HIV CONTROLLERS HAVE LOW FREQUENCIES OF INTACT PROVIRAL DNA

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Introduction

Elite controllers or suppressors (ES) are subjects who control viral replication without antiretroviral therapy. Studies using standard DNA PCR assays or the quantitative viral outgrowth assay (QVOA) have shown that these subjects have smaller viral reservoirs than chronic progressors (CP) on antiretroviral therapy (ART). However, standard DNA PCR assays measure both defective and replication-competent virus and the QVOA measures only a fraction of the replication-competent reservoir.

The objective of this study was to better approximate the size of the latent reservoir in ES by measuring the frequency of CD4+ T cells that contained intact proviral DNA.

Materials and Methods

Total and intact proviral DNA was measured in unfractionated CD4+ T cells from 10 CPs, 9 treatment naïve ES and 2 viremic controllers (VCs, VL < 1000 copies pre-treatment) on ART with the recently described intact proviral DNA assay (IPDA).

CD4+ T cells from 5 ES and the 2 VCs on ART were also cultured in the standard QVOA.

RESULTS

Table 1: Clinical characteristics of ES assessed in this study

	Gender	Years HIV positive	Years on ART	CD4 count	Protective HLA allele	Total proviral DNA per million CD4+ T cells	Intact proviral DNA per million CD4+ T cells	IUPM	Cells cultured (million)
ES3	F	26	NA	1014	HLA-B*57	6.78	< 1.00	<0.04	18
ES5	F	27	NA	918	HLA-B*57	108.1	1.61	<0.03	25
ES6	F	25	NA	916	HLA-B*57	30.41	1.25	< 0.02	29
ES9	F	21	NA	680	HLA-B*27 HLA-B*57	44.34	1.04	ND	ND
ES18	F	7	NA	970	None	19.00	5.58	ND	ND
ES24	M	8	NA	1269	HLA-B*57	372.57	191.31	4.57	5
ES31	F	11	NA	956	HLA-B*27 HLA-B*57	63.14	5.33	ND	ND
ES46	M	13	NA	667	HLA-B*57	9.85	1.95	<0.06	12
ES51	M	13	NA	1015	HLA-B*57	1.45	0.82	<0.03	20

Results

CHRONIC PROGRESSORS HAVE HIGHER FREQUENCY OF TOTAL PROVIRUSES RELATIVE TO VIREMIC CONTROLLERS AND ELITE SUPPRESSORS

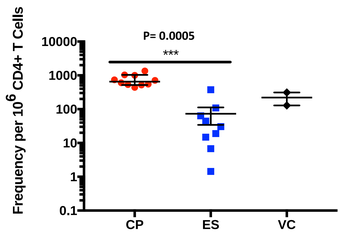


Fig.1: High frequency of total HIV proviruses in CP
Bulk CD4+ T cells from 10 CP, 2 VC and 9 ES were flash frozen and delivered to Accelevir Diagnostics for the IPDA assay from which the frequency of total proviral DNA was quantified and determined to be higher in CP than both VC and ES.

	(Median or average) frequency/million CD4+ T cells
CP	602.52
VC	30.4
ES	220.07

CHRONIC PROGRESSORS HAVE HIGHER FREQUENCY OF INTACT PROVIRUSES RELATIVE TO VIREMIC CONTROLLERS AND ELITE SUPPRESSORS

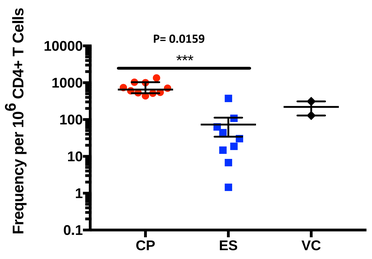


Fig.2: High frequency of intact HIV proviruses in CP
Bulk CD4+ T cells from 10 CP, 2 VC and 9 ES were flash frozen and delivered to Accelevir Diagnostics for the IPDA assay from which the frequency of intact proviral DNA was quantified and determined to be higher in CP than both VC and ES.
For one ES, the frequency of intact proviruses was imputed since it could not be determined via the IPDA (open square).
For another ES, the frequency of intact proviral DNA was higher than all patients studied.

	(Median or average) frequency/million CD4+ T cells
CP	36.95
VC	2.83
ES	1.61
ES24	191

NO SIGNIFICANT DIFFERENCE IN PROPORTION OF INTACT TO TOTAL HIV PROVIRAL DNA IN CHRONIC PROGRESSORS VERSUS VIREMIC CONTROLLERS AND ELITE SUPPRESSORS

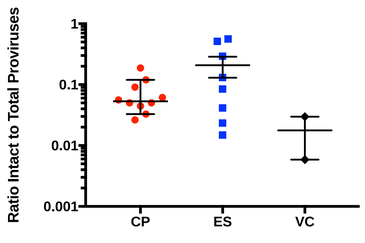


Fig.3: Ratio if intact to total proviral DNA is not significantly different between patient groups
The intact to total HIV proviral DNA ratio was calculated for 10 CP, 2 VC and 8 ES (ES with imputed intact proviral DNA frequency was omitted).
There was no significant difference across the 3 groups.

POSITIVE CORRELATION BETWEEN FREQUENCY OF INTACT AND INDUCIBLE HIV PROVIRAL DNA ACROSS ALL PATIENT GROUPS STUDIED

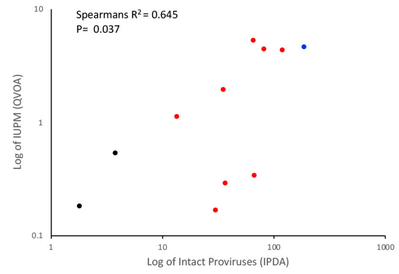
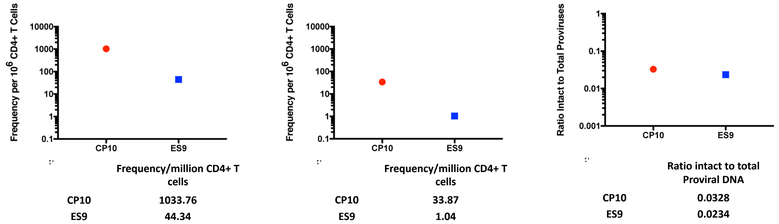


Fig.4: Positive correlation between IUPM and IPDA across all patients
Amongst patients where we had detectable intact viral frequencies from IPDA and inducible reservoir results from QVOA, we plotted a scatter graph and observed a positive correlation between the IUPM and IPDA.

Red- CP
Black- VC
Blue- ES

TRANSMISSION PAIR SHOWS SIMILAR FREQUENCIES (AND RATIO) OF TOTAL AND INTACT PROVIRAL DNA AS NON-TRANSMITTING PAIR PATIENT GROUPS



	Frequency/million CD4+ T cells
CP10	1033.76
ES9	44.34

	Frequency/million CD4+ T cells
CP10	33.87
ES9	1.04

	Ratio intact to total Proviral DNA
CP10	0.0328
ES9	0.0234

Fig.5: Discordant couple also demonstrates higher total and intact HIV proviral DNA in CP relative to ES with no difference in ratio of intact to total
CP10 and ES9 are a discordant couple first described 12 years ago who continue to maintain their clinical statuses.
From both patients, bulk CD4+ T cells were flash frozen and delivered to Accelevir Diagnostics for the IPDA assay from which the frequency of total and intact HIV proviral DNA was quantified and the ratio between the two calculated.
Similar to data observed in other patient groups, CP10 had a higher frequency of total and intact proviral DNA relative to partner ES9. There was however not much difference between the two patients when the ratio of intact to total proviruses was compared.

Conclusions

We show that ES have a median frequency of both intact proviral DNA and total proviral DNA that are both more than 1 log lower than the frequencies seen in CP. These findings suggest that while the absolute frequency of persistent HIV is lower in ES as compared to CP, the relative composition of that pool of persistent proviruses may not differ significantly. Furthermore, this data has implications for HIV cure strategies as it demonstrates that while this small reservoir size may contribute to control in ES, it is not an absolute requirement as one ES had a higher frequency of intact proviral DNA than all of the CP in our study.

Acknowledgements

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