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Subjects Infected With Clade C-containing HIV Exhibit Higher CD4+ T Cell Activation

Priscilla R. Costa[1], Wendy Hartogensis[2], Ricardo S. Diaz[1], Peter Bacchetti[2], Frederick M. Hecht[2], Breno R. Santos[3], AMPLIAR study team, Esper G. Kallas[1], Christopher D. Pilcher[2]

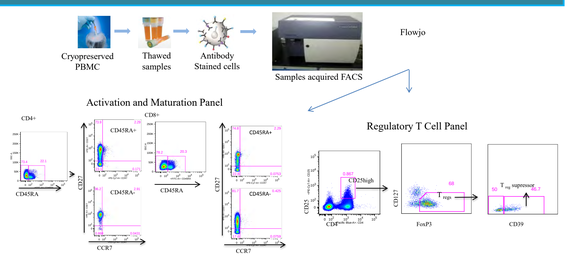
[1]Universidade de São Paulo, Brasil, [2] University of California, San Francisco, [3] Universidade Federal de São Paulo, Brasil, [4] Grupo Hospitalar Conceição, Porto Alegre, Brasil

Background

Cellular activation has been implicated in the progressive immunodeficiency and the development of end-organ disease in HIV-1 infection. Different factors may interfere in immune activation, e.g., host factors (HLA-B27 or B57, presence of CCR5Δ32 mutation), co-infections (GBV-C), and viral factors (tropism to CCR5+ and/or CXCR4+ cells, HIV-1 or HIV-2). Recently, studies have shown that different HIV-1 clades can lead to different clinical outcomes. Patients with different HIV clades may differ in CD4+ T cells loss over time. Clade C viruses are the most transmitted in the world; however, the reasons for such scenario is not well understood. Genetic differences in LTR, Nef, Tat, and Vpu may play a role in potential preferred HIV transmission and immunopathogenesis, in clade C strains over other clades. We hypothesized that individuals infected by clade C (or C-containing recombinant strains) would have different cell activation profile than non-C containing HIV-infected subjects.

Methods

Sixty-three Brazilian subjects recently infected by HIV, from São Paulo (Southeast Brazil) and Porto Alegre (Southern Brazil) were studied and divided into two groups: a "C- containing Subtypes" Group (n=20), with clades C, BC, CU, CK, CRF_31BC, CRF31-like, CRF40-like, and Complex; and a "Non-C- Subtypes" group (n=43), infected by clades B, BF, BF1, F1, and CRF02 AG. From each subject, peripheral blood mononuclear cells (PBMC) were analyzed at acute infection (<3-6 months) and after the viral set point (approximately six months later), all time points prior to initiating antiretroviral therapy (ART). Samples were analyzed to characterize T cell activation and maturation markers (CD38, HLA-DR, CD27, CD45RA, and CCR7) and regulatory CD4+ T Cells (CD4, CD25, CD127, FoxP3 and CD39) using multiparametric flow cytometry. FlowJo software was used for data analyses and the SPSS and Prism softwares were used for statistical analyses; p values < 0.05 were considered statistically significant.



Results

C- Containing Group		Non- C- Group	
Subtypes	%(nº)	Subtypes	% (nº)
C	55.00 (11/20)	B	81.39 (35/43)
BC	15.00 (3/20)	BF	9.30 (4/43)
CU	5.00 (1/20)	BF1	4.65 (2/43)
CK	5.00 (1/20)	F1	2.32 (1/43)
CRF_31BC	5.00 (1/20)	CRF02 AG	2.32 (1/43)
CRF31-like	5.00 (1/20)		
CRF40-like	5.00 (1/20)		
Complex	10.00 (2/20)		

Table 1. Subtype distribution in the two groups.

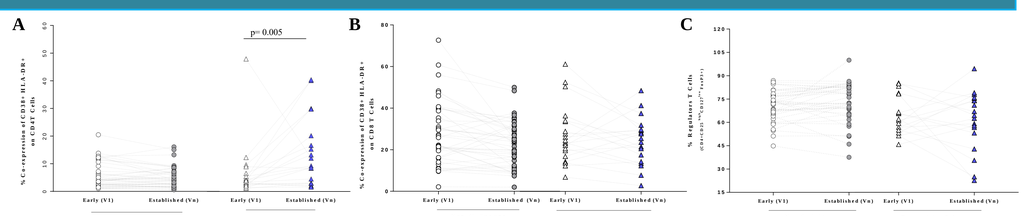


Figure 1. A) Co-expression of CD38+HLA-DR+ on CD4 T Cells was analysed in early and established HIV-1 infection in both groups, C- containing Subtypes and Non- C Subtypes. B) Co-expression of CD38+HLA-DR+ on CD8 T Cells was analysed in early and established HIV-1 infection in both groups, C- containing Subtypes and Non- C Subtypes. C) Frequency of regulators CD4 T Cells (CD4+ CD25 high CD127 low FoxP3+) was analysed in early and established HIV-1 infection in both groups, C-containing Subtypes and Non-C Subtypes

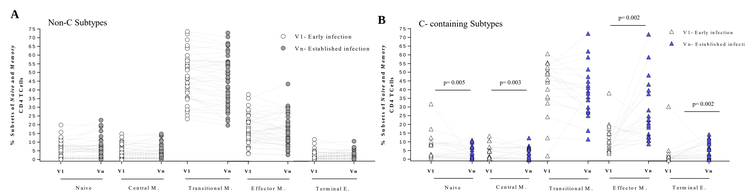


Figure 2. Distribution of naive/memory subsets of CD4 T Cells was compared in early (open symbol) and established (grey and blue symbols) HIV-1 infection in both groups, Non- C Subtypes (A) and C- containing Subtypes (B). There was no statistically significant difference in the distribution of CD8 T Cells naive/memory subsets (data not shown). The classification used for naive/memory subsets was based upon expression of three markers; Naive (CD45RA+CD27+CCR7+); Central Memory (CD45RA-CD27+CCR7+); Transitional memory (CD45RA-CD27+CCR7-); Effector Memory (CD45RA-CD27-CCR7-); Terminal Effector Memory (CD45RA+CD27-CCR7-).

Table 2. Patient characteristics by HIV-1 viral subtype category (Non-C vs. C-containing viral subtype).

Patient Characteristics	Non-C (n=43)	C-containing (n=20)	p-value
Age in years, mean (95%Confidence interval [CI])	33.5 (30.6 to 36.4)	34.4 (28.9 to 40.0)	0.74
Sex, % malc (n/N)	90.7% (39/43)	60.0% (12/20)	0.004
HIV risk factor MSM[1] % (n/N)	81.4% (35/43)	50.0% (10/20)	0.01
CCR5 Delta 32 % (n/N)	7.0% (3/43)	20.0% (4/20)	0.13
GBVC + at Baseline, % (n/N)	14.0% (6/43)	25.0% (5/20)	0.28
Viral Load at Baseline, geometric mean[2] (95%CI)	11437 (6178 to 21172)	7669 (3422 to 17187)	0.44
Viral Load at Follow Up, geometric mean (95%CI)	12078 (6926 to 21063)	2866 (1012 to 8120)	0.008
CD4 at Baseline, geometric mean[3] (95%CI)	613 (542 to 694)	496 (405 to 607)	0.06
CD4 at Follow Up, geometric mean* (95%CI)	545 (479 to 621)	476 (346 to 654)	0.34

Table 3: Regression models for the effect of Non-C HIV-1 viral subtype (compared with C-containing subtypes) on activation in total T cells and T cell maturation subsets. Coefficients (exponentiated into fold-effects where log scale was used) are presented with 95% confidence intervals (CI) and p-values.

Outcome (Marker)			Univariate Non-C Effect Size			Multivariable Adjusted Non-C Effect Size[4]		
	time[2]	Scale[3]	Coefficient or Fold Effect	(95%CI)	(p-value)	Coefficient or Fold Effect	(95%CI)	(p-value)
% CD38+ HLA-DR+ TOTAL CD4+ T cells								
	Baseline	log	0.952	(0.609 to 1.488)	(p=0.83)	0.924	(0.563 to 1.516)	(p=0.75)
	Follow Up	log	0.422	(0.264 to 0.674)	(p<0.001)	0.375	(0.237 to 0.594)	(p<0.0001)
	Change	log	0.443	(0.313 to 0.628)	(p<0.0001)	0.406	(0.266 to 0.618)	(p<0.0001)
% CD38+ HLA-DR+ NAIVE CD4+ T cells								
	Baseline	raw	-0.060	(-0.292 to 0.172)	(p=0.61)	-0.009	(-0.281 to 0.263)	(p=0.95)
	Follow Up	raw	-0.656	(-1.108 to -0.205)	(p=0.005)	-0.724	(-1.211 to -0.237)	(p=0.004)
	Change	raw	-0.596	(-0.943 to -0.249)	(p=0.001)	-0.715	(-1.072 to -0.358)	(p=0.001)
% CD38+ HLA-DR+ CENTRAL MEMORY CD4+ T cells								
	Baseline	raw	0.004	(-0.087 to 0.094)	(p=0.93)	0.020	(-0.084 to 0.125)	(p=0.70)
	Follow Up	raw	-0.460	(-0.753 to -0.168)	(p=0.003)	-0.541	(-0.844 to -0.239)	(p=0.001)
	Change	raw	-0.464	(-0.735 to -0.193)	(p=0.001)	-0.562	(-0.835 to -0.288)	(p=0.001)
% CD38+ HLA-DR+ EFFECTOR MEMORY CD4+ T cells								
	Baseline	log	1.125	(0.645 to 1.964)	(p=0.67)	1.129	(0.613 to 2.077)	(p=0.69)
	Follow Up	log	0.420	(0.233 to 0.759)	(p=0.005)	0.357	(0.195 to 0.655)	(p=0.001)
	Change	log	0.374	(0.241 to 0.580)	(p<0.0001)	0.316	(0.185 to 0.542)	(p<0.0001)
% CD38+ HLA-DR+ EFFECTOR Terminal								
	Baseline	log	1.119	(0.486 to 2.576)	(p=0.79)	0.982	(0.371 to 2.597)	(p=0.97)
	Follow Up	log	0.276	(0.122 to 0.621)	(p=0.002)	0.217	(0.087 to 0.543)	(p=0.002)
	Change	log	0.246	(0.137 to 0.442)	(p<0.0001)	0.221	(0.109 to 0.450)	(p<0.0001)
% CD38+ HLA-DR+ TRANSITIONAL MEMORY CD4+ T cells								
	Baseline	log	1.136	(0.829 to 1.558)	(p=0.42)	1.200	(0.862 to 1.670)	(p=0.27)
	Follow Up	log	0.758	(0.519 to 1.105)	(p=0.15)	0.693	(0.478 to 1.005)	(p=0.053)
	Change	log	0.667	(0.475 to 0.935)	(p=0.02)	0.578	(0.400 to 0.833)	(p=0.004)

[4] Multivariate models included the following covariates: HIV-1 RNA Viral Load at baseline, sex (male vs. female), sexual risk factor (men who have sex with men vs. other), CCR5 delta 32 genotype, GBVC status at baseline, and CD4 count at baseline.
[2] Time: Specimens were drawn at a baseline visit and at a follow-up visit, on average 7 months later. Change is the follow up value minus the baseline value.
[3] Scale: where log transformation of the outcome was needed to ameliorate a skewed distribution (scale=log), coefficients were exponentiated to yield the fold-effect of a Non-C viral subtype on the outcome compared with C-containing viral subtype. Otherwise, raw, untransformed data were modeled (scale=raw), and the presented coefficients are interpreted as the raw difference in the outcome for Non-C viral subtype compared with C-containing viral subtype.

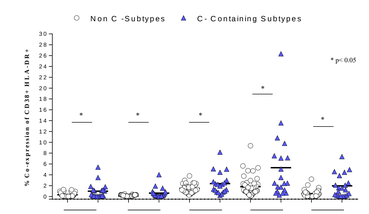


Figure 3. Co-expression of CD38+HLA-DR+ on naive and memory subsets of CD4 T Cells was compared between C- containing Subtypes and Non- C Subtypes group, in established HIV-1 infection. There was no statistically significant difference in the CD38+ HLA-DR+ co-expression of CD8 T Cells naive/memory subsets (data not shown).

Table 4: Regression models for the effect of Non-C HIV-1 viral subtype (compared with C-containing subtypes) on Regulatory CD4+ T cells. Coefficients (exponentiated into fold-effects where log scale was used) are presented with 95% confidence intervals (CI) and p-values.

Outcome (Marker)			Univariate Non-C Effect Size		Multivariable Adjusted Non-C Effect Size[4]	
	Time[2]	Scale[3]	Coefficient or Fold Effect (95%CI) (p-value)		Coefficient or Fold Effect (95%CI) (p-value)	
% CD4+ CD25high FoxP3+ Regulatory T cells						
	Baseline	log	5.241	(-0.878 to 11.360) (p=0.09)	5.770	(-2.292 to 13.832) (p=0.16)
	Follow Up	log	11.269	(2.940 to 19.598) (p=0.009)	8.719	(-1.053 to 18.491) (p=0.08)
	Change	log	6.485	(-1.814 to 14.785) (p=0.12)	2.819	(-7.168 to 12.806) (p=0.57)
% CD4+ CD25high FoxP3+ CD39+ Suppressor T cells						
	Baseline	raw	0.685	(-12.271 to 13.642) (p=0.92)	5.162	(-11.113 to 21.442) (p=0.53)
	Follow Up	raw	-6.538	(-21.181 to 8.105) (p=0.38)	-12.854	(-28.919 to 3.210) (p=0.11)
	Change	raw	-2.500	(-13.677 to 8.678) (p=0.66)	-11.338	(-25.323 to 2.646) (p=0.11)
% CD4+ CD25- FoxP3- CD39+ Inducer T cells						
	Baseline	raw	1.154	(0.689 to 1.931) (p=0.58)	1.119	(0.607 to 2.064) (p=0.71)
	Follow Up	raw	0.866	(0.504 to 1.488) (p=0.60)	0.765	(0.405 to 1.446) (p=0.40)
	Change	raw	0.778	(0.393 to 1.538) (p=0.46)	0.752	(0.333 to 1.694) (p=0.48)

[4] Multivariate models included the following covariates: HIV-1 RNA Viral Load at baseline, sex (male vs. female), sexual risk factor (men who have sex with men vs. other), CCR5 delta 32 genotype, GBVC status at baseline, and CD4 count at baseline.
[2] Time: Specimens were drawn at a baseline visit and at a follow-up visit, on average 7 months later. Change is the follow up value minus the baseline value.
[3] Scale: where log transformation of the outcome was needed to ameliorate a skewed distribution (scale=log), coefficients were exponentiated to yield the fold-effect of a Non-C viral subtype on the outcome compared with C-containing viral subtype. Otherwise, raw, untransformed data were modeled (scale=raw), and the presented coefficients are interpreted as the raw difference in the outcome for Non-C viral subtype compared with C-containing viral subtype.

HIV viral load, CD4+ T cell count and activation (percentage CD38+ HLA-DR+ expression in total CD4+ T cells) were similar between C and non-C groups at baseline. In the C group, but not in the non-C group, CD4+ T cell activation increased substantially between the two time-points. At the follow-up visit, CD4+ T cell activation was statistically significantly higher in the C group in T cell subsets, including naïve (CD45RA+ CD27+CCR7+) (p=0.005), central memory (CD45RA-CD27+CCR7+) (p=0.003), effector terminal (CD45RA+CD27-CCR7-) (p=0.002), and effector memory (CD45RA-CCR7-CD27-) (p=0.005) subsets. In multivariate models, differences in CD4+ CD38+HLA-DR+ expression remained statistically significant after controlling for baseline CD4+ and HIV viral load, gender, and transmission route/risk group.

Conclusions

Differences in CD38+HLA-DR+ expression on CD4+ T cells remained statistically significant after controlling for baseline CD4+ T cell counts, and HIV viral load, gender, and transmission route/risk group. Infection with clade-C-containing was associated with higher levels of T cell activation than infection with non-C containing HIV-1 clades circulating in Brazil. This effect was independent of CD4+ T cell counts, HIV viral load, gender, and infection route. HIV-1 related T cell activation may be clade-specific and the differences emerge upon establishment of chronic phase. Such findings may be involved in differences found in HIV pathogenesis and transmission in regions with prodominant C-clade circulation. Correlation of these findings with disease progression, end-organ disease, or HIV transmission is warranted.

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CAPES

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