SIV Dynamics in the CD8-Depleted vs. Non-CD8-Depleted Rhesus Macaque Model for NeuroAIDS

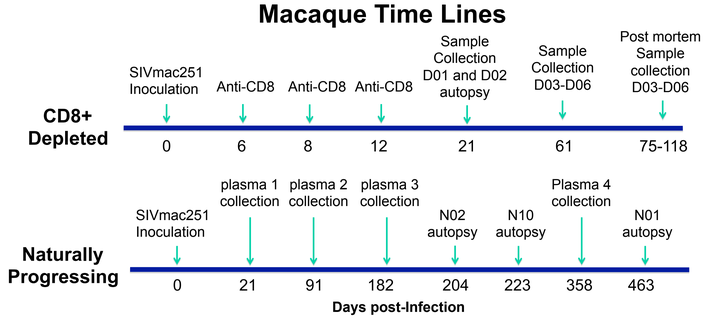
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Background. The CD8⁺ T-cell-depleted and SIV-infected macaque is frequently used for studying the pathogenesis of HIV, especially as it pertains to neuropathogenesis. CD8⁺ T-cell depletion results in the inability to control viral replication and these macaques rapidly progress to SIV-associated encephalitis, which ensures the collection of a large amount of data for neuropathology researchers. However, it is unknown how CD8⁺ depletion impacts viral sequence evolution within a recently infected host. In this study, we compared biological indicators of disease progression, RNA viral loads, and sequences collected at 3 weeks post-infection between naturally progressing and CD8⁺ depleted macaques in order to observe how SIV sequence dynamics may vary between the two models.

Methods. Two groups of macaques were inoculated with the SIVmac251 viral swarm. One group, consisting of six macaques, was CD8-depleted, while another group, consisting of twelve macaques, was allowed to progress naturally with SIV. At three weeks post-infection, SIV envelope sequences were generated from tissue RNA (plasma, CD14, and bronchial lavage) from all macaques using a single genome sequencing approach. Two hundred viral swarm sequences were also generated for comparison to the macaque data. Alignments were performed using Clustal in MEGA4[1] with further optimization performed by hand. Within-sample distance analysis was performed in MEGA4. Maximum-Likelihood phylogenetic trees with 1000 bootstrap replicates were generated for the 3-week and viral swarm sequences using PhyML[2]. Cluster analysis was performed using Clusterer[3]. Selection analysis was performed using fixed effects likelihood method (FEL) on the DataMonkey website[4].

Macaque Time Lines



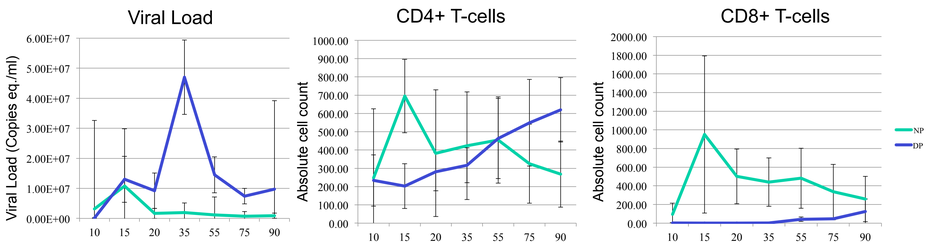


Figure 1. Time lines for experiment, viral load, CD4 and CD8 levels. Viral load and T-cell counts were averaged and are shown with standard deviation up to 90 days post infection (dpi). Unpaired *t* tests were performed to compare results. NP=naturally progressing, DP=CD8 depleted. **Results**. A productive SIV infection is reflected in the viral loads; viral loads were significantly higher in the DP macaques from 20-90 dpi ($p<0.001$), at which time they remained relatively the same as the NP macaques. CD4 counts were significantly different only at 15 dpi ($p<0.001$), although CD8 T-cell counts were significantly different until 55 dpi ($p<0.001$).

Table 1. Macaque Pathology Summary

Macque ID★	Euthaniza-tion Time (dpi)	SIV detected plasma (21 dpi)	SIV detected brain (autopsy)	Brain Pathology	SAIDS	Other Pathology*
D01^	21	Yes	Yes	Encephalitis	Yes	Inflammation
D02^	21	Yes	Yes	Encephalitis	Yes	Inflammation
D03	75	Yes	Yes	Encephalitis	Yes	Lungs
D04	91	Yes	Yes	Encephalitis	Yes	Gut
D05	95	Yes	Yes	Encephalitis	Yes	Eyes
D06	118	Yes	Yes	Encephalitis	Yes	lungs
N01	463	Yes	No	No	Yes	Poor Health
N02	204	Yes	Yes	No	Yes	Gut/Lungs
N03	554	Yes	No	No	Yes	Poor Health
N04	260	Yes	No	No	Yes	Lungs
N05	373	Yes	No	No	Yes	Lungs
N06^	20	Yes	No	No	No	Inflammation
N07^	20	Yes	No	No	No	Inflammation
N08+	>354	Yes	No	No	NA	NA
N09	300	Yes	No	No	Yes	Lymphoma
N10	223	Yes	Yes	Encephalitis	Yes	Lungs/eyes
N11+	>354	Yes	No	No	NA	NA
N12	275	Yes	No	No	Yes	Gut/Lungs

★ D = CD8-depleted and N = naturally progressing. * Macaques were sacrificed upon any indication of poor health. At autopsy other SIV-associated pathologies are indicated. ^Macaques D01, D02, N06 and N07 were sacrificed early, prior to the onset of SAIDS, and to study early viral entry into the brain. +Macaques N08 and N11 are still living.

Within Population Diversity

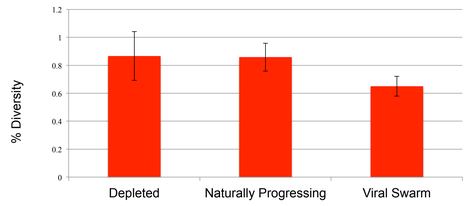


Figure 2. The percent base substitutions per site averaged over all sequence pairs were calculated in MEGA4 using the Tamura 3-parameter model. Standard deviation estimates were obtained by bootstrap (1000 replicates). Unpaired *t* tests were performed to compare results. **Result.** At three weeks post infection, both macaque populations were significantly more variable than the viral swarm sequence population($p<0.001$), but were not significantly different from each other ($p>0.1$).

Representative Phylogenies

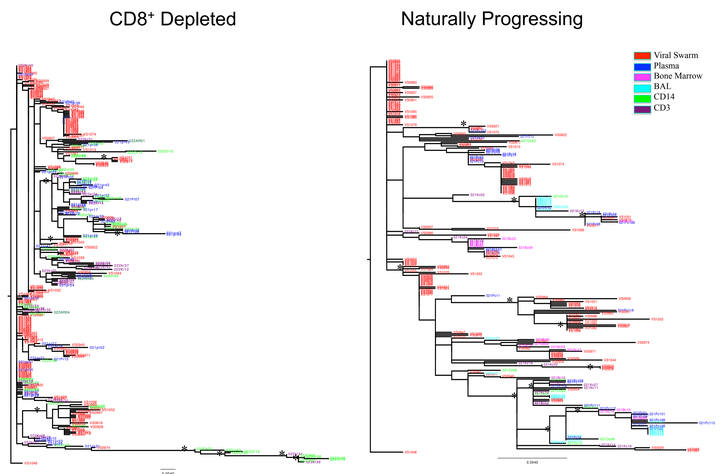
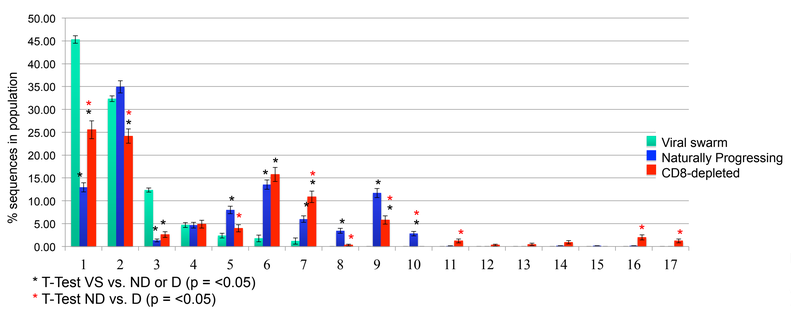


Figure 3. Maximum-likelihood phylogenies were constructed in PhyML for 3 week sequences (plasma, bone marrow, CD3, CD14 and BAL) for all three week sequences within CD8+ depleted and naturally progressing sequence populations. Shown are representative trees for two macaques (D02 and N02). Significant branches are marked with an asterisk. **Result.** In general, viral swarm sequences were mixed with three week samples from different tissues; however, the depleted macaques had one highly differentiated clade with sequences generated from CD14 expressing cells (circulating monocytes) and CD3 cells (T-cells), shown at the bottom of the tree.

Clustering Analysis



* T-Test VS vs. ND or D ($p = <0.05$)
* T-Test ND vs. D ($p = <0.05$)

Figure 4. All sequences were clustered at a level of 95% identity using the program Clusterer. Seventeen clusters were identified. Fisher's exact two-tailed *t* test was used to identify the significant difference between populations within each cluster. **Result.** Similar to phylogenetic analysis, a large degree of overlap was found between macaque sequences and viral swarm sequences. The CD8-depleted sequences appeared in more clusters, suggesting more freedom to evolve due to a weakened immune system.

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Signature Pattern Analysis

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                          V1                                  V2                                  C2          V3                  V4
                 118       142               164       174       189               213       333       410
SIVmac239     TDRWGLTKSI  VDMVNETSSCIAQDN QMISCK GLKRDKKKEY SADLVCEQGNNTGN  TSVIQ WDAIR QPINDRPKQAWCWFGGKWK  WVEDRNT..ANQKPKEQHKR
Cluster 1     --K------S  I----------TH-- ---G-- ------T--- -T--------S-D-  --I-- --T-- --V-E--N--------N--  ------DL..TT-R---R-R-
Cluster 2     --K------S  I----------TH-- ---G-- ------T--- -T--------S-D-  --I-- --T-- --V-E--N--------N--  ------DL..TT-R---R-R-
Cluster 3     --K------S  I----------TH-- ---G-- ------T--- -T--------S-D-  --I-- --T-- --V-E--N--------N--  -------LTLTT--LR-R---
Cluster 4     --R------  V----------TH-- ---G-- ------T--- -T--------S-D-  --I-- --T-- --V-E--N--------N--  ------DL..TT-R---R-R-
Cluster 5     --R------S  I----------TH-- ---G-- ------T--- -T--------S-D-  --I-- --T-- --V-E--N--------N--  ------DL..TT-R---R-R-
Cluster 6     --K------S  I----------TH-- ---G-- ------T--- -T--------S-D-  ------ --T-- --V-E--N--------N--  ------DL..TT-R---R-R-
Cluster 7     --R------S  I-----------N-- ------- ------T--- -T--------S-D-  ------ --T-- --V-E--N--------N--  ------DL..TT-R---R-R-
Cluster 8     --K------S  I----------TH-- ---G-- ------T--- -T--------S-D-  ------ --T-- --V----N--------N--  ------LTLTT--S--R---
Cluster 9     --K------S  I----------TH-- ---G-- ------T--- -T--------S-D-  ------ --T-- --V----N--------N--  -I----L..TT-R---R-R-
Cluster 10(ND)--K------S  I----------TH-- ---G-- ------T--- -T--------S-D-  --I-- --T-- --V----N--------N--  -I----DL..TT-R---R-R-
Cluster 11(D) --K------S  I----------TH-- ---G-- ------T--- -T--------S-D-  --I-- --T-- ------------------  --------..----------
Cluster 12    --R------S  I--I-------VH-- ---G-- ---------- ----------S---  ------ ------ ----------R----  --------..----------
Cluster 13    --K------L  I----------VH-- ------ ------T--- -T--------S-D-  ------ --T-- --V-E--N--------N--  ------DV..T------R-R-
Cluster 14    --R------S  I--I-------VH-- ---G-- ------T--- -T--------S-D-  --I-- --T-- --V-E--N--------N--  ------DL..TT-R---R-R-
Cluster 15    --R------S  IH---D-N--S--- ---G-- ------T--- -T--------S-D-  --I-- --T-- --V-E--N--------N--  ------DV..TT-R---R-R-
Cluster 16(D) --R------I  V---------------- -I---- ---E------ ---------D---  ------ ------ ------------------  --------..----------
Cluster 17(D) --R------I  V---------------- -I---- ---E------ ---------D---  ------ ------ --V-E--N--------N--  ------DL..TT-R---R-R-
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Figure 5. A consensus sequence was generated for each cluster and aligned to the reference SIVmac239 sequence. Shown are excerpts from the full alignment from regions V1, V2, C2, V3 and V4, which contain amino acid changes from consensus. Positional information is provided above sequences. **Result.** A majority of signatures occurred in variable loop domains. Clusters 7, 16 and 17 contain sequence motifs that have been previously suggested to contain macrophage-specific or brain-specific sequence motifs[5].

Positive Selection

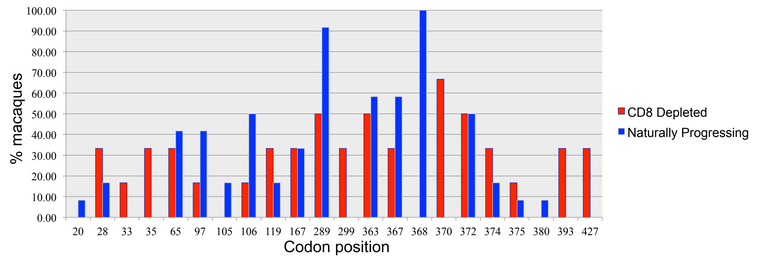


Figure 6. Codon selection analysis was performed using FEL in HyPhy for each macaque. Positively selected positions are listed on the x-axis. The frequency that each selected position was observed in each macaque in the two populations is indicated on the y-axis. **Result.** Overall, the number of positively selected sites between the two groups was about the same; however, some sites were interesting in that they occurred at high rates in the naturally progressing macaques (289, 368), which could be due to the virus adjusting to an intact immune system.

Discussion and Conclusions.

Both the CD8⁺ depleted and naturally progressing macaques maintained sufficient viral loads over time to indicate a productive SIV infection. Despite the fact that CD8⁺ T-cell counts were significantly different from 15-55 dpi, CD4⁺ T-cell counts were only significantly different at day 15 and there was only a short window of time where an altered immune system could impact how the virus was evolving. The study shows that CD8⁺ depletion minimally impacts SIV sequence evolution during early infection, when biological indicators of disease are perturbed; however, some changes were noted, including the emergence of a small number of highly diverse sequences. These genotypes could be explained by the virus evolving in a weakened immune system and have also been suggested to contain neurotropic signatures[5]. The development of neurotropic SIV signatures in macaques that develop encephalitis is in line with HIV studies that have suggested brain-specific forms of HIV evolving in patients that develop neurological disorders[6].

The most important difference between the two populations appeared in Table 1, where only one of the twelve naturally progressing macaques developed encephalitis before required euthanization due to SAIDS; however, in larger studies, about 25% of naturally progressing macaques will develop neuroAIDS within 90-120 days post infection. All of the CD8⁺ depleted macaques in this study developed encephalitis. The CD8⁺-depleted and SIV-infected macaque model increases the possibility of understanding neuroAIDS, as well as reduces costs and time associated with research. Further studies are underway to assess and compare sequence evolution over the full course of infection in the naturally progressing and CD8 depleted macaque populations.

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