LIMITED EVOLUTION OF THE VIRAL RESERVOIR IN A NONHUMAN PRIMATE MODEL OF ELITE CONTROL

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BACKGROUND

- Elite controllers, who control HIV infection in the absence of antiretroviral therapy, may hold the key to developing a cure.
- While this control effectively prevents high-level viremia and disease progression, the latent viral reservoirs persist in these individuals.
- In a recently described nonhuman primate model of elite control, pigtail macaques (PTMs) are infected with a variant of SIVmac239 (termed ΔGY) containing a 2 amino acid deletion in a conserved Env trafficking motif.
- After inoculation into PTMs, this virus produces high-level viremia, but is typically controlled to undetectable levels by host cellular immune responses in the absence of neutralizing antibodies. Animals remain clinically well for months-to-years.
- Because this phenotype resembles elite control of HIV in humans, we are investigating how the latent reservoir persists in this unique context.

METHODS

- We studied 4 male PTMs infected with ΔGY for >5 yrs. 3 maintained undetectable levels of plasma viral RNA (<15 copies/ml; elite controllers), while 1 exhibited low viral blips (>15 to <4300 copies/ml; partial controller).
- We used the SIV intact proviral DNA assay (IPDA) to measure the number of intact proviruses in blood and lymph nodes, 151 and 165 weeks post-infection, respectively.
- We developed a novel assay for full-length sequencing of SIV proviruses that uses 4 overlapping, non-multiplexed PCR amplicons following whole genome amplification (WGA) to analyze how individual proviruses persist.

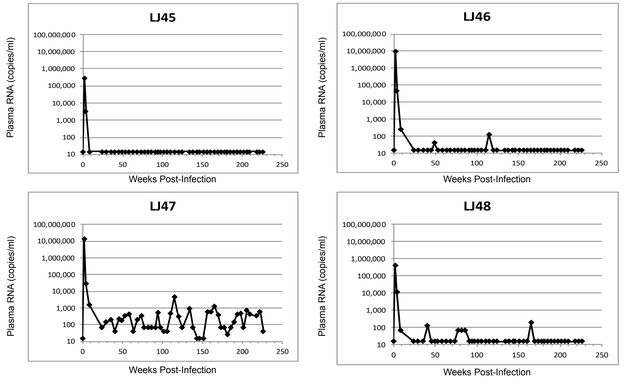


Figure 1: Plasma viral RNA levels for 4 long-term ΔGY-controlling PTMs

Minimal sequence changes in proviruses from ΔGY-elite controllers suggest a marked restriction of productive replication

RESULTS

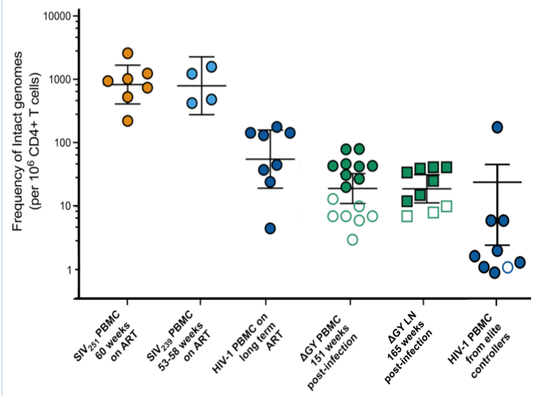


Figure 2: ΔGY-infected pigtail macaques have smaller latent reservoirs than SIVmac251-infected rhesus macaques
The SIV IPDA was used to measure the number of intact proviruses in CD4+ T cells from blood and lymph nodes. The data were compared to published data from SIVmac251-infected rhesus macaques on ART for 60 weeks, with humans on long term ART (A. Bender et al. 2019), and with human elite controllers (A. Kwaa et al. 2020). Open circles represent wells which had intact genomes with a frequency below the limit of detection of the assay and represent the maximum possible number of intact genomes.

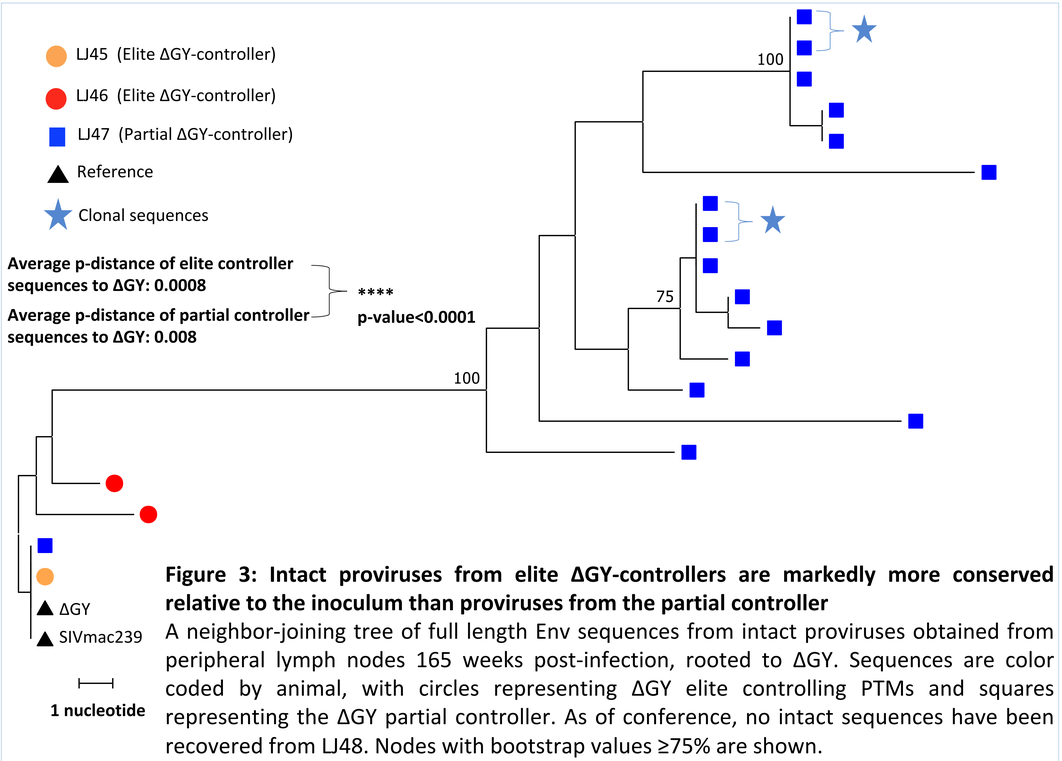


Figure 3: Intact proviruses from elite ΔGY-controllers are markedly more conserved relative to the inoculum than proviruses from the partial controller
A neighbor-joining tree of full length Env sequences from intact proviruses obtained from peripheral lymph nodes 165 weeks post-infection, rooted to ΔGY. Sequences are color coded by animal, with circles representing ΔGY elite controlling PTMs and squares representing the ΔGY partial controller. As of conference, no intact sequences have been recovered from LJ48. Nodes with bootstrap values ≥75% are shown.

Protein	Mutation	Present In	Significance	Notes
Env	R722G	0/3 elite 13/16 partial	Previously found in animals that lost or did not attain control	See M. Breed et al. 2013a, M. Breed et al. 2015
	S727P	0/3 elite 15/16 partial	Previously found in animals that lost or did not attain control	See P. Fultz et al. 2001, M. Breed et al. 2013a, M. Breed et al. 2013b, M. Breed et al. 2015
	R751G	0/3 elite 15/16 partial	SIVmac239 fitness mutation	
Gag	K165R	1/3 elite 15/16 partial	CTL Escape	In KP9 epitope restricted by Mane-A1*084 (shared by all animals)
	P376S	15/16 partial	CTL Escape	In AF9 epitope restricted by Mane-A4*14 (present in LJ47)
Tat	A96T	0/3 elite 15/16 partial	CTL Escape	In KSA10 epitope restricted by Mane-A1*084 (shared by all animals)
	K115E	1/3 elite 15/16 partial	CTL Escape	In KVA10 epitope restricted by Mane-A1*084 (shared by all animals)
	V122M	0/3 elite 1/16 partial	CTL Escape	In KVA10 epitope restricted by Mane-A1*084 (shared by all animals)

Table 1: Mutations of known significance in full length intact ΔGY proviral sequences

CONCLUSIONS

- The smaller reservoirs of ΔGY-elite controllers relative to those of ART-treated noncontrollers is similar to reservoir sizes of human elite controllers compared to those of ART-treated noncontrollers (A. Kwaa et al. 2020).
- The presence of CTL escape mutations in the partial controller demonstrates that the CTL response can be a potent driver of virus evolution in reservoirs.
- The minimal sequence changes in proviruses from ΔGY-elite controllers suggest a marked restriction of productive replication.
- Efforts to identify proviral integration sites in this model are ongoing, as are studies to assess interventions that can impact the size and the persistence of viral reservoirs.

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