in vitro and in vivo systems.

Future Considerations of MVA and NYVAC Vectors as HIV Vaccines

The development of a vaccine against HIV/AIDS has met major difficulties.^{35,36} It has been quite disappointing the failure of the multicentre phase IIb STEP study (HVTN 502) with the Merck vaccine. This study was conducted in 3,000 adults at high risk of HIV infection, receiving three doses of recombinant adenovirus serotype 5 expressing Gag-Pol-Nef of HIV-1 from clade B. The trial was suspended due to a higher incidence of HIV infection in vaccinated individuals with high titres of antibodies to adenovirus.⁴⁵ In spite of finding strong cellular responses to HIV antigens in vaccinees, these responses were non protective.⁴⁶ It is not yet clear why these responses fail to

protect, although explanations have been provided.⁴⁷ Therefore, the development of adeno vectors different from those that infect humans, like adeno serotypes from chimpanzees, to avoid the presence of antibodies in the vaccines, is under intensive investigation. In the case of poxvirus vectors, the population below 35 years of age does not have antibodies to vaccinia virus, as the practice of vaccination against smallpox was discontinued worldwide many years ago and hence, these vectors could be used safely in naïve population. The only phase III clinical trial ongoing is based on the canary poxvirus vector ALVAC that has been administered in Thailand to about 16,000 men and women 18–30 years of age and received a regimen of ALVAC-HIV expressing Env/Gag-Pol from clades B/E given at 0, 1, 3, 6 months plus VaxGen purified protein gp120 given at months 3 and 6. In spite of the critique that this trial received, the Data and Safety Monitoring Board recommended in 2007 that the trial continue. The results will be known in September 2009. Will it be another disappointment in HIV vaccines? This will happen if the vaccine does not show a benefit in the control of HIV infection. Since there were no benefits in previous clinical trials when immunized independently with either ALVAC-HIV or gp120 protein,^{48,49} in principle it will be expected at most a moderate effect of the combined vaccine. If the vaccine confers limited protection in the range of over 30%, the findings might be considered relevant as it will be an indication that a vaccine does something against HIV infection and that improved vaccines could be developed.

From what we now know experimentally, the poxvirus vectors MVA and NYVAC are excellent candidates for the generation of HIV vaccines. These vectors have shown a good safety profile, elicit protective immune responses to SIV in macaques, and in humans trigger strong, broad and durable immune responses to HIV antigens. While in both hosts, monkey and human, NYVAC has been shown to drive preferentially the immune responses towards CD4⁺ T cells, MVA shifted the response more towards activation of CD8⁺ T cells. Both vectors have distinct biological characteristics as

confirmed by gene array analyses, induction of cytokine and chemokines, intracellular signaling pathways and nature of polyfunctional responses. While definition of correlates of protection to HIV remains to be firmly established, there are a number of markers that can be used as potential indicators in the control of HIV infection, like: (1) a requirement for specific activation of CD4⁺ and CD8⁺ T cells; (2) triggering polyfunctional responses; (3) enhanced magnitude and breadth of the immune response; (4) induction of long-term memory cells; (5) production of high titre neutralizing antibodies with broad specificities. The MVA and NYVA vectors already developed fulfil some of the predictions, except that they are largely directed to activate T cell responses. There is a need for the generation of new vectors with the ability to elicit the production of high titer neutralizing antibodies.⁵⁰ Along these lines, generation of MVA and NYVAC vectors with improved B and T cell responses will likely be produced. In fact, MVA vectors with viral genes deleted that antagonize host specific immune responses have been generated and some immunological benefit has been observed.⁵¹ Moreover, enhancement of immune responses of MVA and NYVAC vectors expressing HIV antigens has been obtained through co-expression of certain cytokines⁵² as well as by combining the vectors with adjuvants.⁵³ As more knowledge is gained on the biological properties of genes in the vaccinia virus genome, and on how adjuvants can be used in conjunction with the poxvirus vectors MVA and NYVAC, it is likely that newly developed vectors with enhanced immunogenicity will emerge in the coming years. It will be important to establish in future studies whether anyone of the poxvirus vectors MVA or NYVAC could be used alone or in combination with other vectors as more potent HIV/AIDS vaccines. Experiments with monkeys vaccinated with similar vaccines as those to be used in the clinical trials but expressing SIV antigens will be needed to establish if the immune responses triggered in this animal model correlate with reduction of virus load and protection after challenge with a pathogenic SIV. Further clinical trials will delineate which are the most

potent vectors and/or their combination leading to effective induction of innate and adaptive immune responses to HIV antigens. Undoubtedly, basic understanding of the molecular interaction between the virus vector and the host together with its immunological behavior will provide a rationale for optimal use of MVA and NYVAC vectors as HIV vaccines.

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