

Induction of MVA-specific Antibodies and T-cells in HIV-1 Infected Patients and Healthy Controls by Immunization with a Recombinant MVA-nef.

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Abstract

Background: Vaccinia viruses were highly effective in the eradication of smallpox, but they are associated with rare, but severe side effects, particularly for immune compromised individuals. To improve the safety of smallpox vaccines, attenuated vaccinia vectors have been developed. MVA is a highly attenuated vaccinia virus that replicates poorly, if at all, in mammalian cells. So far, data are lacking regarding the immunogenicity of MVA in HIV-1 infected patients. Therefore, we analyzed both in HIV-1 infected patients and in healthy subjects the induction of MVA-specific antibodies and T-cells after immunization with a recombinant HIV-1 nef expressing MVA.

Methods: 14 HIV-1 infected patients on HAART and 14 HIV-negative subjects were vaccinated s.c. with MVA-nef at a dose of 5×10^8 TCID₅₀ at week 0, 4 and 16. All 14 HIV+ and 4/14 HIV-neg. subjects had received small pox vaccination in their childhood, whereas 10/14 HIV-neg. subjects were vaccinia naïve. MVA specific T-cells were monitored by α -IFN ELISPOT and MVA-specific antibodies by ELISA.

Results: At baseline, MVA-specific T-cells could be detected in 3 patients (120 to 180 SFU/10⁶ PBMC) and in 2 HIV-neg. subjects (70 and 100 SFU/10⁶ PBMC). All subjects developed a MVA-specific T-cell response 2 to 4 weeks after the 3rd vaccination. At week 4, 6 and 16, but not at week 18, the median frequencies of MVA-specific T-cells in HIV-neg. subjects were significantly higher than in the HIV+ patients. The median frequency of MVA-specific T-cells at week 18 was 860 SFU/10⁶ PBMC (range 260-3330) in HIV-neg. subjects and 490 SFU/10⁶ PBMC (range 30-4360) in the HIV+ patients. Depletion experiments performed in 8 patients and in all HIV-neg. subjects revealed that in 6/8 HIV+ patients and in 4/14 HIV-neg. subjects MVA was recognized only by CD8+ T-cells, while in 2/8 patients and in 10/14 HIV-neg. subjects both MVA-specific CD4+ T-cells and CD8+ T-cells could be detected. Prior to vaccination 5/14 HIV+ subjects and 4/14 HIV-neg. subjects had a weak antibody response to MVA. Already 4 weeks after a single immunization 13/14 infected individuals developed MVA-specific antibodies while the remaining patient seroconverted following the 2nd vaccination. All 14 controls seroconverted at week 4.

Conclusions: This study demonstrated that MVA-nef is a safe and immunogenic vector that could induce a strong MVA-specific T-cell and antibody response both in HIV-1 infected patients and in uninfected individuals.

Methods

The analysis for this study is based on the pooled data from two phase-I studies that were performed to evaluate the safety and immunogenicity of a MVA-nef vaccine in HIV-1+ patients and HIV-1 negative healthy volunteers.

Subjects:

The first study included 14 male HIV-1 infected patients with a mean age of 46 years (range 32-61). All patients had been vaccinated in their childhood against smallpox. Inclusion criteria were: asymptomatic HIV-1-infection, HAART for at least 3 months and CD4 cells $\geq 400/\mu$ l (Harrer E et al. Antiviral Therapy, in press). The second study included 14 HIV-1 negative volunteers (13 male and 1 female) with a mean age of 32 (range 19-60 years). Two of the HIV-1 negative subjects had received full smallpox vaccination in their childhood against smallpox and another two have received at least one smallpox immunisation.

MVA-nef vaccine:

The MVA-nef construct was generated by insertion of the gene encoding the HIV-1-LAI Nef gene under the control of the vaccinia early/late promoter P7.5 into the site of deletion II of MVA by homologous recombination using flanking MVA sequences. No antibiotic resistance genes are present in the recombinant construct. MVA-nef was propagated in primary chicken embryo fibroblast cells, followed by centrifugational purification on sucrose cushions and gradients. The vaccine was produced according to current Good Manufacturing Practices (cGMP) by Bavarian Nordic and was provided in aliquots of 5×10^8 TCID₅₀/ml. The MVA-nef vector was developed at the GSF-Forschungszentrum für Umwelt und Gesundheit[†] in Munich, Germany, by Volker Erfle and Gerd Sutter, supported by the European Commission (Genomic HIV-1 vaccine trial: BI04-CT97-2109).

Immunisation protocol:

The subjects were immunised subcutaneously in the upper arm with 5×10^8 TCID₅₀ of the MVA-nef virus at baseline (week 0), then at week 4 and at week 16. Only one patient (#14) received his second immunisation at week 2 instead of week 4. Patient #7 received the 3rd immunisation at week 15, patients #3, #9 and #12 at week 17 instead of week 16. Two weeks after the third immunisation all HIV-1+ patients stopped antiretroviral therapy.

Analysis of MVA-specific T-cells:

MVA-specific T-cells were evaluated using a standard gamma-IFN - ELISPOT using 10⁶ PBMC/well in triplicates. MVA-nef and the control vector MVA were added into the wells at a Multiplicity of Infection (MOI) of 50 TCID₅₀/cell. The plates were incubated for 16 hours at 37° C and then processed according to standard protocols. Spots were counted using a video-based automatic ELISPOT reader (AID, Strassberg, Germany) and the results are expressed as the median of triplicates and calculated as SFU/10⁶ PBMC/well. In the trial with HIV-1 negative subjects an ELISPOT reaction was calculated as positive if the numbers of spot forming units (SFU) in a given well was $\geq 5 \times 10^3$ PBMC after subtraction of the number of SFUs in the negative control. Due to slightly higher background values in the trial with HIV-1 positive patients an ELISPOT reaction was calculated as positive if the number of SFUs was $\geq 10^4/10^6$ PBMC after subtraction of the negative control. CD4-, or CD8-restriction was analyzed with PBMC depleted of CD4+, or CD8+ T-cells, respectively using magnetic beads.

Analysis of MVA-specific antibodies:

MVA-specific IgG antibody responses were measured using a direct ELISA. 96-well TRANSP MAXIS plates (Nunc, Wiesbaden, Germany) were coated overnight at 4°C with 100 μ l (7.5 μ g/ml) of MVA antigen in coating buffer (200 mM Na₂CO₃, pH 9.6). Plates were washed twice and blocked with 200 μ l of PBS-FCS 5% (PAA Laboratories, Linz, Austria) for 30 min at room temperature. The plates were washed a further two times and heat-inactivated (56°C for 30 min). Test sera were titrated in duplicates using 2-fold serial dilutions. Again, the plates were incubated for 1 hour at room temperature and duplicated with 5 times. A goat anti-human horseradish peroxidase (HRP) detection antibody (Sigma-Aldrich) was diluted 1 in 40000 and 100 μ l added to each well. The plates were incubated for 1 hour at room temperature and washed 5 times. O-phenyldiamine dihydrochloride tablets (Sigma-Aldrich) were prepared according to the manufacturer's instructions and 100 μ l of the chromogen was added per well. The plates were incubated in the dark at room temperature for 30 min and the reaction was stopped by adding 50 μ l 3M H₂SO₄ (Merck, Darmstadt, Germany) per well. The Optical Density (OD value) was read at 492 nm, with a reference of 405 nm. The antibody titres were calculated by linear regression and defined as the serum dilution that resulted in an optical density of 0.3.

Introduction

Concerns about smallpox bioterrorism have raised new interest in smallpox vaccines. Vaccinia viruses proved to be highly effective in the eradication of smallpox; however, these vaccines are associated with rare, but severe side effects, particularly for immune compromised individuals. To improve the safety of smallpox vaccines, attenuated vaccinia vectors have been developed. The Modified Vaccinia Virus Ankara (MVA) was attenuated by packaging the vaccinia virus strain CVA for more than 500 times on chicken embryo fibroblasts, resulting in the loss of about 15% of its genome, leading to a virus that replicates poorly, if at all, in mammalian cells. MVA-572 (corresponding to 572 passages) was used as a pre-vaccine in a two-step vaccination program against smallpox and shown to be safe in more than 120,000 primary vaccinees. Vaccines based on MVA have proved to be a safe and a potent stimulator of cellular immunity in a variety of animal models including immune deficient animals. So far, data are lacking regarding the MVA-specific immunogenicity of MVA-vectors in HIV-1 infected patients. In this study, we present data about safety and induction of MVA-specific T-cells and MVA-specific antibodies from two MVA-nef phase-I trials that were performed in HIV-1 infected patients and in healthy volunteers.

Results

A: Safety:

MVA-nef was well tolerated with local injection site reactions in all subjects, and mostly mild, rarely moderate systemic side effects in some subjects: see Figure 1.

B: MVA-specific T-cells:

Although all HIV-1 infected patients had previously been vaccinated against smallpox, MVA-specific T-cells could be detected at baseline only in three patients with a local frequency of 120 to 108 SFU/10⁶ PBMC (patients #5, #7 and #14). However, all patients developed a MVA-specific T-cell response 2 to 4 weeks post the 3rd vaccination, with a median frequency of 490 SFU/10⁶ PBMC (range 30 - 4360 SFU/10⁶ PBMC) at week 18 and 670 SFU/10⁶ PBMC (30 - 2120 SFU/10⁶ PBMC) at week 20 (Figure 2). The presence of circulating T-cells to MVA in the peripheral blood decreased during the follow up, such that at week 42 (median, range 38-43 weeks) an MVA - specific T-cell response could only be detected in 7 patients, with a median frequency of 110 SFU/10⁶ PBMC (range 0 - 590). Depletion experiments performed in 8 patients at a median time of week 23 (range week 20 - week 30) revealed that in 6 patients MVA was recognized only by CD8+ T-cells, while in the remaining two patients we also could detect an MVA-specific CD4+ T-cell response. In the HIV-1 negative subjects low frequencies of MVA-specific T-cells could be detected at screening in two subjects with prior vaccinia immunization (70 and 100 SFU/10⁶ PBMC). MVA-specific T-cells could be detected in 13/14 HIV-1 negative subjects 4 weeks after the 1st immunisation and in all two weeks after the second immunisation (Figure 3). Depletion experiments performed in all HIV-1 negative subjects revealed that in 4/14 subjects revealed that MVA was recognized only by CD8 cells, whereas in 10/14 subjects both MVA-specific CD4+ and CD8+ T-cells could be detected.

At week 4, 6 and 16, but not at week 18, the median frequencies of MVA-specific T-cells in HIV-neg. subjects were significantly higher than in the HIV+ patients (Figure 4) The median frequency of MVA-specific T-cells at week 18 was 860 SFU/10⁶ PBMC (range 260-3330) in HIV-neg. subjects and 490 SFU/10⁶ PBMC (range 30-4360) in the HIV+ patients.

C: MVA-specific antibodies:

Prior to vaccination 5/14 HIV+ subjects and 4/14 HIV-neg. subjects had a weak antibody response to MVA. Already 4 weeks after a single immunization 13/14 infected individuals developed MVA-specific antibodies while the remaining patient seroconverted following the 2nd vaccination. All 14 controls seroconverted at week 4. Only after the 3rd booster the HIV-1 negative volunteers developed significantly higher MVA-antibody titers than the HIV-1 positive patients (Figure 5).

Conclusion

Our study could demonstrate that the MVA-nef vector was safe both in HIV+ patients on HAART with CD4 > 400 and in HIV-1 negative volunteers. MVA-nef could induce MVA - specific T-cells and MVA-specific antibodies in all subjects. This finding indicates that MVA-nef, apart from its potential as a candidate HIV-vaccine, potentially could offer the additional benefit of a safe smallpox vaccine for HIV infected patients. However, as a smallpox protective effect of MVA has not been established in humans, the utility of MVA as a safe smallpox vaccine requires further clinical studies to define surrogate parameters for protection against smallpox.

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Figure 1

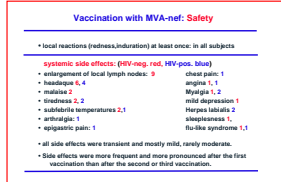


Figure 2

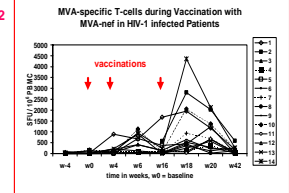


Figure 3

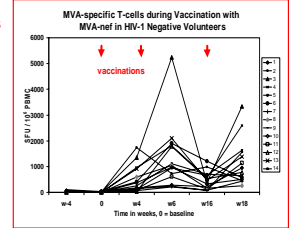


Figure 4

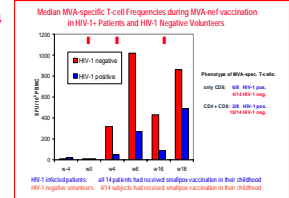
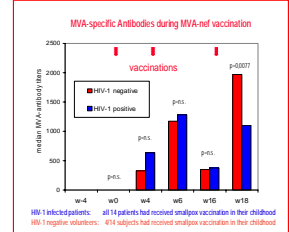


Figure 5



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