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Safety and Immunogenicity of Novel Recombinant BCG and Modified Vaccinia Virus Ankara Vaccines in Neonate Rhesus Macaques▽

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Although major inroads into making antiretroviral therapy available in resource-poor countries have been made, there is an urgent need for an effective vaccine administered shortly after birth, which would protect infants from acquiring human immunodeficiency virus type 1 (HIV-1) through breast-feeding. *Bacillus* Calmette-Guérin (BCG) is given to most infants at birth, and its recombinant form could be used to prime HIV-1-specific responses for a later boost by heterologous vectors delivering the same HIV-1-derived immunogen. Here, two groups of neonate Indian rhesus macaques were immunized with either novel candidate vaccine BCG.HIVA^{401} or its parental strain AERAS-401, followed by two doses of recombinant modified vaccinia virus Ankara MVA.HIVA. The HIVA immunogen is derived from African clade A HIV-1. All vaccines were safe, giving local reactions consistent with the expected response at the injection site. No systemic adverse events or gross abnormality was seen at necropsy. Both AERAS-401 and BCG.HIVA^{401} induced high frequencies of BCG-specific IFN-γ-secreting lymphocytes that declined over 23 weeks, but the latter failed to induce detectable HIV-1-specific IFN-γ responses. MVA.HIVA elicited HIV-1-specific IFN-γ responses in all eight animals, but, except for one animal, these responses were weak. The HIV-1-specific responses induced in infants were lower compared to historic data generated by the two HIVA vaccines in adult animals but similar to other recombinant poxviruses tested in this model. This is the first time these vaccines were tested in newborn monkeys. These results inform further infant vaccine development and provide comparative data for two human infant vaccine trials of MVA.HIVA.

Close to 2.3 million of children globally are infected with human immunodeficiency virus type 1 (HIV-1). The majority of neonatal infections occur *in utero* or intrapartum and, in the absence of preventative interventions, up to 29% of infants breast-fed by infected mothers acquire HIV-1 (6). Furthermore, HIV-1-infected children face a worse prognosis than adults in that, without antiretroviral treatment (ART), 25% of perinatally infected children progress to AIDS within 1 year (10), and the median time to AIDS for the remaining children is less than 7 years (2). It is now clearly established that maternal and extended infant ART can substantially reduce transmission of HIV-1 through breast-feeding (23). However, in a resource-poor setting, many logistical barriers to implementation of the ART-based prevention of mother-to-child-transmission (PMTCT) remain (23). Because nutrition and hygiene makes breast milk an important determinant of infant survival (22, 28), formula feeding as a protective measure against HIV-1 acquisition is recommended only if it is AFASS (acceptable, feasible, affordable, sustainable, and safe). Unfortunately, AFASS it is still not for majority of infected mothers in sub-Saharan Africa. Also, mixed bottle and breast feeding is associated with a 10-fold increase in HIV-1 transmission relative to exclusive breast-feeding (4). Thus, an effective infant vaccine against HIV-1 infection is the best and safest solution for PMTCT of HIV-1 with the added practical option of prolonging breast-feeding.

Neonatal immunity is immature compared to the adult immune system (25). The differences include naivety of the immune cells, a tendency to develop Th2 responses (5) and antigen-presenting cells with inefficient cytokine production (35). For example, human cord blood T cells proliferated poorly and produced low levels of interleukin-2 (IL-2) and gamma interferon (IFN-γ) when endogenous antigen-presenting cells presented the antigen (35, 44). Also, infant myeloid dendritic cells are less efficient in priming Th1 responses because of their decreased responsiveness to Toll-like receptor stimulation, lower levels of surface costimulatory molecules, and lower production of IL-12 (8, 27). In several infections, qualitative and quantitative differences between human newborn and adult responses were detected (1, 9, 26, 37). In contrast, other studies of infants reported proliferation as well as IL-2 and IFN-γ production by T cells equal to that of adults following T-cell receptor-independent activation (21, 46). These latter observations indicate that neonate T cells are not intrinsically

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"locked" into an immature phenotype but, given the correct stimuli, they can develop mature immune responses (25). The requirement for specific stimuli will likely differ for different pathogens and vaccine vectors.

Mycobacterium bovis bacillus Calmette-Guérin (BCG) is commonly delivered at birth as an antituberculosis vaccine as a part of the WHO Expanded Programme on Immunization (EPI). It has been reported by several studies to promote an adultlike Th1 response in newborns (16, 24, 34, 43), although it was also suggested that delaying the BCG delivery to 10 weeks of age benefits the quantity and quality of BCG-induced CD4 T-cell responses (20). BCG and related mycobacterial vectors have been explored as vaccines against other infectious agents, including human and simian immunodeficiency viruses (19), and in adult animals showed immunogenicity and protection (3, 36, 39, 47, 48). The only clinical study of recombinant BCG (rBCG) in adults failed to provide consistent efficacy (7). We have suggested the use of rBCG expressing an HIV-1-derived immunogen as the priming component of a heterologous vaccine platform for PMTCT of HIV-1 through infected breast milk (18), where it is critical to prime HIV-1-specific responses as soon as possible after birth. These responses could be boosted a few weeks later or shortly after the already busy EPI by heterologous vaccines delivering the same HIV-1-derived immunogen. To this extent, we constructed the novel candidate vaccine BCG.HIVA^{401} (36) by inserting a gene coding for the HIV-1 clade A-derived immunogen HIVA (14) into recombinant BCG strain AREAS-401 (40). AERAS-401 is a newly developed strain that displayed enhanced safety (40) and immunogenicity (11, 15) in murine models relative to its parental BCG vaccine strain Danish SSI-1331. Increased safety represents an important feature should the BCG.HIVA^{401} vaccine be deployed in babies born to HIV-1-infected mothers. We showed that BCG.HIVA^{401} in a heterologous combination with recombinant modified vaccinia virus Ankara MVA.HIVA and recombinant ovine atadenovirus OAdV.HIVA induced robust polyfunctional HIV-1-specific T-cell responses in adult macaques (36). Here, we assess the safety and immunogenicity of the BCG.HIVA prime-MVA.HIVA boost regimen in newborn rhesus macaques.

MATERIALS AND METHODS

BCG.HIVA^{401} vaccine construction and preparation. Construction of BCG.HIVA^{401} was described previously (36). The parental *M. bovis* endosomalytic rBCG strain AERAS-401 (40) and BCG.HIVA^{401} were grown at 37°C in protein-free 7H9 medium or in Middlebrook 7H9 medium supplemented with 10% OADC enrichment (BD Biosciences) plus 0.05% (vol/vol) tyloxapol (Sigma), and BCG.HIVA^{401} was analyzed by Western blotting for HIVA expression. The titers and viabilities of the frozen stocks were determined on 7H10 agar (BD Biosciences) plates by serial dilution.

Preparation of MVA.HIVA virus stock. Construction of MVA.HIVA was described previously (14). Working vaccine stocks were grown in chicken embryo fibroblast cells by using Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum, penicillin-streptomycin, and glutamine and then purified on a 36% sucrose cushion, titered, and stored at −80°C until use.

Rhesus macaques, vaccination, and isolation of lymphocytes. Eight 5- to 22-day-old Indian rhesus macaques (*Macaca mulatta*) from a breeding colony in the United Kingdom were housed and treated strictly in accordance with UK Home Office Guidelines. Four macaques per group were vaccinated with 10^7 CFU of parental AERAS-401 or BCG.HIVA^{401} intradermally (i.d.), followed by two intramuscular (i.m.) injections of 5×10^7 PFU of MVA.HIVA 11 and 14 weeks later. A 1-ml portion of peripheral blood was removed on each bleed from the femoral vein and yielded between 0.8×10^6 to 1.7×10^6 peripheral blood mononuclear cells (PBMC) following Lymphoprep cushion centrifugation (Nycomed Pharma). At necropsy, specific lymph nodes were dissected and pressed through a mesh strainer to release residing lymphocytes. These isolated cells were used fresh.

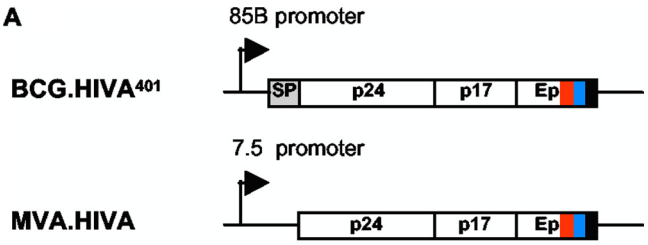
Peptides and preparation of peptide pools. HIVA-derived 15-mer peptides overlapping by 11 amino acid residues were kindly provided by the International AIDS Vaccine Initiative and used as described previously (31). Briefly, peptides were synthesized, purified by high-performance liquid chromatography and confirmed to be >80% pure by using mass spectroscopy (Sigma-Genosys). Individual peptides were dissolved in dimethyl sulfoxide (Sigma-Aldrich) to yield a stock of 40 mg/ml and stored at −80°C. Peptides were combined into five pools, each containing 20 to 23 individual peptides. Pools 1 to 4 corresponded to the Gag p24/p17 regions of HIVA, while pool 5 corresponded to the C-terminal polyepitope region of HIVA. Working pool stocks were prepared by combining 20 μl of each peptide stock and adding phosphate-buffered saline to a final volume of 5 ml; stocks were then sterile filtered, divided into aliquots, and stored at 4°C for up to 1 week before use. Purified protein derivative (PPD) RT49 was purchased from the Statens Serum Institute (Denmark) and used at a concentration of 20 μg/ml in assay wells.

IFN-γ ELISPOT assay. The frequencies of cells that released IFN-γ upon restimulation with HIVA-derived peptides or PPD RT49 were assessed by using an enzyme-linked immunospot (ELISPOT) assay. The procedures and reagents of a commercially available kit (Mabtech) were used throughout. Briefly, 200,000 PBMC were added per well, and the released IFN-γ was captured by monoclonal antibody (MAb) GZ-4 immobilized on the bottom of the assay wells, visualized using a combination of the secondary MAb 7-B6-1 coupled to an enzyme and a chromogenic substrate (NBT-BCIP), and quantified by spot count using the AID ELISPOT reader system (Autoimmun Diagnostika). All assays were carried out in duplicate, and the background counts were subtracted. For data analysis, control counts from each neonate were pooled to form the control group. For each peptide pair, a Wilcoxon test was performed, and *P* values were corrected within each animal by using the Benjamini-Hochberg correction. Any *P* value that was <0.05 postcorrection was deemed significantly different from the control pool. Statistical software was used (http://www.r-project.org).

RESULTS

Vaccination regimens. Eight neonate rhesus macaques between 5 and 22 days old were allocated into groups 1 or 2 receiving either empty parental BCG strain AREAS-401 or BCG.HIVA^{401}, respectively, the latter of which expresses immunogen HIVA derived from consensus African HIV-1 clade A structural proteins p24 and p17 of the Gag capsid coupled to a string of partially overlapping HLA class I-restricted epitopes (14) (Fig. 1A). The animals were then given two doses of MVA.HIVA 11 and 14 weeks later (Fig. 1B). There were two reasons for comparing the empty and recombinant BCG strains. First, in our parallel murine experiments, it is difficult to detect any HIV-1-specific CD8 T-cell responses induced by BCG.HIVA alone: the BCG.HIVA priming was indicated only indirectly by increased HIV-1-specific T-cell responses after the MVA.HIVA boost (18; unpublished data). Therefore, group 1 received empty parental BCG as a control for the BCG.HIVA^{401} prime. Second, two PedVacc clinical trials in Africa test MVA.HIVA delivered to regular BCG-vaccinated human neonates born to either healthy or HIV-1-positive mothers (NCT00982579 and NCT00981695), which serve as the first stage toward possible future trials testing the heterologous rBCG-rMVA boost regimen. The present experiment provided an opportunity for a parallel evaluation of the same regimen in nonhuman primates. We believe it is important to run human and macaque studies in parallel to accumulate comparative data for these two systems.

The BCG.HIVA^{401} prime-MVA.HIVA boost regimen was safe. The first aim of the present study was to assess the safety of the novel recombinant BCG.HIVA^{401} vaccine in neonate



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Time (Weeks)	0*	4	8	11	12	14	15	19	23
AERAS-401/ BCG.HIVA^{401}	X								
MVA.HIVA				X		X			
Phlebotomy	X	X	X		X		X	X	X
Spleen and LN									X

•Animals received the first vaccine at between 5 and 22 days of age

FIG. 1. HIVA immunogen and experimental design. (A) Schematic representation of the HIVA expression cassettes in BCG.HIVA^{401} and MVA.HIVA. The immunogen HIVA consists of consensus HIV-1 clade A Gag p24-p17 domains and a string of partially overlapping epitopes (Ep). The polyepitope region contains epitopes derived from Gag, which are not present in the p24-p17 domains, Pol, Nef, and Env (14). To facilitate the preclinical development of the HIVA vaccines, the polyepitope region also includes immunodominant Mamu-A*01-restricted epitope CTPYDINQM derived from SIV Gag (CM9; residues 181 to 189) (red) (29). In BCG.HIVA^{401} (top), the HIVA transcription is driven by the mycobacterial 85B antigen promoter, and nucleotides coding for the 19-kDa protein signal peptide (SP) are attached to the HIVA open reading frame. In MVA.HIVA (bottom), the gene transcription is controlled by vaccinia virus promoter P7.5 (14). (B) Experimental design. *, The specific ages of neonates at the time of AERAS-401/BCG.HIVA^{401} vaccination were as follows: N3 and N8, 5 days; N1 and N2, 6 days; N4 and N7, 9 days; N6, 20 days; and N5, 22 days.

rhesus macaques. Thus, neonates macaques were injected with 10^7 CFU i.d. of either AERAS-401 or BCG.HIVA^{401}, and the injection sites were closely monitored and documented. For rigorous safety assessment, this dose was 10-fold higher than that of BCG commonly used after birth for human babies as an antituberculosis vaccine. All eight macaques developed a slightly raised erythematous round lesion with induration by week 1 similar to that observed in human neonates, which resolved by week 4 (Fig. 2). In these small groups of neonates, BCG.HIVA^{401} caused inflammatory reactions indistinguishable from the parental BCG strain. The priming vaccination was followed by two doses of 5×10^7 PFU of MVA.HIVA i.m., which is same dose and route as that used in the two PedVacc clinical trials. No adverse reactions were observed from MVA.HIVA vaccination. No abnormal gross pathology was observed in any of the eight neonate macaques at necropsy (week 23). For gross pathological examination, animals were laid supine. A midline incision from sternum to pubic symphysis was made followed by lateral incisions to the anterior shoulder and thigh. Upon opening, the mediastinum appeared normal with no evidence of lung or cardiac pathology. The organs of the peritoneum, kidneys, and adrenals were unremarkable with no lymphadenopathy. Overall, treatment with both the BCG.HIVA^{401} and the MVA.HIVA vaccines was not associated with any systemic toxicological changes, and the findings of local inflammation at the injection sites are considered to be consistent with a predicted response to the vaccine administration.

Neonate responses to PPD were strong and similar in both groups receiving the parental AERAS-401 and BCG.HIVA^{401}. Next, we evaluated the BCG-induced response in peripheral blood to PPD as a measure of the anti-*M. tuberculosis* re-

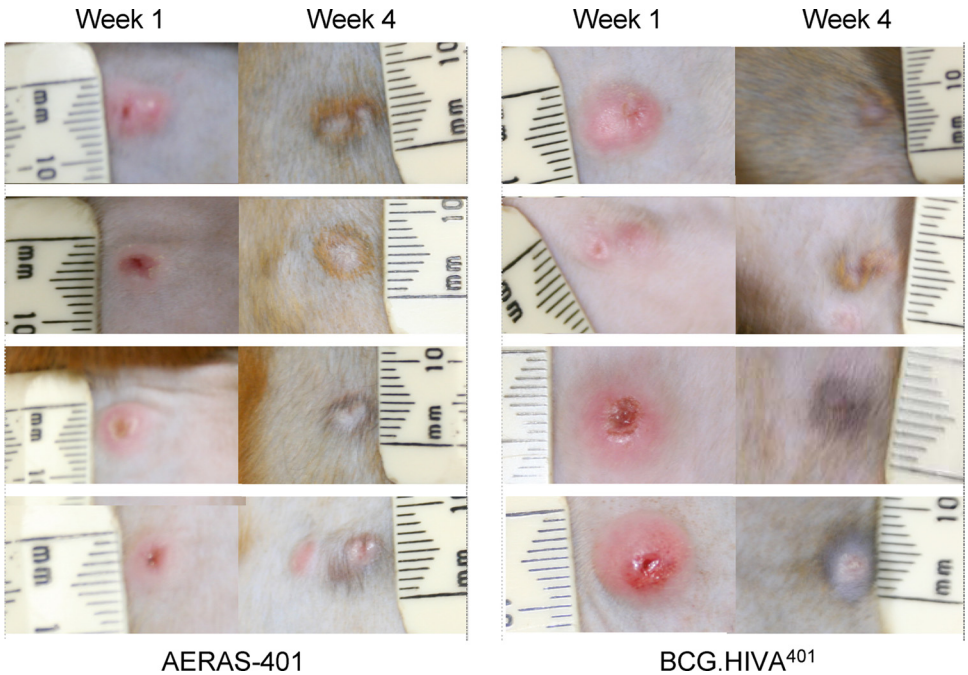

FIG. 2. Local reaction observed on infant macaques at the site of AERAS-401/BCG.HIVA^{401} administration. Neonatal rhesus macaques were given 10^7 CFU i.d. of either AERAS-401 or BCG.HIVA^{401}, i.e., 10× the human infant dose. Pictures of the injection sites were taken at 1 and 4 weeks after the vaccine injection.

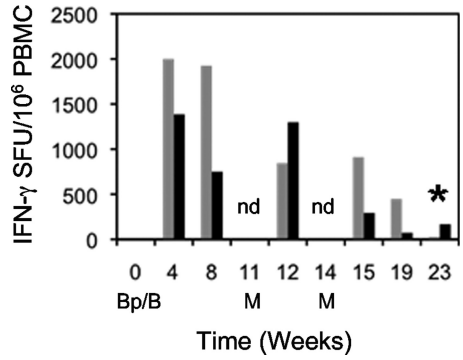

FIG. 3. BCG-specific T-cell responses elicited by AERAS-401/BCG.HIVA^{401} administration to infant macaques. Two groups of four infant rhesus macaques were immunized with 10^7 CFU i.d. of either empty parental AERAS-401 or BCG.HIVA^{401}, and the responses elicited to BCG were determined in an ex vivo IFN-γ ELISPOT assay using PPD as the antigen. The times of vaccinations are shown below in weeks. Bp, parental AERAS-401; B, BCG.HIVA^{401}; M, MVA.HIVA. The panel shows median responses for AERAS-401 (gray bars) or BCG.HIVA^{401} (black bars); the mock-stimulated background yielded a vast majority of wells with no spots. *, Statistically significant difference ($P = 0.015$) in a two-tail Student *t* test, which was not reached at any other time points. nd, not done.

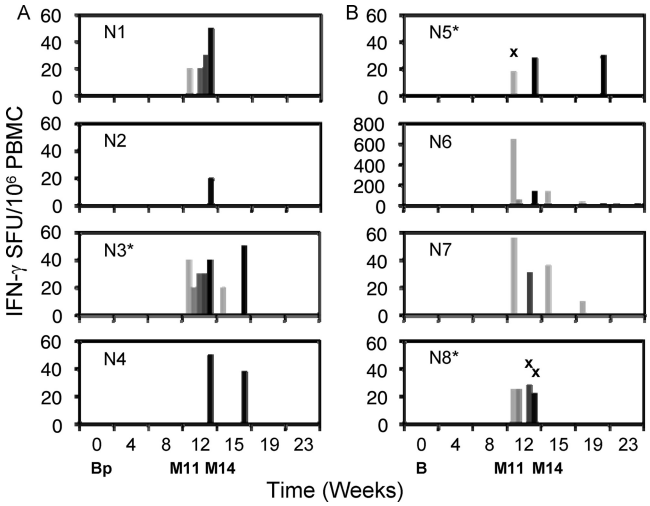

FIG. 4. HIV-1-specific T-cell responses elicited by HIVA. Two groups of four infant rhesus macaques were immunized with 10^7 CFU i.d. of either empty parental AERAS-401 (Bp; panel A) or BCG.HIVA^{401} (B; panel B) at week 0 and boosted by 5×10^7 PFU of MVA.HIVA i.m. at weeks 11 and 14 (M11 and M14). HIV-1-specific T-cell responses were measured in an *ex vivo* IFN-γ ELISPOT assay using HIVA-derived peptide pools 1 to 5 (from light gray to black bars). Note that Pool 5 contains Mamu-A*01-restricted epitope CM9. For the ELISPOT data analysis, control counts from each neonate were pooled to form the control group. For each peptide pair, a Wilcoxon test was performed, and *P* values were corrected within each animal using Benjamini-Hochberg correction. Any *P* value that was <0.05 postcorrection was deemed significantly different from the control pool. Only significant values were represented in the graph, with the exception of bars marked by "x". Software was used for the statistical analysis (http://www.r-project.org). *, Mamu-A*01$^+$ animals.

sponse, mostly mediated by CD4$^+$ T cells by using an ex vivo IFN-γ ELISPOT assay. The PPD responses peaked for both groups at 4 weeks postvaccination and declined thereafter (Fig. 3). On several occasions, the number of IFN-γ spot-forming units (SFU) was too high to be counted accurately and was assigned a best estimate frequency of 2,000 SFU/10^6 PBMC. Therefore, median frequencies are used for the description of these results. At peak, medians of 2,000 (all four animals) and 1,387 (range, 710 to 2,000) SFU/10^6 PBMC were detected after the AERAS-401 and BCG.HIVA^{401} administrations, respectively. The PPD responses induced by these two BCG strains were similar and reached a statistically significant difference ($P = 0.015$) only at the last time point at week 23, enumerating medians of 25 (range, 0 to 45) versus 167 (range, 110 to 326) SFU/10^6 PBMC for AERAS-401 and BCG.HIVA^{401}, respectively. This is not considered likely to be of any immunological sequelae. The PPD-specific responses were not boosted by the MVA.HIVA vaccinations. Thus, both parental strain AERAS-401 and vaccine BCG.HIVA^{401} alone elicited a strong BCG-specific response in recipient neonate macaques.

Weak HIV-1-specific T-cell responses were only detected after the MVA.HIVA boost. HIV-1-specific responses in the circulating blood induced in groups 1 and 2 of neonate macaques were determined prior to and throughout the immunization schedule in a standardized IFN-γ ELISPOT assay using five pools of 15-mer peptides overlapping by 11 amino acids spanning the entire HIVA immunogen sequence (32). The same HIVA peptide pools were used in previous nonhuman primate studies (17, 33, 36, 45) and clinical trials (reviewed in reference 13) of the HIVA vaccines. Here, a single administration of BCG.HIVA^{401} into neonates did not elicit any HIV-1-specific responses detectable in an *ex vivo* IFN-γ ELISPOT assay 4 and 8 weeks later. The first HIV-1-specific T-cell responses were detected in all eight animals 1 week after the first MVA.HIVA boost (week 12). With the exception of animal N6, the responses of which totaled 837 SFU/10^6 PBMC, the frequencies detected in the other seven neonates were between 20 (N2) and 160 (N3) SFU/10^6 PMBC in total (Fig. 4). It is noteworthy that animals N6 and N7 had enlarged lymph nodes in the left axilla at day 28, which resolved by day 56. Taking advantage of five HIVA peptide pools, we estimated that the neonates responded to at least a median of 2.5 (range, 1 to 5) epitopes. Because the blood volumes taken from macaque neonates were limited (1 ml per bleed), we did not perform any tests to discriminate between CD4$^+$ and CD8$^+$ T cell-mediated responses. The second MVA.HIVA administration boosted IFN-γ responses to single peptide pools in three animals (N3, N4, and N5) (weeks 15 or 19). This is likely due to the anti-vector antibody and T-cell responses induced by the first rMVA immunization, which dampens the insert-specific T-cell stimulations. All animals were screened for the immunodominant allele Mamu-A*01, and neonates N3, N5, and N8 were found to be positive. This did not influence the magnitude of the vaccine-induced response. Overall, given the animal numbers used in the present study, we did not observe any benefit of BCG.HIVA^{401} priming, which would be reflected in augmented responses detected after the MVA.HIVA boost, i.e., there was no difference between the group 1 and 2 elicited HIV-1-specific IFN-γ responses.

Vaccine-elicited responses were detected in regional tissues. It has been shown in the simian immunodeficiency virus (SIV)/rhesus macaque model that after oral inoculation of newborn

TABLE 1. BCG.HIVA^{401}-MVA.HIVA regimen-induced tissue-specific responses in neonate rhesus macaques[a]

Animal	Tissue	Response (SFU/10^6 cells)					
		HIVA peptide pool					PPD
		1	2	3	4	5	
N5	Spleen	0	0	0	0	0	0
	Submandibular LN	0	0	0	0	0	30
	Retropharyngeal LN	ND	ND	ND	ND	ND	ND
	Axillary LN	0	0	0	0	0	30
N6	Spleen	0	0	0	0	0	0
	Submandibular LN	0	0	0	0	28	26
	Retropharyngeal LN	0	0	0	0	25	36
	Axillary LN	0	0	0	0	0	45
N7	Spleen	0	0	0	0	0	40
	Submandibular LN	ND	ND	ND	ND	ND	ND
	Retropharyngeal LN	0	0	0	0	0	15
	Axillary LN	0	0	0	0	0	30
N8	Spleen	0	0	0	0	0	0
	Submandibular LN	ND	ND	ND	ND	ND	ND
	Retropharyngeal LN	ND	ND	ND	ND	ND	ND
	Axillary LN	0	0	0	0	0	35

[a] Responses were determined in an *ex vivo* IFN-γ ELISPOT assay. LN, lymph node; ND, not done.

animals, the virus spreads rapidly within days from the oral mucosa to regional and peripheral lymph nodes (30). Thus, at postmortem examination (week 23), lymphocytes from several lymphoid organs of group 2 neonate macaques N5-N8 were isolated and analyzed for vaccine-induced T cells in a standard *ex vivo* IFN-γ ELISPOT assay. Although weak PPD responses between 15 and 45 SFU/10^6 cells were detected in all animals at least in one of the examined tissues (spleen and submandibular, retropharyngeal, and axillary lymph nodes), HIV-1-specific responses of 25 and 28 SFU/10^6 cells were detected in at two sites only in neonate N6 (Table 1), which responded with the most vigorous IFN-γ response in the PBMC (Fig. 4). Note that at week 23, no HIV-1-specific responses were detectable in the peripheral blood.

DISCUSSION

In the course of this work, we assessed the safety and immunogenicity of a heterologous prime-boost regimen consisting of one dose of a novel BCG.HIVA^{401} strain, followed by two doses of MVA.HIVA vaccines in rhesus macaque neonates, an immunologically, technically, and ethically challenging model. We showed that both vaccines were safe causing only local reactions consistent with injection and induction of immune responses. BCG.HIVA induced strong BCG-specific responses but failed to elicit any directly or indirectly (through enhancement of boost) detectable HIV-1-specific IFN-γ-producing T-cell responses. MVA.HIVA induced weak, but consistent anti-HIV-1 responses. This is the first time that AERAS-401 strain of BCG and both the BCG.HIVA^{401} and the MVA.HIVA vaccines have been tested in macaque neonates.

Vaccination with the parental AREAS-401 and recombinant BCG.HIVA^{401} strains induced high frequencies of IFN-γ-producing PBMC responding to the mycobacterium PPD stimulation, and these frequencies were similar for both strains. However, cellular responses elicited by BCG.HIVA^{401} specific for the HIV-1-derived passenger immunogen HIVA were undetectable in *ex vivo* and cultured (not shown) IFN-γ ELISPOT assays. Although similar results were obtained in adult mice, BCG.HIVA^{401} induce low, but definite and boostable HIV-1-specific responses in adult rhesus macaques (36). Thus, the T-cell immunogenicity already decreased by somewhat inefficient production and/or processing of rBCG-expressed HIVA observed in adult animals was further compromised by the relative immaturity of the infant immune system. In 10-week-old human neonates vaccinated with BCG at birth, responses to BCG were mediated mainly by $CD4^+$ T cells, displayed complex cytokine patterns, and suggested that relying on IFN-γ production alone may underestimate the magnitude and complexity of infant response (38). Thus, the focus in the present study on the standard IFN-γ detection could have also contributed to the failure to detect HIV-1-specific responses induced by BCG.HIVA^{401} vaccination.

With the exception of macaque N6, HIV-1-specific T-cell responses elicited by the MVA.HIVA vaccination were weak, but detected after the first MVA.HIVA vaccination in all eight neonates. The second MVA.HIVA administration reboosted these responses in three of eight of these animals. This is again in contrast to adult rhesus macaques, in which MVA.HIVA induced strong HIV-1-specific T-cell responses (17, 33, 36, 45). The immunogenic and protective properties of rMVAs in nonhuman primate infants was investigated by Van Rompay et al. (41, 42). In the first study, neonate rhesus macaques were vaccinated with MVA-SIVgpe expressing SIV Gag, Pol, and Env and challenged orally with pathogenic SIVmac251. The vaccinees survived longer and displayed better antibody responses than members of the control group, while only two vaccinees had detectable SIV-specific IFN-γ responses after the MVA.SIVgpe vaccination (42). In a subsequent study, delivering SIV Gag, Pol, and Env using both canarypox and MVA vectors to neonate macaques prior to oral SIVmac251 challenge reduced SIVmac251 acquisition and decreased viremia in infants that became infected (41). Here, the HIVA immunogen is designed for humans and, as such, consists of HIV-1 Gag protein coupled to a string of HLA-restricted epitopes. Because HIV-1 does not replicate in rhesus macaques, the neonates in the present experiment were not challenged. In any case, the protective efficacy in the SIV/macaque model has not been validated by human protection, and its predictive value to the vaccine's performance in humans is at present unclear (12).

In contrast to vaccine efficacy, the SIV/macaque model has been extremely useful for following the dissemination of infection after oral challenge. It was shown that the virus passes from the oral mucosa to the submandibular and retropharyngeal lymph nodes, followed by the axillary lymph nodes and the Peyer's patches within 4 days. A key factor involved in the PMTCT of HIV-1 is likely to be the local immunity at the port of entry, where the vaccine-induced T cells may have to respond. The results of the BCG.HIVA^{401} neonate rhesus macaque trial demonstrated that recall IFN-γ ELISPOT responses to HIVA peptides were detected in cells derived from the retropharyngeal and submandibular lymph nodes at necropsy (week 23) in the best responding animal N6. Indeed,

stronger PPD-specific responses were detected more consistently at the studied immunological sites of these neonates.

In conclusion, we tested two candidate HIV-1 vaccines, BCG.HIVA^{401} and MVA.HIVA, for the first time in rhesus macaque infants. We found the vaccines safe, but less immunogenic for T cells than when administered to adult animals. These results inform further development of vaccines against mother-to-child transmission of HIV-1 through breast milk and complement two MVA.HIVA infant trials in Africa.

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