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MAJOR ARTICLE

Frequent Development of Broadly Neutralizing Antibodies in Early Life in a Large Cohort of Children With Human Immunodeficiency Virus

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Background. Recent studies have indicated that broadly neutralizing antibodies (bnAbs) in children may develop earlier after human immunodeficiency virus (HIV) infection compared to adults.

Methods. We evaluated plasma from 212 antiretroviral therapy-naive children with HIV (1–3 years old). Neutralization breadth and potency was assessed using a panel of 10 viruses and compared to adults with chronic HIV. The magnitude, epitope specificity, and immunoglobulin (Ig)G subclass distribution of Env-specific antibodies were assessed using a binding antibody multiplex assay.

Results. One-year-old children demonstrated neutralization breadth comparable to chronically infected adults, whereas 2- and 3-year-olds exhibited significantly greater neutralization breadth ($P = .014$). Likewise, binding antibody responses increased with age, with levels in 2- and 3-year-old children comparable to adults. Overall, there was no significant difference in antibody specificities or IgG subclass distribution between the pediatric and adult cohorts. It is interesting to note that the neutralization activity was mapped to a single epitope (CD4 binding site, V2 or V3 glycans) in only 5 of 38 pediatric broadly neutralizing samples, which suggests that most children may develop a polyclonal neutralization response.

Conclusions. These results contribute to a growing body of evidence suggesting that initiating HIV immunization early in life may present advantages for the development of broadly neutralizing antibody responses.

Keywords. antibodies; broad neutralization; pediatric HIV.

In 2019, 460 000 human immunodeficiency virus (HIV) infections occurred in young adults aged 15–24 [1]; and adolescents represent the only age group with increased numbers of acquired immune deficiency syndrome (AIDS)-related deaths over the last decade [2]. A vaccine that can be administered before sexual debut to protect during the vulnerable window of adolescence and generate lifelong immunity is therefore a priority for the field. Such a vaccine will probably need to induce broadly neutralizing antibodies (bnAbs) because their protective role has been established in animal models [3]. However, traditional vaccine approaches have failed to induce bNAbs [4]. Furthermore, only a subset of adults with HIV develops bnAbs after several years of infection [5], and these bnAbs frequently demonstrated unusual traits such as high levels of somatic hypermutation (SHM), nucleotide insertions and deletions, long complementary determinant region 3 (CDR3) lengths, and restricted variable gene use [6].

Recent studies have indicated that children may develop neutralization breadth earlier [7] or more frequently [8] than adults. Epitope mapping of neutralizing antibodies (NAbs) in children suggested that bnAbs from children and adults target the same key epitopes, but plasma neutralization in children seems to be polyclonal [9], whereas in adults it is typically attributed to a single or a few distinct epitope specificities [10]. Despite these recent findings, our knowledge of the development of Env-specific Ab responses in pediatric settings remains incomplete. Although several studies have investigated the ontogeny and immunoglobulin (Ig)G subclass distribution of HIV-specific Abs in adults with HIV [11], few studies have been conducted in young children [12]. Further characterization of Env-specific binding responses and IgG subclass distribution in children might guide the design of vaccines targeting the early life period.

Previous studies investigating neutralization breadth development in children have limitations such as small cohort size [7] or focus on nonprogressor children [8]. To define the frequency and kinetics of bnAb development in children, we acquired plasma samples from 212 antiretroviral therapy (ART)-naive children infected either in utero or at the time of

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Table 1. Cohort Clinical Summary[a]

	Adult Cohort	Pediatric Cohort		
		1-year-old	2-year-old	3-year-old
Clinical characteristics	n = 117	n = 91	n = 62	n = 59
Clade Infected	Multiple clade-infected (with subset of 44 clade B-infected)	Clade B-infected		
Duration of infection	Chronically infected	Infected in utero or at birth		
ART exposure	ART-naive	ART-naive		

Abbreviations: ACTG, AIDS Clinical Trials Group; ART, antiretroviral therapy.

[a]Pediatric cohort samples were provided by completed IMPAACT Studies ACTG 152, 300, 382, and 390. Adult clade B-infected plasma samples were provided by the Neutralization Serotype Discovery Project.

delivery from the International Maternal, Pediatric, Adolescent AIDS Clinical Trials (IMPAACT) repository and from 44 ART-naive HIV chronically infected adults from the Neutralization Serotype Discovery Project (NSDP) study [13]. We compared the magnitude, specificity, and IgG subclass distribution of HIV-1 Env-specific Abs between children and adults. In addition, we compared neutralizing antibody responses in children and adults using a global panel of HIV-1 strains [14], and we defined the epitope specificity of pediatric neutralizing antibodies. To our knowledge, this study represents the most comprehensive analysis of HIV neutralizing antibody responses in a large cohort of young children conducted to date.

MATERIALS AND METHODS

Samples

Plasma samples from ART-naive children (n = 212) who were enrolled in the completed studies AIDS Clinical Trials Group (ACTG) 152, 300, 382, and 390 [15–18] were obtained from the IMPAACT biospecimen repository. These children were assumed to be infected with clade B HIV-1 at birth or in utero because they were born to women living in the United States. Adult plasma samples (n = 44) were obtained from ART-naive adults with chronic clade B HIV-1 infection who participated in the Neutralization Serotype Discovery Project [13]. A summary of the clinical characteristics of the study populations is provided in Table 1.

Binding Antibody Multiplex Assay for the Measurement of Env-Specific Immunoglobulin G

A previously described binding antibody multiplex assay (BAMA) [19] was used to measure IgG binding to a panel of 17 HIV-1 antigens (Supplemental Table 1). Based on pilot experiments, a dilution of 1:100 was used for all antigens except for gp140s, Bio-V3B, and recMNgp41 that were tested at 1:2000. Antigen-specific IgG was detected with a mouse antihuman IgG phycoerythrin-conjugated Ab (Southern Biotech) at 2 μg/mL. Human immunodeficiency virus-1 human hyperimmune immunoglobulin (HIVIG) was used as positive control, and normal human serum was used as negative control. Binding was measured using a Bio-Plex 200 instrument (Bio-Rad Laboratories, Inc.). Immunoglobulin G responses were expressed as mean fluorescence intensity (MFI) and were blank bead subtracted, except for gp70_B.CaseA_1V2 and gp70 MNV3, which were subtracted by the MFI of beads coupled with a control gp70 construct (MuLVgp70). A response was considered positive if the MFI was above a positivity cutoff determined as the highest of either (1) the mean plus 3 standard deviations MFI of a panel of 20–30 HIV-negative plasma or (2) the lower detection limit of 100 MFI. To ensure consistency between assays, 50% effective concentration and maximum MFI values of HIVIG control were tracked by Levey-Jennings charts [20]. Env-specific IgG subclass antibodies were measured using a modified BAMA as previously described [21].

Neutralization Assays

Neutralization was measured in TZM-bl cells as previously described [22] against a panel of 10 viruses from the global neutralization panel ([14]) (Table 2). Results were reported as the 50% inhibitory dilution (ID_{50}), which is the dilution of plasma resulting in 50% reduction in luminescence compared to that of virus control wells. To map neutralizing epitopes, a panel of 6-11 HIV-1 Env epitope knockout pseudoviruses (strains BJOX002, 25710, or TRO.11) was used (Supplemental Table 4). Panel choice was dependent on which parent virus was most potently neutralized by the plasma sample.

Table 2. Frequency of Neutralization Response in Adults vs Children[a]

Virus	Clade	Adults (%)	Children (%)	*P* Value
25710	C	91	90	
TRO11	B	68	78	*P* = .048
X2278	B	75	86	*P* = .015
BJOX2000	CRF07_BC	60	55	
X1632	G	63	28	*P* < .001
CE1176	C	62	51	
246F3	AC	48	71	*P* < .001
CH119	CRF07_BC	67	88	*P* < .001
CE0217	C	46	40	
CNE55	CRF01_AE	34	41	

[a]Proportion of adult and pediatric samples with 50% inhibitory dilution >100 for each virus. The pediatric cohort demonstrated comparable or superior neutralization frequency to adults in 9 of 10 viruses tested. The adult cohort had higher neutralization frequency against only 1 virus, X1632. Statistically significant *P* values included as determined by Fisher test.

Statistical Analysis

All statistical analyses were performed in the R statistical computing and graphics environment. We used magnitude-breadth (MB) curves [23] to summarize the neutralization activities of a subject across viruses. An MB curve is a cumulative distribution function that represents the proportion of viruses neutralized with potency no less than x for every point along the x-axis. To determine the neutralization score, we computed the area under the MB curve, which equals to the average potency across a set of viruses. For 2-sample tests, we used Mann-Whitney rank-based tests. All *P* values are 2-sided. The Benjamini and Hochberg [24] approach was used to adjust for multitesting. Positive response rates were compared between groups by Fisher's exact tests [25]. Median regression was conducted using the quantreg package [26]. Titers of NAbs among responders were compared between groups by 95% confidence intervals (CIs) about the ratio of geometric mean titers. Equality of the overall distribution of $\log_{10}$ NAb titers between 2 groups was tested as described [27], using 10 000 permutated data sets to compute a *P* value. The false discovery rate (FDR) was used to determine tests that remained statistically significant after adjustment for the multiple hypothesis tests. The FDR method was performed at level 0.05.

RESULTS

Human Immunodeficiency Virus-1 Env-Specific Antibody Binding Responses in the Pediatric Cohort in Comparison to Adults

A panel of 17 HIV-1 antigens was used to assess the breadth and epitope-specificity of Env-specific IgG in children (n = 212) and adults (n = 44) with HIV. More than 90% of children and adults had antibodies that bind to the majority of the Env glycoproteins (Supplemental Table 1). Overall, the magnitude and breadth of Env-specific antibodies were comparable between children and adults, although children had higher levels of gp41-specific antibodies (Figure 1). It is interesting to note that the magnitude of IgG binding against most antigens increased from 1 to 2 years of age, but it was comparable between 2- and 3-year-old children (Supplemental Figure 1), suggesting that Env-specific antibody levels progressively increase during the first 2 years of life.

Env-specific antibodies from adults and children generally bound to the same epitopes, with only slight differences observed between the 2 groups (Supplemental Table 2): children had higher IgG levels against the constant region 1 ([C1] adjusted $P < .001$) and the membrane-proximal external *region* (MPER), but the difference in the magnitude of MPER-specific antibody responses was not significant after adjustment for multiple comparisons. Adults had higher levels of antibodies against the constant region 5 ([C5]

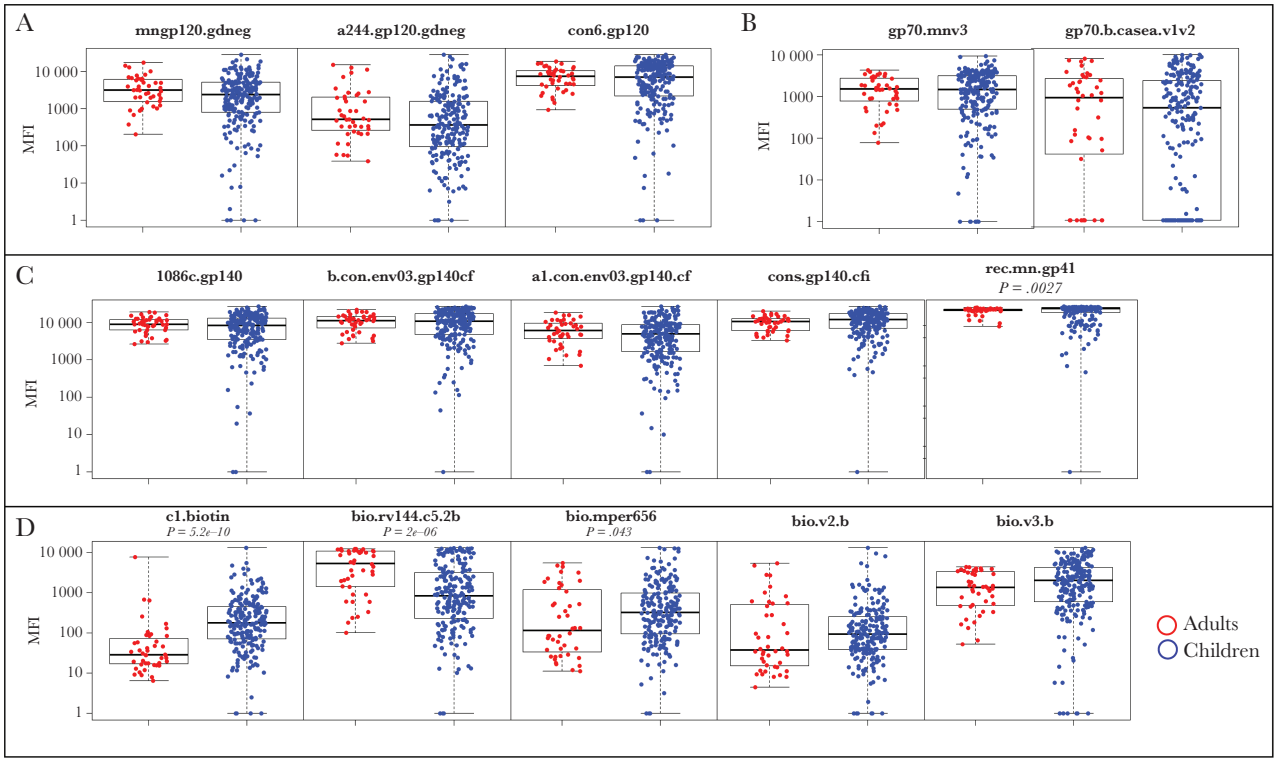

Figure 1. Human immunodeficiency virus (HIV)-1 Env-specific total immunoglobulin (Ig)G. Total IgG for select HIV-1 Env epitopes were measured by binding antibody multiplex assay in adult and pediatric cohorts. Epitopes include gp120 (a), variable loops (b), gp140/gp41 (c), and peptides (d). Significant difference between adult and pediatric cohorts by Wilcoxon as noted.

adjusted $P < .001$). Likewise, a higher percentage of children had detectable MPER-specific (79% vs 61%, $P = .017$) and C1-specific IgG (58% vs 11%, $P < .001$), whereas the frequency of C5-specific antibodies was higher in adults (77% vs 42%, $P < .001$).

Human Immunodeficiency Virus (HIV)-1 Env-Specific Immunoglobulin G Subclass Distribution in Adults and Children With HIV

We then assessed the subclass distribution of Env-specific IgG. Children tended to have lower magnitude gp120-specific IgG1 responses compared to adults ($P = .042$), but this difference was not significant after adjustment for multiple comparisons. Both adults and children had very high magnitude of gp41-specific IgG1 (Figure 2). Children also had lower magnitude gp41-specific IgG3 (adjusted $P = .030$) and lower levels of Env-specific IgG4 than adults (adjusted $P < .001$ for gp140, gp120, and gp41). In contrast, children had higher magnitude gp41-specific IgG2 antibodies (adjusted $P = .011$). Similar to total IgG, the levels of gp120-specific IgG1 increased with age, whereas low levels of other IgG subclass were observed across the age groups (Supplemental Figure 2).

Although the majority of adults and children had detectable levels of Env-specific IgG1, slight differences in the frequency of the other subclasses were observed between adults and children (Supplemental Table 3). Most adults and children had IgG3 and IgG4 against gp41, but a higher proportion of children had gp41-specific IgG2 (87% vs 48%, $P < .001$). In contrast, gp120-specific IgG4 were detected more frequently in adults than in children (48% vs 22%, $P = .002$). Approximately half of the adults and children had gp120-specific IgG3 and approximately 20% had gp120-specific IgG2. The proportion of adults and infants with detectable levels of IgG subclass antibodies against the variable loop 2 (V2) and the variable loop 3 (V3) was comparable.

Human Immunodeficiency Virus (HIV) Neutralizing Antibody Responses in Children With HIV Compared to Adults

The ability of pediatric samples to mediate broad neutralization was assessed against a panel of 10 HIV-1 pseudoviruses from the global neutralization panel [14] and compared with previously reported data of 117 chronically infected adults from the NSDP [13]. Overall, 69% of children and 68% of adults neutralized at least 50% of the viruses in the panel. Broad neutralization frequency slightly increased with age, because only 60% of 1-year old children but 76% of 2-year-old children were able to neutralize 50% of the viruses. Using an ID_{50} of 100 to define robust neutralization, we observed that 26% of children but only 19% of adults neutralized 50% of the viruses with an ID_{50} ≥100. The percentage of children with robust neutralization was comparable across the age groups (23% for 1-year-old, 29%

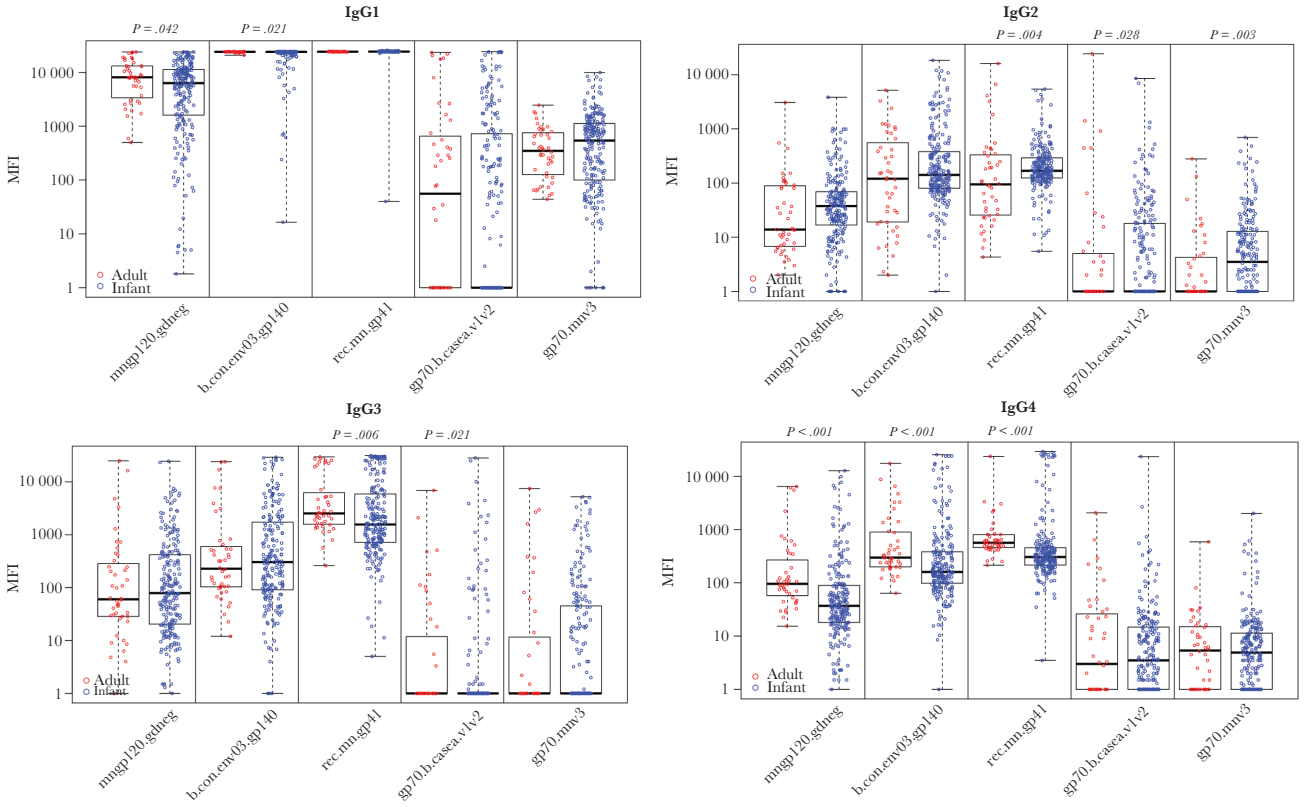

Figure 2. Human immunodeficiency virus (HIV)-1 Env-specific immunoglobulin (Ig)G subclass. Individual IgG subclasses for select HIV-1 Env epitopes were measured by binding antibody multiplex assay in adult and pediatric cohorts. Significant difference between adult and pediatric cohorts by Wilcoxon as noted.

for 2- and 3-year-olds). There was no statistical difference in the percentage of children and adults that neutralized 5 of the 10 tested viruses (Table 2), whereas a higher percentage of children than adults were able to neutralize 4 viruses (HIV TRO11; HIV X2278; HIV 246F3; HIV CH119). A higher percentage of adults than children neutralized HIV X1632. Children also demonstrated comparable or superior neutralization potency (Figure 3). The median neutralization titer was higher in children than in adults for 4 viruses tested, whereas adult samples demonstrated higher neutralization potency against one virus.

Neutralization scores were generated as the area under the magnitude-breath curve for all tested viruses as previously described [23]. Overall, the neutralization score was higher in children than in adults ($P = .014$) (Figure 4a). As with other antibody measurements, the neutralization score increased with age in children ($P = .014$) (Figure 4b), but, by 1 year of age, the plasma neutralization activity in perinatally infected children is comparable to that of chronically infected adults ($P = .44$) (Figure 4c).

Because the neutralization score is influenced both by the proportion of the viruses neutralized and the potency with which these viruses are neutralized, the relative contribution of these factors was examined using magnitude-breadth curves (Figure 4d). The average ID_{50} of the pediatric cohort was significantly greater than that of adults with a similar proportion of viruses neutralized, indicating that the higher neutralization score observed in children is mostly driven by a superior neutralization potency.

Association Between Neutralization Breadth and Clinical Factors in Children With Human Immunodeficiency Virus

We performed linear regression analyses to define associations between neutralization and patient $CD4^+$ T-cell percentage and absolute counts (Supplemental Figure 4). We found a modest positive association between neutralization breadth score and $CD4^+$ T-cell percentage (slope = 0.01, $P = .002$) as well as with $CD4^+$ T-cell counts (slope = 0.07, $P = .003$). However, no significant association was observed between viral load and neutralization breadth (slope 0.10, $P = .28$), although this analysis was limited by the small number of children with available viral load (n = 15). The impact of other clinical factors such as the timing of infection (intrauterine versus perinatal) could not be evaluated due to the paucity of information in the database. Nevertheless, our results suggest that factors other than $CD4^+$ T cells likely contribute to drive neutralization breadth development in children.

Epitope Specificity of Neutralizing Antibodies in Children With Human Immunodeficiency Virus

The epitope specificity of neutralizing antibodies was mapped in pediatric samples that neutralized ≥5 viruses with an ID_{50} ≥100 (n = 38). Neutralization assays were performed against a virus from the global panel that the plasma sample neutralized potently (either HIV TRO11, 25710, or BJOX002) and against a panel HIV-1 pseudoviruses with selective mutations to abrogate the neutralization potency of a specific class of bnAbs [28] (Supplemental Table 4). In addition, the samples were tested against mutant pseudovirus TRO11.W672A to assess the presence of MPER-specific antibodies. A total of 5 of 38 plasma samples demonstrated at least a 3-fold reduction in ID_{50} against one mutant compared to the parent virus (Table 3). In 3 samples neutralization was mediated by antibodies against V3 glycan-epitope, whereas in 1 sample it was mediated by antibodies against the CD4 binding site, and the last one showed neutralization activity against V2 glycan-dependent epitope.

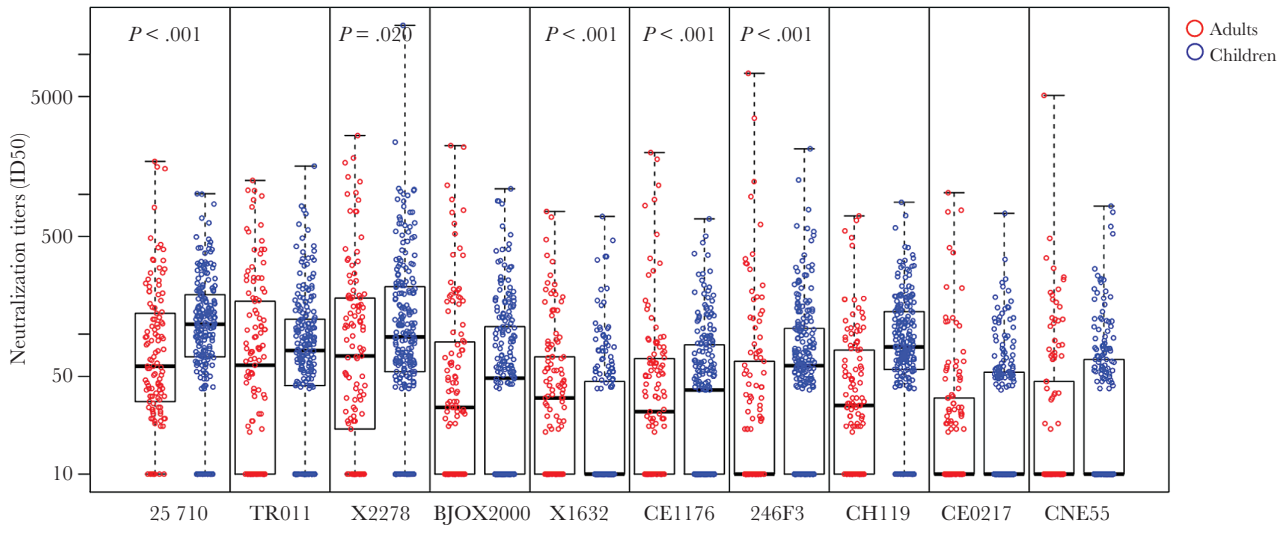

Figure 3. Neutralization potency in adults versus. children. The neutralization titers of the pediatric samples were calculated for each virus and compared with data from the historical adult cohort. Children demonstrated higher neutralization potency against 4 of 10 viruses, and adults demonstrated higher neutralization potency against 1 of 10 viruses. Statistical significance determined by Wilcoxon test as noted. ID50, 50% inhibitory dilution.

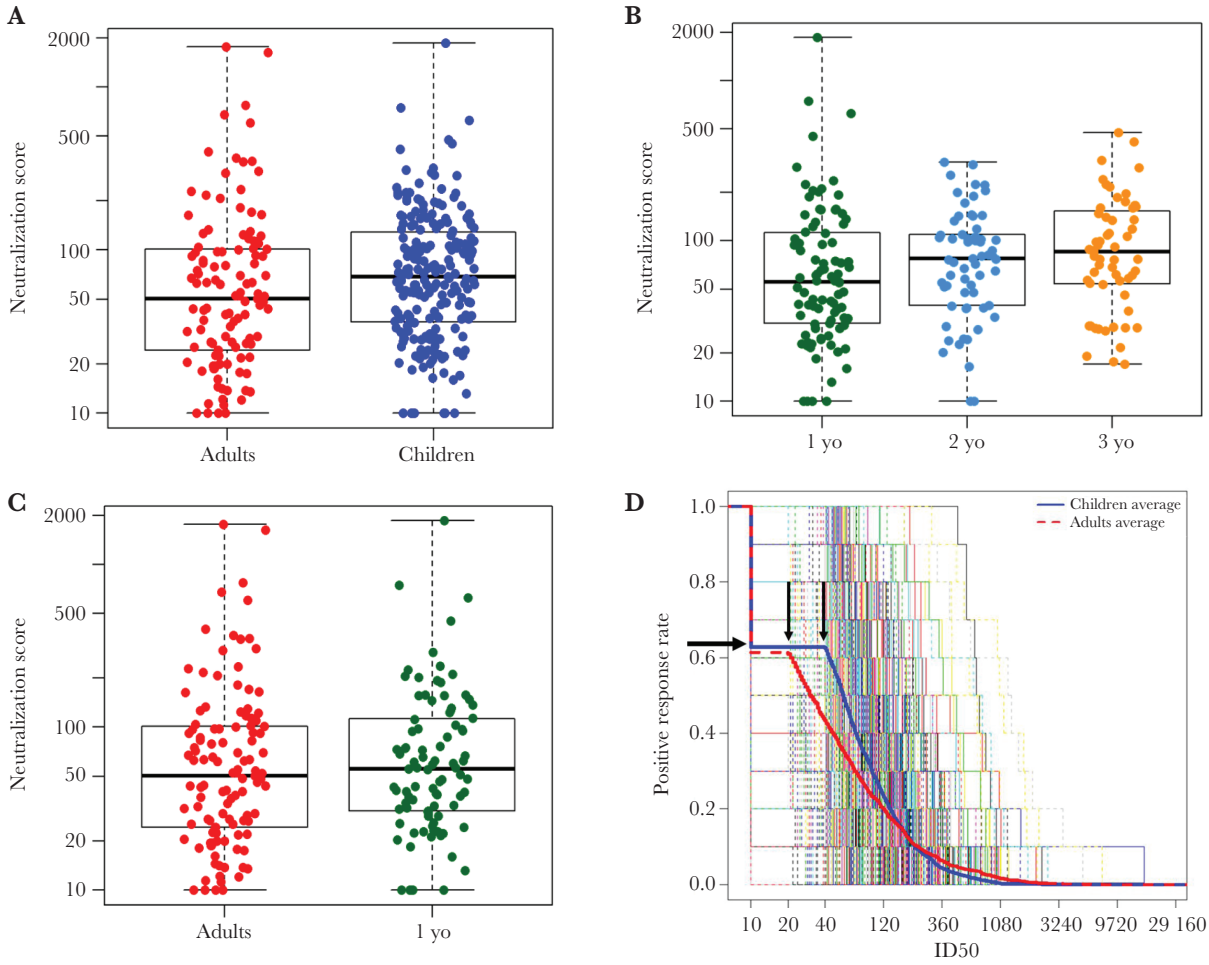

Figure 4. Neutralization score in adults versus children. Neutralization score was determined as the area under the curve of the neutralization curve for all tested viruses (a). Overall, the neutralization score was greater in children than in chronically infected adults ($P = .014$, Wilcoxon) (b). Neutralization score increased with age ($P = .014$, median regression analysis) (c). The neutralization score of the 1-year-old (yo) cohort was comparable to that of adults ($P = .44$, Wilcoxon) (d). Magnitude-breadth curve comparing adults versus children. The average 50% inhibitory dilution (ID50) value for the pediatric cohort was significantly greater than that of the adult cohort ($P = .013$, Mann-Whitney *U*) as indicated by the vertical arrows, whereas a similar proportion of viruses were neutralized in the 2 cohorts as indicated by the horizontal arrow.

Association Between Binding Antibody Responses and Neutralization Epitope Specificity

We then examined associations between neutralization and binding antibody responses (Supplemental Table 4) and observed a marginally statistically significant correlation between total gp120 and gp140 IgG and neutralization score. Moreover, gp140 IgG4 and gp41 IgG4 levels were weakly associated with neutralization score.

We also examined the association between the levels of epitope-specific binding antibodies and the neutralization specificity. Child 2 (from Table 3) in which neutralization specificity mapped to a V2-glycan epitope demonstrated moderately high V1V2-specific IgG and IgG1 responses ranking at the 65th and 56th percentile. No V1V2-specific IgG2, IgG3, or IgG4 was detected in this child (Supplemental Figure 5). The 3 children (3–5) in which neutralization specificity mapped to V3 glycan dependent epitope demonstrated high V3-specific IgG binding ranking at the 75th, 85th, and 83rd percentile. They also demonstrated high V3-specific IgG1 ranking at the 61st, 79th, and 75th percentile for the IgG1 distribution, whereas the levels of the other IgG subclass were variable. Thus, although high levels of V1V2- or V3-specific IgG or IgG1 antibodies are not indicative of V2- or V3-specific neutralizing antibody responses, when children exhibited neutralizing antibodies to a particular site they tended to have high binding antibodies to the same site.

DISCUSSION

Recent reports indicating (1) that HIV vaccination can elicit robust and durable antibody responses in infants [29, 30] and (2) that children may develop broad neutralization more frequently and earlier than adults [9] suggest that the early life immune system could present advantages to achieve neutralization breadth through vaccination. Only a few studies have assessed neutralizing antibody responses in children. Goo et al [7] investigated neutralization in a small cohort (n = 28) of clade

Table 3. Epitope Specificity Of Broadly Neutralizing Pediatric Samples

			Epitope Targeted by Mutant											
			CD4 bs	CD4 bs	CD4 bs	CD4 bs	2G12 Sensitive	V3 Glycan	V2 Glycan	gp120-gp41 (35022)	gp120-gp41 (35022)	gp120-gp41 (PGT151)	Putative Epitope Specificity
Child ID	Age	WT ID50	N279A	N280D	G458Y	N276Q	N295V	N332A	N160K	N88A	N625A	N611A	
1	1	331	1550[a]	2531[a]	2428[a]	<50[b]	NA	476[a]	474[a]	628[a]	745[a]	NA	CD4 bs
2	1	1268	2111[a]	3516[a]	2108[a]	754[c]	1100[c]	1083[c]	370[b]	1444[a]	928[c]	923[c]	V2 glycan
3	2	415	NA	NA	NA	NA	171[d]	80[b]	362[c]	322[c]	338[c]	328[c]	V3 glycan
4	3	471	1486[a]	5411[a]	938[a]	196[d]	167[d]	120[b]	605[a]	397[c]	260[c]	427[c]	V3 glycan
5	3	890	1571[a]	4097[a]	1223[a]	503[c]	1954[a]	115[b]	1098[a]	1420[a]	677[c]	972[a]	V3 glycan

Abbreviations: bs, binding site; ID_{50}, 50% inhibitory dilution; NA, not applicable; PTD, protein transduction domain; WT, wild type.

NOTE: Neutralization of mutant variants in the 5 pediatric samples that neutralized ≥5 of the 10-virus global panel with an ID_{50} ≥100 and demonstrated at least a 3-fold reduction in ID_{50} against 1 mutant pseudovirus.

[a] <1-fold decrease.

[b] >3.00-fold decrease.

[c] 1.0- to 1.99-fold decrease.

[d] 2.00- to 2.99-fold decrease.

A-infected children from Kenya and reported that broad neutralization could be detected in infants as early as 1 year after infection. Subsequently, Muenchhoff et al [8] reported higher frequency of broad neutralization in South African children aged >5 years compared to chronically infected adults. Our study focused on a large cohort of children with HIV aged 1 to 3 to specifically define the kinetics of the development of broad neutralization in children, because adults with HIV usually develop neutralization breath after several years of infection [31]. This age range excluded the possibility that passive maternal antibodies contributed to the measured neutralization because before 1 year of age, it would be difficult to decipher the contribution of maternal versus infant antibodies. The uniqueness of these historical pediatric ART-naive samples is worth noting because the World Health Organization now recommends initiation of ART at the time of diagnosis.

In contrast to the findings from Muenchhoff et al [8], who reported more frequent bnAb development in children than in adults (75% vs 19%), we found similar proportions of children and adults who neutralized 50% of the viruses (69% vs 68%). Comparable frequencies were still observed when we only considered children and adults who neutralize 50% of viruses with ID_{50} >100 (26% vs 19%, $P = .13$). Difference in age between the 2 cohorts could have contributed to these divergent results. Although we did not test longitudinal samples, we found that neutralization scores increased with age (Figure 4). Makhdoomi et al [32] have also reported that the neutralization breadth in children with HIV from India aged 5 to 17 years increased over time. Furthermore, our cohort was United States-based (predominant clade B infections), whereas Muenchhoff et al [8] studied South African children (predominant clade C infections). The South African cohort also focused on the specific subpopulation of nonprogressors [8], and the virus panels used to assess neutralization breadth in the 2 studies were different. Finally, it is possible that differences in transmission modes contributed to the slightly divergent results [33]. In our US-based cohort, transmission occurred in utero and perinatally, whereas breast milk transmission is also an important mode of transmission in South Africa. Despite these differences, it is worth noting that our results combined with those of previous studies establish that children are able to rapidly develop broad neutralizing antibody responses after HIV infection.

Previous studies indicated that increased levels of HIV-specific IgG2 and IgG4 during early HIV infection in adults correlate with development of broad neutralization [34] and that high levels of IgG3 are associated with neutralization [35]. We therefore investigated whether a distinct IgG subclass profile in children contributed to explain the early development of broad neutralization. Overall, the IgG subclass profiles of the pediatric and adult cohorts were grossly similar with only a few statistically significant differences. Most notably, the majority of children mounted a detectable gp41-specific

IgG2 response, whereas this response was seen in less than half of chronically infected adults. The clinical significance of this difference is unclear because there was no association between this response and neutralization breadth. The ability of children to produce IgG2 is usually delayed compared to other subclasses [36], thus the observation that young children develop more robust IgG2 responses than adults is somewhat surprising. Nevertheless, because low levels of gp41-specific IgG2 are usually associated to later stage disease [37], the higher levels of gp41-specific IgG2 in children may simply be a marker of more recent infections. We were also interested to define whether higher HIV-specific IgG3 were observed in children based on the recent report that IgG3 enhances the neutralization activity of an HIV V2-specific bnAb [16]. For most antigens, there was no difference in the magnitude of IgG3 responses between adults and children, except for gp41 and V1V2, which were higher in adults than in children. Thus, overall, our results suggest that IgG subclass distribution may not contribute to the early development of broad neutralization in children.

In adults, broad neutralization is frequently mediated by a single or a few antibody specificities [38, 39]. Doria-Rose et al [40] used a fingerprinting computational approach to define the epitope specificity of 143 donors with neutralization breadth >30%, and they reported that the neutralization antibodies mapped to known or potential new specificities in 53% of the donors. Our studies and others suggest that polyclonal responses may occur more frequently in children. It is notable that Goo et al [7] reported that the plasma neutralization in 7 children with broad neutralization could not be mapped to known dominant neutralization epitopes. Moreover, in a cohort of 16 clade C-infected nonprogressor children from South Africa, Ditse et al [9] observed that the majority of children had antibodies targeting multiple known neutralization epitopes. In contrast, Mishra et al [27] reported that only 2 of 10 infant elite neutralizers demonstrated polyclonal responses. Although it is possible that our selection of mutant pseudoviruses failed to identify antibodies targeting a known epitope—becauseas the most common bnAb targets [9] were represented in our panel—our results likely indicate that breadth was mediated either by a polyclonal response or that these pediatric nAbs target novel neutralization epitopes. In adults, it has been proposed that the development of bnAbs results from cooperation between multiple B- cell lineages, which selects for escape mutants and allows the continual stimulation of the bnAb lineage [6]. Our findings may suggest that the abundance of naive B cells in children allows for activation of multiple B-cell lineages upon HIV infection leading to a polyclonal response. Thus, plasma broad neutralization in children could be mediated by the additive activity of a collection of antibodies with limited to moderate neutralization.

CONCLUSIONS

Elucidating the mechanisms through which children achieve broad neutralization will be critical to guide vaccine development. More importantly, analysis of 2 V3-glycan-dependent bnAbs isolated from children indicated that breadth was acquired through a pathway distinct from that of adult antibodies from the same class [41, 42] with notably lower levels of somatic hypermutation and early accumulation of critical improbable mutations [43]. Identifying and characterizing more Abs from children is imperative to determine whether low levels of SHM is a consistent feature of pediatric nAbs. This knowledge will ultimately guide HIV vaccine strategies aiming at inducing broad neutralization with the goal of achieving protective immunity before sexual debut.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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