

CD8+ T Cells Exert Potent Selective Pressure in Young HIV-Infected Infants

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Abstract

Introduction: Few studies have characterized virus-specific CD8+ T cells in infants, particularly during the first few months of life. An important question is whether the CD8+ T cell responses generated by young infants are capable of controlling viral replication or exerting selective pressure in vivo over the first year of life.

Methods: RNA was extracted from plasma obtained from 5 untreated, HIV-1 infected infants at 1-3, 4-6, and 12-15 months of age. Gag and nef cDNAs were amplified and 10 clones per gene per patient were sequenced. Molecular HLA class I typing was performed. A peptide-based IFN- γ ELISPOT assay was used to detect and measure the frequencies of epitope-specific CD8+ T cells.

Results: Intra patient genetic distances and the ratios of non-synonymous/synonymous (dN/dS) amino acid substitutions increased over time, suggesting diversification of infant sequences. dN/dS ratios were highest in nef and gag p15. Seventy five percent of all amino acid (aa) substitutions observed in the nef gene and nineteen percent of amino acid substitutions in gag p15 were either within epitopes corresponding to infant HLA alleles or within regions previously described as targets for HIV-1 specific CD8+ T cell responses. Analysis of sequences within potential epitopes restricted by infant HLA alleles revealed evidence of CD8+ T cell escape as early as 2-3 mo of age. For example, in an HLA A24+ infant, Q192H substitution was detected within an A24-restricted nef epitope (aa 186-194; DSRLAFQHIM) by 2 months. In another HLA A24+ infant, F135Y substitution was detected in an A24-restricted nef epitope (aa134-143; RFLPTFGWCF) as early as 3 months of age. Reduced recognition of the variant sequences compared with wild type sequences was demonstrated by

Objectives

Conclusions: Our findings demonstrate that young infants are capable of generating CD8+ T cell responses that exert potent selective pressures in vivo and that these CD8+ T cell responses shape viral evolution over the first year of life.

Methods

- Characterize viral evolution in gag and nef genes over the first year of life.
- Determine potential selective pressures exerted by CD8+ T cells on viral sequences in early pediatric HIV-1 infection.

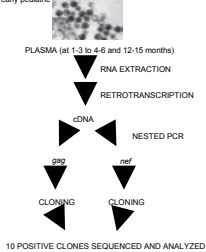


Table 1. Characteristics of infants studied

Infant	HIV-1 Genotype	HLA type	Time from infection	Admission to therapy (age at initiation)	CD4 (cells/μl)	CD8 (cells/μl)	CD8/CD4 ratio
P-1024	integrated	A*24, A*11, B*37, B*39, Cw*4, Cw*7	2 mos 6 mos 11 mos	No Yes ZDV (4-7 mos)	3522 2923 1190	4506 2546 1006	All All Child at 12 mos
P-1031	integrated	A*2, A*10, B*37, B*39, Cw*4, Cw*5	2 mos 6 mos 11 mos	No No ZDV (1 mo)	2109 2043 1368	839 1196 562	NS NS Child at 12 mos
P-1189	integrated	A*24, A*23, B*37, B*39, Cw*4, Cw*7	2 mos 6 mos 12 mos	No No No	2576 2338 1880	3446 2423 2405	NS AC AC
P-1115	integrated	A*23, A*2, B*37, B*39, Cw*4	1 mo 3 mos 6 mos 12 mos	No No Yes ZDV (1 mo)	1588 1370 1368 1880	3916 4201 2402 2402	NS NS NS Child at 12 mos
P-1026	integrated	A*24, A*12, B*37, B*39, Cw*4	3 mos 13 mos 18 mos	No Yes ZDV (10 mos)	2006 1387 111	1737 542 401	NS NS Child at 18 mos

*ZDV, zidovudine; *NVP, nevirapine; *AZT, didanosine; *TDF, tenofovir; *CD8, classification system's Human Immunodeficiency Virus (HIV) infection in children under 13 years of age; MMR, September 30, 1991 (4); 10-10

Table 2. Intra patient genetic distances and Shannon entropy values

Infant	Sample	Time (mo)	Mean pairwise genetic distance (dN/dS) (n=1000)				Mean pairwise genetic distance (dN/dS) (n=1000)				
			gag p55	p17	p24	p15	nef	gag p55	p17	p24	p15
P-1024	2 mos	0-7	0.1	0.1	0.2	0.0	1	0.2	0.1	0.1	0.0
		8-11	0.1	0.1	0.2	0.0	1	0.2	0.1	0.1	0.0
		12 mos	0.1	0.1	0.2	0.0	1	0.2	0.1	0.1	0.0
		18 mos	0.1	0.1	0.2	0.0	1	0.2	0.1	0.1	0.0
P-1031	2 mos	0.0	0.3	0.2	0.1	0.0	0.0	0.4	0.3	0.2	0.1
		6 mos	0.0	0.3	0.2	0.1	0.0	0.0	0.4	0.3	0.2
		11 mos	0.0	0.3	0.2	0.1	0.0	0.0	0.4	0.3	0.2
		12 mos	0.0	0.3	0.2	0.1	0.0	0.0	0.4	0.3	0.2
P-1189	2 mos	0.0	0.3	0.1	0.1	0.0	0.1	0.2	0.2	0.1	0.1
		6 mos	0.0	0.3	0.1	0.1	0.0	0.1	0.2	0.2	0.1
		11 mos	0.0	0.3	0.1	0.1	0.0	0.1	0.2	0.2	0.1
		12 mos	0.0	0.3	0.1	0.1	0.0	0.1	0.2	0.2	0.1
P-1115	1 mo	1.4	0.3	0.2	0.1	1.1	0.1	0.3	0.3	0.2	0.1
		3 mos	1.0	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.0
		6 mos	1.0	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.0
		12 mos	1.0	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.0
P-1026	3 mos	0.2	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.0	
		13 mos	0.2	0.1	0.1	0.0	0.1	0.1	0.1	0.0	
		18 mos	0.2	0.1	0.1	0.0	0.1	0.1	0.1	0.0	
		18 mos	0.2	0.1	0.1	0.0	0.1	0.1	0.1	0.0	

*NS, No data.
†The dN/dS ratio values were calculated according to the Nei and Li method. The normalized Shannon entropy was calculated as $- \sum_{i=1}^n (p_i \ln p_i)$ in which p_i is the frequency of each nucleotide in the sequence and n is the total number of sequences compared. A Shannon entropy value of 1 means that all positions in the sequence have the same four nucleotide frequency (tetranucleotide). Values above 1 mean that all positions are identical (tetranucleotide).

Table 3. Infant dN/dS ratio values in gag and nef proteins

Infant	Interval (age in months)	Gag nucleotide substitutions, Mean dN/dS ^a				Nef nucleotide substitutions, Mean dN/dS ^a				
		gag p55	p17	p24	p15	nef	gag p55	p17	p24	p15
P-1024	2 to 4 mos	0.88	0.34	0.86	2.17	1.2				
	4 to 15 mos	0.40	0.19	0.24	1.66	0.81				
P-1031	2 to 4 mos	1.37	1.70	1.13	1.15	4.07				
	4 to 11 mos	0.78	0.88	0.5	1.26	0.79				
P-1189	2 to 6 mos	0.86	0.66	0.9	1.09	1.14				
	6 to 12 mos	0.45	2	0.25	0.76	1.45				
P-1115	1 to 3 mos	0.38	0.27	0.52	0.36	0.28				
	3 to 12 mos	0.37	0.21	0.45	0.45	0.79				
P-1026	3 to 13 mos	ND ^b	ND	ND	ND	3.18				
	13 to 15 mos	1.14	1.25	0.95	1.37	1.80				

^adN/dS values higher than 1 are in bold.
^bND, No Data.

Results

Figure 1. Global infant distribution of non-synonymous mutations (dN) over time in gag and nef proteins

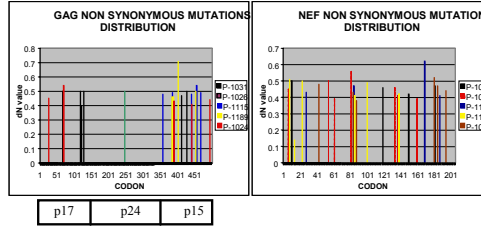
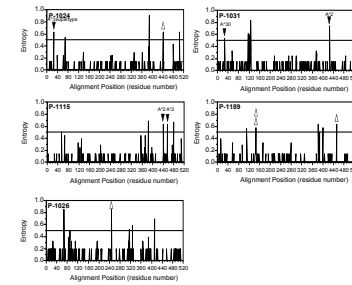
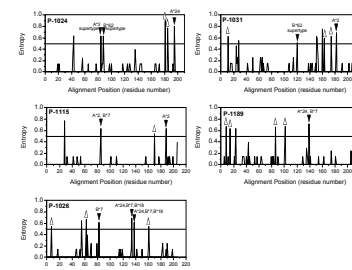


Figure 2. Infants amino acid variability in gag gene



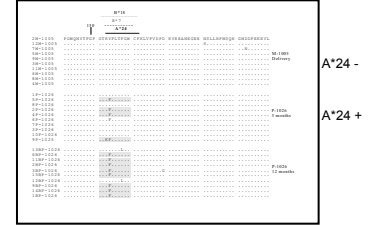
Shannon entropy values from the amino acid alignments of the three time points per infant in the gag gene were calculated. An entropy cut off value of 0.5 (amino acid frequency substitution at a determined position higher than 0.5) was established. Amino acid substitutions within epitopes corresponding to epitopes restricted by the infant's HLA alleles and Amino acid substitutions within epitopes corresponding to epitopes described in the HIV-1 Immunology Database as recognized by humans.

Figure 3. Infants amino acid variability in nef gene



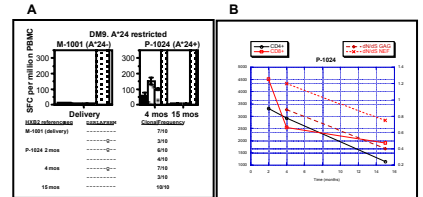
Shannon entropy values from the amino acid alignments of the three time points per infant in the nef gene were calculated. An entropy cut off value of 0.5 (amino acid frequency substitution at a determined position higher than 0.5) was established. Amino acid substitutions within epitopes corresponding to epitopes restricted by the infant's HLA alleles and Amino acid substitutions within epitopes corresponding to epitopes described in the HIV-1 Immunology Database as recognized by humans.

Figure 4. CD8+ T cell selective pressure in an A24-restricted epitope (RFLPTFGWCF) in the nef gene in infant P-1026



Detailed amino acid alignment of the epitope and flanking regions with maternal (M-1001) at delivery and infant's (P-1026) at 3 and 12 months of age. CD8+ T cell selective pressure drove appearance of an escape mutant (Y135F). This mutant has been previously described in Furtakci, T et al 2004. Frequent transmission of cytosolic-T-lymphocyte escape mutants of human immunodeficiency virus type 1 in the highly HLA-A24-positive Japanese population. *J Virol* 78:8437-8445. Other overlapping epitopes restricted by the infant's HLA B*7 and B*18 are shown.

Figure 5. Transmutation, selection and reversion of an A24 escape mutant (DSRLAFQHIM) in the nef gene in infant P-1024.



A. Elispot assays were performed with maternal and infant autologous epitope sequences. HXB2 optimal epitope sequences were used as a wild type. PBMC from mother (M-1001) at delivery and from infant (P-1024) at different time points were used. HLA class I epitope restriction, maternal and infant HLA class I alleles, epitopes varying over time, and dN/dS frequencies for each variant tested are also shown. Background level (PBMC and B-LCL + peptide) with no peptide, PBMC or PBMC + B-LCL with wild type, PBMC or PBMC + B-LCL with epitope variant and PBMC + PHA. B. Decline over time of CD4+ T cells, CD8+ T cells counts and dN/dS ratios in gag and nef from 4 mos to 15 mos.

Conclusions

- Diversification of plasma gag and nef gene sequences was observed over the first year of life.
- Sequence changes were detected as early as 2-3 months of age and commonly occurred within previously described CD8+ T cell epitopes.
- These data provide support for the use of vaccines to prevent or modify the course of HIV-1 infection in young infants.

