Differences in Evolution of HIV-1 Subtype C Reverse Transcriptase Between Children and Adults Likely Explained by Maturity of Cytotoxic T-Lymphocyte Responses

Shanmugam Saravanan,¹ Selvamurthi Gomathi,¹ Bagavathi Kausalya,¹ Jayaseelan Boobalan,¹ Kailapuri G Murugavel,¹ Pachamuthu Balakrishnan,¹ Sarvode N. Mothi,² Sunil S. Solomon,^{1,3} Nagalingeswaran Kumarasamy,¹ Poongulali Selvamuthu,¹ Suniti Solomon,¹ and Davey M. Smith^{4,5}

Abstract

In this study HIV-1 subtype C-infected adults demonstrated higher purifying selection on their viral populations in reverse transcriptase (RT) than infected children. This difference is likely explained by more mature cytotoxic T-lymphocyte responses in adults, which may have implications for the development of drug resistance in the RT region.

SLECTION FOR GENETIC MUTATIONS IN HIV-1 allows the virus to evade recognition by cytotoxic T-lymphocytes (CTL).^{1,2} These CTL responses vary in their antiviral efficacies, timing of their response, and targeting of viral epitopes.³ The reverse transcriptase (RT) protein of HIV-1 is an important target of CTL responses in infected individuals,⁴ and more than 40 different RT-specific CTL epitopes have been identified.⁴ The RT enzyme is also a target for most approved antiretroviral therapies,⁵ and similar to CTL evasion, selection for genetic mutations in HIV-1 RT decreases the susceptibility to these antiretroviral therapies.⁵ Understanding the antigenicity, immunogenicity, and evolution of CTL epitopes that incorporate antiretroviral (ART) drug resistance mutations (DRM) is perhaps a first step toward developing immune strategies against the emergence and transmission of drug-resistant HIV strains.^{1,6}

The ability to mount a potent CTL response differs between children and adults in that HIV-infected children have a weaker CTL response than HIV-infected adults.⁷ Generally, HIV-infected children usually need to be older than 3 years of age and have a sufficient number of circulating CD4 T cells to mount a strong and antigen-driven CTL response against HIV.⁶ It has been suggested that this weakness of the CTL response against HIV⁶ in infants is caused by antigen exhaustion and induction of Th2-like immune responses in early life.⁸ Teasing out the mechanisms responsible for any differ-

ences in CTL responses between adults and children is complicated, since pediatric HIV infection results in a complex pattern of qualitative and quantitative defects in the immune system⁹ and shared HLA-mediated selective pressures on the virus in a vertical transmitting mother–infant pair may undermine future HLA-mediated viral control in the child.³

Here, we investigated differences in CTL response between adult and children by measuring mutational changes and selection pressure in HIV-1 subtype C RT from 56 vertically infected children and 490 horizontally infected adults who were receiving first-line ART [nevirapine (NVP) or efavirenz (EFV)+zidovudine (AZT)/stavudine (d4T)+lamivudine (3TC)] and were failing to control their infection. (The patients who fail to improve on CD4 count as per WHO guidelines are recommended for drug resistance testing to identify possible treatment failure.)

From the blood collected from our study populations, we generated bulk sequences of HIV-1 RT (645 bp; GenBank accession numbers for children sequences: KP993550–KP993603, KR013206, KR013207; adult sequences: KP993604–KP994093), as previously described.¹⁰ Sequences were aligned (Clustal W) and subtyped using REGA v2,¹¹ Nucleotide diversity, entropy (PHYML and HIV LANL),¹² selection by REL in HyPHY¹³ (each sequence was analyzed to a subtype C consensus reference created from our subtype C sequences), and drug resistance (IAS-USA)¹⁴ were measured.

¹YRG CARE, Chennai, India.

²Asha Kirana Hospital, Mysore, India.

³Johns Hopkins University, Baltimore, Maryland.

⁴University of California San Diego, La Jolla, California.

⁵Veterans Affairs Healthcare System, San Diego, California.

FIG. 1. Frequency of HIV-1 reverse transcriptase (RT) drug resistance-associated mutations (DRM) in children and adults.

Mutations were mapped to known epitopes of HLA allele selection and compared between groups. Comparisons used ANOVA and Tukey's multiple posttests (Prism v5.03).

At the time of sampling, the mean age of the children was 8.2 ± 2.8 years and of the adults was 36 ± 12 years. Overall, the median CD4 count was low for both: 200 cells/ μ L in children and 165 cells/ μ L in adults. The most common first-line regimen in both groups was d4T/3TC/NVP or EFV with the rest on AZT/3TC/NVP or EFV. Drug resistance patterns were similar for both groups (Fig. 1): M184V/I (78.6% children, 81.5% adults), T215Y/F (28.5% children, 42.6% adults), K103N (32.1% children, 37.1% adults), Y181C (32.1% children, 33.2% adults), and G190A (32.1% children, 31.4% adults). Overall, nonsynonymous to synonymous mutation ratios (dN/dS) were different between children ($dN/dS = 0.290$) and adults ($dN/dS = 0.305$). Specifically, adaptive ($dN/dS > 1$, $p = 0.005$) and purifying selection ($dN/dS < 1$, $p = 0.02$) was higher in adults, while neutrally evolved codons were higher ($dN/dS = 1$, $p < 0.001$) among children (Fig. 2).

Though 27 codons demonstrated positive selection, only 10 were present in children whereas all 27 were present in adults, with both groups having positively selected codons that were associated with drug resistance. The codon positions 69, 74, 98, 101, 181, 188, 190, 215, and 221 are the common DRMs observed in RT that are associated with positive selection. The frequencies of positively selected codons 74 (12.5% in children vs. 10.4% in adults), 101 (25% in children vs. 18.7% in adults), and 188 (12.5 in children vs. 6.9% in adults) are higher in children compared to adults while 69 (3.6% in children vs. 9% in adults), 98 (1% in children vs. 17.7% in adults), 181 (33.9% in children vs. 36.4% in adults), 215 (28.5% in children vs. 47.7% in adults), and 221 (16% in children vs. 17.1% in adults) are higher in adults.

Epitope mapping revealed that HLA alleles A2, A30, B18, B35, and B51 could influence the selection observed at codons 178, 179, 181, 188, and 190 in adults, and only codon 178 demonstrated positive selection in the children while alleles A11 and A3 could influence the selection of codons 98

FIG. 2. HIV-1 RT nucleotide delta nonsynonymous to delta synonymous substitution (dN/dS) ratio.

and 101, which shows an extensive difference in the frequency between adults and children. Alleles A68, B57, and B51 could exert positive selection pressure on drug resistance codons 181, 215, and 221, which shows a higher frequency in adults.

Statistical associations between a patient's HLA and mutations that accumulated in either the protease or the RT genes during virological failure of ART have previously indicated that the CTL response could influence the emergence of drug resistance.¹⁵ In this study of HIV-infected adults and children with ART failure and drug resistance, we demonstrated that CTL responses to HIV-1 subtype C are different between the two groups, with the adults having higher adaptive and purifying selection than the children. The observed difference could represent differences in duration of infection, transmitted CTL escape variants during vertical transmission, maturity of CTL responses, or any combination of these factors.

Concerning the duration of infection, we did not have good estimates for either adults or children groups; however, the median age of the children was 8.2 ± 2.8 years and they were infected throughout their life and the mean age for the adults was 36 ± 12 years and they were infected through sexual exposure, likely within 10 years of sampling. Therefore, these infection times might be comparable given that both groups had similar CD4 counts. Since the virus that is transmitted vertically comes from a genetically related source, i.e., infected mother, the mother and the child would share HLA alleles, which would determine the CTL response. Therefore, it is likely that the virus transmitted from the mother to the child may have already escaped from at least some of the potential CTL responses in the child. In other words, less positive selection would have been observed because the transmitted viruses may already have resistance to potential CTL responses that could develop in the child. Another possible explanation is that there are differences in CTL maturity and response between adults and children⁶ and the lack of a strong CTL response in children may be due to intrauterine exposure to HIV that results in maturational or developmental abnormalities in CD4 and CD8 cells.

Understanding how CTL responses mature across ages could be important in understanding the HIV-1 mutational patterns of ART resistance and understanding how CTL responses vary with age may explain in part the differences observed in HIV pathogenesis between adults and children.

Acknowledgments

We are most grateful to the clinical and laboratory staff at the YRG Centre for AIDS Research and Education, VHS, Chennai, India, for their facilitation of the study. We would like to thank the Fogarty AIDS International Training and Research Program (AITRP), Brown University, Rhode Island, for financial support, thus providing us with an opportunity to present the poster at the XIX International AIDS Conference in Washington DC, July 22–27, 2012. Dr. Smith has received research support by grants from the National Institutes of Health: AI100665, MH062512, DA034978, AI036214, and EB015365 and the James B. Pendleton Charitable Trust.

Author Disclosure Statement

No competing financial interests exist.

References

1. Larsson M, Jin X, Ramratnam B, *et al.*: A recombinant vaccinia virus based ELISPOT assay detects high frequencies of Pol specific CD8 T cells in HIV-1-positive individuals. *AIDS* 1999;13:766.
2. Moore CB, John M, James IR, *et al.*: Evidence of HIV-1 adaptation to HLA-restricted immune responses at a population level. *Science* 2002;296:1439.
3. Tiemessen CT and Kuhn L: Immune pathogenesis of pediatric HIV-1 infection. *Current HIV/AIDS Rep* 2006;3:13–19.
4. Menéndez-Arias LUIS, Mas A, and Domingo E: Cytotoxic T-lymphocyte responses to HIV-1 reverse transcriptase (review). *Viral Immunol* 1998;11(4):167–181.
5. Imamichi T: Action of anti-HIV drugs and resistance: Reverse transcriptase inhibitors and protease inhibitors. *Curr Pharm Design* 2004;10(32):4039–4053.
6. Sandberg K, *et al.*: HIV-specific CD8+ T cell function in children with vertically acquired HIV-1 infection is critically influenced by age and the state of the CD4+ T cell compartment. *J Immunol* 2003;170(8):4403–4410.
7. Shalekoff S, *et al.*: Identification of human immunodeficiency virus-1 specific CD8+ and CD4+ T cell responses in perinatally infected infants and their mothers. *AIDS (London, Engl)* 2009;23(7):789.
8. Wasik TJ, Jagodzinski PP, Hyjek EM, *et al.*: Diminished HIV-specific CTL activity is associated with lower type 1 and enhanced type 2 responses to HIV-specific peptides during perinatal HIV infection. *J Immunol* 1997;158(12):6029–6036.
9. Burns DN and Mofenson LM: Paediatric HIV-1 infection. *Lancet* 1999;354:1–6.
10. Saravanan S, Vidya M, Balakrishnan P, *et al.*: Evaluation of two human immunodeficiency virus-1 genotyping systems: ViroSeq 2.0 and an in-house method. *J Virol Methods* 2009;159:211–216.
11. Alcantara LCJ, Cassol S, Libin P, *et al.*: A standardized framework for accurate, high-throughput genotyping of recombinant and non-recombinant viral sequences. *Nucl Acids Res* 2009;37:634–642.
12. www.hiv.lanl.gov/.
13. Kosakovsky Pond SL, Frost SDW, and Muse SV: HyPhy: Hypothesis testing using phylogenies. *Bioinformatics* 2005;21(5):676–679.
14. Johnson VA, Calvez V, Gunthard HF, *et al.*: Update of the drug resistance mutations in HIV-1: March 2013. *Top Antivir Med* 2013;21(1):6–14.
15. Lamhamedi-Cherradi S, Culmann-Penciolelli B, Guy B, *et al.*: Qualitative and quantitative analysis of human cytotoxic T-lymphocyte responses to HIV-1 proteins. *AIDS* 1992;11:1249.

Address correspondence to:

Shanmugam Saravanan
Y.R. Gaitonde Centre for AIDS Research
and Education (YRG CARE)
Voluntary Health Services (VHS) Hospital
Taramani, Chennai 600 113
India

E-mail: saravanan@yrgcare.org