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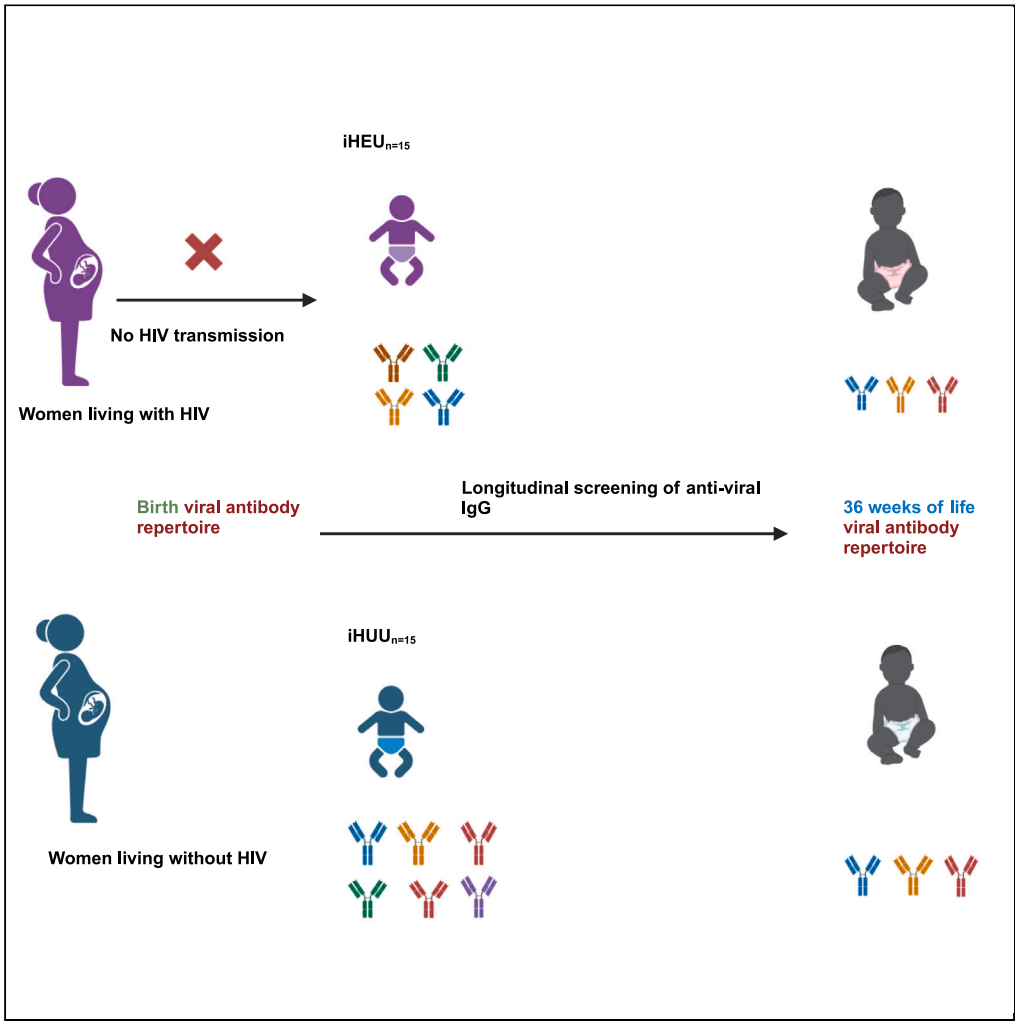

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Highlights

Antibodies against herpesviruses dominated infants' anti-viral repertoire at birth

iHEU had reduced anti-viral IgG birth repertoire compared to iHUU

Enteroviral IgG dominated at 36 weeks following loss of herpesviral antibodies

Similar anti-viral IgG repertoire breadth and diversity in both groups at 36 weeks

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Reduced anti-viral IgG repertoire in HIV-exposed but uninfected infants compared to HIV-unexposed infants

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SUMMARY

Infants who are HIV exposed but uninfected (iHEU) have higher risk of viral infections compared to infants who are HIV unexposed (iHUU). We explored the effect of intrauterine HIV exposure on the infant antibody repertoire by quantifying plasma immunoglobulin (Ig) G against 206 eukaryote-infecting viruses using phage immunoprecipitation sequencing (PhiPSeq) in iHEU and iHUU at birth and 36 weeks of life. Maternal HIV infection altered the infant IgG repertoire against eukaryote-infecting viruses at birth, resulting in significantly lower antibody breadth and diversity among iHEU compared to iHUU. Neonatal anti-viral IgG repertoire was dominated by antibodies against viruses belonging to the Herpesviridae family, although, by 36 weeks, this had shifted toward antibodies against enteroviruses, likely due to waning of maternal-derived antibodies and polio vaccine-induced antibody responses as expected. The observed reduced anti-viral IgG repertoire breadth and diversity acquired at birth in iHEU might contribute to the increased rates of viral infections among iHEU during early life.

INTRODUCTION

Universal access to antiretroviral treatment (ART) has drastically reduced perinatal transmission of HIV from 25%–40% in the early 2000s to a global rate of less than 7% in the option B+ era.[1,2] However, the prevalence of HIV among women of childbearing age remains high, resulting in an increasing population of iHEU.[2] Sub-Saharan Africa carries the highest number of these infants, having an estimated population of 13.2 million children born to women living with HIV.[3] Since iHEU have higher risk of poor health outcomes marked by increased risk of gastrointestinal tract and lower and upper respiratory diseases compared to iHUU, it is important to determine immunological mechanisms contributing to these outcomes.[4–6]

The etiological agents of infections among iHEU are often viral, such as human respiratory syncytial virus (RSV). RSV has been reported to cause higher rates of hospitalization with prolonged hospital stays and mortality in iHEU compared to iHUU.[7] Multiple factors potentially explaining these observed health outcome disparities between iHUU and iHEU have been suggested, including long-term immunological differences which are possibly a result of *in utero* and postnatal immune activation, although the exact mechanism leading to these alterations is not clearly established.[8,9] Evidence of immunological differences in iHEU includes impaired T cell functionality characterized by a state of chronic activation and lower production of pro-inflammatory cytokines following stimulation with vaccine and polyclonal antigens.[10–13] Moreover, altered innate immune responses, such as lower expression of perforin and interferon-gamma (IFN-γ) in natural killer (NK) cells which are pivotal in controlling and fighting viral infections, have been reported.[14]

Passive immunity derived from maternal antibodies is crucial in supplementing immune responses during early infancy. Maternal HIV infection has been demonstrated to negatively impact transplacental antibody transfer to newborns.[15,16] However, other studies that investigated whether maternal HIV infection impairs humoral immunity in iHEU have reported contradicting findings, suggesting that iHEU have similar or higher antibody responses to vaccines compared to iHUU.[17–19] One of the shortcomings of these studies is the limited number of antigens investigated, mostly excluding antibodies to non-vaccine antigens. However, since the current early-childhood vaccines do not target most common viral pathogens causing disease in iHEU, a comprehensive analysis of anti-viral antibodies is needed.

We therefore characterized the anti-viral immunoglobulin (Ig) G repertoire in iHEU and iHUU at birth and 36 weeks of life using VirScan, a pan-analysis method for human anti-viral antibodies.[20] Here, we show that the anti-viral IgG repertoire breadth and diversity differ between iHEU and iHUU at birth with dominance of viral antibodies against herpesviruses. However, at 36 weeks of life, infant anti-viral antibody

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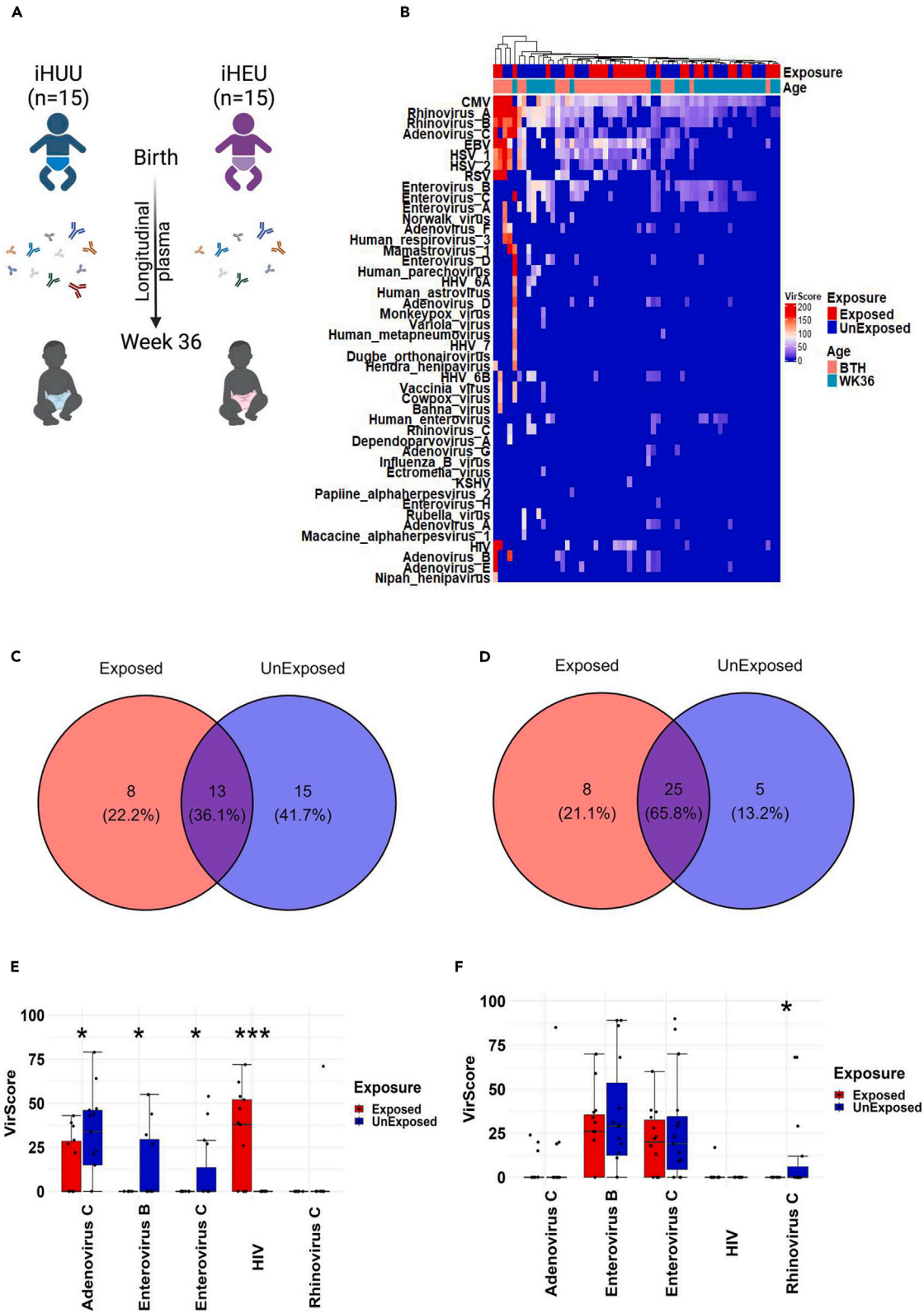
A
iHUU
(n=15)
iHEU
(n=15)
Birth
Longitudinal plasma
Week 36
B
Exposure
Age
CMV
Rhinovirus_A
Rhinovirus_B
Adenovirus_C
EBV
HSV_1
HSV_2
RSV
Enterovirus_B
Enterovirus_C
Enterovirus_A
Norwalk_virus
Adenovirus_F
Human_respirovirus_3
Mamastrovirus_1
Enterovirus_D
Human_parechovirus
HHV_6A
Human_astrovirus
Adenovirus_D
Monkeypox_virus
Variola_virus
Human_metapneumovirus
HHV_7
Dugbe_orthonairovirus
Hendra_henipavirus
HHV_6B
Vaccinia_virus
Cowpox_virus
Bahna_virus
Human_enterovirus
Rhinovirus_C
Dependoparvovirus_A
Adenovirus_G
Influenza_B_virus
Ectromelia_virus
KSHV
Papiine_alphaherpesvirus_2
Enterovirus_H
Rubella_virus
Adenovirus_A
Macacine_alphaherpesvirus_1
HIV
Adenovirus_B
Adenovirus_E
Nipah_henipavirus
VirScore
200
150
100
50
0
Exposure
Exposed
UnExposed
Age
BTH
WK36
C
Exposed
UnExposed
8
(22.2%)
13
(36.1%)
15
(41.7%)
D
Exposed
UnExposed
8
(21.1%)
25
(65.8%)
5
(13.2%)
E
VirScore
100
75
50
25
0
*
*
*
***
Adenovirus C
Enterovirus B
Enterovirus C
HIV
Rhinovirus C
Exposure
Exposed
UnExposed
F
VirScore
100
75
50
25
0
*
Adenovirus C
Enterovirus B
Enterovirus C
HIV
Rhinovirus C
Exposure
Exposed
UnExposed

Figure 1. Antibodies against herpesviruses and enteroviruses dominated birth and 36-weeks infants' viral antibody repertoire, respectively

(A) Paired infant plasma samples were collected at birth and 36 weeks of life.

(B) Heatmap of forty-six viral antibodies detected in iHEU and iHUU at birth (BTH) and 36 weeks of life.

(C and D) Anti-viral antibody specificities detected in iHEU and iHUU at birth (C) and 36 weeks of life (D).

(E and F) Boxplots of VirScores that differed significantly between iHEU and iHUU at birth (E) and 36 weeks of life (F), with 1 VirScore as limit of detection. *** (p value < 0.001). ** (p value <0.01). * (p value < 0.05). BTH (Birth). WK36 (36 weeks of life). iHEU (red) (n = 15). iHUU(blue) (n = 15).

repertoire was mainly composed of antibodies against enteroviruses without differences in viral antibody repertoire breadth and diversity between HIV-exposure groups.

RESULTS

Cohort characteristics

We analyzed and compared anti-viral IgG repertoire between iHEU (n = 15) and iHUU (n = 15) at birth and 36 wereks of life. There were no differences in infant sex and birth weight between groups; although all infants were born at term (≥37 weeks gestation), the median gestational age at delivery for iHUU (39.9 weeks) was higher than for iHEU (38.8 weeks, p = 0.041; Table S1). No major differences were observed in the reported administration of early-childhood vaccines. Mothers of iHEU were older compared to iHUU (30.0 vs. 25.6 years, p = 0.034) and had a greater number of previous pregnancies (p = 0.055). All mothers living with HIV were virally suppressed (<1,000 copies/mm^3) with a mean CD4 count of 584 cells/mm^3. The reported duration of breastfeeding was similar between mothers with and without HIV (p = 0.435; Table S2).

Lower anti-viral IgG breadth and diversity in iHEU compared to iHUU

We first explored the impact of maternal HIV infection on infant anti-viral IgG repertoires cross-sectionally by infant age (Figure 1A). In the 30 infant plasma samples analyzed at birth, we detected distinct antibodies against 36 out of 206 viral species contained in the library. The most frequently detected antibodies across all infants at birth were herpesvirus 4, commonly known as Epstein-Barr virus (EBV: n = 30, 100%); herpesvirus 5, also known as cytomegalovirus (CMV: n = 28; 93.3%); herpes simplex virus 1 (HSV-1: n = 27; 90%); rhinovirus A (n = 23; 76.7%); herpes simplex virus 2 (HSV-2: n = 22; 73.3%); and adenovirus C (n = 19; 63.3%) (Figure 1B and Table S3). Among iHEU, anti-HIV IgG was detected in 10 (66.7%) infants at birth, while, as expected, none of the iHUU had detectable antibodies against HIV. Specificities of IgG to 21 viruses were identified in iHEU, whereas iHUU had IgG specificities to 28 viruses, with an overlap of specificities to 13 viruses between the groups (p = 0.129, Figure 1C). When antibody detection was parsed between iHEU vs. iHUU at birth, the most frequently targeted viruses in iHEU were EBV (n = 15, 100%), CMV (n = 13, 86.7%), HSV-1 (n = 12, 80%), HSV-2 (n = 11, 73.3%), HIV (n = 10, 66.7%), and rhinovirus A (n = 9, 60%). In iHUU, antibodies against CMV, EBV, and HSV-1 were detected in 100% (n = 15) of the infants. Thereafter, anti-rhinovirus A (n = 14, 93.3%), adenovirus C (n = 12, 80%), HSV-2 (n = 11, 73.3%), and rhinovirus B (n = 9, 60%) were the most common (Figure 1B). The proportion of infants with detectable antibodies against enterovirus B was lower in iHEU (0/15, 0%) compared to iHUU (5/15; 33.33%, p = 0.042) at birth; however, following multiple comparisons correction the differences were not statistically significant (adjusted p value [p.adj] = 0.892) (Table S3). IgG measured at 36 weeks in all infants targeted 38 unique viruses. In the iHEU, 33 unique viruses were targeted, compared to 30 in iHUU, with an overlap of 25 viral specificities (p = 0.542, Figure 1D, Table S4).

VirScan is a semi-quantitative assay, and the computed VirScores have been previously shown to correlate well with antibody titers measured by ELISA.[21] We therefore compared anti-viral specific IgG VirScores between iHEU and iHUU. Using this approach, iHUU had higher VirScores (a surrogate for antibody levels) against enterovirus B (p = 0.018) and C (p = 0.038) as well as human adenovirus C (p = 0.048) at birth compared to iHEU. However, these differences were not statistically significant when adjusted for multiple comparisons (Figures 1E and S1). As expected, iHEU had higher anti-HIV antibodies compared to iHUU (p=<0.001, p.adj = 0.001; Figures 1E and S1). We observed higher levels of anti-rhinovirus C IgG in iHUU when compared to iHEU at 36 weeks, albeit not significant after adjusting for multiple comparisons (p = 0.038 Figures 1F and S2). Specifically, anti-rhinovirus IgG were not detected in any of the iHEU samples, while seropositivity among iHUU was 6.7% at birth and increased to 26.7% at 36 weeks of life (Tables S3 and S4). The analysis revealed an overall RSV seropositivity rate of 36.7% (11/30) at birth and 6.7% (2/30) at 36 weeks of life (Figure 1B, Tables S3 and S4). RSV has been implicated in the increased morbidity and mortality in iHEU.[7] However, the proportion of participants with antibodies against RSV and corresponding anti-RSV antibody levels did not differ between iHEU and iHUU at birth and 36 weeks of life.

High-dimensionality reduction analysis using non-metric multi-dimensional scaling (nMDS) of Bray-Curtis distances was employed to compare differences in anti-viral antibody repertoire between iHEU and iHUU using the VirScores of the targeted viruses for each infant at birth and 36 weeks of age. Overall, infant anti-viral IgG repertoire clustered by age (permutational multivariate analysis of variance [PERMANOVA] p = 0.001; Figure 2A), with 25.6% of the variation in the viral antibody repertoires attributable to age. Within the birth samples, nMDS revealed distinct clustering between iHEU and iHUU (p = 0.001; Figure 2A) with HIV exposure explaining 11.7% difference, while at 36 weeks no significant clustering was observed (R^2 = 2.7%; p = 0.598).

We further compared anti-viral IgG repertoires between the infant samples by measuring Shannon diversity of the VirScores. At birth, the diversity of the IgG repertoire of iHEU was significantly lower compared to that of iHUU (p = 0.015; Figure 2B). This lower IgG repertoire in iHEU translated to an overall median seropositivity count of 6 unique targeted viruses per infant compared to median seropositivity count of 9 unique targeted viruses per infant among iHUU at birth (p = 0.020; Figure 2C). Although the diversity (Figure 2B) and count (Figure 2C) of the repertoire of infant IgG generally decreased with infant age, these differences were not statistically significant between time points. When the

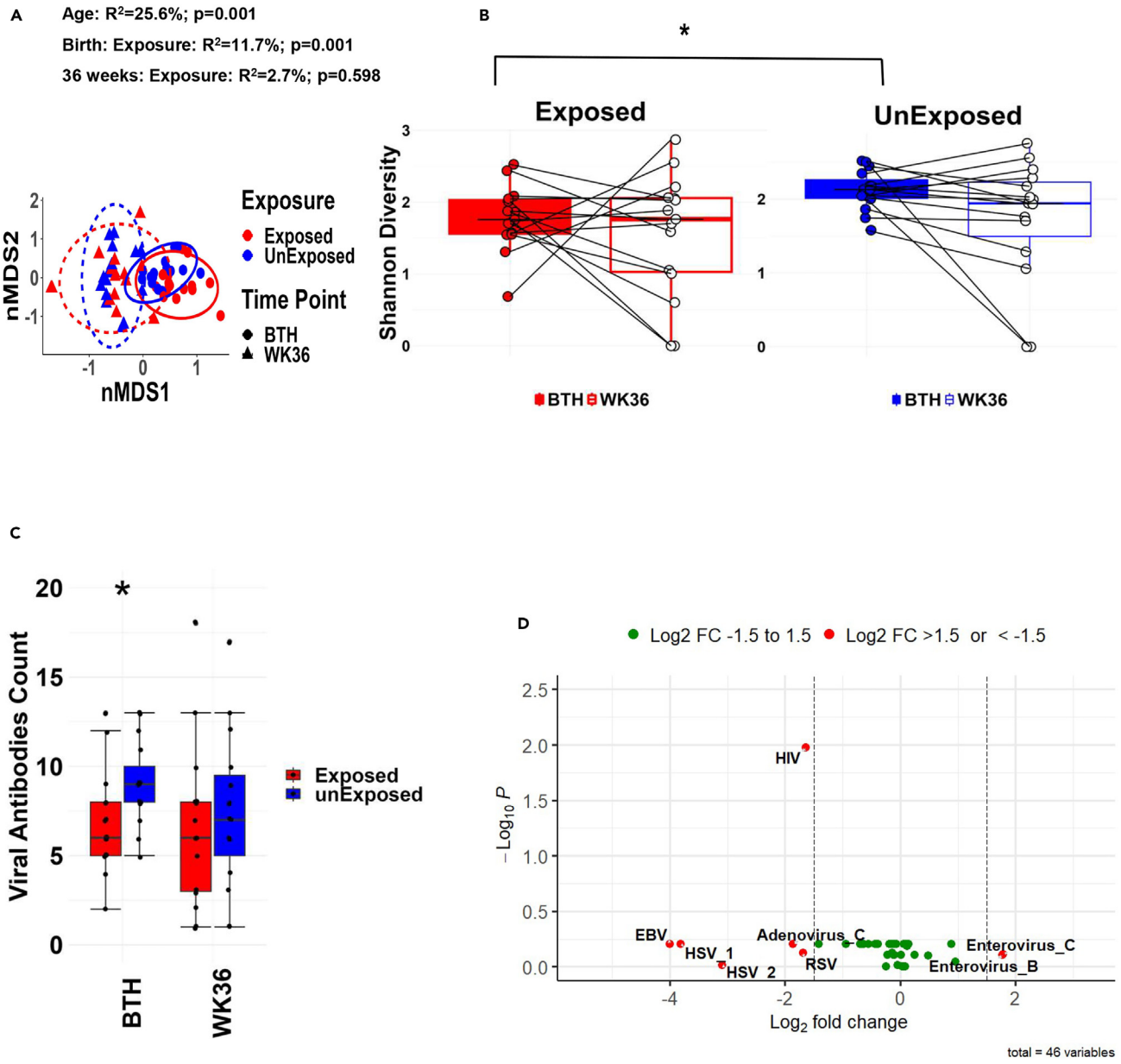

Figure 2. iHEU had decreased viral antibody repertoire diversity and breadth at birth compared to iHUU

(A) HIV exposure- and age-associated clustering of non-metric multi-dimensional scaling (nMDS)—Bray-Curtis dissimilarity distances of viral antibodies in iHEU and iHUU at birth (BTH) and 36 weeks of life (WK36).

(B) Shannon diversity of iHEU and iHUU at birth and 36 weeks of life.

(C) Average number of unique viral antibodies detected at birth and 36 weeks of life in iHEU and iHUU per participant.

(D) Log-2-fold change of viral antibodies levels detected at birth and 36 weeks of life. BTH (Birth). WK36 (36 weeks of life). Circle (birth), Triangle (weeks 36 of life). iHEU (red) (n = 15). iHUU (blue) (n = 15). *** (p value < 0.001). ** (p value <0.01). * (p value < 0.05).

diversities in the two groups were compared longitudinally using generalized estimating equations (GEE), on average iHUU had a 0.241 higher (95% confidence interval [CI] −0.081–0.563, p = 0.142) Shannon diversity index compared to iHEU, although this did not reach statistical significance. No differences in diversity and seropositivity count were observed at 36 weeks between iHEU and iHUU (Figures 2B and 2C).

Loss of IgG against herpesviruses and emergence of anti-enteroviral antibodies between birth and week 36 of life

We next investigated the temporal changes in antibody levels by calculating mean fold change (MFC) between VirScores measured at 36 weeks of age and birth. Overall, independent of HIV exposure status, the highest decrease in IgG VirScores was observed for antibodies

targeting herpesviruses, including EBV (MFC = −4.00), HSV-1 (−3.81), HSV-2 (−3.09), and CMV (−1.41), as well as adenovirus C (−1.86), RSV (−1.69), and HIV (−1.64). In contrast, there was an increase in antibodies against enteroviruses, including enterovirus A (MFC = 0.95), B (1.77), and C (i.e., poliovirus, 1.78) and human enteroviruses without assigned species level annotation (0.88) (Figure 2D). Of the 10 iHEU that had detectable anti-HIV IgG at birth, only one retained detectable antibodies to HIV at 36 weeks of age. The difference in MFC against all viruses, however, was not statistically significant between iHEU and iHUU (Figure 2D).

DISCUSSION

In this study, we investigated the effect of maternal HIV infection on anti-viral antibody repertoires during the first nine months of life. The altered anti-viral antibody repertoire in iHEU at birth was characterized by lower anti-viral antibody diversity and breadth, which could be one of the contributing factors to the increased risk of viral infections in iHEU compared to iHUU during the first year of life.[22,23] Anti-viral antibody repertoire at birth was dominated by antibodies against viruses belonging to the Herpesviridae family, which decreased with advancing infant age as antibodies against enteroviruses emerged. Consistent with our findings, Pou and colleagues also reported that birth plasma of iHUU was dominated by antibodies targeting herpesviruses 1, 2, 4, and 5; rhinovirus A; and adenovirus C; however, they did not evaluate later time points.[21]

Although we did not measure transplacental antibody transfer in this study, antibodies measured at birth were presumed to be maternally derived.[24,25] This was corroborated by the observation that anti-HIV-specific IgG were only detected in iHEU, further validating the specificity of the VirScan assay as previously described elsewhere.[20] Women living with HIV (WLH) have been observed to have poor efficiency in transplacental antibody transfer compared to women living without HIV.[16,26] The implicated mechanisms responsible for reduced transplacental antibody transfer efficiency in WLH include HIV-associated hypergammaglobulinemia resulting in high concentrations of non-specific Ig being produced, reduced antigen-specific antibodies levels, altered glycosylation patterns of antigen-specific antibodies, and neonatal fragment crystallizable receptor (FcRn) affinity.[15,16,27–29] In our study, we observed reduced anti-viral antibody repertoire in iHEU at birth, which was characterized by lower levels of antibodies to enterovirus C and B as well as adenovirus C compared to iHUU. Other studies have shown increased incidence of enterovirus- and adenovirus-associated lower respiratory tract infections (LRTIs) in iHEU compared to iHUU.[7] Therefore, whether the low levels of antibodies to these viruses at birth are associated with the increased incidence of enteroviruses and adenovirus in iHEU in the first 6 months of life should be investigated further. Other studies have also demonstrated altered antigen-specific antibody levels in iHEU arising from perturbed humoral immunity in WLH. These altered antibody levels translated to higher antibodies against herpesvirus and lower antibodies against childhood vaccines including mumps and polio vaccines in cord blood of iHEU compared to iHUU.[15,30] Inefficient transplacental antibody transfer against specific antigens such as RSV, measles, rotavirus, and hepatitis B in WLH has been reported.[16,26] Therefore, although our study is limited by the absence of paired maternal antibody data, the differences observed in anti-viral IgG repertoire at birth between iHEU and iHUU are most likely to be driven by either altered anti-viral antibody levels in WLH and/or impaired transplacental antibody transfer efficiency. These findings warrant further investigations to determine the mechanisms associated with altered anti-viral IgG repertoire and its association with increased risk of viral infections reported among iHEU.

As part of the Expanded Program on Immunization (EPI), infants receive childhood vaccines including polio, hepatitis B, rotavirus, and measles as viral antigens. Apart from the measles vaccine, most infants would have received their primary immunization within the first 36 weeks of life, resulting in >95% seropositivity.[17,31] In this study, however, we did not detect antibodies against some of these viral vaccine antigens. A possible explanation for this outcome could be due to the limitation of the VirScan assay in discriminating antibodies targeting closely related viral species having homologous proteins used in the assay.[20,32] For instance, in our study, 70% of the infants at 36 weeks of life had antibodies against enterovirus C species, which consist of multiple subtypes including polioviruses. Antibodies targeting enterovirus C increased with infant age, suggesting seroconversion following poliovirus immunization. We did not detect HIV antibodies in one-third of the iHEU at birth, which could be explained by either low HIV antibodies in maternal blood or failure of the VirScan assay to detect these antibodies in infant plasma. Previously, Xu and colleagues also reported decreased sensitivity of the VirScan assay when detecting antibodies at lower concentration and those against viruses with smaller genomes as well as conformational epitopes.[20] This reduced sensitivity of the VirScan assay could account for the lack of seropositivity against hepatitis B and rotaviruses in the current study despite infants receiving Hepatitis B and rotavirus vaccines. Antibodies against CMV, EBV, HSV-1, and HSV-2 dominated anti-viral IgG repertoire at birth, with more than 70% of infants harboring these specificities. This highlighted the ubiquitous presence of CMV, EBV, HSV-1, and HSV-2 infections in adult population in Africa as previously reported.[33,34] It is also worth noting that CMV, EBV, HSV-1, and HSV-2 are large-genome viruses, and that would increase detection probability as they contain more epitopes when compared to other viral targets contained in the VirScan library. Therefore, the increased breadth of epitopes could also explain their dominance of the birth anti-viral IgG repertoire. Previously when Xu et al. validated VirScan assay with known HSV-1- and HSV-2-positive samples, confirmed by ELISA, the VirScan achieved greater than 90% sensitivities and specificities for antibodies against these pathogens.[20] Therefore, we are certain that, in the current study, the analysis was able to differentiate between different herpesviruses particularly HSV-1 and HSV-2. However, careful considerations should be adopted when employing VirScan for serologically profiling viruses associated with risk to infection.

The decay of maternally derived antibodies during infancy before the maturation of infant B cells poses a risk to infection early in life.[35,36] Similarly, suboptimal B cell response to infections and/or vaccines could also contribute to the burden of viral disease among iHEU. Indeed, abnormalities in the B cell compartment for iHEU have been documented, including an increased proportion of exhausted B cells and altered B cell homeostasis potentially affecting the antigen-specific antibody production.[30,37] In the current study, we noted waning for most of the antibodies detected at birth by 36 weeks, with a significant decline observed for antibodies targeting EBV, HSV-1 and HSV-2, and RSV. This

decay of viral antibodies detected from birth to 36 weeks suggests a lack of exposure to these viral pathogens in this cohort. However, we did not perform more analyses to ascertain the incidence of viral infections. There were no differences in the longitudinal change of viral antibodies levels between the two groups, implying that iHEU generated comparable antibody responses to iHUU, as previously demonstrated.[17,18] Anti-rhinovirus C IgG was, however, lower in iHEU compared to iHUU at 36 weeks. Previously iHEU have been shown to have increased incidence of rhinovirus-associated LRTI compared to iHUU. Even though we did not find differences in infectious morbidity and mortality between iHEU and iHUU in our current study cohort,[38] the decreased levels of antibodies against rhinovirus C could indicate impaired antibody immunity in iHEU, which could subsequently increase susceptibility to RTI. It could also represent differences in exposures between the two groups. RSV remains the main contributor of LRTI in <6months infants, and the incidences of RSV-associated LRTI have been shown to be higher in iHEU, partially contributing to the 12-fold increase in deaths compared to iHUU.[7] Our study however did not observe differences in the proportion of participants harboring RSV antibodies and corresponding levels between iHEU and iHUU at birth and 36 weeks. These findings deviate from other studies that reported decreased transplacental transfer of anti-RSV IgG in iHEU compared to iHUU which could be attributed to the semi-quantitative nature of VirScan assay used in the current study.[26,39] It was also difficult to ascertain if the similarities in anti-RSV IgG between the two groups at 36 weeks of life was due to dissimilarities in viral exposure rates or comparable antibody responses following RSV exposure, although the latter is more likely given that the infants were from the same geographical location. Future studies would need to investigate the association between viral exposure and antibody responses in infants and the relation to maternal HIV infection.

Limitations of the study

Limitations in our study included a small sample size for a comparison of differences between iHEU and iHUU thus limiting the power of detecting differences between the two groups when multiple comparisons were considered. A large sample size would therefore be required to corroborate the findings observed in this study while controlling for confounding variables including gestational age, birth weight, sex, and vaccine uptake. When the baseline characteristics were compared between iHEU and iHUU, only the gestational age was significantly different between the two groups; however, all infants included in the study were delivered at term, thus attenuating the potential bias of decreased antibody transfer associated with preterm delivery. Similarly, while the EPI vaccines coverage was <100% in both groups, it is worth noting that it did not differ between groups; therefore differences in IgG levels observed in the current study could not be explained by differences in vaccine uptake. Although the VirScan affords high-throughput measure of antibodies in small samples volumes, the technology is limited in detecting antibodies at very low concentrations especially against viruses with small genomes and those with cross-reactive epitopes.[20] The sensitivity of the assay is further limited by the measurement of linear epitopes only, therefore underestimating the levels of IgG against viruses that present conformational epitopes. Moreover, VirScan assay has also demonstrated decreased specificity to detecting antibodies against closely related viruses such as enteroviruses and adenovirus, thus limiting our ability to refine our detection up to species level in the case of antibodies against polioviruses. Lastly the VirScan assay only detects the IgG isotype; thus other isotypes including IgM, which is produced during acute infections, and IgA, a predominant isotype in isotype in mucosal immunity, would be missed. However, we were able to demonstrate that maternal HIV infection significantly alters the anti-viral IgG repertoire breadth and diversity in neonates. Although the mechanism for this is unclear, it likely reflects impairment of the IgG repertoire in their mothers,[15,21,40] as well as reduced transplacental antibody transfer.[17,18] Since we also observed a broad decay of anti-viral antibodies during infancy, it is important to determine how HIV exposure influences adaptive immunity to pathogens following infection, and whether this could explain the health outcome disparities observed in iHEU compared to the iHUU counterpart.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
  - Participants characteristics
  - Ethics statement
- METHOD DETAILS
  - Whole blood collection
  - Laboratory analysis
  - Data analysis
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.110282.

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AUTHOR CONTRIBUTIONS

R.G., S.D., and H.J. conceptualized the study. R.G. performed the data analysis. S.D. and H.J. provided project supervision. A.-U.H., B.A., C.M.G., and H.J. oversaw the project administration. R.G. drafted the original manuscript. S.D., H.J., C.M.G., A.-U.H., and B.A. reviewed and edited the manuscript for publication.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
T7Select 10-3b DNA	Millipore	70548
Biological samples		
Plasma	This study	NA
Chemicals, peptides, and recombinant proteins		
Dynabeads Protein A	Invitrogen	10002D
Dynabeads Protein G	Invitrogen	10004D
NaHCO3	Sigma-Aldrich	S5761
Na2CO3	Sigma-Aldrich	223530
Phosphate Buffered Saline	Thermo Fisher Scientific	10010023
Fetal Bovine Serum	Corning	35-011-CV
NaCl	Sigma-Aldrich	S7653
Tris-HCL	Sigma-Aldrich	RES3098T-B7
NP-40	Sigma-Aldrich	492016
Tween 20	Sigma-Aldrich	P1379
dNTPs	Applied Biosystems	N8080261
Agarose	Sigma-Aldrich	9012-36-6
1 kb Plus DNA ladder	Thermo Fisher Scientific	10787018
Critical commercial assays		
T7Select Packaging Kit	Millipore	70014–3
NucleoSpin Gel and PCR Clean-up	Macherey–Nagel	740609.50
KAPA Library Quantification Kit	KAPA Biosystems	KK4828
DNA Clean & Concentrator Kit	Zymo Research	D4005
Herculase II polymerase	Agilent	600679
Deposited data		
Raw fastq data	NCBI SRA database: PRJNA1091874	
Oligonucleotides		
Primer for Phip-Seq	Mohan et al., 2018[41]	Table 1 and Table S1 of Mohan et al.,2018
Software and algorithms		
Python		https://www.python.org/
Pepysn	Mohan. et al. 2018[41]	https://github.com/lasersonlab/pepsyn
Bowtie	Langmead and Salzberg, 2012[42]	http://bowtie-bio.sourceforge.net/index.shtml
Samtools	Li. et al. 2009[43]	https://github.com/samtools
edgeR	Robinson. et al. 2009[44]	https://doi.org/10.18129/B9.bioc.edgeR
AVARDA	Monaco. et al. 2022[32]	https://github.com/drmonaco/AVARDA
R		https://www.r-project.org/

RESOURCE AVAILABILITY

Lead contact

Further requests and enquires regarding resources and reagents should be directed to and will be fulfilled by the lead contact Sonwabile Dzanibe (s.dzanibe@uct.ac.za).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Raw sequence reads generated have been deposited at the NCBI SRA database and are publicly available as of the date of publication. The accession numbers are listed in the key resources table.
- This paper does not report any original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Participants characteristics

We selected a convenient sample of 15 iHEU and 15 iHUU (Figure 1A, Tables S1 and S2) who were enrolled between 2014 and 2019 within an ongoing prospective cohort study previously described.[38] The selected individuals were based on the availability of the plasma in the biorepository. Briefly, pregnant women ≥18 years living with or without HIV were enrolled, together with their respective newborn infants. To rule out *in utero* and/or perinatal HIV transmission, HIV-DNA PCR tests were performed for all infants at 6 weeks of age and repeated at 9 months of age, or 6 weeks after breast-feeding cessation. Infants testing HIV DNA PCR positive were excluded from the study and referred for care. This study analysis was limited to infants delivered at ≥37 weeks completed gestational age with a birth weight of ≥2.4 kg. Additionally, only infants delivered vaginally after an uncomplicated pregnancy or labor were included. All mothers living with HIV and their infants were provided with standard of care regimens according to South African (SA) guidelines, including administration of antiretrovirals for pregnant mothers as well as anti-viral and cotrimoxazole prophylaxis for their respective babies.[45,46] All infants received routine vaccinations following the SA EPI vaccination guidelines.[47,48] Since this study was conducted within the public sector of South Africa few if not none of the women included in this study received maternal vaccines such as tetanus, pertussis or influenzae administered during pregnancy.

Ethics statement

The study was granted ethical approval by the University of Cape Town Human Research Ethics Committee (UCT-HREC) under project numbers 285/2012 and 613/2022.

METHOD DETAILS

Whole blood collection

Blood was collected from infants at birth and 36 weeks of life in heparinized tubes and plasma isolated within 6 h after collection and stored at −80°C until analysis.

Laboratory analysis

Anti-viral antibodies were measured using PhiPSeq by CDI LABS (Baltimore, Maryland,USA) as previously described.[41] This utilizes a bacteriophage library displaying linear viral peptides created from protein sequence of eukaryotic-viruses infecting humans available on the Uniprot database.[49] Viral-specific IgG levels in plasma samples were quantified by mixing 1 mL of library aliquot at a concentration of 1 × 10^10 plaque-forming units (pfu) with 2 μg of diluted plasma. Eight beads-only "mock" controls were included to correct for non-specific binding. Each participant sample underwent a single test. All IgG-phage complexes were immunoprecipitated using protein A/G affinity beads. The precipitates were amplified in a 20 cycle PCR reaction using Herculase II Fusion Polymerase kit (Agilent, CA, USA) followed by addition of sample-specific indices and Illumina P5/P7 adapters. Libraries were pooled for sequencing on Illumina NextSeq to generate 50 cycles of single end reads.

Data analysis

Sequencing reads were mapped to the original library sequences using Bowtie[42] and read counts for each peptide per sample was calculated using SAMtools.[43] The R software package edgeR version 3.18[44] was used to compare the reads in each sample against the "mock" (beads only) immunoprecipitations using a negative binomial model to obtain test statistic and fold-change value for each peptide. Peptides (positive hits) were deemed significantly enriched if they contained hit counts, *p*-values, and fold changes of at least 15, 0.001, and 5, respectively as previously described.[32] Enriched hits for each peptide were used to calculate the Virus Score for each virus targeted in the sample using the Anti-viral Antibody Response Deconvolution Algorithm (AVARDA) pipeline.[32] Briefly, peptide hits were aligned to a database containing all the known eukaryote-infecting viral genomes translated in all six reading frames to establish enriched peptide-virus relationships. A sequence homology-based network was created for each virus' enriched peptide with required alignment E values <100 between all VirScan peptides contained in the sample. This network was used to define the response breadth (minimum number of independent specificities) for each virus, herein referred to as VirScore. Finally, using a null model, each peptide was assigned to its most likely associated viral genome. The limit of detection for each viral antibody was 1 VirScore.

QUANTIFICATION AND STATISTICAL ANALYSIS

R software (version 4.0.2, R Core Team, Vienna, Austria) was used to perform statistical inferences and generate associated graphs. Viral antibody repertoire diversity was assessed using Shannon indices as well as Bray-Curtis distance matrices with 999 permutations in the R package vegan version 2.6–4.[50] Non-metric multi-dimensional scaling (nMDS) was performed to visualize the infant IgG viral repertoires. Permutational multivariate analysis of variance (PERMANOVA) was applied to determine significant dissimilarities between infant groups. The model included timepoint of sample collection, HIV exposure as well as the random effect for each participant. Wilcoxon signed-rank tests for unpaired data were used to compare antibody levels repertoire between the two groups. False discover rate (FDR) was used to correct for multiple comparisons. Fisher's exact method was used to test the role of HIV exposure on the presence or absence of viral antibodies. Generalized estimating equations (GEE) were used to compare diversity of the anti-viral antibody repertoires between iHEU and iHUU longitudinally. Significance level for all analyses were considered at p-value <0.05.