Abstract # 217

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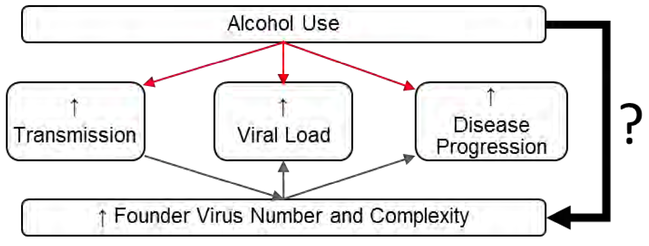
# CHRONIC ALCOHOL ABUSE ACCELERATES SIV DISEASE PROGRESSION THROUGH ESTABLISHMENT OF DIVERSE VIRUS POPULATIONS AND INCREASED VIRAL EXPRESSION IN TISSUE RESERVOIRS

**S. Robichaux, A.M. Amedee**

Department of Microbiology, Immunology and Parasitology, Louisiana State University Health Sciences Center, New Orleans, LA

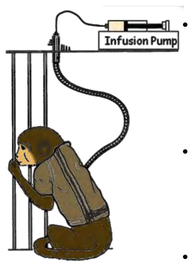
## INTRODUCTION

- Alcohol abuse adversely affects multiple aspects of HIV disease including, increasing HIV transmission, decreasing antiretroviral therapy (ART) efficacy and accelerating disease progression.
- The prevalence of alcohol abuse is approximately 2x greater among those with HIV compared to the general population.
- The biological mechanism(s) by which alcohol abuse modulates HIV transmission and subsequent establishment of persistent reservoirs remain unresolved.
- The chronic binge alcohol (ALC) treated, SIV-infected rhesus macaque provides an ideal model to investigate the effects of ALC consumption on acute virus and host interactions

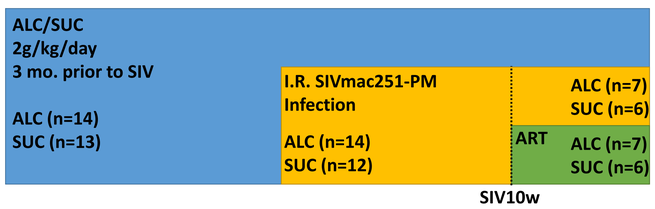



- Recent studies have shown that the number of transmitted founder viral genotypes and complexity of the founder virus population are related to viral load and the rate of disease progression; with increased complexity associated with increased viral loads and accelerated disease progression.
- Linking these observations, we hypothesize that alcohol consumption effects SIV disease progression by directly altering transmission.

## STUDY DESIGN




- Three months prior to intra-rectal SIV inoculation, animals were treated with daily infusions of ethanol (n=14), or sucrose (SUC; n=12) as control; ethanol treatment achieved a 50mM peak blood ethanol concentration post administration.
- Following SIV inoculation, blood samples were obtained longitudinally for measures of SIV levels in plasma and PBMC. Peripheral lymph node and gut biopsies were obtained at viral set point (SIV6w-10w), and mononuclear cells were isolated for SIV RNA and DNA measures.
- In first positive plasma samples, founder viral genotypes were identified by gp160 single genome amplification (SGA) and V1-V2-targeted next generation sequencing (NGS) approaches.
- Viral RNA and DNA levels were measured by TaqMan real-time RT-qPCR and qPCR assays that specifically target the SIV gag gene.




## Acute SIV Viremia

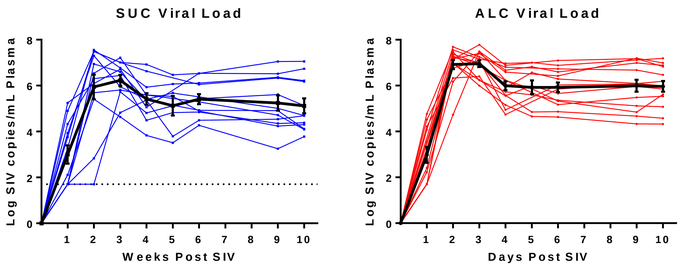



**Figure 1.** Plasma SIV RNA levels over the first 10 weeks post SIV-infection in 12 SUC (Blue) and 14 ALC (Red) treated macaques. At viral set point (6-10 weeks post SIV), animals administered alcohol showed significantly higher viremia (mean Log 5.95 SIV copies/mL plasma) relative to sucrose treated controls (mean Log 5.22 SIV copies/mL plasma) (p<0.05).

## Stock & Founder Viral Genotypes

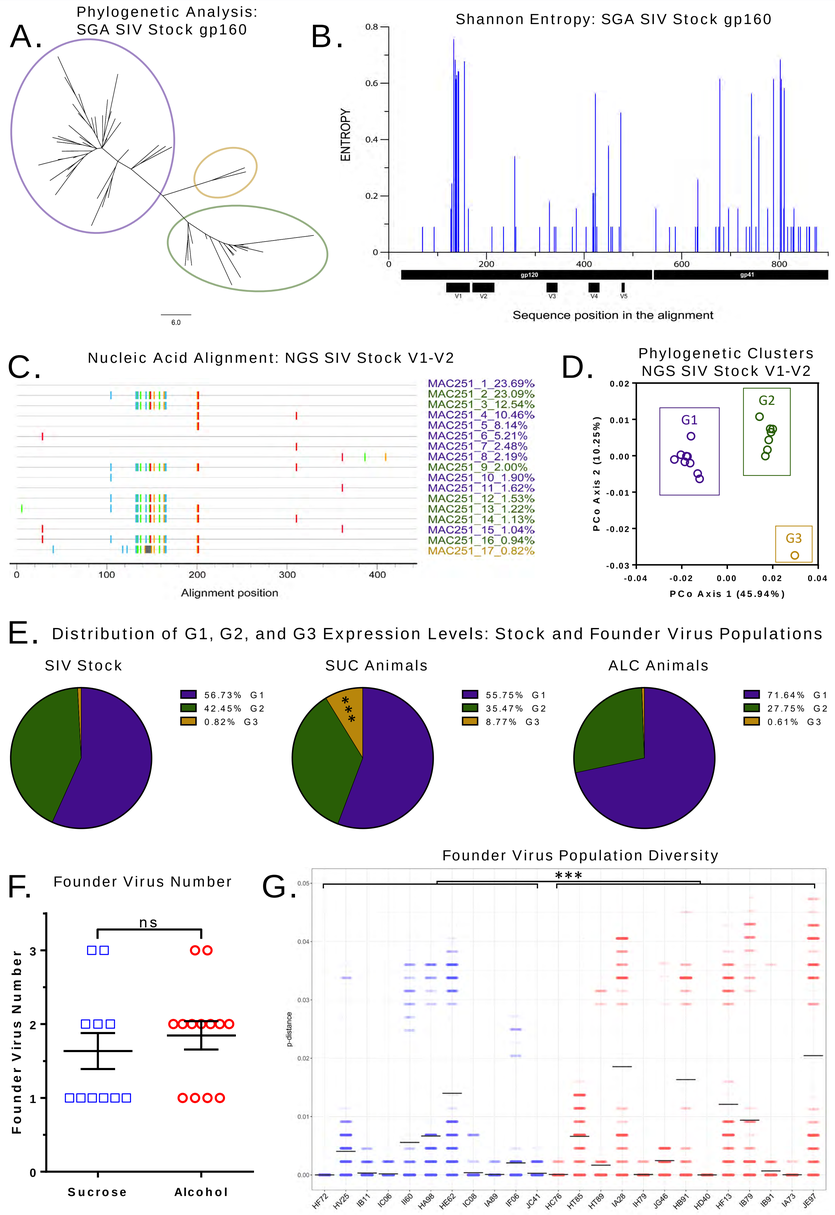



**Figure 2. Founder (First Positive Plasma) Virus Analysis**. **(A)** Maximum parsimony tree (*PAUP4.0a150*) constructed from 56 SGA $SIV_{mac251}$ inoculum gp160 sequences; circles designate *de novo* OTU clusters (>1% average distance) (*Mothur*). **(B)** Shannon entropy inoculum gp160 sequences demonstrating V1 hyper-variability. **(C)** NGS $SIV_{mac251}$ inoculum V1V2 sequence alignment (~30,000 sequences; Q score ≥ 20) and **(D)** principal coordinate plot demonstrating 3 distinct V1V2 OTU clusters (>3% average distance) (*Mothur*). **(E)** Percent expression of G1, G2, G3 OTUs in SIV inoculum, sucrose animals, and alcohol animals; the selective transmission and expression of minor stock genotype, G3, was significantly pronounced in SUC animals, while the transmission and expression profile of ALC animals was more analogous to the inoculum. **(F)** Number of founder viral V1V2 genotypes in sucrose and alcohol animals. **(G)** Diversity of founder virus V1V2 populations in 11 SUC and 13 ALC animals; founder virus populations of ALC animals were significantly more diverse relative to controls with alcohol animals exhibiting a >2-fold higher (0.31% Sucrose vs 0.68% Alcohol) diversity. *** p<0.001.

## Set Point Virus Diversity

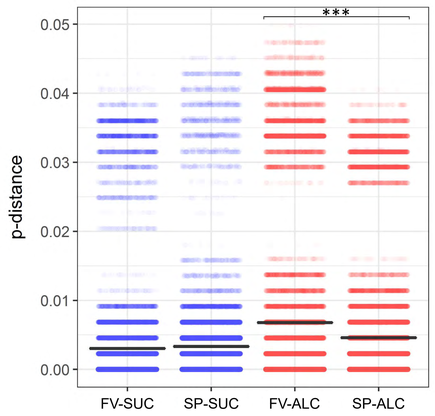



**Figure 3. Set Point (SIVw10 Plasma) Virus Analysis**. Diversity of set point virus V1V2 populations in 11 SUC and 13 ALC animals (0.33% Sucrose vs 0.45% Alcohol). Concurrent with a ~5.5 fold increase in viremia, ALC animals exhibited a significant, 35% reduction in V1V2 population; while, sucrose controls showed a modest, 10% increase in diversity over the same time course. *** p<0.001

## Set Point Viral Replication Kinetics

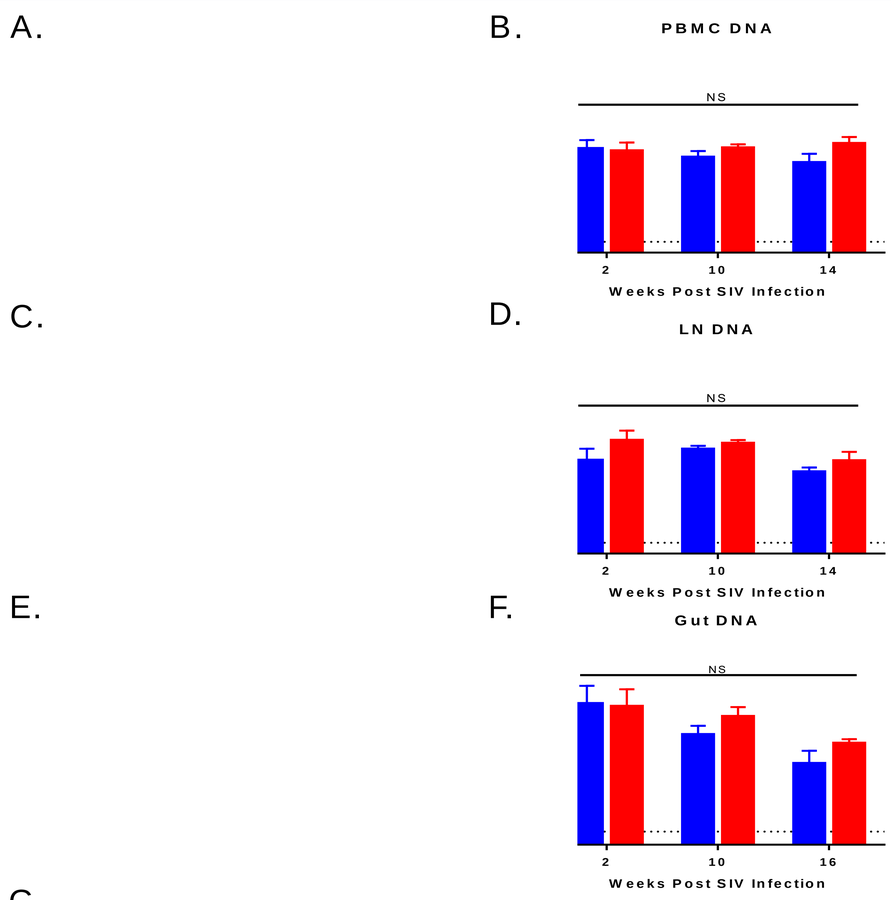



**Figure 3. SIV Replication Kinetics.** Mean SIV RNA levels in **(A)** plasma, **(C)** peripheral lymph nodes, and **(E)** duodenal tissues at SIVw2 (peak viremia), and set-point (SIV10w & SIV14-16w). For each tissue, one-way ANOVA revealed a significant effect of alcohol consumption on SIV RNA levels. Tissue and time-matched mean SIV proviral burdens in **(B)** PBMC, **(D)** peripheral lymph nodes, and **(F)** duodenal tissues. Tissue specific, one-way ANOVA did not reveal a significant effect of alcohol on proviral burden, indicating similar numbers of SIV-infected cells in ALC and SUC animals. Together, these analyses suggest that increased SIV RNA levels in ALC animals is a consequence of increased SIV replication kinetics. **(G)** 11 ALC animals and 10 SUC animals which showed a predominant expression (>90%) of G1, G2, or G3, and 3 (2 ALC; 1 SUC) animals with mixed G1/G2 FV populations. One-way ANOVA showed a significant affect of set-point genotypes on set-point viral load, indicating the transmission and selective expression of specific genotypes impacts set-point viral load. *p<0.05, **p<0.01.

## SUMMARY

- Utilizing single genome amplification of gp160 and next generation sequencing of V1-V2, we characterized the founder virus population in 13 ALC and 11 SUC animals intra-rectally inoculated with SIVmac251. The stock quasispecies was comprised of three main genotypic clusters (G1, G2, G3).
- Although similar numbers of founder virus types were transmitted in ALC and SUC animals, SUC animals expressed higher numbers of sequences mapping to the minor stock genotype G3 as compared to ALC animals.
- The diversity of founder viral populations in ALC animals was higher than those observed in SUC controls (0.31% Sucrose vs 0.68% Alcohol)
- At viral set-point, the viral population in SUC animals revealed a modest 6.45% increase in diversity relative to founder virus populations. In contrast, ALC animals exhibited a 32.35% reduction in diversity.
- At viral set point, similar numbers of infected cells (SIV DNA) were found in peripheral blood as, well as peripheral lymph nodes and gut biopsies from SUC and ALC animals. Concurrently, significantly higher viral RNA expression was observed in plasma, peripheral lymph node and gut tissue reservoirs of ALC animals compared to controls.
- These observations suggest alcohol consumption:
  - mitigates early host selective pressures to influence the founder viral population.
  - promotes the selective expression of specific genotypes early in the disease course.
  - Results in higher viremia and more rapid disease progression from the greater expression of specific viral genotypes, rather than an increased number of infected cells.