

# VIGOROUS HIV-1-SPECIFIC CTL RESPONSES IN LONG TERM SURVIVORS OF NEONATAL HIV-1 INFECTION

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## BACKGROUND

- The capacity to mount broad and potent adaptive immune responses develops progressively during infancy and early childhood
- Many children who acquired HIV-1 infection prior to 1990 are now reaching adulthood, despite the unavailability of potent antiretroviral therapy during this key period of immunological ontogeny
- HIV-1-specific CD8<sup>+</sup> T lymphocyte (CTL) responses are a key factor maintaining asymptomatic infection, but these responses in children remain understudied compared to infected adults
- Because persons infected prior to 1990 were not treated with fully suppressive antiretroviral therapy during their early immunologic development, we hypothesized that clonal exhaustion might lead to a skewed or narrowed CTL response to HIV-1

## METHODS

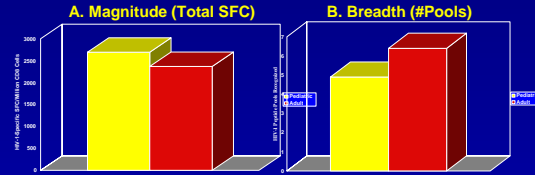
- 17 perinatally-infected subjects averaging age 18y (range 14-22)
- All treated with combination drug therapy at the time of study; 12/17 had undetectable plasma viremia (<50 or <400 RNA copies/ml), 4/17 had detectable viremia <1000, and 1/17 had viremia of 1209
- 10/17 had been categorized as CDC disease stage C, 6/17 stage B, and 1/17 stage A
- HIV-1-specific CTL responses were examined by IFN- $\gamma$  ELISpot assay using 53 pools of consecutive peptides (15mers overlapping by 11, NIH AIDS Repository) based on Clade B consensus sequences of all 9 viral proteins, with polyclonally-expanded CD8<sup>+</sup> PBMC
- 10 chronically infected adult subjects (not on drug treatment) were evaluated with the same method for comparison
- T cell receptor recombination excision circle (TREC) PCR quantitation and flow cytometric analysis of lymphocyte markers were performed using unexpanded PBMC.

## RESULTS

Subject	Sex	Age	Stage	VL	CD4	CD8	Ratio	SFC	Pools
AP-04	F	16	B	<400	915	1049	0.87	5470	12
AP-07	F	20	C	1209	129	128	1.01	8249	11
AP-08	M	14	B	<50	309	333	0.93	915	2
AP-10	F	16	C	<50	515	751	0.69	328	0
AP-11	F	18	B	247	658	1360	0.48	3928	10
AP-13	F	18	C	162	163	432	0.38	12920	17
AP-15	M	15	A	<50	732	1009	0.73	3310	8
AP-16	M	19	C	<50	1026	687	1.49	490	0
AP-19	M	21	C	56	663	935	0.71	818	2
AP-20	M	21	C	51	877	926	0.95	5151	12
AP-21	F	14	B	<400	793	1201	0.66	925	4
BP-02	M	18	C	<50	395	347	1.14	820	3
BP-06	M	17	C	<50	516	383	1.35	236	0
BP-07	M	22	C	<400	339	1653	0.21	496	1
CP-01	M	21	C	<50	698	729	0.96	553	0
CP-04	F	18	B	<50	847	920	0.92	230	0
CP-06	M	17	B	<50	473	328	1.44	945	2

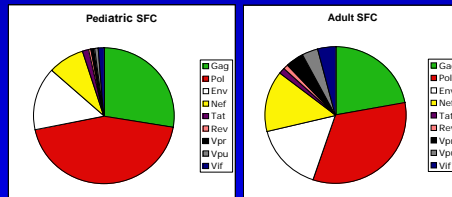
**Table 1. Characteristics of the pediatric cohort and ELISpot responses.** Listed for each subject are sex, age, CDC clinical stage, viremia (RNA genomes/ml), absolute CD4/CD8 counts and ratios, and total ELISpot responses against HIV-1 (SFC/10<sup>6</sup> CD8<sup>+</sup> T lymphocytes and total peptide pools recognized >100SFC/10<sup>6</sup> of 53 tested)

## RESULTS (continued)



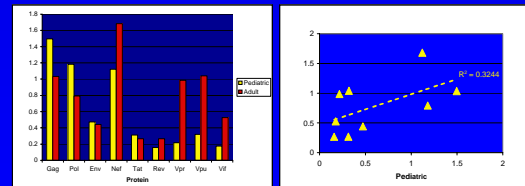
**Figure 1. The magnitude and breadth of HIV-1-specific CTL responses is similar in pediatric and adult subjects.** A. The mean total ELISpot response against all HIV-1 peptide pools for the pediatric subjects (range 230-12920) is compared to 10 chronically-infected, untreated adults (range 131-3920). B. The mean number of peptide pools (of 53 total) recognized by ELISpot (>100SFC/million CD8<sup>+</sup>T lymphocytes) for the pediatric subjects (range 0-17) is compared to 10 chronically-infected, untreated adults (range 0-14).

Although the pediatric subjects are generally being treated with suppressive antiretroviral combination therapies (as opposed to the adults who are untreated), these vigorous CTL responses suggest ongoing viral replication and antigenic stimulation in these subjects.



**Figure 2. Gross targeting of CTL in pediatric and adult subjects is predominantly directed at Gag, Pol, Env, and Nef.** The mean targeting of CTL as determined by ELISpot is plotted as percentages of the total SFC response for both pediatric and adult subjects.

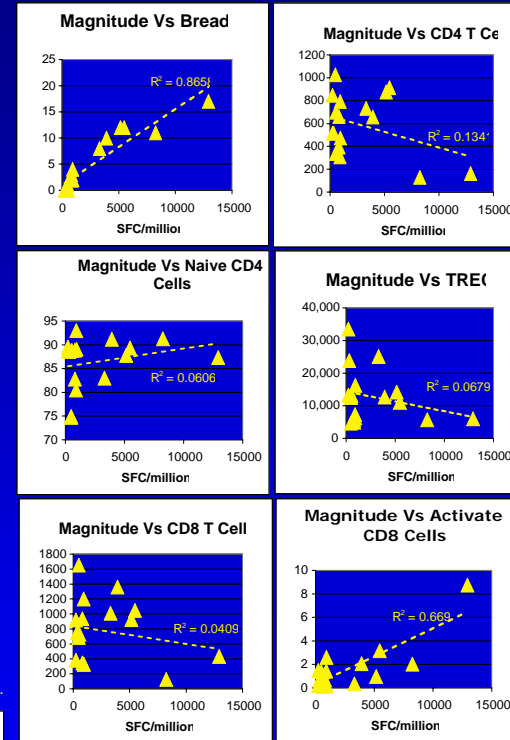
These data show grossly similar overall targeting of CTL in both groups.



**Figure 3. Size-adjusted targeting of CTL in pediatric and adult subjects is predominantly directed at Gag, Pol, and Nef.** A. The mean targeting of CTL as determined by ELISpot and adjusted by the amino acid size of the HIV-1 proteins is plotted for both pediatric and adult subjects. B. The mean pediatric and adult values for each protein are plotted against each other.

These data again show similar overall targeting of CTL in both groups, but demonstrate possible differences. The pediatric subjects appear to target the more conserved structural proteins in preference to more variable "accessory" proteins, as compared to the adults (whose targeting is similar to that published by Addo et al, J. Virol. 2003, 77:2081-92). The significance of this trend is unclear.

## RESULTS (continued)



**Figure 4. The magnitude and breadth of CTL targeting are directly related, and linked to levels of CD8 cell activation but not indirect measures of thymic function.** The total magnitude of HIV-1-specific CTL responses detected by ELISpot for each pediatric subject is plotted against: breadth of CTL (number of peptide pools recognized of the 53 screened), total CD4 T cell count, percentages of naive CD4 T cells (as reflected by CD27+CD31+CD45RA<sup>+</sup> staining), T cell receptor excision circle frequency (TREC), total CD8 T cell count, and percentage activated CD8 T cells (as reflected by DR+/CD38<sup>+</sup> staining).

These results show a tight correlation of CTL magnitude versus breadth, lack of correlation of CTL to: overall levels of CD4 or CD8 T cells, measures of thymic function (naive CD4 T cell levels or TREC). CTL activity is correlated to levels of CD8 cell activation as reflected by DR+/CD38<sup>+</sup> expression (as well as each marker individually, not shown).

## CONCLUSIONS

- Despite long term (neonatal) infection during immunologic development, many subjects demonstrated surprisingly vigorous CTL responses
- Despite suppressive antiretroviral therapy (reported to reduce CTL responses in adults), the magnitude and breadth of these responses was similar to that of untreated, chronically infected adults
- The targeting of these responses was grossly similar to that for adults, but there was a trend towards more targeting of conserved structural proteins and less targeting of variable "accessory" proteins, possibly reflecting more escape over the longer course of infection in pediatric subjects, or greater susceptibility of CTL in these subjects to escape (e.g. narrower TCR breadth)
- The magnitude and breadth of the CTL response does not show any direct relationship to CD4 cells or thymic output
- The CTL response is directly related to the level of CD8 cell activation, likely reflecting ongoing viral replication
- Combination antiretroviral therapy instituted years after perinatal infection and symptomatic disease may therefore preserve or reconstitute the CTL response against HIV-1, but fail to provide full antiviral suppression
- Overall these data suggest adequate immunologic reserve in the intermediate term after neonatal HIV-1 infection; it remains to be seen whether this reserve will be adequate for the long term, and whether the trends for differences between the pediatric and adult subjects reflect early immunologic exhaustion



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