# Low Social Rank Prior to SIV or SHIV Infection Associates with Higher Viral Load in Chronic Infection

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## ABSTRACT

**Background:** The tempo of disease progression in HIV-infected humans and SIV-infected macaques is quite variable among individuals, with high levels of virus replication predicting shorter survival. Prolonged exposure to stress may alter immune system function even in the absence of HIV or SIV infection, and disruptions in housing environment, a known stress stimulus, shorten survival time in SIV-infected macaques. Macaques housed in social groups establish a relatively stable matriarchal hierarchy. Within these hierarchies, subordinate animals consistently demonstrate elevated markers of chronic stress compared to dominant ones. In the current study we tested the hypothesis that stress history, as dictated by the subject's social rank prior to study assignment, predicts viral load during chronic SIV infection.

**Methodology:** All rhesus macaques originated from social groups housed at Yerkes National Primate Research Center with established rank systems prior to isolation for further studies involving SIV or SHIV infection. Viral loads from five previous studies (four involving SIVmac and one involving SHIVsf162p3) were measured by RTPCR as copies of SIV RNA/ml of plasma, and the relative data were retrospectively compiled and analyzed. All animals' social and medical histories prior to study assignment were obtained from colony records. Retrospective individual animal meta-analysis was conducted on viral load data across the acute, early chronic, and late chronic phases of infection.

**Results:** A total of 62 macaques infected with SIV or SHIV were stratified based on the pre-infection social rank, and then evaluated longitudinally throughout infection for viral load in plasma. We found that, in the acute phase of SIV or SHIV infection, the level of virus replication was not predicted by social rank prior to study assignment (p=0.37). Interestingly, in both early and late chronic phases of the infection, social rank prior to study assignment significantly influenced the level of virus replication (p=0.01 and p=0.002 for subordinate vs. dominant/mid). In the early chronic phases of infection copies of SIV RNA/ml of plasma in subordinate subjects (mean = 5.202 $\log_{10}$ (copies/ml) was elevated compared to subjects that originated from dominant/mid ranks (mean = 4.488 $\log_{10}$ (copies/ml). Also during late chronic infection, higher viral loads were observed in subordinate subjects (mean = 5.806 $\log_{10}$ (copies/ml) compared to subjects from dominant/mid social ranks (mean = 4.213 $\log_{10}$ (copies/ml).

**Conclusions:** These data demonstrate that social history prior to SIV or SHIV infection can influence viral load in the chronic phases of the disease, with increased exposure to stress being associated with higher levels of virus replication during chronic infection.

### Table 1. Subjects

| ID | Virus | Dose | Route of Infection | Sex | Social Rank | ID | Virus | Dose | Route of Infection | Sex | Social Rank |
|---|---|---|---|---|---|---|---|---|---|---|---|
| RCm6 | SIVmac251 | 300 | IR | F | H | RHz8 | SHIVsf162p3n | 300 | IV | F | H |
| RCq6 | SIVmac251 | 300 | IR | F | L | RIh9 | SHIVsf162p3n | 300 | IV | F | H |
| RMn9 | SIVmac251 | 300 | IR | F | L | RIw12 | SHIVsf162p3n | 300 | IV | F | M |
| RNr10 | SIVmac251 | 300 | IR | M | M | ROp8 | SHIVsf162p3n | 300 | IV | F | M |
| RNt7 | SIVmac251 | 300 | IR | F | L | ROz12 | SHIVsf162p3n | 300 | IV | F | M |
| RQk10 | SIVmac251 | 300 | IR | F | L | RPo12 | SHIVsf162p3n | 300 | IV | F | H |
| RUf9 | SIVmac251 | 300 | IR | F | L | RQm6 | SHIVsf162p3n | 300 | IV | F | H |
| RVi10 | SIVmac251 | 300 | IR | M | H | RQu8 | SHIVsf162p3n | 300 | IV | F | H |
| RZu10 | SIVmac251 | 300 | IR | M | L | RWf12 | SHIVsf162p3n | 300 | IV | F | H |
| RAy10 | SIVmac239 | 1,000 | IR | M | L | RYa12 | SHIVsf162p3n | 300 | IV | M | L |
| REf10 | SIVmac239 | 10,000 | IR | F | M | RZt8 | SHIVsf162p3n | 300 | IV | F | M |
| REw10 | SIVmac239 | 1,000 | IR | M | H | RAd9 | SIVmac239 | 3,000 | IV | F | L |
| REw9 | SIVmac239 | 1,000 | IR | F | H | RAk9 | SIVmac239 | 3,000 | IV | F | H |
| REz10 | SIVmac239 | 10,000 | IR | M | H | RAy8 | SIVmac239 | 3,000 | IV | F | M |
| RGd11 | SIVmac239 | 10,000 | IR | M | L | RDa10 | SIVmac239 | 3,000 | IV | F | M |
| RGn10 | SIVmac239 | 10,000 | IR | F | H | RDz10 | SIVmac239 | 3,000 | IV | F | H |
| RHa9 | SIVmac239 | 10,000 | IR | M | H | RFn7 | SIVmac239 | 3,000 | IV | F | L |
| RIf11 | SIVmac239 | 10,000 | IR | F | H | RFs12 | SIVmac239 | 3,000 | IV | F | H |
| RJz10 | SIVmac239 | 10,000 | IR | M | M | RHd10 | SIVmac239 | 3,000 | IV | F | M |
| RKe11 | SIVmac239 | 10,000 | IR | F | L | RJm7 | SIVmac239 | 3,000 | IV | F | M |
| ROf11 | SIVmac239 | 1,000 | IR | M | H | RKv7 | SIVmac239 | 3,000 | IV | F | L |
| RPt10 | SIVmac239 | 1,000 | IR | M | M | RLf8 | SIVmac239 | 3,000 | IV | F | M |
| RPz10 | SIVmac239 | 10,000 | IR | M | M | RLw10 | SIVmac239 | 3,000 | IV | F | H |
| RVv9 | SIVmac239 | 1,000 | IR | F | L | RNw10 | SIVmac239 | 3,000 | IV | F | H |
| RWb11 | SIVmac239 | 10,000 | IR | M | M | RZc9 | SIVmac239 | 3,000 | IV | F | H |
| RWm10 | SIVmac239 | 1,000 | IR | F | H | RBf7 | SIVmac239 | 3,000 | IV | F | H |
| RYe11 | SIVmac239 | 10,000 | IR | M | H | RBt10 | SIVmac239 | 3,000 | IV | F | M |
| RYn10 | SIVmac239 | 1,000 | IR | F | L | RFe9 | SIVmac239 | 3,000 | IV | F | L |
| RBh11 | SHIVsf162p3n | 300 | IV | F | L | RHm13 | SIVmac239 | 3,000 | IV | M | H |
| RBz8 | SHIVsf162p3n | 300 | IV | M | L | RYq6 | SIVmac239 | 3,000 | IV | F | M |
| REl9 | SHIVsf162p3n | 300 | IV | F | H | RZo10 | SIVmac239 | 3,000 | IV | F | M |

Dose: tissue culture infectious doses 50 (TCID50): Route of Infection: IR = intrarectal, IV = intravenous; Social Rank: H = high or dominant, M = middle, L = subordinate or low

## Frequency of Aggression Received Determines Social Rank

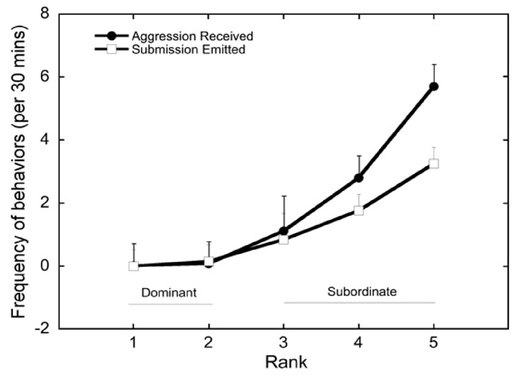


**Figure 1.** Each subject's relative dominance (or social) rank within her social group is determined based on outcomes of dyadic agonistic interactions in which a subordinate female is one who unequivocally emits a submissive behavior in response to an approach or to an actual aggressive act from a more dominant animal (Bernstein 1976). Thus, although dominant rank can be determined by who aggresses whom, it is more frequently determined by who submits to whom, and categorized into high (dominant), medium and low (subordinate) ranks. Each animal's relative rank was calculated as the ratio of her rank to the total number of animals in her group, exclusive of animals <12 months old. This figure is from a previous study (Michopoulos et al, 2011) and is shown here to illustrate the frequency of aggression and how the animals are assigned rank.

## RESULTS

## Subordinate Social Status Prior to Infection Predicts Higher Viral Loads During Chronic Infection

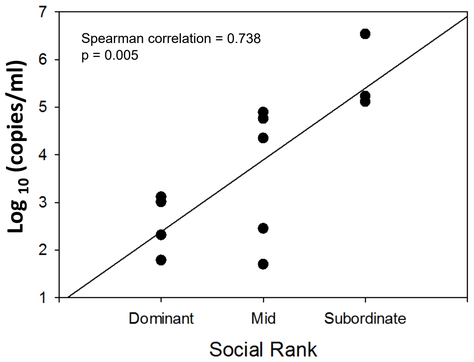


**Figure 2.** Initial retrospective assessment of the effects of social rank prior to study assignment on viral load correlated social rank and viral load on day 65 post-infection. Subjects in this analysis originated from the same study and had equivalent experimental parameters. In addition, all subjects in this analysis were adult females.

These data demonstrate that there is a significant correlation between the social rank of the subject prior to study assignment on the viral load as measured 65 days after infection with SIV. All subjects are individually housed after leaving Yerkes Field Station and the time between leaving their social groups and this day 65 viral load measure is approximately 3-4 months.

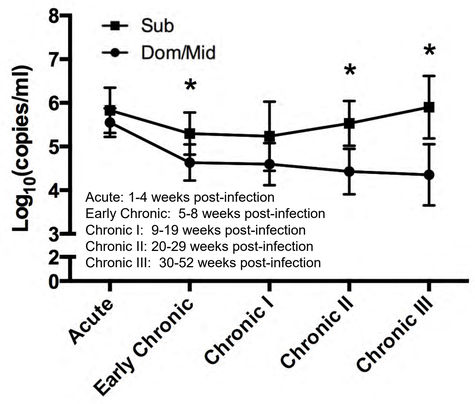


**Figure 3.** Given the effects of social rank on viral load in the study presented in Figure 2, a larger analysis was pursued gathering retrospective data from 6 separate studies. General study parameters are pictured in Table 1.

Individual animal meta-analysis demonstrated that social rank prior to study assignment predicted viral load in early chronic and chronic stages of infection, but did not influence viral load in the acute stages of the infection. Data are represented as group means with upper and lower 95% confidence intervals.
* indicates $p < 0.05$

Neither age or sex significantly influenced viral load in this analysis.

## Subjects from Subordinate Social Ranks Have Lower CD4+ T Cell Counts during Chronic SIV Infection

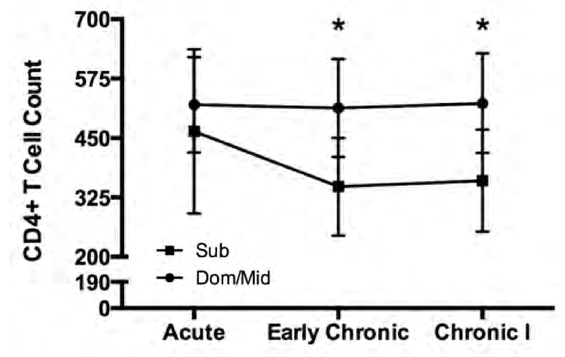


**Figure 4.** CD4+ T cell counts reflected similar dynamics to those of viral load throughout the course of infection. Mean CD4+ T cell counts did not differ between the groups until the chronic phase of infection (mean = 348 cells in the subordinate group compared to mean = 513 cells in the dominant/mid group, p=0.03). Data are presented as group means with upper and lower 95% confidence intervals. Acute: 1 – 4 weeks post-infection, Early Chronic: 5 – 8 weeks post-infection, Chronic 1: 9 – 19 weeks post-infection

## Subjects from Subordinate Social Ranks Have a Greater Increase in CD4+ T Cell Activation over Time

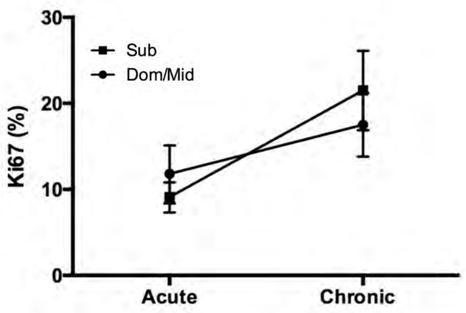


**Figure 5.** Preliminary analysis of a small subset of subjects demonstrate that CD4+ T cells have a greater mean difference in activation between the acute and chronic stages of infection in the subordinate subjects (12.4% increase) as compared to the dominant subjects (5.7% increase; difference in delta was p = 0.0097). Data are presented as group means with upper and lower 95% confidence intervals.

## CONCLUSION

These data demonstrate that social history prior to SIV or SHIV infection can influence viral load as well as CD4+ T cell counts in the chronic phases of the disease, with increased exposure to stress being associated with significantly higher levels of virus replication and lower numbers of CD4+ T cells during chronic infection.

## ACKNOWLEDGMENTS

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