

Innate and Adaptive Immunity in Treatment Naive HIV-infected Pediatric Patients in Chennai, India

Abstract
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ABSTRACT

Title: Innate and Adaptive Immunity in Treatment Naive HIV-infected Pediatric Patients in Chennai, India

Background: Little is known of the state of innate and adaptive immunity in HIV-infected children not on ARV in relation to disease stage and disease progression.

Methods: A prospective study of 62 perinatally HIV-infected children naive to ARV, age range 3-13 yrs, median plasma virus load 91,300 HIV RNA copies/ml (range 4,490-750,000) was conducted in Chennai, India to investigate phenotypic markers of T cells and phenotype/functions of myeloid dendritic cells (mDC) and plasmacytoid dendritic cells (pDC) by flow cytometry. Patients were classified into immune category (IC-1) [CD4% >25; n=20 (32.5%)], IC-2 [CD4% 15-25; n=33 (53.5%)] and IC-3 [CD4% <15; n=9 (14%)]. Median age was similar (6-7 yrs) in the 3 groups. Six age-matched healthy controls were included.

Results: Among the 3 groups, patients in IC-3 manifested significantly lower proportion of naive (CD45RA+ CD62L+) CD4 and CD8 T cells as compared to IC-1, IC-2 and controls (p < 0.002 for all), with expansion of activated (CD38+ HLA-DR+) CD4 and CD8 T cells (p < 0.05). CD8 T cells manifested higher activation than CD4 (p=0.022). Central memory (CD45RA-CD62L+) population was expanded in the IC-3 subgroup as compared to IC-1 in CD4 (p=0.008) & CD8 effector memory T subset was higher in IC2 & 3 compared to controls (p=0.035, p=0.003 resp.). Upon stimulation with TLR-7/8 agonist resiquimod (RSQ), upregulation of maturation marker (CD83) was less in all groups compared to healthy controls in mDC (p=0.05) and pDC (p<0.001). RSQ induced upregulation of CD80 and CCR7 was equivalent in mDC and pDC in all patient groups in comparison with controls. IC1 had a higher percentage of CD80 in pDC compared to controls (p=0.039). RSQ-induced cytokines, IFN-α in pDC and TNF-α in mDC and pDC were equivalent in all patient groups and controls. Over a 9 month follow-up, 8 patients were started on ARV and rapidly normalized CD4 T cells, with virologic suppression to undetectable levels in 5 of 9; those not on therapy remained stable.

Conclusion: This cohort of HIV-infected children manifested strong mDC and pDC numbers and function, and only subjects in IC-3 manifested an isolated defect in stimulus induced upregulation of maturation marker, CD83. Depletion of naive CD4 T cells and increased immune activation remained the most predictable markers of disease progression. Intact DC function may contribute to disease stability in this group of older HIV-infected children.

AIMS

To investigate markers of innate and adaptive immunity in relation to immunological status based on CD4 T cells in children with perinatal HIV infection, and to evaluate immunologic changes over time.

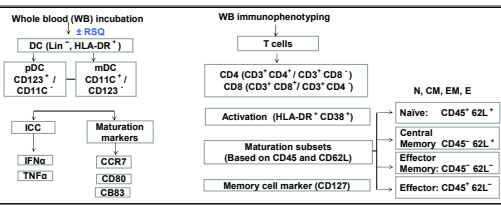
METHODS

Study subjects: In this ongoing study, HIV+ children in outpatient care at TRC, Chennai, are enrolled into after informed consent, and followed at 3 month intervals. 62 children have been enrolled to date. Baseline values for all and 9 month data for 47 children are presented. 6 healthy children (Age 3-13yr; median 4.95) served as controls.

Immunologic assays: Whole blood specimens were split for DC assays and T cell immunophenotyping and processed as shown below. Table 1 shows fluorochrome labeled monoclonal antibodies utilized. Isotype controls were used. Flow cytometry was performed on a FACs Calibur (BD Biosciences, CA) followed by analysis with FLOWJO software (Tree Star, San Carlos, CA v4.6.2) in the TRC Immunology Laboratory.

Ab Combo	FITC	PE	PerCP	APC
1	Lin 1	CD83	HLA-DR	CD11c
2	Lin 1	CD123	HLA-DR	IFN-α
3	Lin 1	CD123	HLA-DR	TNF-α
4	Lin 1	CD123	HLA-DR	CCR7
5	CD3	CD127	CD4	CD8
6	CD3	CD38	HLA-DR	CD8
7	CD45RA	CD62L	CD3	CD4

Table 1:
Antibody Panel



Statistical Analysis was performed using a general linear model with planned contrasts to compare means among the 3 CD4 groups. SAS version 9.1 was used for all analyses. Graph Pad Prism software (version 4.1) was used to plot the graphs

RESULTS

Patient characteristics at baseline

Category	Total subjects	IC 1	IC 2	IC 3
	62	20	33	9
CD4% median	23	29.5	22	10
range	5-44	27-44	15-25	5-13
Age median	6.59	6	7	6
range	3-13	3-13	3-12	3-13
VL (log) median	4.96	4.7	4.97	5.32
range	3.65-5.87	3.65-5.88	3.75-5.88	4.73-5.69

Fig 1: Analysis of cytokine expression in dendritic cells

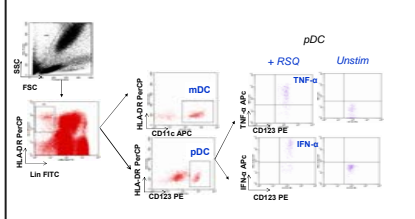
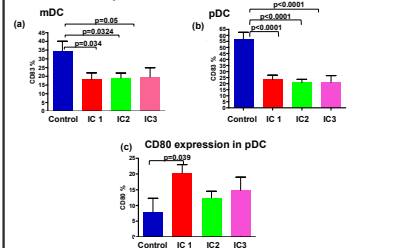
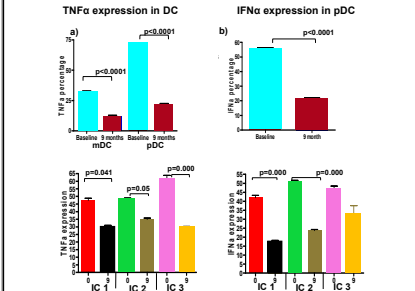


Fig 2: Patients have decreased induction of CD83 in both DC subsets upon RSQ stimulation, but not in CD80



Maturation marker (CD83) and activation marker (CD80) expression in DC identified as a) mDC (CD11c+ / CD123+) or b) pDC (CD11c- / CD123+) in WB samples of HIV-infected patients and healthy controls cultured with and without RSQ for 5 hours.

Fig 3: Cytokine expression in DC subsets with RSQ stimulation at baseline and at 9 months



Intracellular TNF-α or IFN-α expression was determined in DC identified as myeloid (CD11c+ / CD123+) or plasmacytoid (CD11c- / CD123+) in WB samples cultured with and without RSQ for 3 hours in patients and healthy controls. Data for RSQ stimulated cultures is shown. (a) TNF-α expression in mDC and pDC of HIV patients at baseline and 9 months. (b) IFN-α expression in pDC of HIV patients at baseline and 9 months.

RESULTS

Fig 4: CD4 and CD8 T cell maturation subsets at baseline

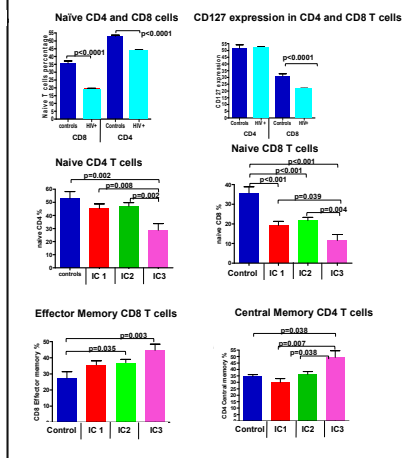


Fig 5: Expression of activation markers in CD4 and CD8 T cells at baseline

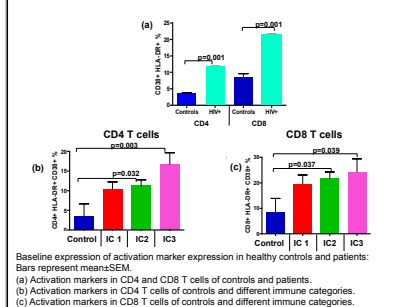
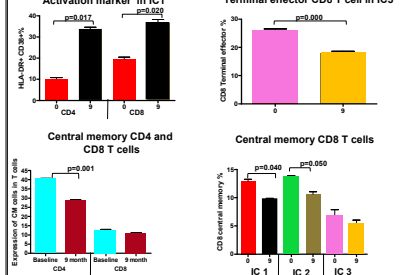


Fig 6: 9 month follow up showing changes in T cell markers



SUMMARY and CONCLUSIONS

Table 2: Summary of immunologic characteristics

	Baseline compared to controls			At 9 months, compared to baseline		
	Total	IC groups		Total	IC groups	
DENDC	↔	↔	↔	↔	↔	↔
pDC CD80	↔	↑	↔	↔	↓	↔
mDC CD83	↓	↓	↓	↔	↔	↓
pDC CD83	↓	↓	↓	↔	↔	↓
pDC IFNα	↔	↔	↔	↓	↓	↔
mDC TNFα	↔	↔	↔	↓	↔	↔
pDC TNFα	↔	↔	↔	↓	↓	↓
CD4 DR+ CD38+	↑	↔	↔	↔	↑	↔
CD8 DR+ CD38+	↔	↔	↔	↔	↔	↔
CD4 Naive	↔	↔	↔	↔	↔	↔
CD8 Naive	↓	↓	↓	↔	↔	↔
CD4 CM	↔	↔	↔	↓	↔	↔
CD8 CM	↔	↔	↔	↓	↓	↔
CD4 EM	↔	↔	↔	↔	↑	↔
CD8 EM	↑	↑	↑	↓	↔	↔
CD4 Effector	↔	↔	↔	↔	↔	↔
CD8 Effector	↔	↔	↔	↔	↔	↓

↔ no difference, ↑ increase, ↓ decrease

General Summary of Cohort:

- This study has characterized the immune status of a cohort of 62 perinatally infected HIV infected children in South India: median age 6.7 years, (range 3 to 12 years), median VL, 4.96 log HIV RNA copies/ml & median CD4, 23%.
- Clinically the children were mostly stable over period of 9 month of follow-up and fell into WHO classes I (40%), II (18%), III (16%) and IV (5%) at baseline.
- Immune Categories based on CD4%: IC1, (n=20), IC2, (n=33) and IC3, (n=9).
- Only 8 children were started on antiretroviral treatment during this study, (4 in IC3, 4 in IC2); Median duration of ARV was 7 months (range 2-9 mo.).

Innate immunity:

- Proportions of both pDC and mDC were normal at baseline and over 9 months.
- Stimulation with TLR7/8 agonist Resiquimod (RSQ) resulted in reduced induction of maturation marker CD83 in mDC & pDC. Although induction of cytokines IFN-α in pDC and of TNF-α in both pDC and mDC were normal at baseline, cytokine induction deteriorated over 9 months, predominantly in pDC.

Adaptive Immunity:

- Among T cells, proportions of naive CD4 and CD8 T cells were reduced whereas memory subsets were altered mainly in the CD8 subset, with increase in effector memory and decrease in central memory over time.
- Activation markers CD38 and HLA-DR were increased in CD4 and CD8 subsets and remained high over follow-up.

This cohort of largely clinically stable HIV-infected children had demonstrable immunological defects dominated by deficits in naive T cells and ongoing immune activation, with progressive but subtle defects of innate immunity, involving mainly pDC. Predictive role of early identification of immunologic defects is under investigation.

Acknowledgements

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