Published in final edited form as:
Vaccine. 2005 September 7; 23(38): 4711–4719. doi:10.1016/j.vaccine.2005.01.145.

Modified vaccinia Ankara expressing HIVA antigen stimulates HIV-1-specific CD8 T cells in ELISpot assays of HIV-1 exposed infants☆

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Abstract

Recombinant modified vaccinia virus Ankara expressing HIV-1 antigens (MVA.HIVA) was used in ELISpot assays to monitor HIV-1-specific T cell responses in infants. Responses to MVA.HIVA and HIV-1 peptides were examined in 13 infected and 81 exposed uninfected infants in Nairobi, Kenya. Responses to MVA.HIVA (38%) and peptide stimulation (38%) were similar in frequency ($p = 1.0$) and magnitude (mean 176 versus 385 HIVSFU/10^6, $p = 0.96$) in HIV-1 infected infants. In exposed uninfected infants, MVA.HIVA detected more positive responses and higher magnitude responses as compared to peptide. MVA.HIVA ELISpot is a sensitive method for quantification of HIV-1-specific CD8+ T cell responses in HIV-1 exposed infants. These results demonstrate the relevance of HIV-1 clade A consensus-derived immunogen HIVA for the viruses currently circulating in Nairobi.

Keywords

Enzyme-linked immunospot assay; Exposed seronegatives; Mother to child transmission

1. Introduction

Highly attenuated modified vaccinia virus Ankara (MVA) is used as a vector to deliver recombinant human immunodeficiency virus type 1 (HIV-1) antigens in vaccine models [1,2]. Multiple passages of MVA through chicken embryo fibroblast cells produces a highly attenuated virus capable of high expression of recombinant antigens yet with limited ability

☆Presented in part: 2003 IAS Conference on HIV Pathogenesis and Treatment (abstract no. 408).

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Conflict of interest: The authors have no conflict of interest with any commercial or other association in conjunction with the research presented herein.

to replicate in mammalian cells [3,4]. Attenuated MVA lacks genes responsible for poxvirus immune evasion strategies, including the production of receptors for TNF, IFN-γ, IFN-α/β, and C–C chemokines [5]. These modifications result in improved immunogenicity as compared to other poxvirus vectors and have made MVA an attractive vector for use in human vaccines.

A prime-boost HIV-1 vaccine currently undergoing clinical evaluation in Nairobi, Kenya and Oxford, England includes a recombinant MVA vector containing HIV-1 $p17^{gag}$ and $p24^{gag}$ genes fused to a string of partially overlapping HIV-1 cytotoxic T lymphocyte (CTL) epitopes (MVA.HIVA) [6–8]. The *gag* gene region of the construct is designed from a consensus of HIV-1 A clade sequences of East African isolates. The epitopes contained in the polyepitope region include clade A *gag*, *env*, *pol*, and *nef* epitopes presented by class I HLA alleles common in East Africa. The immunogenicity of the vaccine is currently undergoing evaluation in low risk volunteers.

Vaccinia viruses expressing recombinant antigens have previously been used in IFN-γ ELISpot assays to study CD8+ T cell responses in HIV-1 infected individuals [9]. Recombinant vaccinia virus stimulated ELISpot assays are especially useful in studies involving very young infants since a small number of wells are needed prior knowledge of HLA type is unnecessary. Recombinant MVA vectors have been used in vitro to restimulate influenza and HIV-1-specific lysis in chromium release cytotoxicity assays [10,11]. MVA may be a more sensitive vector for ELISpot assays because it lacks expression of IFN-γ receptors which otherwise may reduce detection of secreted IFN-γ [5]. Recombinant vaccinia viruses expressing HIV-1 genes are being used in a number of clinical vaccine trials [12–14]. Processing and presentation of vaccinia-expressed HIV-1 antigens by peripheral blood mononuclear cells (PBMCs) in ex vivo assays may be most representative of how these recombinant proteins are utilized in vivo. We used the IFN-γ ELISpot assay to evaluate the MVA.HIVA construct as an antigen. Responses to optimized individual HIV-1 peptide and MVA.HIVA stimulation were compared in PBMCs of HIV-1 infected and exposed uninfected infants and adults in Kenya.

2. Materials and methods

2.1. Patients

We examined HIV-1-specific CD8+ cell responses to class I HIV-1 peptide and MVA.HIVA stimulation in 13 HIV-1 infected infants, 81 HIV-1 exposed uninfected infants, 7 HIV-1 uninfected adults (2 women, 5 men) at low risk for HIV-1 infection, and 13 infected women. Women and infants in this study were part of an ongoing vertical HIV-1 transmission study, in which HIV-1 infected women were enrolled during pregnancy and received zidovudine during the last month of gestation for the reduction of HIV-1 transmission [15]. Prior to delivery, women were counseled on infant feeding options, and infants were fed according to maternal preference. As part of the transmission study, mother–infant pairs were followed for 1 year after delivery and infant blood was obtained at birth, months 1, 3, 6, 9 and 12 for ELISpot and HIV-1 DNA PCR assays. Infant HIV-1 infection status was determined at each time point by nested PCR techniques amplifying HIV-1 *gag* DNA from dried blood spotted on filter paper [16]. Two consecutive tests positive for HIV-1 DNA was used to diagnose infection. “HIV-1 exposed uninfected” infants were classified as infants exposed to HIV-1 in utero and peripartum in whom we were unable to detect HIV-1 DNA at any time. In some infants, additional exposure occurred postpartum via breast-feeding. CD4+ and CD8+ T cell counts and percentages were determined at each visit following the identification of infection using TriTest CD3FITC/CD8PE/CD45PerCP antibodies(BD Biosciences, San Jose, California, USA) and FACScan analysis with CELLQuest Software (BD Biosciences).

2.2. HLA Typing and peptides

Infant DNA was extracted from peripheral blood mononuclear cells with the Puregene DNA Isolation Kit (Puregene, Minneapolis, Minnesota, USA) and used in amplification refractory mutation system-PCR [17] to determine infant HLA types. Peptides were selected on the basis of HLA type from a panel of optimized CTL epitopes previously used for studies of CTL responses in Kenyan sex workers [18].

2.3. ELISpot Assay

We used the IFN-γ ELISpot assay to measure HIV-1-specific CD8+ T cell responses. Investigators were blinded as to the HIV-1 infection status of the samples while conducting the ELIspot assays. All ELISpots were performed on freshly isolated cells obtained from EDTA anticoagulated blood. PBMC were isolated by density gradient centrifugation and resuspended in RPMI medium supplemented with 10% heat inactivated fetal bovine serum (R10). Cells expressing CD8 cell surface antigens were depleted from PBMCs using anti-CD8 monoclonal antibodies (Mab) coated Dynabeads according to the manufacturer's instructions (Dynal Inc., Lake Success, New York, USA). PBMC or CD8+ depleted cells were used in ELISpot assays. Lymphocyte subset depletions were verified by flow cytometry using TriTest antibodies (BD Biosciences) and analyzed on a FACScan using CELLQuest Software (BD Biosciences).

The ELISpot assay was performed as previously described [19] with modifications described in [18]. Briefly, 96-well Millipore plates (Millipore Corp, Bedford, UK) were coated with 1-DIK Mab at 15 μg/ml (Mabtech, Nacka, Sweden) and incubated at 37 °C for 2 h. Plates were washed six times with sterile RPMI then blocked for 30 min with R10. PBMC from HIV-1 infected or exposed uninfected subjects were added to the plates in duplicate at 2×10^5/well and stimulated with either PHA at 20 μg/ml (positive control, Murex Biotech, Dartford, UK), R10 media alone (peptide control), 20 μM peptide, MVA wild type (wt) as a vector control, or MVA.HIVA at an MOI of 1. The construction of the multi-CTL epitope construct HIVA is described elsewhere [6,20]. The plated cells were incubated at 37 °C and 5.0% CO_2 overnight. Following incubation, plates were washed six times with PBS plus 0.05% Tween-20 (Sigma Chemicals Co., St. Louis Missouri, USA) and coated with biotinylated anti-IFN-γ-Mab 7-B6-1 at 1 μg/ml (Mabtech) for 3 h at room temperature. Plates were washed six times with PBS-Tween followed by the addition of streptavidin-conjugated alkaline phosphatase (1:1000, Mabtech) for 1.5 h at room temperature. Plates were washed again and developed with an alkaline phosphatase-conjugate substrate kit (Bio-Rad Laboratories, Hercules, California, USA) for 10 min or until blue spots were clearly visible in the PHA control wells with the naked eye. The reaction was stopped by washing with tap water. Spots were counted with the AID ELISpot reader and software (Autoimmun Diagnostika, Straβerg). Spot forming units (SFU) was defined as the average number of spots in duplicate wells, and HIV-1-specific SFU (HIVSFU) was defined as SFU minus the average number of spot forming cells in the negative control wells. A positive response was defined by an assay meeting the following criteria: (1) strong response in the PHA-stimulated positive control wells, (2) HIVSFU at least 50 HIVSFU/10^6 PBMC after subtraction of no peptide control for HLA-matched peptides or subtraction of MVA wild type for HIVA.MVA assays and (3) SFU at least twice the value of the appropriate negative control.

2.4. Statistical analysis

Statistical analyses were performed using SPSS for Macintosh software v.10 (SPSS Inc., Chicago, IL, USA). All tests were performed on pair-wise data where MVA.HIVA and peptide ELISpots were conducted concurrently on the same infant sample. For infants with assays performed at multiple visits, only the first visit is used in the comparative analysis.

Wilcoxon signed ranks tests were used for comparison of cohort characteristics (age, number of visits, number of peptides tested). Paired *t*-tests for comparisons of HIVSFU frequencies obtained from the two assay methods were performed on $\log_{10}$-transformed data. McNemar and kappa (κ) statistics and matched odds ratios were used for the description of assay concordance and agreement of responses.

3. Results

3.1. Cohort characteristics

The median age of HIV-1 infected and exposed uninfected infants studied was 6 months. HIV-1 infected and exposed uninfected infants had similar numbers of sample visits, peptides screened, and ELISpot assays (Table 1). CD4 data were available for 11 HIV-1 infected infants. The median CD4/CD8 ratio was 0.61 (range 0.18–2.92). One infant included in this analysis showed signs of immunosuppression at the time of ELISpot assays. This infant was 12 months old when tested and had a CD4/CD8 ratio of 0.18 and a viral load of 6.6 $\log_{10}$ copies/ml. The majority of infants included in this study were breastfed until 6 months of age, and similar percentages of HIV-1 infected and exposed uninfected infants were breastfed. None of the infants participating in this study have a history of smallpox vaccination.

3.2. MVA.HIVA stimulates HIV-1-specific CD8+ T cells

To determine which cells were responding to MVA.HIVA stimulation, we compared the response from whole PBMC and CD8+ depleted PBMC in IFN-γ ELISpot assays. Since cell numbers were limited in infant samples, we used PBMCs obtained from HIV-1 infected women for the depletion experiments. In 5 HIV-1 infected women, a mean depletion of 99.2% CD8+ T cells was verified with FACS (range 98.8–99.8%). In the CD8+ depleted samples, we observed a 96.8% loss of responding cells, corresponding to a loss of 2106 SFU/10^6 PBMC (range in decrease: 375–3155 SFU/10^6 cells, Fig. 1). These data indicate that the IFN-γ production observed in the MVA.HIVA ELISpot assay is primarily released from CD8+ T cells. However, CD8+ depleted PBMC from three women still had weak detectable responses, indicating that there may be a small contribution of non-CD8+ T cells to the observed response.

3.3. HIV-1-specific responses in infected individuals

Infants were examined with both MVA.HIVA and HIV-1 peptides to enable comparisons of responses in the two assay methods. Comparisons were conducted for peptides contained in the HIVA construct, including $p17^{gag}$ or $p24^{gag}$ epitopes and the HIV-1-specific peptides in the HIVA polyepitope tail [6]. The panel of HIV-1 derived peptides used in the peptide ELISpot assays is shown in Table 2. Among 13 infants infected with HIV-1, 38% (5/13) had positive responses in peptide ELISpot assays and 5/13 (38%) had positive responses in MVA.HIVA ELISpot assays (Table 3). Among those infants tested, 11/13 (85%) had matching positive (4 infants) or negative (7 infants) results by both MVA.HIVA and peptide ELISpots, indicating good agreement between the two methods ($\kappa = 0.68$, $p = 0.02$). Discordant responses were not biased toward more positives in either assay (McNemar $p = 1.0$).

3.4. HIV-1-specific responses in uninfected and exposed uninfected subjects

Adults not infected with HIV-1, who were at low risk for HIV-1 exposure were used as negative controls, and 13 HIV-1 infected women were used as positive controls. We detected no positive responses to MVA.HIVA in the six uninfected adults (four men two women, mean 2.92 HIVSFU/10^6 PBMC). However, 12/13 HIV-1 infected women had significant HIV-1-specific IFN-γ release (mean 896 HIVSFU/10^6) in response to

stimulation with MVA.HIVA, indicating immune recognition of antigens present in the construct and suggesting the response to MVA.HIVA is mediated by cells specific for HIV. In 81 HIV-1 exposed uninfected infants, MVA.HIVA peptide stimulation resulted in significantly more positive responses than HIV-1 peptides (52% versus 15%, OR = 8.5, CI = 3.0, 24). In this group of infants, results from the two assay methods showed poor agreement, with only 53% of assays returning similar results (35 infants were negative in both assays, 8 were positive in both assays, $\kappa = 0.09$ $p = 0.3$). The discordant responses were primarily positive in response to MVA.HIVA stimulation and negative in response to peptide stimulation (38 discordant responses, 34/38 (89%) MVA.HIVA positive and peptide negative, McNemar $p < 0.001$).

3.5. Magnitude of HIV-1-specific T cell responses

In order to determine the contribution of responses to the MVA vector in MVA.HIVA assays, we examined the frequency of unstimulated cells and cells responding to the MVA wild type vector, which does not contain the HIVA immunogen. The mean response in unstimulated cells was 43.5 SFU/10^6 (range 0–168) in HIV-1 infected infants, and 43.3 SFU/10^6 (range 0–533/10^6) in exposed uninfected infants. The mean response to stimulation with the MVA wt vector (after subtracting the non-stimulated background values) was 4.61 wtSFU/10^6 (range 0–113/10^6) in infected infants and 12.4 wtSFU/10^6 (range 0–275/10^6) in exposed uninfected infants.

We calculated the magnitude of those responses fitting our criteria as positive (Fig. 2). The mean magnitude of positive responses to HIV-1 peptide stimulation was greater in HIV-1 infected infants (852 HIVSFU/10^6, range 128–2195/10^6) compared to exposed uninfected infants (174 HIVSFU/10^6, range 50–735/10^6, $p < 0.001$). There was also a trend for stronger positive responses to MVA.HIVA stimulation in HIV-1 infected infants (413 HIVSFU/10^6, range 97.5–770/10^6) compared to exposed uninfected infants (198 HIVSFU/10^6, range 50–955/10^6, $p = 0.1$). There was no difference in the magnitude of positive responses detected to MVA.HIVA and HIV-1 peptide stimulation in either HIV-1 infected infants ($p = 0.2$) or exposed uninfected infants ($p = 0.9$) in pairwise comparisons of the two assay methods.

3.6. Specificity of responses

The MVA.HIVA vaccine construct being evaluated in clinical trials contains two regions with the potential to stimulate immune responses: a string of class I HIV-1 epitopes and a p24/p17^{gag} subtype A consensus sequence. We were unable to directly determine the epitope specificity of the responses directed towards the fusion protein. However, since we included discrete HIV-1 peptides derived from p17 and p24 and also peptides contained in the polyepitope region in our screening, it was possible to measure peptide-specific responses in infants who responded to stimulation with MVA.HIVA. The number of gag-derived peptides and polyepitope-derived peptides tested was similar between HIV-1 infected and exposed uninfected infants (Table 1). Of four HIV-1 infected infants with responses to stimulation with p24/p17^{gag} peptides not contained in the polyepitope tail, all had positive responses to MVA.HIVA stimulation, suggesting that the responses observed to MVA.HIVA may result from processing and presentation of the gag consensus region. In nine HIV-1 exposed uninfected infants with responses to stimulation with p24/p17^{gag} peptides, six also had responses to MVA.HIVA stimulation.

In three HIV-1 infected infants with responses to single peptides that were included in the polyepitope region of HIVA, two had responses to MVA.HIVA, suggesting that epitopes included in the polyepitope tail can also be processed and presented to T cells. Six HIV-1 exposed uninfected infants had responses to peptides included in the polyepitope region, and three of these infants also had responses to MVA.HIVA stimulation.

We also examined the magnitude of positive responses to stimulation with p24/17^{gag} peptides and peptides contained in the polyepitope tail of the HIVA construct (Fig. 2). The positive response to p24/17^{gag} peptides was 821 HIVSFU/10^6 (128–2195/10^6) in HIV-1 infected infants and 173 HIVSFU/10^6 (50–553/10^6) in exposed uninfected infants, and the mean response to HIV-1 peptides included in the polyepitope tail was 499 HIVSFU/10^6 (97.5–770/10^6) in HIV-1 infected infants and 111 HIVSFU/10^6 (50–298/10^6) in exposed uninfected infants.

4. Discussion

Poxviruses expressing recombinant viral genes have been used extensively to deliver antigens in HIV-1 HIV-1 vaccine models [12–14] and in vitro cellular assays [9,10,21–23]. MVA.HIVA has been shown to be highly immunogenic in preliminary primate studies [1,2,24–26], and may thus be an effective vaccine in human clinical trials. Recent studies have demonstrated that recombinant vaccinia virus vectors are effective delivery systems for HIV-1 antigens in ELISpot assays [9,23]. Since the entire set of viral antigens can be included in the recombinant construct, it is also possible to screen for responses to unidentified viral epitopes. Though the use of pooled 15-mer peptides spanning entire HIV-1 genes can also be used in ELISpot assays with few cells and without the need for HLA typing, recent studies have cast doubt upon the sensitivity of this method in detecting CD8+ T cells responses. Beattie et al. [27] have recently shown that the sensitivity of responses to single epitopes may be affected by its placement in the 15-mer, and that epitopes that were placed central to the 15-mer were less likely to be antigenic than if they were placed at the N- or C-terminus. In this scenario low-level responses were often lost altogether. Moreover, Goulder and colleagues noted responses to a peptide in vitro that was unable to bind class I MHC in vivo [28]. We hypothesized that the MVA.HIVA construct might be a biologically representative antigen in ex vivo T cells studies, since antigens must be processed and presented as they would be in vivo. We used the IFN-γ ELISpot assay to determine whether the construct can be used to monitor HIV-1-specific responses to natural HIV-1 infection and exposure.

The MVA.HIVA construct was a powerful stimulator of CD8+ T cell responses in HIV-1 infected adults in this study. We have not identified the viral subtypes of the women and infants participating in this analysis, however, in a previous study conducted in Nairobi, subtypes were determined for 347 women participating in a mother to child transmission cohort [29]. On the basis of env sequences 70.3% of the women were determined to be infected with HIV-1 subtype A. Since the women in the present study had similar demographics, we expected most of the subjects to be infected with subtype A virus, and therefore to have had the potential to recognize epitopes contained within the HIV construct. Nearly all the HIV-1 infected adult women in the control group had responses to MVA.HIVA stimulation (92%). MVA.HIVA assays conducted in HIV-1 infected infants correlated well in both frequency and magnitude with parallel assays conducted with peptides included in the HIVA immunogen.

HIV-specific CD8+ T cells have been described in a number of HIV-1 exposed individuals in whom there is no detection of virus, including sexworkers [11,18], uninfected partners in discordant couples [30,31], occupationally exposed healthcare workers [32], and uninfected infants born to HIV-1 infected women [33]. In exposed uninfected infants, we were able to detect stronger and more frequent responses following stimulation with MVA.HIVA compared to peptides. Higher sensitivity of the construct in response to MVA.HIVA stimulation compared to our panel of peptides may be explained in a variety of ways. (1) There is evidence that exposed uninfected individuals preferentially respond to a different repertoire of HIV-1 peptides than infected individuals [18], and uninfected vaccine

recipients do not always recognize the same epitopes as infected individuals [34,35]. Seventy-nine percent of MVA.HIVA assays in HIV-1 exposed uninfected infants were not confirmed by peptide assays, suggesting that unidentified HIV-1 epitopes contained in the gag gene may be recognized by exposed uninfected individuals. (2) The MVA.HIVA construct includes multiple antigens that are simultaneously delivered to cells within the ELISpot assay. The higher frequency and magnitude of responses in the MVA.HIVA assays may also be attributed to the collective responses to multiple epitopes which may be undefined and which may be less immunodominant individually. (3) Since the HIV-1 gag gene was included in the HIVA immunogen, it is also possible that a portion of the responses observed in our ELISpot assays are attributable to CD4+ T or NK cells. Though the ELISpot assays using CD8+ depleted PBMCs indicated that the great majority (97%) of cells responding to MVA.HIVA stimulation were CD8+, there was a residual level of IFN-γ release, suggesting non-CD8+ T cells may contribute to the measured IFN-γ secretion. Unfortunately, we were unable to use infant PBMC for depletion experiments. Lymphocytosis is observed in both HIV-1 infected and healthy infants, and the majority of the infants in this study had normal CD4/CD8 ratios. Higher CD4 percentages in infants could result in a higher contribution of this subset to the MVA.HIVA response than in adults in chronic infection, who may have depleted CD4s following years of infection. Several studies have demonstrated strong HIV-1-specific IFN-γ production by CD4 T cells in young infected infants with high viral loads [36–38]. Further studies into the phenotype of responding cells in infected and exposed uninfected individuals will be necessary to better describe the effect of MVA.HIVA stimulation in different T cell subsets. (4) Finally, the MVA.HIVA construct contains vaccinia epitopes as well as HIV-1 epitopes, which may affect the hierarchy and amount of HIV-1 epitopes that are processed and presented. However, the frequency and magnitude of the two assays were similar in HIV-1 infected infants suggesting the amount of available antigen did not vary largely between the two assay methods in this group. The difference in the sensitivity of the assays in the exposed uninfected infants may be partially explained by better antigen processing and presentation in exposed uninfected infants compared to infected infants. One of the immune evasion strategies exploited by HIV-1 is subversion of antigen processing and presentation. HIV-1 nef downregulates class I MHC expression [39,40], mutations in epitope flanking regions prevent antigen processing [41,42] and host generated anti-HIV-1 antibodies may inhibit proteolysis of gp120 [43].

MVA.HIVA is being tested in adults as a candidate HIV-1 vaccine. Based on IFN-γ ELISpot assays using overlapping peptide pools, immunogenicity of MVA.HIVA vaccination in HIV-1 uninfected volunteers has been demonstrated in small pilot adult trials [44]. Subsequent larger trials using standard assays suggested responsiveness in about 20% of vaccinated volunteers [45], however, preliminary data from an on-going trial which uses a number of T cell assays with the emphasis on in vitro lymphocyte expansion suggest responses in a majority of adults [46]. The majority of MVA.HIVA vaccinated HIV-1 infected subjects on HAART also demonstrated increase in their baseline pre-vaccination immune responses following the MVA.HIVA administration (Lucy Dorrell, TH and AMcM, unpublished). However, it is important to keep in mind that there are qualitative differences in T cells measured in present study and the trials above. HIV-1-specific T cells in HIV-1 infected individuals are, or had been before HAART, chronically exposed to HIV antigens, while T cell responses in vaccine trials in healthy volunteers were evoked by a limited number brief pulses of the HIVA immunogen delivered by non-replicating vectors. While it is not clear to which antigen the infants in this study were exposed, i.e. a brief HIV-1 replication, infected cells, ‘dead’ HIV-1 or gag protein, the intensity of exposure is likely to be somewhere between the chronic HIV-1 infection and vaccination. This could be reflected in the sometimes transient nature of these responses, their different resistance to freezing–thawing procedures and sometimes marginal functionality.

The data we present here suggest that stimulation with the MVA.HIVA construct may be a useful tool in the planned ELISpot evaluations of vaccine recipients as well as individuals naturally exposed to HIV-1. Further investigations will be necessary to determine the functional status of responding cell populations, and to determine the relative contribution of the different parts of the construct to the in vitro immunogenicity of MVA.HIVA. Finally, these data show that recombinant synthetic sequences can be processed and presented by cells in vitro. Although we were unable to determine specifically what antigens were processed and presented in the cells stimulated with MVA.HIVA, we were able to determine that both the consensus gag sequence and the polyepitope tail contributed to the responses we observed. These data indicate that the HIVA construct is highly immunogenic in vitro in eliciting HIV-1-specific T cell responses in exposed uninfected individuals, reinforcing our confidence in the ability of the synthetic construct to be utilized in vivo.

Acknowledgments

Informed consent was obtained from all women for participation in this study. Human experimentation guidelines of the US Department of Health and Human Services and the Institutional Review Boards of the University of Washington and the University of Nairobi were followed in the conduct of this study.

This research was supported by an NIH research grant: HD 23412. B.L. and J.S. were scholars in the International AIDS Research and Training Program, supported by the Fog-arty International Center, National Institutes of Health (D43-TW00007). G.J.-S. is a Pediatric AIDS Foundation Elizabeth Glaser Scientist.

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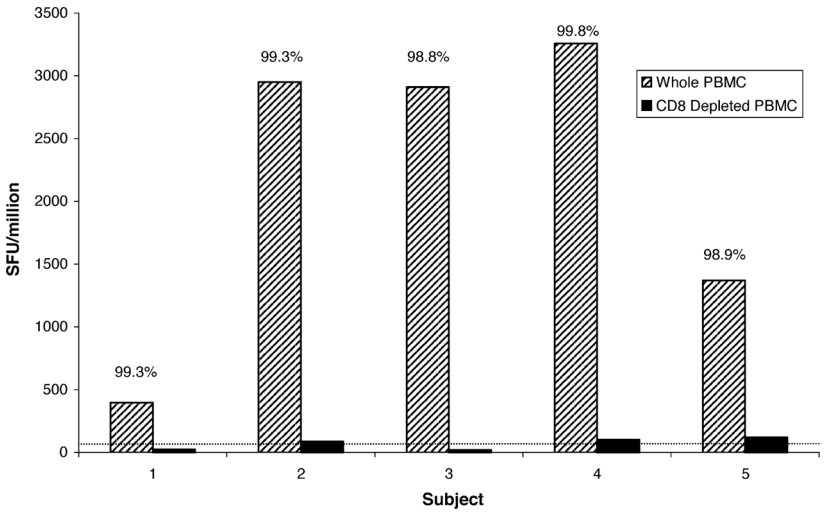

Fig. 1.

MVA.HIVA ELIspot asays in PBMCs from HIV-1 infected women. Cells expressing CD8 cell surface antigens were depleted from PBMCs using anti-CD8 monoclonal antibody-coated magnetic beads. Percentages above bars indicate percent CD8+ T cell depletion for each individual. A dotted line indicates where the HIVSFU/10^6 = 50.

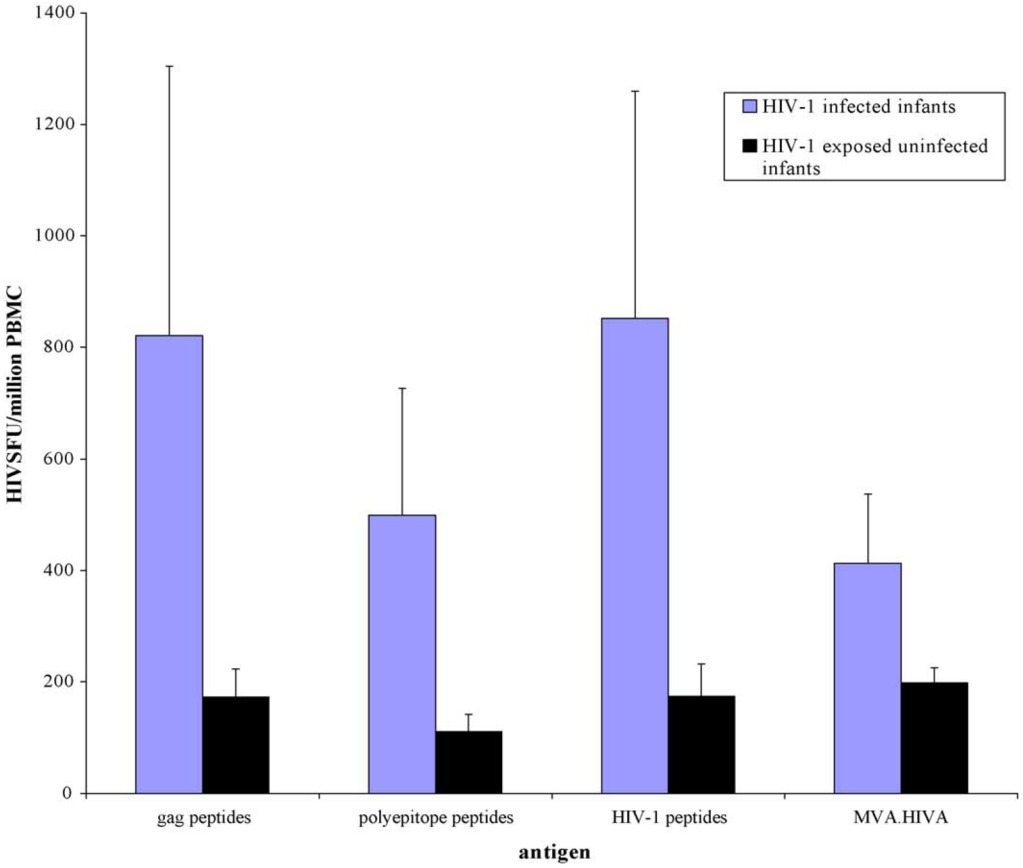

Fig. 2.

Mean magnitude of HIV-1-specific T cell responses in HIV-1 infected and exposed uninfected infants. The mean HIVSFU/10^6 PBMC is shown for assays meeting our criteria as positive. Gag peptides = p24/p17 peptides not included in the polyepitope region of HIVA. Polyepitope peptides = peptides included in the polyepitope region of HIVA. HIV-1 peptides = peptides included in either the p24/p17 or polyepitope region of HIVA. Error bars show standard error of the mean.

Table 1

Characteristics of HIV-1 infected and exposed uninfected infants in the study cohort

	HIV-1 infected infants	HIV-1 exposed uninfected infants
Number of infants[a]	13	81
Breastfed to M6 (%)	67	55
Ever breastfed (%)	85	69
Age at time of assay	6 (1–12)	6 (1–12)
Number of HIV-1 peptides sampled	5.5 (1–11)	6 (1–16)
p24/17^{gag} peptides	3 (0–9)	3 (0–10)
peptides in polyepitope tail of HIVA	2.5 (0–6)	3 (0–9)
CD4 (%)	24 (13–35)	
CD4/CD8 ratio at 6 months	0.61 (0.18–2.9)	

[a]Numbers and percentages are provided for categorical variables, and medians and ranges for continuous variables. Infected and exposed uninfected infants did not differ significantly in mode of feeding, age, or number of peptides sampled.

Table 2

HIV-1 peptides included in the peptide ELIspot assays

Peptide[a]	MHC class I restriction	HXB2 CTL epitope position
TVYYGVPVWK	**HLA-A3**	**GP120 (GP16037–46)**
YLKDQQLL/YLRDQQLL	**HLA-A24, B8**	**GP41 (GP160586–593)**
ERYLKDQQL	**HLA-B14**	**GP41 (GP160584–592)**
ALKHRAYEL	**HLA-A2**	**NEF (190–198)**
QVPLRPMTYK	**HLA-A3, A11**	**NEF (73–82)**
DLSHFLKEK	**HLA-A3, A31, A33**	**NEF (86–94)**
VPLRPMTY	**HLA-B35**	**NEF (72–79)**
TPGPGVRYPL	**HLA-B7**	**NEF (128–137)**
FLKEKGGL	**HLA-B8**	**NEF (90–97)**
GSEELRSLY	A1	P17 (71–79)
SLFNTVATL/SLYNTVATL	A2	P17 (77–85)
KIRLRPGGK	A3	P17 (18–26)
RSLYNTVATLY	A30	P17 (76–86)
GGKKKYRL/GGKKKYKL	B8	P17 (24–31)
KYRLKHLVW	Cw4	P17 (28–36)
TLNAWVKVI/TLNAWVKVV	A2	P24 (19–27)
YVDRFFKTL	A26	P24 (164–172)
DLNTMLNTV/DLNMMLNIV	B14, Cw8	P24 (51–59)
RAEQASQEV/RAEQATQEV	B14, Cw8	P24 (173–181)
TPQDLNMML/TPQDLNTML	B42, B53, B*8101	P24 (48–56)
QATQEVKNW	B53	P24 (176–184)
VKNWMTETLL	B53	P24 (181–190)
ASQEVKNWM/ATQEVKNWM	B53	P24 (177–185)
DTINEEAAEW	B53, A25	P24 (71–80)
ISPRTLNAW/LSPRTLNAW	B57, B*5801	P24 (15–23)
KAFSPEVIPMF	B57, B*5801	P24 (30–40)
TSTLQEQIGW/TSTLQEQIAW	B57, B*5801	P24 (108–117)
SPRTLNAWV	B7	P24 (16–24)
GPGHKARVL	B7, B42	P24 (223–231)
ATPQDLNTM	B7, B*8101	P24 (47–55)
DIYKRWII/EIYKRWII	B8	P24 (128–135)
QASQEVKNW	Cw4	P24 (176–184)
DRFFKTLRA/DRFYKTLRA	**HLA-B14, Cw8**	**P24 (166–174)**
FRDYVDRFYK/FRDYVDRFFK	**HLA-B18, B49**	**P24 (161–170)**
KRWIILGLNK/KRWIIMGLNK	**HLA-B27**	**P24 (131–140)**
PPIPVGEIY	**HLA-B35**	**P24 (122–130)**
RDYVDRFYKTL/RDYVDRFFKTL	**HLA-B44, A24**	**P24 (162–172)**
ITLWQRPLV	**HLA-A*6802, A74**	**Pro (3–11)**
ILKEPVHGV/ILKDPVHGV	**HLA-A2**	**RT (309–317)**

Peptide[a]	MHC class I restriction	HXB2 CTL epitope position
VIYQYMDDL	**HLA-A2**	**RT (179–187)**
AIFQSSMTK/SIFQSSMTK	**HLA-A3,A11,A33**	**RT (158–166)**
HPDIVIYQY/NPDIVIYQY	**HLA-B35**	**RT (175–183)**

[a]Peptides included in the polyepitope tail of the HIVA construct are shown in bold.

Table 3

Summary of HIV-1-specific CD8+ T cell responses in infants

HIV-1 infection status	Stimulus	Infants with positive responses	Concordant responses[a]	Discordant responses[b]
Infected	MVA.HIVA	38% (5/13)	85% (11/13)	15% (2/13)
Infected	HIV-1 Peptides	38% (5/13)	$\kappa = 0.68$, $p = 0.02$	OR = 1 (0.06, 16), McNemar $p = 1.00$
Exposed uninfected	MVA.HIVA	52% (42/81)	53% (43/81)	47% (38/81)
Exposed uninfected	HIV-1 peptides	15% (12/81)	$\kappa = 0.09$, $p = 0.3$	OR = 8.5 (3.0, 24), McNemar $p < 0.001$

[a] Both tests positive or negative. κ statistic is shown for comparisons of concordant responses.

[b] One test positive and one test negative. Odds ratio and McNemar statistics are shown for comparison of discordant responses of MVA.HIVA and HIV-1 peptide assays.