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VACCINES AND ANTIVIRAL AGENTS

HIV-Exposed Infants Vaccinated with an MF59/Recombinant gp120 Vaccine Have Higher-Magnitude Anti-V1V2 IgG Responses than Adults Immunized with the Same Vaccine

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ABSTRACT In the RV144 vaccine trial, IgG responses against the HIV envelope variable loops 1 and 2 (V1V2) were associated with decreased HIV acquisition risk. We previously reported that infants immunized with an MF59-adjuvanted rgp120 vaccine developed higher-magnitude anti-V1V2 IgG responses than adult RV144 vaccinees. To determine whether the robust antibody response in infants is due to differences in vaccine regimens or to inherent differences between the adult and infant immune systems, we compared Env-specific IgG responses in adults and infants immunized with the same MF59- and alum-adjuvanted HIV envelope vaccines. At peak immunogenicity, the magnitudes of the gp120- and V1V2-specific IgG responses were comparable between adults and infants immunized with the alum/MNrgp120 vaccine (gp120 median fluorescence intensities [FIs] in infants = 7,118 and in adults = 11,510, $P = 0.070$; V1V2 median MFIs of 512 [infants] and 804 [adults], $P = 0.50$), whereas infants immunized with the MF59/SF-2 rgp120 vaccine had higher-magnitude antibody levels than adults (gp120 median FIs of 15,509 [infants] and 2,290 [adults], $P < 0.001$; V1V2 median FIs of 23,926 [infants] and 1,538 [adults]; $P < 0.001$). Six months after peak immunogenicity, infants maintained higher levels Env-specific IgG than adults. Anti-V1V2 IgG3 antibodies that were associated with decreased HIV-1 risk in RV144 vaccinees were present in 43% of MF59/rgp120-vaccinated infants but only in 12% of the vaccinated adults ($P = 0.0018$). Finally, in contrast to the rare vaccine-elicited Env-specific IgA in infants, rgp120 vaccine-elicited Env-specific IgA was frequently detected in adults. Our results suggest that vaccine adjuvants differently modulate gp120-specific antibody responses in adults and infants and that infants can robustly respond to HIV Env immunization.

IMPORTANCE More than 150,000 pediatric HIV infections occur yearly, despite the availability of antiretroviral prophylaxis. A pediatric HIV vaccine could reduce the number of these ongoing infant infections and also prime for long-term immunity prior to sexual debut. We previously reported that immunization of infants with an MF59-adjuvanted recombinant gp120 vaccine induced higher-magnitude, potentially protective anti-V1V2 IgG responses than in adult vaccinees receiving the moderately effective RV144 vaccine. In the present study, we demonstrate that the robust response observed in infants is not due to differences in vaccine regimen or vaccine dose between adults and infants. Our results suggest that HIV vaccine adjuvants may differentially modulate immune responses in adults and infants, highlighting the need to conduct vaccine trials in pediatric populations.

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The widespread availability of antiretroviral (ARV) prophylaxis has significantly reduced the overall incidence of HIV-1 mother-to-child transmission (MTCT), but due to implementation and social barriers, as well as poor adherence and drug resistance in resource-poor areas, more than 150,000 infants continue to acquire HIV via MTCT annually (1). Breastfeeding is responsible for nearly half of these infant infections. Even with optimal implementation of ARV prophylaxis, HIV vertical transmission will still occur in as many as 5% of HIV-exposed infants due to acute maternal infections during pregnancy and breastfeeding and to a residual risk of transmission despite prophylaxis (2, 3). The alternative of replacement feeding is not a viable strategy to reduce breast milk transmission in resource-poor areas since the high infectious disease burden, high formula costs, and lack of potable water and refrigeration make breastfeeding crucial to infant survival. Novel strategies such as a safe, effective pediatric HIV vaccination could address these gaps in protection and contribute to the elimination of pediatric HIV infections (2). In addition, infant immunization could be boosted later in life to achieve broad immunity prior to sexual debut.

Because of the developmental stage of the infant immune system, it is traditionally believed that infants mount poor immune responses to vaccines compared to adults (4, 5). Nevertheless, several studies have demonstrated that immunization in childhood can lead to long-lasting immunity (6–8). Only a few studies have compared adjuvanted vaccine-elicited antibody responses between adult and pediatric populations. Fukase et al. (9) assessed the safety and immunogenicity of MF59-adjuvanted influenza vaccines in children (aged 6 months to 18 years) and adults and reported comparable frequencies and antibody responses between the two groups. Comparable antibody responses were also reported between children (3 to 17 years old) and adults immunized with an alum-adjuvanted influenza vaccine (10). Moreover, an association between younger age at the time of vaccination and better antibody responses has been reported for vaccines such hepatitis B and human papillomavirus vaccines (11, 12). Thus, young children can mount robust antibody responses following vaccination.

Previous phase I/IIa infant vaccine trials have demonstrated that HIV-1 immunization of infants is safe and can induce both cellular and humoral immune responses (13, 14). Nevertheless, because no infant HIV vaccine efficacy trial has been conducted to date, it is unclear whether these vaccine-elicited responses are protective. Insights into the mechanisms of vaccine protection can be gained from adult vaccine efficacy trials such as a moderately successful adult RV144 vaccine trial that showed 31% efficacy 3 years after the last vaccination (15). Analysis of immune correlates of risk in this trial indicated a correlation between reduced risk of HIV-1 acquisition and an increased plasma concentration of IgG against the HIV-1 envelope variable loops 1 and 2 (V1V2), whereas the HIV-1 Env-specific IgA levels correlated with increased risk of infection in vaccinees (16, 17). Importantly, our group recently demonstrated that infants vaccinated with a recombinant HIV gp120 protein adjuvanted with MF59 developed higher-magnitude and longer-lasting anti-V1V2 IgG antibody responses than did adult RV144 vaccine recipients (18). At peak immunogenicity, these pediatric V1V2 responses were 22 times greater than those detected in the RV144 vaccinees. Moreover, 6 months after peak immunogenicity, the magnitude of the responses in infants remained higher than in adults at peak immunogenicity. Infant vaccine-elicited antibody responses were durable: V1V2-specific IgG was detected more than 18 months after immunization in most infant vaccinees (18). Since the vaccine efficacy of the RV144 vaccine 1 year after the first vaccination was 60% (19), a vaccine eliciting protective responses similar to RV144 in infants could protect 60% of HIV-exposed infants during the first year of life, when breastfeeding takes place. Therefore, if a pediatric vaccine induces higher levels of antibodies than the RV144 vaccine and these antibodies are present for a longer duration, the efficacy of the pediatric vaccine might be even greater than 60%.

The higher magnitude of potentially protective anti-V1V2 IgG in vaccinated infants

TABLE 1 Patients from PACTG 230 and AVEG 201 included in the study

Treatment	Dose (μg)	No. of patients	
		Adults (AVEG 201)	Infants (PACTG 230)
Chiron	5	0	17
	15	0	13
	50	42	15
Total		42	45
VaxGen	30	0	14
	100	0	19
	300	49	14
Total		49	47
Placebo		10	16

compared to adult RV144 vaccinees could be due either to intrinsic differences between the adult and infant immune systems or to differences in the vaccine regimens. In these previously compared trials, infants were immunized with a recombinant gp120 (rgp120) vaccine using MF59 as an adjuvant, whereas adults were vaccinated with an ALVAC prime/AIDSVAX boost (vcp1521 + AIDVAX B/E) regimen using alum as adjuvant. It was recently reported that adults vaccinated with an ALVAC prime/MF59-adjuvanted protein boost regimen did not develop higher-magnitude V1V2-specific IgG responses than those immunized with an ALVAC prime/alum-adjuvanted protein boost regimen (20). However, use of the MF59 adjuvant has augmented the efficacy of trivalent influenza vaccine in children (21), suggesting that MF59 could differently modulate vaccine responses in adults and children. To determine whether the impressive vaccine response elicited by MF59/rgp120 in infants is a response unique to this regimen, we compared humoral responses elicited by the same alum/rgp120 (VaxGen) and MF59/rgp120 (Chiron) vaccines in adults (AVEG 201 vaccine trial) (22) and infants (the Pediatric AIDS Clinical Trial Group Protocol 230 [PACTG 230] trial) (23). Our results demonstrate that MF59/rgp120 uniquely elicited robust Env-specific responses in infants, suggesting differences in the modulation of HIV vaccine responses in adults and infants.

RESULTS

Higher-magnitude V1V2-specific IgG responses in MF59-adjuvanted rgp120-vaccinated infants than adults. In the RV144 vaccine trial, V1V2 IgG responses were associated with a reduced risk of HIV acquisition (17). Thus, we first compared the levels of V1V2 IgG in adult (AVEG 201) and infant (PACTG 230) vaccine recipients (Table 1). The sequences of the V1V2 construct used in binding assays and of the V1V2 region of the vaccine strains are presented in Fig. 1. At peak immunogenicity, the magnitude of IgG binding antibodies against the gp70 B case A V1V2 construct was 10-fold higher in MF59-adjuvanted rgp120 (Chiron)-vaccinated infants than in adults (median MFIs of 23,926 [infants] and 1,538, $P < 0.001$; Fig. 2). In contrast, there was no difference in magnitude of this response between infants and adults immunized with the alum-adjuvanted rgp120 (VaxGen) vaccine (median MFIs of 512 [infants] and 804 [adults], $P = 0.50$). The high level of V1V2-specific IgG in infants immunized with the MF59-

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B case A V1V2      CVTLNCIDLRNATNATSNSNTTNTTSSSGGLMMEQGEIKNCSFNITTSIRDKVQKEYALFYKLDIVPIDNPKNS...TNYRLISC
B case A V1V2 169K CVTLNCIDLRNATNATSNSNTTNTTSSSGGLMMEQGEIKNCSFNITTSIRDKKQKEYALFYKLDIVPIDNPKNS...TNYRLISC
B case A V1V2 mut3 CVTLNCIDLRNATNATSNSNTTNTTSSSGGLMMEQGEIKNCSFNITTSIRDKKQKVHALFYKLDIVPIDNPKNS...TNYRLISC
A244 V1V2          ----H-TNANLTKANLT..-VN-R-NV-NIIGNITD-VR-----M--EL---K--VH----------EDNND-...SE----N-
1086C V1V2         ------TNVKGNES.................DTSEVM-----KA--ELK--KH-VH-------V--LNGNSS-..SGE----N-
SF2 V1V2           ------T--GK---TN-SN..........WKEEIK----------------I---N---RN--V-----ASTTTNY------H-
MN V1V2            ------T----T--TNNSTDNN-SN...SEGTIKG--M-----------G---------L-----ES---DS.....-S------
Bio.V2.B           ..........................................KKK-----------------V--.................
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FIG 1 Amino acid sequence of the Env region of the vaccine strains and V1V2 constructs in binding assays. The SF2 V1V2 sequence has a deletion of 7 amino acids and an insertion of 5 amino acids compared to MN V1V2 sequence. Overall, there is 56% analogy in amino acids between the two vaccine strains V1V2 sequences. There is less than 50% analogy in amino acids between the clade C 1086 and the clade AE A244 V1V2 region and B case A V1V2. The Bio.V2.B peptide sequence is similar to the B case 1 V1V2 sequence, except at one amino acid position.

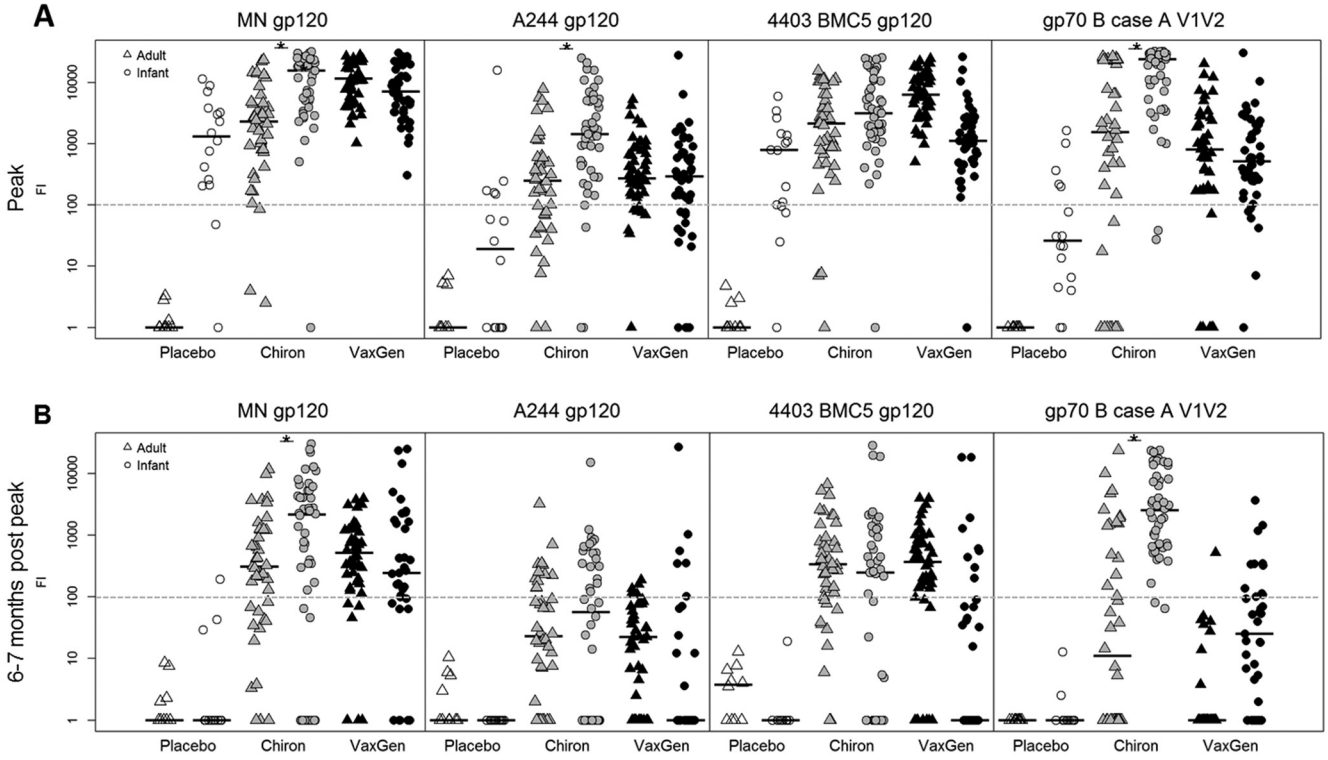

FIG 2 Env-specific IgG response in adult and infant rgp120 recipients at peak immunogenicity and 6 to 7 months later. (A) At peak immunogenicity, the magnitude of the Env-specific binding antibody response was higher in infants (circles) than in adults (triangles) immunized with the MF59/rgp120 (Chiron) vaccine (gray) against MN gp120 (clade B) and gp70 B case A. (B) Seven months after the peak, undetectable to low levels of gp120-specific and V1V2-specific IgG were measured in adults and infants immunized with the alum/rgp120 vaccine (black), whereas the magnitude of these responses remained high in MF59/rgp120 infant vaccinees. The dashed line represents the positivity cutoff. Asterisks indicate statistically significant differences.

adjuvanted rgp120 vaccine compared to the other vaccine groups suggest that the MF59 adjuvant elicits robust, potentially protective, V1V2 IgG antibodies in early life.

Since most HIV vaccines tested in adults to date induce short-lived Env-specific antibody responses (24, 25), we compared the durability of V1V2-specific IgG responses in MF59- and alum-adjuvanted rgp120 adult and infant vaccine recipients by measuring antibody levels 6 to 7 months after peak immunogenicity (Fig. 2). Only a small percentage of either MF59-adjuvanted rgp120 adult vaccinees (38%) or alum-adjuvanted rgp120-vaccinated infants (27%) and adults (2%) still had detectable levels of V1V2-specific IgG responses, whereas 95% of infants vaccinated with the MF59/rgp120 vaccine still had a detectable response. The magnitude of the V1V2-specific IgG response was also higher in infants immunized with the MF59-adjuvanted rgp120 vaccine than in adults at this later time point (median FIs of 2,523 [infants] and 11 [adults], $P = 0.02$). In addition, the half-lives of V1V2-specific antibodies were significantly longer in infants than in adults after the administration of either alum/rgp120 (infants [46 days] versus adults [20 days], $P = 0.03$) or MF59/rgp120 (infants [88 days] versus adults [42 days], $P = 0.004$) vaccination. There was little correlation between the V1V2 IgG half-life and the peak magnitude V1V2 IgG responses (the rank correlation coefficient for MF59/rgp120-treated infants was $r = -0.01$, with a 95% Monte Carlo confidence interval [CI] of -0.32 to 0.31; for MF59/rgp120-treated adults it was $r = -0.02$ [95% Monte Carlo CI $= -0.33$ to 0.30], and for alum/rgp120-treated infants it was $r = 0.18$ [95% Monte Carlo CI $= -0.13$ to 0.46]). Interestingly, the magnitude of the V1V2 IgG response in MF59/rgp120-immunized infants 7 months after peak immunogenicity was comparable to that of adults at peak immunogenicity (median FIs of 2,523 in infants 6 months after peak and 1,538 in adults after peak). Thus, the robust V1V2-specific IgG responses elicited by the MF59/rgp120 vaccine in infants were also more durable than the adult responses.

TABLE 2 Percentages of adult and infant vaccine recipients with detectable HIV Env-specific IgG at peak immunogenicity

	Recipients (%) with detectable HIV Env-specific IgG				
	Chiron		VaxGen		Placebo
Vaccine	Adults	Infants	Adults	Infants	Infants
MNgp120	93	98	100	100	88
A244 gp120	67	91	84	72	31
4403 BMC5	93	98	100	98	69

HIV gp120 vaccination elicits robust Env-specific binding IgG responses in both adults and infants. We next measured antibody responses against the vaccine clade B strain MNgp120, the clade AE strain A244 gp120 (RV144 vaccine strain), and the clade C strain 4403 BMC5 gp120 (infant transmitted/founder envelope) in vaccinated adults and infants at peak immunogenicity. The majority of vaccine recipients had IgG responses against the clade-matched vaccine strain MNgp120, as well as against A244 gp120 and 4403 BMC5 gp120 (Table 2). Most of the infant placebo recipients also had detectable levels of Env-specific IgG at 24 weeks, indicating that maternally acquired antibodies were still present in infants at the peak immunogenicity time point. However, the level of Env-specific IgG was $10^{0.5}$- to 10-fold lower among placebo-treated recipients than in vaccine recipients (Fig. 2), suggesting that vaccine-elicited antibodies were predominant in the vaccinated infants. As expected, adults and infants immunized with the alum/rgp120 vaccine (VaxGen) showed a high-magnitude MNgp120-specific IgG binding response, and there was no statistically significant difference in the magnitude of the response between the two groups (median FIs of 11,510 [adults] and 7,118 [infants], $P = 0.07$; see Fig. 2). In contrast, adults immunized with the MF59/rgp120 vaccine had lower-level MNgp120 IgG responses than adult alum/rgp120 recipients (median FIs of 2,290 [MF59/rgp120] and 11,510 [alum/rgp120], $P < 0.001$), whereas there was no statistically significant difference in the magnitude of the IgG response between infants immunized with the two vaccines (median FIs of 15,509 [MF59/rgp120] and 7,118 [alum/rgp120]; $P = 0.09$). Moreover, the magnitude of the MNgp120-specific IgG binding response was also significantly higher in MF59/rgp120-vaccinated infants than in adults (median FIs of 15,509 [infants] and 2,290 [adults], $P < 0.001$). Similarly, MF59/rgp120 vaccinated infants had higher magnitude IgG binding responses against A244gp120 than adults (median MFIs of 1,426 [infants] and 250 [adults], $P < 0.001$), but the magnitude of the IgG response against 4403 BMC5 was comparable between the two groups (median FIs of 2,124 [adults] and 3,122 [infants], $P = 0.070$). Adults and infants immunized with the alum/rgp120 vaccine showed comparable binding IgG responses against A244 gp120 (median FIs of 270 [adults] and 291 [infants], $P = 0.45$), but the magnitude of the response against 4403 BMC5 was higher in adults than in infants (median FIs of 6,264 [adults] and 1,113 [infants], $P < 0.001$). Overall, our results suggest that the MF59-adjuvanted rgp120 vaccine elicited high-magnitude gp120-specific IgG responses in infants that were distinct from the responses elicited in adults.

We assessed the levels and frequencies of MNgp120-specific IgG in adult and infant vaccine recipients 6 to 7 months after peak immunogenicity. At this time point, Env-specific IgG were rarely detected in infant placebo recipients, indicating that maternal antibodies had waned (Fig. 2). In contrast, most adult and infant vaccinees still had detectable levels of antibodies against MNgp120 (frequency of responders, MF59/rgp120 [infants, 77%; adults, 69%] and alum/rgp120 [infants, 68%; adults, 85%]); however, the magnitude of the response was ~10-fold lower than at peak immunogenicity in both adult and infant vaccinees. Comparably low levels of gp120-specific IgG were detected in alum-adjuvanted rgp120 adult and infant vaccinees (median FIs of 512 [adults] and 242 [infants], $P = 0.31$), whereas the magnitude of the response remained higher in infants than adults immunized with MF59-adjuvanted rgp120 vaccine (median FIs of 306 [adults] and 2,145 [infants], $P = 0.02$). Nevertheless, there was no statistical difference in the half-lives of MNgp120 antibodies between adults

and infants immunized with the MF59-adjuvanted rgp120 vaccine (63 days [infants] versus 69 days [adults], $P = 0.9$). There was little correlation between the MNgp120-specific IgG peak levels and half-lives in all of the vaccine groups (rank correlation coefficients for MF59/rgp120-treated infants of 0.18 [95% Monte Carlo CI= −0.14 to 0.46] and MF59/rgp120-treated adults of −0.16 [95% Monte Carlo CI = −0.45 to 0.16]; rank correlation coefficients for alum/rgp120-treated infants of 0.20 [95% Monte Carlo CI = −0.12 to 0.48] and alum/rgp120-treated adults of 0.01 [95% Monte Carlo CI = −0.27 to 0.29]). In contrast, there was a strong correlation between the half-life of IgG against MNgp120 and the half-lives of the other tested other gp120 formulations (A244 gp120 and 4403 bmC5 gp120) in infant and adult vaccinees (the rank correlation coefficient ranged from 0.41 to 0.80 in infants and was >0.70 for all adults). The half-life of V1V2- and MNgp120-specific IgGs showed weak to moderate correlations in MF59/rgp120-vaccinated infants (rank correlation coefficient of 0.44 [95% Monte Carlo CI = 0.15 to 0.66]; for MF59/rgp120-vaccinated adults, the rank correlation coefficient was 0.40 [95% Monte Carlo CI = 0.08 to 0.64], and for alum/rgp120-vaccinated infants, the rank correlation coefficient was 0.56 [95% Monte Carlo CI = 0.31 to 0.74], but in the alum/rgp120-vaccinated adults, the rank correlation coefficient was −0.09 [95% Monte Carlo CI = −0.38 to 0.20]). These results indicate that the half-lives of different Env gp120-specific antibodies are more closely correlated to each other than between gp120- and V1V2-specific antibodies.

Higher-magnitude Env-specific and V1V2-specific IgG responses in MF59-adjuvanted rgp120-vaccinated infants compared to adults is not due to differences in vaccine doses used in adult and infants. Infants immunized with the MF59-adjuvanted rgp120 (Chiron) vaccine received a dose of 5, 15, or 50 μg, whereas all adult vaccine recipients were immunized with 50 μg of rgp120. To assess whether vaccine dose helps to explain the differences in vaccine-elicited antibody responses between adults and infants, we compared the magnitudes of the rgp120-specific and V1V2 IgG responses between adults and infants immunized with different vaccine doses at peak immunogenicity. All infant groups had higher-magnitude MNgp120-specific IgG responses than did the adult vaccine recipients (the median FIs in infant groups and the corresponding *P* values compared to adults were determined at various doses: 5 μg, median FI of 5,633, $P = 0.008$; 15 μg, median FI of 21,932, $P < 0.001$; 50 μg, median FI of 19,299, $P < 0.001$ [Fig. 3]). Similarly, the V1V2-specific IgG response was higher in infants than in adults across the vaccine dose groups (the median FIs in infant groups and the corresponding *P* values compared to adults were determined at various doses: 5 μg, median FI of 26,230, $P < 0.001$; 15 μg, median FI of 21,527, $P = 0.009$; 50 μg, median FI of 10,986, $P = 0.005$). Our analyses uncovered differences in response levels between the different infant vaccine groups. Notably, infants immunized with the 5-μg dose had lower-magnitude MNgp120 IgG responses ($P = 0.037$) but tended to have higher-magnitude levels of V1V2 IgG ($P = 0.06$) than infants immunized with the 50-μg dose. We also assessed the impact of vaccine dose in infants immunized with the alum/rgp120 (VaxGen) vaccine and observed no difference in the response magnitude of either MNgp120- or V1V2-specific IgG between those immunized with 30, 100, or 300 μg of rgp120. Thus, our results demonstrate that the difference in IgG responses between adults and infants immunized with the MF59-adjuvanted rgp120 vaccine is not due to different vaccine doses but indicates that the vaccine dose impacts the magnitude of the vaccine-elicited IgG response in infants.

Higher frequency of V1V2 IgG3 responders in MF59/rgp120-vaccinated infants than in adults. In the RV144 immune correlate analysis, V1V2-specific IgG3 responses were strongly associated with reduced HIV acquisition risk (26). We therefore measured IgG3 responses against rgp120 and against the gp70 B case A V1V2 construct at the peak immunogenicity time point (Fig. 4). IgG3 responses against MNgp120 were rarely detected in vaccinated infants (MF59/rgp120, 9%; alum/rgp120, 2%) or adults (MF59/rgp120, 14%; alum/rgp120, 35%). Similarly, only a small proportion of adults (MF59/rgp120, 12%; alum/rgp120, 10%) and alum-adjuvanted rgp120 infant vaccinees (4%) had detectable levels of V1V2-specific IgG3. However, nearly half of

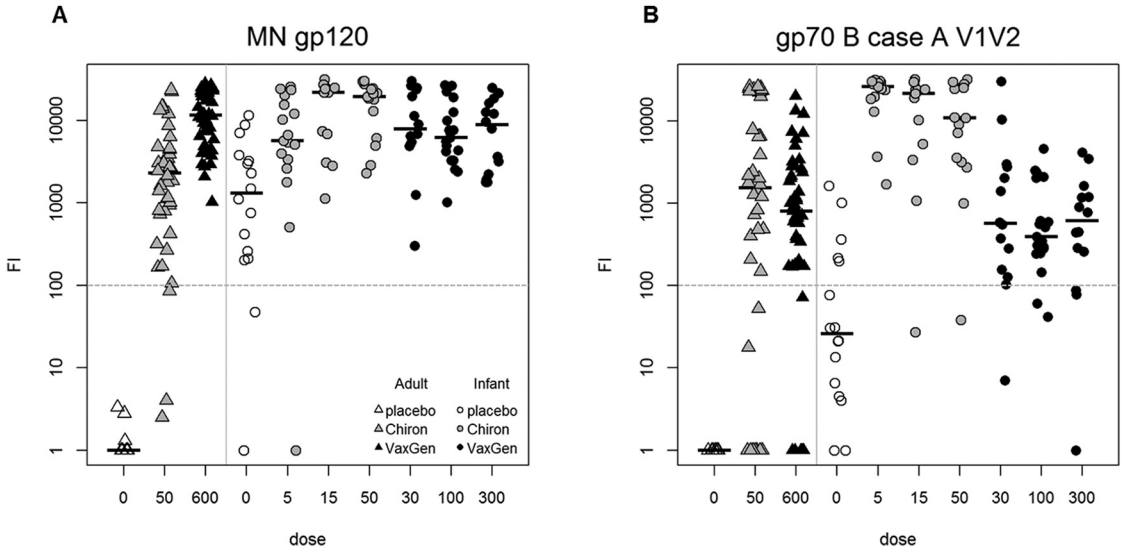

FIG 3 Influence of vaccine dose on the levels of vaccine-elicited IgG at peak immunogenicity in adults and infants immunized with the rgp120 vaccines. Infants immunized with MF59/rgp120 received either with 5, 15, or 50 μg of rgp120, whereas adults were immunized with 50 μg of protein. Infants immunized with alum/rgp120 received 30, 100, or 300 μg of protein, whereas adults were immunized with 600 μg of protein. The levels of gp120-specific IgG (A) and V1V2-specific IgG (B) were higher in vaccinated infants than in adults, irrespective of the vaccine dose. The dashed line represents the positivity cutoff.

MF59/rgp120 vaccinates infants (43%) had a detectable potentially protective V1V2 IgG3 responses ($P = 0.018$). There was no difference in the response levels of detectable V1V2-specific IgG3 between adults and infants.

Breadth of V1V2 IgG in MF59/rgp120 adult and infant vaccinees. The breadth of the V1V2 IgG response was assessed by determining the percentage of adults and infants immunized with the MF59-adjuvanted rgp120 vaccine who had detectable levels of antibodies against a panel of V1V2 constructs at peak immunogenicity (Table 3). Although the majority of adults and infants had a detectable response against all of the tested constructs, a higher proportion of infants than adults had a response against

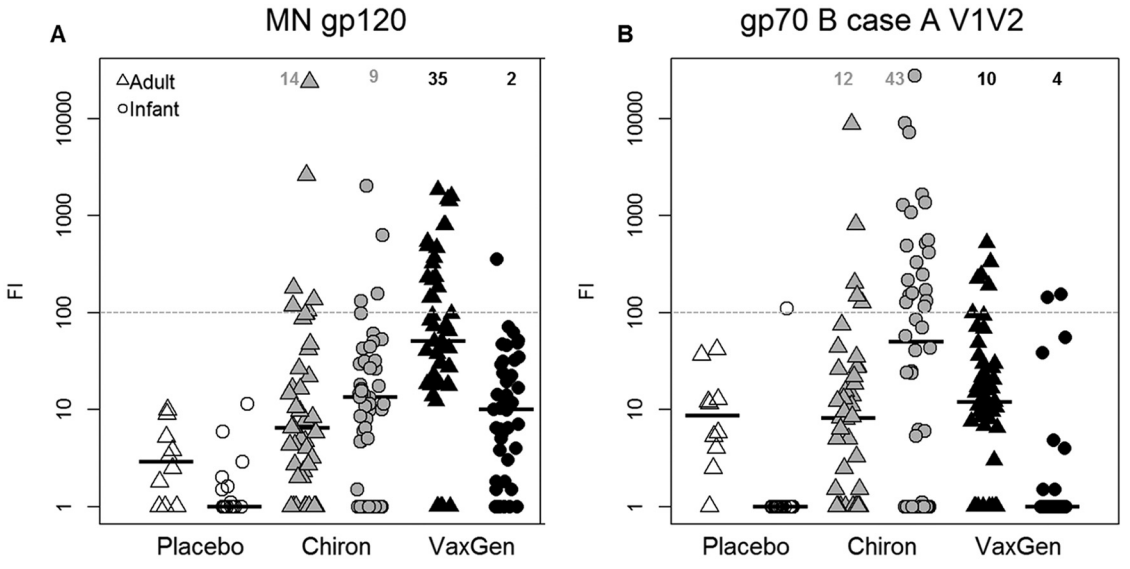

FIG 4 Magnitude and frequency of Env-specific IgG3 responses in adult and infant rgp120 vaccinees. Vaccinated infants had a low frequency of gp120-specific IgG3 (A), but the frequency of V1V2-specific IgG3 responders was higher among MF59/rgp120 infant vaccinees than in adults (B). The numbers in the figure represent the proportions of responders, and the dashed line represents the positivity cutoff.

TABLE 3 Percentages of adults and infants immunized using MF59/rgp120 vaccine (Chiron) with detectable V1V2 IgG at peak immunogenicity

V1V2 IgG	Recipients (%) with detectable V1V2 IgG		*P*
	Adults	Infants	
gp70 B case A V1V2	74	96	0.005
gp70 B case A V1V2 169K	74	93	0.018
gp70 B case A V1V2 mut 3	71	89	0.056
gp70 1086c V1V2	67	87	0.040
AE A244 V1V2 tags	74	83	0.437

the clade B gp70 B case A V1V2 construct ($P = 0.005$), against the clade C gp70 1086c V1V2 construct ($P = 0.040$), and against gp70 B case V1V2 with a V169K mutation ($P = 0.018$). Infant vaccinees also trended to have higher response frequency against gp70 B case V1V2 with V169K/E172V/Y173H mutations ($P = 0.56$) and had a higher response frequency against the clade AE A244 V1V2 construct, although this difference did not reach statistical significance. Overall, these results suggest that MF59/rgp120 vaccination may elicit a greater V1V2 IgG response breadth in infants than in adults.

The magnitude and avidity of the V2-specific IgG response are comparable between adult and infant vaccinees. Peptide array analysis has demonstrated an association between IgG against V2 linear peptides and reduced risk of HIV acquisition in RV144 vaccinees (27). We therefore assessed V2-specific IgG responses in adult and infant rgp120 vaccinees. At peak immunogenicity, the magnitude of V2-specific IgG response was higher in adults and infants immunized with the MF59/rgp120 vaccine than in those immunized with the alum/rgp120 vaccine (median ODs of 1.6 versus 0.2 and $P < 0.001$ [infants] and 0.8 versus 0.32 and $P = 0.008$ [adults]; Fig. 5). However, there was no difference between adults and infants immunized with the MF59/rgp120 vaccine (infant median OD of 1.6 versus adult median OD of 0.8 [$P = 0.10$]). We then compared the avidities of vaccine-elicited V2-specific IgG responses between the vaccine groups (Fig. 5). Interestingly, there was no difference in the avidities of V2-specific antibodies between adults and infants immunized with either vaccine regimens (avidity index

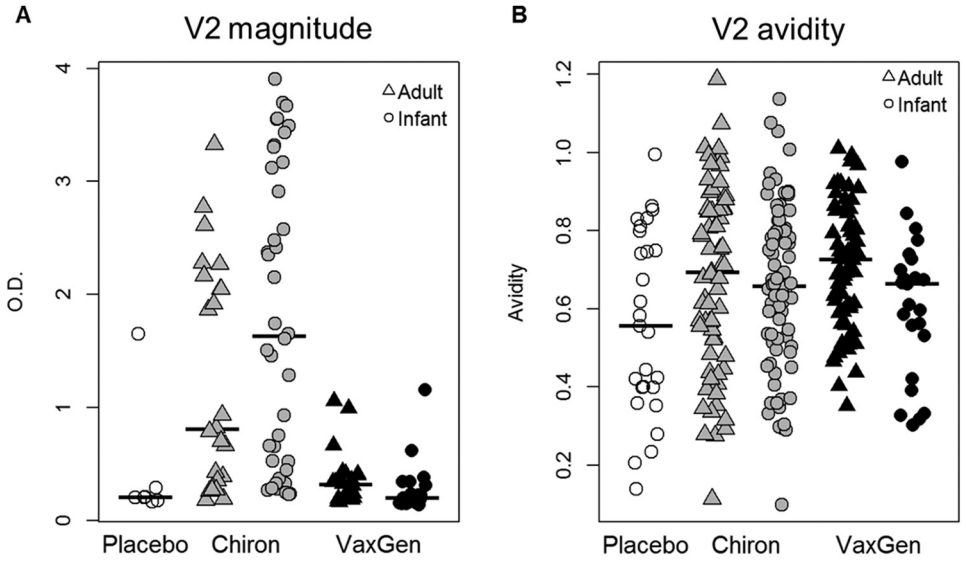

FIG 5 Magnitude and avidity of V2-specific IgG responses in vaccinated adults and infants. (A) Higher-magnitude V2-specific IgG responses in adults and infants immunized with MF59/rgp120 than in those immunized with alum/rgp120. (B) There were no differences in the avidity indices between adults and infants immunized with either the MF59/rgp120 (Chiron) or the alum/rgp120 (VaxGen) vaccine. The avidity index was calculated only for samples with detectable V2-specific IgG binding response. CH58, a V2-specific MAb isolated from a RV144 vaccinee, was used as positive control and had an average avidity index of 0.4.

TABLE 4 Percentages of AVEG 201 adult vaccine recipients with detectable HIV Env-specific IgA

	Recipients (%)[a]					
	Placebo ($n = 10$)		Chiron ($n = 42$)		VaxGen ($n = 49$)	
HIV antigen	Visit 2	Visit 11	Visit 2	Visit 11	Visit 2	Visit 11
A1 con gp140	0	0	0	2	0	4
B con gp140	0	0	0	14	2	22
C1 peptide	0	0	0	7	6	6
MN gp120	0	0	0	19	2	8

[a]Visit 2 was preimmunization, and visit 11 took place 2 weeks after the last vaccine dose.

values: MF59/rgp120 vaccine, 0.60 [infants] versus 0.56 [adults], $P = 0.46$; alum/rgp120 vaccine, 0.66 [infants] versus 0.64 [adults], $P = 0.59$). Moreover, there was no difference in avidity between V2-specific IgG measured at birth (maternally acquired) and values measured at peak immunogenicity (avidity index values measured at birth: MF59/rgp120, 0.68, $P = 0.053$; alum/rgp120, 0.72, $P = 0.37$). Our results indicate that after four vaccine doses, the avidity of V2-specific vaccine-elicited antibodies in infants is comparable to that of vaccinated and chronically infected adults.

Low-frequency Env-specific IgA in MF59/rgp120- and alum/rgp120-treated adult vaccinees. We previously reported that infants immunized with the MF59/rgp120 and alum/rgp120 vaccines rarely developed low levels of Env-specific IgA (18), a response that was associated with increased HIV acquisition risk in the RV144 vaccine trial (17). In the present study, we measured Env-specific IgA in adults immunized with the MF59- and alum-adjuvanted rgp120 vaccines (Table 4). None of the placebo recipients had a detectable response, but a small proportion of alum/rgp120 vaccine recipients had low levels of Env-reactive IgA prior to immunization. The frequency of IgA responders increased in the adult alum/rgp120 group postimmunization, with the highest frequency detected against B con gp140 (22%). Less than 10% of the alum/rgp120-vaccinated adults showed an IgA response against the autologous MNgp120 or against the C1 peptide or A1 con gp120, the two antigen-specific IgA responses most strongly associated with acquisition risk in the RV144 trial. None of the MF59/rgp120-vaccinated adults had detectable levels of Env-specific IgA before immunization, whereas 19% had a response against MNgp120 at peak immunogenicity. Overall, our results suggest that in contrast to infants in whom gp120 vaccine-elicited IgA is only rarely detected, adult immunization with MF59- and alum-adjuvanted rgp120 induces Env-specific IgA in a small proportion of adults.

DISCUSSION

Gaps in current ARV-based strategies to prevent MTCT and the residual risk of MTCT in women who receive ARV underline the need to develop easy-to-implement, safe, immune-based strategies to prevent infant HIV-1 acquisition, such as a pediatric HIV vaccine. To date, only a few HIV vaccine trials have included pediatric populations. We recently analyzed vaccine-elicited antibody responses in HIV-exposed HIV-vaccinated infants from the PACTG 230 vaccine trial and observed that infants immunized with a MF59/rgp120 vaccine developed robust Env-specific IgG and severalfold-more potentially protective V1V2-specific antibodies than did adults from the moderately protective RV144 vaccine trial (18). In the present study, we sought to determine whether the high-magnitude response in infants was due to differences in vaccine regimens or to intrinsic differences between adult and infant immune systems by comparing vaccine-elicited responses between infants from the PACTG 230 trial and adults from the AVEG 201 trial. In both studies, two different vaccines were administered: an alum-adjuvanted rgp120 (MN strain, VaxGen vaccine) and an MF59-adjuvanted rgp120 (SF-2 strain, Chiron vaccine). Interestingly, we observed a significantly higher magnitude response of gp120- and V1V2-specific IgG in infants vaccinated with the MF59-adjuvanted regimen compared to adults, whereas no differences were observed between the

alum-adjuvanted groups. Several factors could account for this difference in the levels of antibody between adults and infants, including distinct properties of adult and infant immune responses, the presence of maternally acquired antibodies in HIV-exposed infants, different vaccine intervals, and distinct effects of the vaccine adjuvant.

In PACTG 230, infants were immunized with 5, 15, or 50 μg of the MF59-adjuvanted Chiron vaccine or with 30, 100, and 300 μg of the alum-adjuvanted VaxGen vaccine (23), whereas in AVEG 201, adults were immunized with 50 μg of MF59/rgp120 or 600 μg of alum/rgp120 (22). To test whether the observed difference between adults and infants was due to the vaccine dose, we compared the levels of vaccine-elicited antibodies between infants immunized with different vaccine doses and adults (Fig. 3) and found that the level was higher in all groups of infants immunized with the MF59/rgp120 compared to adults immunized with the same vaccine, even among infants immunized with the same vaccine dose as the adults. It is important to note that at the equivalent vaccine dose, the dose-to-weight ratio is significantly higher in infants than in adults. Nevertheless, the fact that infants immunized with 1/10 of the adult dose still had a higher-magnitude response than did the adults strongly suggests that the difference observed between adults and infants is not due to the vaccine dose. Previous studies have indicated that longer intervals between vaccine doses can improve vaccine immunogenicity (28). Although distinct vaccine schedules were used in the adult and infant studies, it is unlikely that it fully explains the observed differences because: (i) the difference was observed only in the MF59-adjuvanted group, although the two infant groups were immunized with the same schedule, and (ii) the interval between the vaccine doses was shorter in infants than in adults. In fact, infants were immunized either at 0, 4, 12, and 20 weeks of life (regular schedule) or at 0, 2, 8, and 12 weeks of life (accelerated schedule). In contrast, adults were immunized at days 0, 28, 168, and 364. Thus, differences in vaccine dose and in the schedule of vaccine administration do not explain the higher-magnitude response of Env-specific antibodies observed in vaccinated infants.

At peak immunogenicity, the majority of HIV-exposed infants immunized with placebo had detectable levels of Env-specific antibodies, indicating that maternal antibodies acquired via the placenta were still present. Although we cannot rule out that these maternal antibodies contributed to the high level of Env-specific antibodies detected in vaccinated infants, it is worth noting that the levels of Env-specific IgG in vaccinees were significantly higher than in placebo recipients and remained higher in infants than in adults after the levels of maternal antibodies had waned (Fig. 2B). Importantly, although maternal antibodies are important to protect infants from pathogens until they are able to mount their own immune responses, these antibodies can also interfere with infant immune responses to vaccines (29, 30). In our previous analysis of PACTG 230 samples, we found no correlation between the levels of gp120- and V1V2-specific IgG at birth (maternally acquired IgG) and at week 52 (postvaccination infant IgG response), suggesting that maternal antibodies did not interfere with or enhance the infant response (18). Surprisingly, although most studies indicate that the presence of maternal antibodies inhibits infant responses, it has been reported that preexisting virus-specific antibodies enhances B cell responses in SHIV-infected infant rhesus macaques (31). The mechanism of this enhancement remains unclear, and whether preexisting maternal antibodies could trigger the same mechanism in the setting of vaccination is unknown.

The optimal adjuvant for inducing protective immune responses after HIV vaccination remains unknown. In the moderately effective RV144 vaccine trial, alum was used as an adjuvant; however, in the ongoing HVTN 702 vaccine trial, alum was replaced by the more immunogenic MF59 adjuvant. Nevertheless, the results from recent studies have challenged the hypothesis that MF59 is the optimal adjuvant for HIV immunization in adult populations. A pox prime protein boost MF59-adjuvanted vaccine did not induce robust or durable potentially protective anti-V1V2 IgG antibodies (20). Moreover, an RV144-like SIV vaccine was associated with protection when adjuvanted with alum but not with MF59 (32). Interestingly, studies in mice have shown that alum and MF59 stimulate distinct immunologic pathways and lead to different cytokine profiles

(33, 34). Moreover, in SIV-vaccinated monkeys, these two adjuvants differentially recruited the RAS pathway, which is important for B cell development and maturation. It is also possible that these two adjuvants elicit distinct glycoforms of antibodies since recent studies have demonstrated differences in antibody glycosylation following immunization with distinct vaccine regimens (35). Our finding that the MF59-adjuvanted vaccine is associated with enhanced Env-specific responses in infants but not in adults suggests that distinct adjuvants may be required to achieve robust protective immunity after HIV vaccination in adults and infants.

Our results strongly suggesting differences in vaccine-elicited antibody responses between adults and infants corroborate previous findings demonstrating differences in the pathogenesis and immune response to HIV infection between adults and children, such as possible differences in the development of antibody responses following adult and pediatric HIV infection (36–38). Overall, this body of data indicates that vaccine findings in adult populations may not predict infant responses, emphasizing the need to test promising HIV vaccine strategies in pediatric populations. It is important to keep in mind that a pediatric HIV vaccine could be important not only to protect from breast milk transmission but also could be boosted later in childhood to provide robust immunity prior to sexual debut. Recent epidemiologic surveys have identified young women living in high-HIV-incidence areas as a population at high risk of contracting the disease, and in this group HIV-1 acquisition is often contemporaneous with sexual debut (39, 40). Importantly, recent analysis of the coevolution of virus and Env-specific antibodies in individuals who developed broad neutralizing antibody responses (41–44) led to the hypothesis that the sequential administration of different Env immunogens over a prolonged period of time may be required for a vaccine to elicit broad neutralization (45). Initiation of such strategies in infancy will allow the required time for the immune response to mature and should be tested in future clinical trials.

In conclusion, the results from this study corroborate our previous observations that MF59-adjuvanted rgp120 vaccination uniquely elicits robust, long-lasting Env-specific responses in HIV-exposed infants and advocates for the need to test novel adjuvants and HIV vaccine constructs in pediatric populations.

MATERIALS AND METHODS

Study population. We included deidentified samples from two historic vaccine trials in which adults and infants were immunized with the same rgp120 protein vaccines. The Pediatric AIDS Clinical Trial Group Protocol 230 (PACTG 230) enrolled infants born to HIV-infected mothers between August 1993 and November 1996 in the United States to determine the safety and immunogenicity of recombinant, monomeric gp120 subunit protein vaccines (23). The trial began before the implementation of ARV prophylaxis for the prevention of MTCT, so 30% of the infants did not receive ARV. All other infants received zidovudine during the first 6 week of life. Infants were randomized to receive either Chiron rgp120 (SF-2 strain) with MF59 adjuvant or VaxGen rgp120 (MN strain) absorbed into aluminum hydroxide adjuvant (alum). Initial cohorts of infants were given escalating doses of vaccine within 72 h of birth and then at weeks 4, 12, and 20. Final cohorts received an optimum dose of vaccine at birth and at weeks 2, 8, and 20. AVEG 201 was a United States-based phase II trial to evaluate the immunogenicity and reactogenicity of SF-2 rgp120 in MF59 adjuvant (Chiron vaccine) and MN rgp120 in alum adjuvant (VaxGen vaccine) in four high-risk and two lower-risk adult populations (22). Participants included in this study were immunized at days 0, 28, 168, and 364. The total numbers of patients tested are reported in Table 1. Samples were included based on availability from the IMPAACT and HVTN repositories. The peak immunogenicity was determined empirically as the sample collection time point 2 to 4 weeks after the last immunization. In PACTG 230, this corresponded to week 24 (4 weeks after last vaccine dose), and in AVEG 201 this corresponded to visit 11 (2 weeks after last vaccine dose).

BAMA. The levels of HIV-1 Env-specific IgA and IgG were determined with a customized HIV-1 binding antibody multiplex assay (BAMA) as previously described (46, 47). Samples were tested at a 1:100 dilution for IgG, at a 1:40 sample dilution for IgG3, at 1:50 for the V1V2 breadth, and at a 1:10 dilution for IgA. These dilutions were predetermined to be within the linear range of the assay based on testing serial dilutions of a small subset of plasma samples. For IgG, the antigen panel included proteins 4403 BMC5 gp120 (23), gp70 B. CaseA_V1V2 (29) A244 gD-gp120 (15), B.con env03 gp140 (15), and MN gp120 gDneg (15 [provided by Hua-Xin Liao, Duke University]). For V1V2 breadth, the panel included gp70 B case V1V2, gp70 B case V1V2 169 K, gp70 B case V1V2 mut 3 (V169K/E172V/Y173H), AE A244 V1V2 tags, and gp70 B 1086c V1V2 (provided by Barton Haynes and Hua-Xin Liao, Duke University). The amino acid sequence of the V1V2 constructs tested and that of the vaccine strains is presented in Fig. 1. For IgA, the antigen panel included biotinylated linear peptide C1 (KKKMQEDVISLWDQSLKPCVKLTPLCV) and the proteins A1.con env03 gp140, B.con env03 gp140, and MN gp120 gDneg. Blank beads were used in all

assays to account for nonspecific binding. The positive control used was HIV immunoglobulin (HIV-IG; NIH AIDS Reagent Program), and the negative control was normal human serum (Sigma-Aldrich). The results were expressed as fluorescence intensity (FI). All assays included tracking of the HIVIG standard by Levey-Jennings charts. The IgG positivity cutoff was defined as the mean MFI plus three standard deviations of a panel of 15 negative-control samples, and preimmune samples were used to determine the IgA positivity cutoff. The preset assay criteria for sample reporting were coefficient of variation per duplicate values of ≤20% for each sample, and ≥100 beads were counted per sample.

Avidity assay. Enzyme-linked immunosorbent assay (ELISA) plates were coated with a clade B V2 peptide (Bio-V2.B), diluted to 3 μg/ml with 0.1 M $NaHCO_3$, and then incubated at 4°C overnight. The Bio.V2.B peptide sequence is similar to the B case 1 V1V2 sequence, except at one amino acid position, and differs from the SF2 and the MN V1V2 sequences at seven and six amino acid positions, respectively (Fig. 1). The plates were then washed once, blocked for 1 h with Superblock solution (1× PBS^+ [i.e., PBS plus 4% whey protein, 15% goat serum, and 0.5% Tween 20]), and then washed again. Adult and infant samples were diluted 1:100 with Superblock solution, added to ELISA wells in quadruplicate, and incubated at room temperature for 1 h. The plates were then washed twice, and 1× PBS^+ was added to half of the wells, with 7 M urea added to the other half, followed by a 5-min incubation at room temperature. A 3× wash was then performed, followed by the addition of 1:5,000 dilution of horseradish peroxidase-conjugated anti-human goat IgG (Jackson Immunoresearch) for 1 h at room temperature. After four washes, SureBlue Reserve TMB Microwell peroxidase substrate (VWR International) was added to each well for 7 min, followed by the addition of TMB stop solution, and the plates were read at 450 nm. Avidity indices were calculated according to the following formula: (mean OD in urea-treated wells/mean OD in PBS-treated wells) × 100. Avidity indices were only calculated for samples with OD values above the positivity cutoff, which was defined as 3× the average OD of the blank wells. CH58, a V2-specific monoclonal antibody isolated from an RV144 vaccinee, was used as a control in this assay.

Statistical analysis. To evaluate immune responses in the placebo groups, we combined the two placebo arms in AVEG 201 and the two placebo arms in PACTG 230. Peak immunogenicity (2 to 4 weeks after the 4th dose) analyses were performed using visit 11 (day 378) for AVEG 201 and week 24 for PACTG 230, whereas durability analyses were performed using visit 14 (day 546) for AVEG 201 and week 52 for PACTG 230. Two-sample comparisons of the immune response magnitude were performed using the Wilcoxon-Mann-Whitney test. Comparisons of responder frequencies were performed using the Fisher exact test. All *P* values were two-tailed. Half-lives are computed by fitting a linear model on the log-fluorescence-intensity [log(FI)] scale. Subjects with higher than half of the peak readout at the durability time point were eliminated from the analysis. Kaplan-Meier curves are used to estimate survival curves, and log rank tests were used to determine two-sample survival curves comparisons testing the null hypothesis that the distributions of the half-lives were the same between two groups. Rank-based correlation coefficients were used to compare half-lives between the different antigens and to assess the association between half-life and peak magnitude. All statistical procedures were implemented using the R language and environment for statistical computing and graphics.

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