

CD4 T cell depletion, and not age, may be a driver of abnormal CD4 cell compartments in HIV-infected children initiating ART in Uganda



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ABSTRACT

Background: The naïve CD4 T cell pool is maintained by thymic production of new cells, proliferation within the naïve pool and cell loss through death or differentiation to memory cells. The homeostatic mechanisms operating to maintain naïve and memory pools are not fully understood in healthy children; even less is known in HIV infection, particularly in resource-limited settings when ART is often initiated with advanced immunodeficiency.

- Methods: 1210 ART-naive children meeting WHO criteria for ART in Uganda/Zimbabwe were enrolled into the ARROW trial and started combination ART. 199 children in Uganda (54% girls, aged 5 months-18 years) underwent CD4 immunophenotyping at ART initiation using a combination of CD4, CD45RA and CD31
- Results As expected, CD4 and CD4-for-age z-score varied significantly with age at ART initiation (see table), as did the percentage of CD4 cells in the 'Recent Thymic Emigrant' (RTE. CD45RA+CD31+), Central Naive (CN, CD45RA+CD31-) and memory (M, CD45RA-CD31-) compartments (p=0.001, 0.01, <0.001 respectively). However, multivariable modelling showed this relationship with age was predominantly the result of the lower CD4-for-age in older children (after adjusting for CD4-for-age, p(age)=0.13, 0.40, 0.70 respectively). Every 1 unit lower CD4-for-age was associated with 4.4% smaller RTE, 2.1% greater CN and 3.6% greater M subpopulations at ART initiation (p<0.001). There was no impact of sex on the CD4 subpopulations (p>0.5), but there was a trend towards children with lower weight-for-age having greater CN subpopulations (0.7% greater for every 1 unit lower weight-for-age, adjusted p=0.12).

Age at ART	Children	Median CD4	Median %RTE	Median %CN	Median %M
initiation (yrs)		(CD4-for-age)	CD4 cells	CD4 cells	CD4 cells
0.5-2	75	778 (-2.3)	41%	11%	30%
3-6	49	458 (-3.2)	35%	11%	42%
7-12	50	256 (-5.3)	35%	14%	39%
13-18	25	215 (-7.3)	24%	19%	42%

Conclusions: In all agegroups, the cell proportions in these 3 CD4 compartments were lower than have been reported in healthy Caucasian children. CD4 count seems to be an important driver or consequence of lower RTEs and higher central naïve/memory populations, with a far stronger association than age alone. In children surviving without ART, there may be a shift to maintaining the CD4 cell pool through the relative expansion of naïve and memory pools at some childspecific point, possibly representing declining capacity of the thymus to keep pace with CD4 loss. The long-term consequences of this for ART response are unclear.

BACK	GROU	ND

- & The naïve CD4 T cell pool is maintained by
- thymic production of new cells proliferation within the naïve pool
- cell loss through death or differentiation to memory cells¹

CD4 homeostasis



R The homeostatic mechanisms operating to maintain naïve and memory pools are not fully understood in healthy children

ART is often initiated with advanced immunodeficiency.

- 1210 ART-naive children meeting WHO criteria for ART in Uganda/Zimbabwe were enrolled into the ARROW trial (www.ar vtrial.org) and started combination ART
- immunophenotyping at ART initiation using a combination of CD4, CD45RA and CD31

· We investigated three CD4 cell subpopulations CD45RA+CD31+ Recent Thymic Emigrants (RTE) CD45R4+CD31-Central Naive (CN)

> CD45RA-CD31-Memory (M)

RESULTS - CD4 cell subpopulations vary with age at ART initiation RTF CN M 🗖



* CD4, CD4-for-age z-score and the percentage of CD4 cells in the 'Recent Thymic Emigrant' (RTE, CD45RA+CD31+) population decreased significantly with age at ART initiation (all p≤0.001)

(median CD4 cells/mm³, CD4-for-age, n)

The percentage of CD4 cells in the Central Naive (CN, CD45RA+CD31-) and Memory (M, CD45RA-CD31-) populations increased significantly with age at ART initiation (p=0.01, <0.001 respectively).

> However, multivariable modelling showed this relationship with age was predominantly the result of the lower CD4-for-age in older children

METHODS

- EDTA anti-coagulated peripheral blood from each child was incubated with anti-CD4- PerCP mAb, and with combinations of the following antibodies: anti-CD45RA-APC mAb, anti-CD45RA-FITC mAb, anti-CD45RO-FITC mAb, anti-CD31-PE mAb, anti-HLA-DR-PE mAb and Ki67-FITC.
- After incubating with the antibody cocktail, RBCs were lysed using FACSLyse and washed off with a solution of PBS containing 0.5% BSA and 0.1% sodium azide. The remaining leukocytes were fixed with 4% PFA and acquired on a flow cytometer.
- · For intracellular staining the fixed leukcocytes were permeabilised with saponin and then incubated with the Ki67 antibody.

RESULTS - pre-ART CD4 depletion is a better predictor of CD4 cell subpopulations than age

		Effect (95%CI) of pre-ART factors on*				
	At ART initiation	%RTE	%CN	% M		
1	1 unit lower CD4-for-age	-4.4% (-5.4%,-3.5%) p<0.001	+2.1% (+1.5%,+2.7%) p<0.001	+3.6% (+2.6%,4.6%) p<0.001		
1	1 year older age	+0.4% (-0.2%,+1.0%) p=0.16*	-0.1% (0.4%,+0.3%) p=0.78*	-0.2% (-0.8%,+0.4%) p=0.55*		
	1 unit lower weight-for-age**	-0.0% (-1.5%,+1.4%) p=0.96	+0.7% (-0.2%,+1.6%) p=0.12	-0.5% (-2.0%,+0.9%) p=0.47		
	Girl (vs boy)	+0.6% (-3.6%,+4.9%) p=0.76	+0.7% (-1.9%,+3.4%) p=0.57	+0.0% (-4.3%,+4.3%) p=1.00		

* multivariable model including all factors shown plus centre. P values for age including only CD4-for age and age p=0.13, p=0.40, p=0.70 respectively (as abstract) * similar impact including height-for-age rather than weight-for-age in models

SUMMARY

& In all agegroups, the cell proportions in these 3 CD4 compartments were lower than have been reported in healthy Caucasian children².

- this was largely due to a reduction in the RTEs and increase in CD31+ memory cells (data not shown)

& Total CD4 count seems to be an important driver or consequence of lower RTEs and higher central naïve/memory populations, with a far stronger association than age alone.

& In children surviving without ART, there may be a shift to maintaining the CD4 cell pool through the relative expansion of central naïve and memory pools at the expense of RTE at some child-specific timepoint. This may indicate that with time, the capacity of the thymus to keep pace with CD4 loss is diminished.

 the long-term consequences for subsequent ART response are unclear but will be investigated in this ongoing study

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- 199 children in Uganda (54% girls, aged 5 months-18 years) underwent CD4