

Studies of a prophylactic HIV-1 vaccine candidate based on modified vaccinia virus Ankara (MVA) with and without DNA priming: Effects of dosage and route on safety and immunogenicity[☆]

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Abstract

Background: Two parallel studies evaluated safety and immunogenicity of a prophylactic HIV-1 vaccine in 192 HIV-seronegative, low-risk volunteers. Modified vaccinia virus Ankara (MVA) and plasmid DNA (pTHr) expressed HIV-1 clade A gag p24 and p17 fused to a string of 25 overlapping CD8+ T cell epitopes (HIVA).

Methods: These studies compared intramuscular, subcutaneous, and intradermal MVA at dosage levels ranging from 5×10^6 – 2.5×10^8 pfu. In Study IAVI-010, DNA vaccine was given as a prime at months 0 and 1, followed by MVA as a boost at months 5 and 8. In Study IAVI-011, MVA alone was given at months 0 and 2. Regular safety monitoring was performed. Immunogenicity was measured by the interferon (IFN)- γ ELISPOT assay on peripheral blood mononuclear cells (PBMC).

Results: No serious adverse events were attributed to either vaccine; most adverse events were mild or moderate, although MVA resulted in some severe local reactions. Five vaccine recipients had at least one positive IFN- γ ELISPOT response, but none were sustained.

Conclusion: This HIV-1 vaccine candidate was in general safe and well-tolerated. Local reactions were common, but tolerable. Detectable immune responses were infrequent.

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Keywords: Prophylactic HIV-1 vaccine; safety; immunogenicity; prime-boost; MVA (modified vaccinia virus Ankara); DNA vaccine

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1. Introduction

The development of a safe and effective preventive HIV-1 vaccine remains a global priority. Plasmid DNA Vaccines alone have produced weak CD8+ responses in macaques, but have primed for much stronger responses when gene components have been inserted into viral vectors [1–3]. A number of viral vectors have been developed as recombinant DNA HIV vaccines, including canarypox [4–6], fowlpox [7], replication-deficient adenovirus-5 [1], Semliki Forest virus [8], and Venezuelan equine encephalitis virus [9], and the vector we have used in the study we report below, modified vaccinia virus Ankara (MVA). Over 55 phase I/II trials of HIV candidate vaccines, and one phase III trial have tested about 30 different candidate vaccines worldwide. At least another 27 trials are ongoing, comprising 27 phase I, four phase II and one phase III study. (<http://www.iavireport.org/specials/OngoingTrialsOfPreventiveHIVVaccines.pdf>). To date the immune responses in these clinical trials have been small when compared with responses in macaques to the same vaccines and the phase III trial that has been completed has not demonstrated protection from infection [10]. The aim now is to improve immune responses in man by, for instance, increasing the dose and number of immunizations or by testing different routes of immunization. Vaccine candidates based on (MVA), a virus-derived vector, have induced SIV-specific [11] and HIV-1 specific CD8+ T cell responses in rhesus macaques [2,3,12] and enabled protection against SIV disease in macaques [13–15]. These results supported development of several DNA- and MVA-vectored vaccine constructs [16]. Human studies have indicated a favorable safety profile for DNA and MVA vaccines against HIV-1 [16–18].

The selection of immunogens for HIV vaccines has been hampered by the lack of correlates of protection against HIV-1 infection and progression to AIDS. In chronically HIV-infected persons, there is evidence of a correlation between high levels of T helper responses specific for gag and decreased viraemia [19]. Likewise, there is a positive correlation between gag-specific CD4+ T-cell responses and concentrations of gag specific CD8+ T cell precursors, and an inverse correlation of these markers with plasma HIV-1 RNA levels [20]. Hence, an immunogen was designed to induce HIV-1 specific T-cell responses to gag and selected epitopes from other HIV-1 proteins [21]. Attempts to induce neutralizing antibodies were not part of this vaccine design. The immunogen was designed for areas where HIV-1 clade A predominates.

By 2000, pTHr.HIVA DNA and MVA.HIVA vaccine constructs were ready for clinical trials. Initial studies demonstrated acceptable safety at the dosage levels and routes tested [17] (W. Jaoko, personal communication; F. Nakwagala, personal communication). Overlapping studies were planned to rapidly assess safety and immunogenicity of higher dosages of the MVA.HIVA vaccine administered by three different routes, either with or without pTHr.HIVA

DNA priming. These were conducted as two separate trials, but are reported together because of their complementary design and objectives.

2. Materials and methods

2.1. Recruitment and study population

Male and female volunteers in Kenya, South Africa, Switzerland and the UK were recruited by presentations to members of community organizations, hospitals, colleges, and by advertisements to the general public. Volunteers were eligible if they were free of significant medical conditions by history, physical examination and routine laboratory parameters (hematology, clinical chemistry, urinalysis); 18–60 years old; HIV-1 vaccine-naïve; and not infected with HIV-1 or HIV-2. Lactating or pregnant females were excluded. Sexually active volunteers were required to use contraception until at least 4 months after their last vaccination. Serum HIV antibody tests, with pre- and post test counseling, were performed at regular intervals using a standard ELISA method.

Enrollment in IAVI-010 commenced in April 2003 and was completed by February 2004. Enrollment in IAVI-011 commenced November 2003, and was completed in March 2004.

2.2. Regulatory issues, data monitoring and trial management

The clinical trials were conducted according to ICH Good Clinical Practice (GCP) guidelines and with appropriate national regulatory approvals and ethical approvals. Written, informed consent was obtained from all volunteers.

The Data Coordinating Centre (DCC) at The EMMES Corporation (Rockville, MD, USA) provided randomization schemes. Study site staff, laboratory staff and volunteers were blinded to the allocation of placebo or vaccine but not to the route of MVA.HIVA or dosage level. Data were entered by an Internet-based Data Entry System (IDES) and assessed at the DCC for accuracy, completeness, consistency, and validity. A Trial Steering Committee supervised the studies and an independent Data Monitoring and Ethics Committee reviewed the safety data.

2.3. Investigational vaccines

The pTHr.HIVA DNA and recombinant MVA.HIVA vaccines were developed in partnership between the University of Nairobi, the Medical Research Council (MRC) Oxford, and the International AIDS Vaccine Initiative (IAVI) [21]. Both vectors express the same consensus sequence of HIV-1 clade A gag p24 and p17 fused to a string of 25 partially overlapping CD8+ T cell epitopes (HIVA) [21,22]. Cobra Bio-Manufacturing (Keele, UK) manufactured the pTHr.HIVA DNA vaccine and matching

placebo. The DNA placebo consisted of the DNA vaccine buffer (10 mM Tris–HCl, pH 8.0, 1 mM EDTA, 0.9% NaCl). The MVA.HIVA vaccine, and matching placebo, consisting of MVA vaccine buffer (10 mM Tris–HCl, pH 7.7, 140 mM NaCl), were manufactured by Impfstoffwerk Dessau-Thornau GmbH in Rosslau, Germany. The vaccines were manufactured according to Good Manufacturing Practice (GMP).

2.4. Study design

Both trials were double-blind, randomized, placebo-controlled, dose-escalation designs and both investigated the effect of dosage and route of injection for MVA.HIVA. The major difference in design was that Study IAVI 010 was a DNA prime-MVA-boost trial, while Study IAVI-011 tested only MVA.HIVA. The interval between MVA.HIVA injections was one month longer in the prime-boost study (Table 1).

For Study IAVI-010, volunteers received 0.5 mg of pThr.HIVA DNA or placebo at months 0 and 1 by intramuscular deltoid injection (1.0 ml/dose), followed by MVA.HIVA or placebo at months 5 and 8. Volunteers received either both active vaccines, or both placebos. MVA.HIVA was given at three escalating dosage levels (low-dosage, LD: 5×10^6 pfu; mid-dosage, MD: 5×10^7 pfu; high-dosage, HD: 2.5×10^8 pfu), intramuscularly (IM) or subcutaneously (SC). A MD intradermal (ID) group was included to allow comparison with previous studies. LD and MD received 0.1 ml/dose and HD received 0.5 ml/dose. A satisfactory safety review was required before proceeding to a higher dosage. For Study IAVI-011, MVA.HIVA was given at two dosage levels (LD: 5×10^6 pfu; MD: 5×10^7 pfu) at 0.1 ml/dose.

2.4.1. Safety assessments

Vital signs, injection sites, and medical history were assessed by study staff immediately prior to each adminis-

tration of study vaccine, 30–60 min post vaccination, and at follow-up visits. Local reactions (tenderness, erythema/skin discoloration, skin damage, induration, formation of scab or crust) and systemic events (fever, chills, headache, nausea, vomiting, malaise, myalgia) were prospectively recorded for at least 10 days after each vaccination. Safety assessments included routine laboratory parameters at predetermined time-points. Adverse events were graded for severity as mild, moderate, severe, or very severe, using predefined criteria and were assessed for their relationship to study vaccines by the trial physician and principal investigator. Safety endpoints included a four-fold increase of antibodies to double stranded DNA.

2.5. Immunogenicity assessments

HIV-1 specific T-cell responses were quantified by a validated IFN- γ ELISPOT assay. The ELISPOT assay performed on fresh PBMC's was used to detect the number of T-cells releasing IFN- γ . Synthetic peptides (Anaspec, USA) covering the HIV gene insert were provided by the IAVI Core Laboratory at Imperial College London. Peptides (15-mers overlapping by 11aa; HPLC purified >90%) were used in peptide pools of 23 peptides or one large pool consisting of 90 peptides. In brief, 96-well PVDF membrane (MAIPS4510 Millipore) plates were coated and incubated with Mouse anti-human IFN γ monoclonal antibody (10 μ g/ml; MabTech clone 1-D1K) in sterile PBS. The plates were washed with sterile PBS and blocked with R10. 200,000 PBMCs were added in either quadruplicate wells for mock and HIV peptides or duplicate wells for positive controls. Peptides were then added at a final concentration of 2 μ g/ml. Phytohaemagglutinin (PHA) and a pool of 25 CTL epitopes from influenza/EBV/CMV (FEC) were added separately as positive controls. For a negative control, quadruplicate wells containing the Mock (R10 with DMSO at final concentration 0.45% DMSO in R10) were used in addition to a single

Table 1
Study design

IAVI-010				IAVI-011		
Number	Months 0 and 1	Months 5 and 8		Number	Months 0 and 2	
Vaccine/placebo	DNA	MVA		Vaccine/placebo	MVA	
	Route	Dosage	Route		Dosage	Route
12/3	IM	LD	SC	12/3	LD	SC
12/3		LD	IM	12/3	LD	IM
18/3		MD	ID	18/3	MD	ID
12/3	IM	MD	SC	12/3	MD	SC
12/3		MD	IM	12/3	MD	IM
12/3	IM	HD	SC			
12/3		HD	IM			
90 Vaccine/21 Placebo				66 Vaccine/15 Placebo		

DNA (pThr.HIVA): 0.5 mg.

LD: MVA.HIVA Low Dose: 5×10^6 pfu MVA; MD: MVA.HIVA Mid Dose: 5×10^7 pfu MVA; HD: MVA.HIVA High Dose: 2.5×10^8 pfu MVA; SC: subcutaneous; IM: intramuscular; ID: intradermal.

well containing R10 medium only. The plate was incubated at 37°C, 5% CO₂ overnight, washed with PBS/0.1% Tween 20 (Sigma) and the production of IFN γ by T-cells was assessed by addition of 100 μ l of 1 μ g/ml biotinylated mouse-anti human IFN- antibody (MabTech clone 7-B6-1) for 2–4 h. ABC peroxidase-avidin-biotin complex (Vector labs PK6100) was added for 1 h at room temperature and spots were developed with addition of filtered AEC substrate solution for 4 min. Plates were read using an automated AID ELISPOT reader (AutoImmun Diagnostika, Germany).

At several predetermined time-points, fresh or frozen peripheral blood mononuclear cells (PBMC's) were stimulated with pooled, overlapping peptides representing both gag and the CD8+ T cell epitopes. The assays were performed in Nairobi, Johannesburg and at the IAVI Core Laboratory in London in accordance with Good Clinical Laboratory Practice (GCLP) and IAVI standard operating procedures using standard reagents [23]. The IAVI Core Lab provided supervision, standardization and quality control.

2.6. Statistical considerations

Primary safety endpoints were the severity and frequency of local and systemic reactogenicity events, and the frequency of other adverse events including laboratory abnormalities graded as severe or very severe and serious adverse events. For local and systemic reactogenicity events, the maximum severity of each event in a volunteer was reported. For other adverse events, the endpoint was defined as any severe or very severe event within 28 days that was judged to be possibly, probably, or definitely related to the study vaccine.

Fisher's exact 2-tailed test was used to evaluate the effects of site, dosage and route of administration on the proportion of volunteers with an event. The Cochran-Armitage Trend Test was used to evaluate the effect of dosage. Statistical significance was indicated by *p*-values <0.05. Analyses were performed using SAS statistical software.

The primary immunogenicity endpoint was defined as percentage of volunteers with HIV-1 specific T-cell responses quantified by the ex-vivo IFN- γ ELISPOT assay.

3. Results

For Study IAVI-010: 115 volunteers (90 males, 25 females) were enrolled, 70 in Nairobi and 45 in London, with median age of 24 years (range 18–59). One hundred and one (88%) volunteers received all four vaccinations. In no volunteer were vaccinations discontinued due to a reactogenicity event or related adverse event. Eighty-one volunteers (32 males, 49 females) were enrolled in Study IAVI-011, 35 in South Africa and 46 in Europe (Switzerland and UK). Ninety two percent and 99% of volunteers completed all protocol visits for 010 and 011, respectively. Enrollment into Study 011 commenced in November 2003, and was temporarily halted in March 2004 while new preclinical data were being

reviewed. By the time the trial was allowed to proceed, the immunogenicity data from Study IAVI-010 and other studies were available and a decision was made to limit further clinical development of the candidate vaccines for prophylactic use. Study 010 was completed as planned. Vaccination in Study IAVI-011 was not resumed, but follow-up was completed. All but one volunteer in the LD group received the two vaccinations as planned. In the MD group, all volunteers received the first vaccination as planned, but only two volunteers received the second injection. The planned HD group was not enrolled.

3.1. Safety and tolerability

3.1.1. Local reactogenicity

3.1.1.1. Study IAVI-010: pThr.HIVA DNA. The overall rates of local reactions to the DNA vaccine were not significantly different between vaccine and placebo groups. Six (6.4%) vaccine recipients had moderate, and one (1.1%) had severe local pain while there was no moderate or severe pain in placebo recipients.

3.1.1.2. MVA.HIVA boost. By dosage: Following MVA.HIVA, there was a strong statistically significant relationship between dosage group and proportion of volunteers with any moderate or severe local reaction (*p* = 0.0007). The highest rate of events (69%) was in the HD group, followed by 38% in the MD group and 22% in the LD group (Table 2). There was no significant difference in moderate or severe local reactions between LD and MD. In both MD and HD groups, moderate or severe local reactions were less frequent after second dose compared to the first. There were no moderate or severe events in the placebo group.

By route: There was a statistically significant (*p* = 0.0005) difference between routes of injection in the proportions of volunteers with moderate or severe events. The SC route was associated with most events (67%), followed by ID (44%) and IM (20%) (Table 3). The difference between the IM and SC route was statistically significant only in the HD group (*p* = 0.0014).

By route and dosage: In the low dose group, the maximum severity of most local reactions was mild regardless of route. In the MD group, 60% SC recipients had moderate or severe reactions, whereas amongst ID and IM recipients, none and 44%, respectively, had moderate or severe local

Table 2
Local reactions by dosage for MVA.HIVA in IAVI-010

Severity	Placebo	Low dose	Mid dose	High dose	Total
None/mild	20	18	23	8	69
Moderate/severe	0	5	14	18	37
	100%	78.3%	62.2%	30.8%	
	0.0%	21.7%	37.8%	69.2%	
Total	20	23	37	26	106

Table 3
Local reactions by route for MVA.HIVA in IAVI-010

Severity	Placebo	ID	IM	SC	Total
None/mild	20 100%	10 55.6%	28 80.0%	11 33.3%	69
Moderate/severe	0 0%	8 44.4%	7 20.0%	22 66.7%	37
Total	20	18	35	33	106

reactions ($p=0.0140$). In the HD group, 92% of SC volunteers and 46% of IM volunteers had moderate to severe reactions ($p=0.0302$). There were no moderate or severe local reactions in placebo recipients.

By symptom: Moderate or severe pain and tenderness occurred in only one of the 60 LD and MD recipients, but both were fairly common (19%), though transient, at the high dosage. Severe induration (defined as diameter >3 cm and/or a duration >4 weeks) or moderate induration (1.5–3 cm diameter and/or 2–4 weeks duration) occurred at all MVA.HIVA dosage levels and by all routes except IM in the MD group; the proportion of recipients with events, regardless of route, increased with increasing dosage ($p<0.0009$) LD (5/23: 22%), MD (13/37: 35%), HD (15/26: 58%). In 15 volunteers (5 MD, 10 HD), induration persisted at the 14 day post-vaccination visit. No ulcers or secondary infections occurred.

Residual skin discoloration at the injection site was observed in 21% and 23% of volunteers in MD and HD respectively and in none of the LD volunteers. No skin discoloration was observed at the Nairobi site, while 19% of the predominantly Caucasian London volunteers had skin discoloration. There was no association of skin discoloration with route of administration.

By study site: Moderate or severe local reactions, overall, occurred in more volunteers from London (74%) than from Nairobi (26%) ($p<0.0001$) (Table 4). There was no statistically significant difference between the two study sites in the number of subjects assigned to each dosage. The frequency of events increased with increasing dosage at both sites, but in Nairobi the association was statistically significant ($p=0.0038$). The most frequent reaction, induration, was recorded in 65% of volunteers at the London site compared to 24% at the Nairobi site ($p<0.0004$) (data not shown).

Table 4
Moderate and severe local reactions in all MVA.HIVA vaccine and placebo recipients in IAVI 010 by route and site

Reaction	Fisher's exact 2-tail test	Nairobi	St Thomas	Total
Placebo	Any route	0/11	0/9	0/20
	IM	1/22	6/13	7/35
	$P=0.0059$	5%	46%	
MVA	SC	10/21	12/12	22/33
	$P=0.0021$	48%	100%	
	ID	3/12	5/6	8/18
	$P=0.0430$	25%	83%	

3.1.1.3. Study IAVI-011: MVA.HIVA alone. In this study, MVA.HIVA was administered only at LD and HD (Table 1). No moderate or severe local reactogenicity was reported in the placebo or LD groups. Of the 42 volunteers who received MD MVA.HIVA, 4 (10%) reported moderate and 3 (7%) reported severe local reactions: one volunteer had severe pain and tenderness, one had severe induration and moderate discoloration, and another had severe pain. There were no statistically significant differences between placebo, LD and MD, regardless of route. Observations in the UK, Switzerland and South Africa were not significantly different.

3.1.2. Systemic events

3.1.2.1. Study IAVI-010: pTHr.HIVA DNA. Systemic Events: No severe or very severe systemic events were reported after receipt of the DNA vaccine. The maximum severity was mild or less in 95% and 96% of the placebo and vaccine recipients, respectively, and moderate in 4 (4.3%) vaccine and one (4.8%) placebo recipient (not statistically significant).

3.1.3. Study IAVI-010 MVA.HIVA boost

Following the MVA.HIVA boost, there was no significant difference between dosage groups in the proportion of volunteers with moderate or severe events, although more events occurred in the HD group (19%) compared to the LD, MD and placebo groups (4%, 5% and 10% respectively). Two severe headaches were reported: one placebo recipient and one HD MVA recipient. There was no effect of route.

3.1.4. Study IAVI-011: MVA.HIVA alone

As with local reactogenicity, moderate systemic events were observed only in the MD group. There were no severe systemic events. In the ID group, one volunteer had a moderate headache, and in the IM group, one volunteer also had a moderate headache whilst another had moderate headache, nausea and vomiting. No significant differences were observed between the three dosage groups (placebo, LD, MD), clinical sites, or between routes of administration.

3.1.5. Other adverse events (AEs)

In Study IAVI 010, there were four unsolicited adverse events graded severe and considered related to vaccination; all were induration at the site of MVA.HIVA injection that persisted beyond 14 days post-vaccination.

There were no severe, very severe or serious unsolicited adverse events that were possibly, probably, or definitely related to vaccine, in either study. There were no events, such as sustained chest pain, dyspnea, palpitations or tachycardia that were suggestive of pericarditis or myocarditis.

3.1.6. Laboratory results

There was no consistent pattern of laboratory abnormalities associated with the investigative vaccines throughout either study. There was no induction of antibodies to double stranded DNA. No volunteer demonstrated a false-positive HIV antibody test at any time during the studies as a result of

vaccine-induced antibodies, except one individual who tested positive on one HIV-1 ELISA at the final study visit at month 18. Confirmatory HIV tests including PCR showed that this volunteer was not HIV-infected.

3.2. Immunogenicity

3.2.1. Ex vivo IFN- γ ELISPOT responses following vaccination or placebo

Immune responses were rare, and when they did occur they were not sustained. There was no relationship between immune response and route of delivery or dosage used.

Study IAVI-010: Three vaccine recipients had immune responses, two at M9, one month after the second MVA.HIVA vaccination, and one at M5, four months after the second DNA vaccination but prior to administration of MVA.HIVA. Each responded to at least two peptide pools, with responses ranging in magnitude from 33–200 spot forming cells (SFC)/ 10^6 PBMC. Two of the volunteers received MD (both ID), and one received LD (IM) of MVA.HIVA. One placebo recipient had positive responses to three different peptide pools on a single occasion at M6. There were no sustained responses. One vaccine recipient scored positive by IFN- γ ELISPOT in the CD4 T-cell subset only; the other two responses could not be characterized.

Study IAVI-011: Two volunteers in the MD arm (8%) had immune responses, one from the SC group and one from the ID group. Both showed responses one month after the second vaccination and these responses were not sustained. Each volunteer responded to 2 of 5 peptide pools (31–40 SFC/ 10^6 PBMC).

3.3. Association between immune responses and vaccine reactions

There was no significant difference in mean elispot responses (based on overlapping 95% confidence intervals

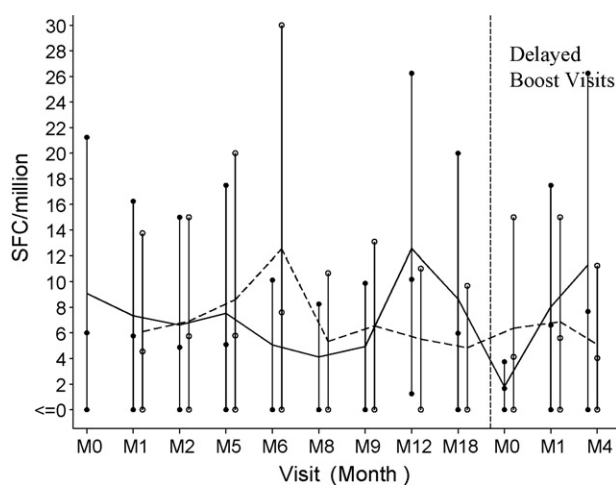


Fig. 1. Protocol 010. Mean ELISPOT response, in subjects with (—) and without (---) any mild or worse local or systemic immunization reaction since the last vaccination.

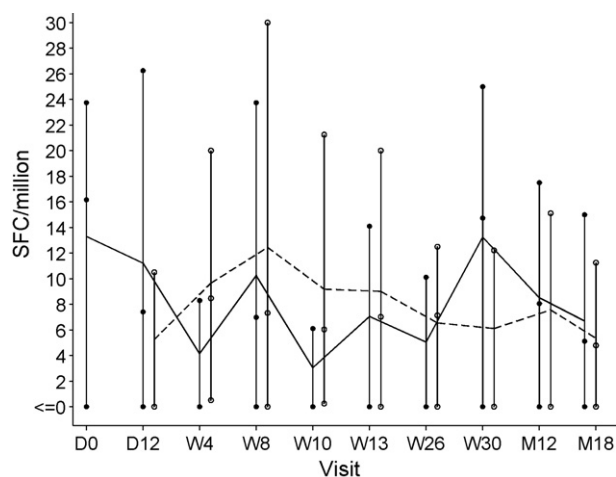


Fig. 2. Protocol 011. Mean ELISPOT response, in subjects with (—) and without (---) any mild or worse local or systemic immunization reaction since the last vaccination.

at each visit—data not shown) between volunteers with reactions and volunteers without reactions (see Figs. 1 and 2 for protocols 010 and 011, respectively). In the 4 volunteers with ELISPOT responses in Study IAVI-010, no systemic or local reactions were seen prior to the blood sampling. For study IAVI 011, there were immune responses in two volunteers, but in these subjects the local reactions following the vaccination prior to blood draw were mild only, and there were no systemic reactions.

4. Discussion

These two clinical trials were part of a program to study safety, tolerability and immunogenicity of different dosage levels and routes of the MVA.HIVA vaccine with or without pThr.HIVA DNA as prime. This program established a firm collaboration between sites in Africa and in Europe, including a standardization of clinical trial and laboratory procedures.

In the two studies, no safety concerns arose either for MVA.HIVA or pTHR.HIVA DNA. Frequent but transient local reactions were associated with MVA.HIVA. Amongst those, induration was the most often observed. This is in accordance with results from earlier trials. Almost all participants in small Phase I trials conducted in the UK experienced local reactogenicity such as redness or induration after MVA.HIVA MD intradermally [17], and similarly, in two small Phase I trials conducted in Kenya, all volunteers had some local reactogenicity, most commonly induration and skin discoloration following MVA.HIVA MD intradermally (W. Jaoko, personal communication). At the highest dosage of MVA.HIVA, moderate systemic events were common, predominantly headache; again, these were transient.

In Study 010, there was a statistically significant relationship between increasing dosage of MVA.HIVA and local reactions for the Nairobi site, but this was not observed in London. We consider it unlikely that there was a difference

in the manner of administration between the two sites, and the excess of induration observed at the London site compared to Nairobi could represent an ascertainment bias. Host differences reflecting the different ethnic composition between the two sites is another possibility, but was not apparent on review of the HLA class I and II results. Such differences were not observed between the European and South African sites in Study IAVI-011, albeit there was no HD group.

The safety of the pThr.HIVA DNA vaccine is comparable with findings for other DNA vaccines [17–20]. The safety of the MVA.HIVA vaccine candidate mirrors the generally good safety profile reported for other recombinant vector based HIV-1 vaccines [28,29].

An initial concern with DNA vaccines was whether they would lead to autoimmune complications due to induction of antibodies against double stranded DNA. There has been no substantive evidence for this in our trials or other clinical trials with DNA vaccines, and therefore less extensive safety monitoring of this aspect is now considered acceptable (www.fda.gov/cber/gdlns/plasnavac.htm).

In the two studies, there was little evidence of detectable *ex vivo* IFN- γ ELISPOT assay response to the pThr.HIVA DNA or MVA.HIVA. However, in a small recent trial, employing higher dosages of both, the DNA- and MVA-based candidate vaccines and measuring immune responses at one week after vaccination rather than 2 or 4 weeks, higher frequency and magnitude of responses were observed with the IFN- γ ELISPOT assay in primed and boosted volunteers [23]. A more sensitive immunogenicity assay, in which PBMC were cultured for 11 to 13 days in the presence of cytokines and antigen, showed HIV-1-specific CD4+ T cell responses in 8 out of 8 recipients of pThr.HIVA DNA plus MVA.HIVA. No responses were observed in placebo recipients. These recent findings suggested that the pThr.HIVA vaccine, when given at a sufficiently high dose, consistently primed immune responses in HIV-uninfected individuals, and that MVA.HIVA consistently provided a boost. This evidence, whilst not sufficient to revise current expectations of the pThr.HIVA and MVA.HIVA vaccines, should lead to optimized measurements of immune responses in future HIV-1 vaccine studies.

There was no evidence that the vaccine related local or systemic reactions that were observed in our studies were due to immune activation, as there was no correlation between immune responses (number of spot forming units) by IFN- γ ELISPOT assay and vaccine reactions among the volunteers.

MVA.HIVA has been tested as a “therapeutic vaccine” and further therapeutic studies with MVA.HIVA are being planned. In a small study of HIV-1 infected volunteers receiving antiretroviral drug therapy, MVA.HIVA specifically increased *ex-vivo* IFN- γ ELISPOT frequencies by an average of over 1000 SFC/10⁶ PBMC in 16 out of 16 vaccine recipients [30,31].

Overall, the immunogenicity of pThr.HIVA and MVA.HIVA in clinical trials has been lower than in pre-clinical studies. This is because the animal models, including

the non-human primate models, differ in several important respects [32]. It might also partly be due to the lower vaccine dosages used in humans as compared to experimental animals. For example, per body surface area, a 6-mg dosage of DNA used in a 2-kg rhesus macaque would translate into approximately 120 mg of DNA used in an 80-kg man. Most other studies of prophylactic HIV DNA candidates have reported similar immunogenicity to our two studies. The recombinant adenovirus vector vaccines, two of which are now in advanced clinical development, have evoked much greater immune responses, and appear to be the most immunogenic candidate HIV vaccines undergoing clinical trials [33].

5. Conclusions

The two HIV-1 vaccine candidates tested were in general safe and well tolerated. Local reactions following MVA.HIVA were common, but tolerable. Detectable immune responses by INF- γ ELISPOT were infrequent. Therefore, clinical development of these vaccine candidates for prophylaxis is currently not being pursued. The studies, however, should provide valuable reassurance on the safety of MVA-vectored vaccines, and the main impetus will be to retain this safety profile while improving immunogenicity.

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