Extracorporeal Inactivation of SIV infected Blood Using the Energex Systems Hemo-Modulator

Project 3370

Study conducted by

Preston A. Marx
Professor of Tropical Medicine

Meredith Hunter
Tessa Williams
Technologists

at the
Tulane National Primate Research Center
Division of Microbiology
18703 Three Rivers Rd.
Covington, Louisiana

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Overall Objective: The overall objective of this study was to test the efficacy of the Hemo-Modulator in boosting virus specific immunity in Simian Immunodeficiency Virus (SIV) -infected rhesus monkeys and to reduce the viral burden in the blood.

Summary: Simian Immunodeficiency Virus (SIV) infection of rhesus macaque monkeys is the most widely accepted AIDS animal model for *in vivo* studies of immune responses and clinical progression to AIDS. The basis for this acceptance is that SIV infects the same types of cells as found in HIV infected humans and the clinical AIDS that develops is the same as that seen in the human disease.

The animal model was used in this study to test for immune stimulation after treatment with the Hemo-Modulator (HM). The HM is an extracorporeal device capable of treating whole blood with UV irradiation and re-infusing the treated blood. The hypothesis is that UV-inactivated SIV will serve as an autologous vaccine and boost the immune system of infected animals against their own strain of virus.

Three monkeys were used for the experiment [T687, AV89 and CN85]. CN85 was infected with SIVmac and received 5 HM treatments between 13 and 16 weeks post-infection. T687 and AV89 had 7 HM treatments between weeks 51 and 55. This strategy allowed us to treat animals at times representing early infection and relatively late in infection. The immunological assay used was ELISPOT, a measure of cell-mediated immunity. The measure of clinical progression was bDNA. bDNA measures the amount of virus in the blood (referred to as the plasma virus load) and is a key marker for progression to AIDS.

Monkeys T687 and AV89 showed an increase in cell mediated immunity (CMI) against SIV. This increase was detected shortly after the treatments. Concomitant with this boost in immunity was a significant decrease in plasma virus load. No deleterious effects were observed that could be associated with HM treatment. There was weight loss during the treatment, however this change in weight was consistent with an anesthesia effect or a heavy viral burden in the case of AV89. When monkeys are anesthetized several times per week, they typically lose weight. Weights returned to normal after treatment. All blood work data were consistent with SIV infection and were not attributed to the HM treatments.

In contrast to the previous two monkeys, CN85 did not show a change in PVL. The results of ELISPOT tests for CN85 are in progress.

Based on results thus far the HM showed promise in this animal model system as demonstrated by a stimulated immunity in 2 of 2 monkeys and a plasma virus load decreased in 2 of 3.

Introduction: Simian Immunodeficiency Virus infection of rhesus macaque monkeys is the most widely accepted AIDS model for *in vivo* immunologic studies and AIDS pathogenesis. SIV is an ideal model for such studies because it infects the same types of cells as HIV in humans and has the same outcome, namely clinical AIDS. Typically, AIDS develops in these infected monkeys in 1 to 4 years after infection.

It is noteworthy that the SIV model for AIDS is a particularly stringent test for immunotherapies, even more so than HIV in humans. The virus loads in SIV infected monkeys are generally higher compared to HIV infections in humans. Moreover, AIDS develops in less than five years compared to a 7 to 12 year course in untreated humans. SIV is also profoundly immunosuppressive so that any changes in immunity against the virus would be viewed as highly significant.

For these reasons, this model was chosen to test Hemo-Modulator efficacy. The Energex HM offers a unique approach to AIDS therapy. The HM is theoretically capable of inactivating SIV in the blood of an infected monkey. The blood is passed through a UV light path and re-infused into the infected animal. The hypothesis is that inactivated virus will serve to stimulate the immune system of the infected monkey by priming antigen processing cells with native virus. Other mechanisms of action are not ruled out at this point, but antigen stimulation is one of the possible mechanisms for producing a positive immunological effect.

In this ongoing study, three monkeys were infected with SIV, a close simian relative of the HIV-AIDS virus. The most important correlates of successful treatment were examined; they are changes in virus load and stimulation of the immune system. These outcomes are viewed as important in both HIV infected persons and in monkey AIDS. The virus load is the amount of virus in the blood. The higher the virus load the shorter the time course to AIDS and death. Therefore, the most important criteria for assessing a successful new anti-HIV drug, or in this case a new medical device, would be a decrease in virus load. Changes in virus load may also reflect boosts in the immune system. Measurement of the cell-mediated immune response to SIV infected cells was also done to directly test for immune stimulation.

Preliminary *In Vitro* Study: Before committing monkeys to *in vivo* studies, a preliminary *in vitro* study was carried out. In this preliminary experiment, SIV- infected tissue culture fluids were passed three times through the HM. The starting concentration of live virus was 45,708 TCID50. The TCID50 is a statistical calculation that measures the number of live particles present. It is defined as the amount of virus required to infect 50% of the tissue cultures.

Table 1. UV Inactivation of SIV Infected Tissue Culture Fluids using the Hemo-Modulator

Number of Treatments	TCID50
Untreated	45708.8
One	3072
Two	1536
Three	192

The untreated sample was an aliquot of SIV infected fluids that was handled identically to the treated virus fluids. Virus was transported in parallel with the treated material and held the same temperature. After treatment all samples were re-titered on CEMx174 cells, a B cell/T cell somatic hybrid cell line used for titration of SIV. The results are shown in Table 1.

The live virus titer was 45708 TCID50 in the untreated sample and was reduced over 93% (3072 TCID50/ml) after one treatment cycle. A second treatment

further reduced the titer by 50% to 1536 TCID50 and the third treatment reduced the titer an additional 87.5% from 1536 TCID50 to 192 TCID50. The overall reduction in live virus titer was >99.9%. Therefore, HM treatment of infected fluids was shown to be highly effective for inactivation of live infectious SIV. The procedure was deemed appropriate for testing *in vivo*.

Results: Two approaches were taken for testing the efficacy of the HM in the SIV macaque model. The first approach was to treat two monkeys that had a long term relatively stable virus load.

AV89 History: This is a female Indian rhesus macaque (born 1993) that is Herpes B-virus, Simian Retrovirus, Simian T Cell Leukemia Virus (STLV1) and SIV negative at the start of the study. This female was assigned to the Estrogen study 3224. The animal was treated with 2 ml of 1.0mg/ml E3 cream vaginally 2x/week for 5 weeks. She was inoculated with 640 TCID50 SIVmac251 vaginally on 11/20/02 (week -75) and her viral loads were below the detectable limits. Late in 2003 she was transferred to Dr. Stephen Smith's Dendritic Cell (DC) vaccination project #3332. She was vaccinated six times with her own DC pulsed with SIVmac239 and then on 4/27/04 (week 0) she was inoculated with SIVmac239 intravenously. By Day 7 post-challenge her virus load was ~200,000 copies/ml. By day 10 the plasma virus loads were over 20 million copies/ml. The virus loads declined slowly to about 75,000 copies/ml on Day 42 post-challenge. After this point, virus loads increased to over 36 million copies/ml by Day 98 (week 14) post-challenge. Since then plasma virus loads have stabilized between 5 and 10 million copies/ml. During this time, other than normal physical exam comments (e.g. gingivitis, alopecia, etc.), her physical examinations were within normal limits.

On 3/22/05 at 49 weeks post-infection AV89 was transferred to the Energex project #3370. She received seven HM treatments beginning in week 51 and again on week 74 as shown in Fig 1. Week 76 treatments are in progress at the time of the writing of this report.

During clinical history on 4/6/05 (week 49) an enlarged spleen was detected by palpation. Epistaxis was observed in week 57 and she was treated with Procaine Penicillin G for 7 days. Severe splenomegaly was also recorded in week 57. She weighed 6.4 kg prior to the Hemo-Modulator procedures and held this weight until week 57 when she dropped to about 6.1kg. She has maintained her weight between 5.5 and 6.2 kg since that time. As of week 72 she weighed 6 kg. Her body condition has remained on average 3 out of 5. All of these clinical signs are consistent with an active SIV infection. Weight loss is routinely seen due to effects of frequent anesthesia. No unexpected deleterious effects were observed during the 7 HM treatments.

T687 Case History: This is a female Indian rhesus macaque (born 1995) that is Simian Retrovirus and STLV1 negative. This female was assigned to the Estrogen study #3224 and was treated with 2 ml of 1.0mg/ml E3 cream 2x/week for 5 weeks. She was challenged with 640 TCID50 SIVmac251 vaginally on 11/20/02 (week 75) and her virus loads were below the detectable limits. Late in 2003 she was transferred to Dr. Smith's DC vaccination project #3332. She was an unvaccinated control on the Smith project. She was vaccinated 6 times with her own DC and then on 4/27/04 (week 0) she was challenged with SIVmac239 intravenously as was AV89. By Day 7 post-challenge her viral load was ~32,000

copies/ml. By Day 10 her virus loads were 1,100,000 copies/ml. Viral loads were at a maximum on Day 14 (14,000,000 copies/ml) after which they decreased and stayed below 1 million copies/ml but were varied with time. On Day 98 post-challenge her virus load was ~9,000 copies/ml. During this time, other than normal physical exam comments (e.g. gingivitis, alopecia, etc.), her physical examinations were within normal limits.

On 3/22/05 (week 47) T687 was transferred to the Energex project and received the same treatments in parallel with AV89.

On 4/20/05 (week 51) it was noted that she had a blood-tinged discharge from both nostrils. A nasal swab was taken and she was started on Procaine Penicillin G for 10 days. On 4/25/05 (week 52), after the blood collection for the HM procedure she became pale and experienced some labored breathing. Pulse oximetry and blood pressure were monitored at that point. Blood was returned to the animal as normal with the procedure. PO2 dropped to 40% and blood pressure was within normal range. The animal was then put on positive pressure ventilation and administered oxyglobin in an attempt to correct the low oxygenation. After several minutes of ventilation, the animal's PO2 level rose to 80%+ and continued to rise over time. The animal was allowed to recover as normal. All other procedures with this animal took place with no complications. 5/2/05 (week 53) it was noted she had bi-lateral nasal discharge and a swab was taken. Again on 6/20 (week 60) it was noted that she had bilateral epistaxis with mucopurlent discharge. She was also observed sneezing in her cage on this date and 6/24 (week 60). Respiratory auscultation was within normal limits and a nasal swab was taken. On 7/7/05 (week 62) the vets made a note of chronic epistaxis, a swab was taken and the animal was started on Kefzol. Physical exam on 8/15/05 (week 69) again made note of dried blood mucous on both nares.

This animal weighed between 9.5 and 10.7 kg prior to the start of the procedures. Since being assigned to this project in 4/05, she has weighed between 10.45 and 11.25 kg. Her body condition has consistently been either 4 out of 5 or 5 out of 5 and many times was not possible for the veterinarians to be able to palpitate her lymph nodes and/or spleen due to her obesity. As of 9/26/05 (week 75) she weighed 10.45 kg. Animal weights are shown in Fig. A.

The time line for treatment and immunologic testing post-infection is shown in Figure 1 below.

CN85 Case History:

This is a male Indian rhesus macaque (born 1995) that is B-virus, Foamy virus positive and SRV, STLV1 negative. He was assigned to Energex on 6/28/04 (week -18) having previously been primarily on holding and breeding projects but for a short time on one of Dr. Kenneth Soike's viral project at which time the animal was given a yellow fever vaccine sub-cutaneously. On 10/20/05 (week -1) the Hemo-Modulator procedure was run on this animal to ensure it's safety with a healthy, uninfected rhesus. On 10/29/04 (week 0) he was inoculated with 20 TCID50 SIVmac251 intravenously. His virus loads peaked at 7.5 log copies/ml on Day 20 (11/19/04; week 3) post-challenge and then stabilized to just over 5 log copies/ml on Day 66 (1/3/05; week 9) post-challenge. Other than normal PE comments (e.g. gingivitis, alopecia, etc) nothing unusual was noted

during the exams. The animal care staff did make note of soft or fluid stools on 7/26/04 and 8/13/04 (weeks -14 and -12 respectively).

This animal was treated five times with the Hemo-modulator machine with procedures occurring on 1/28/05, 1/31/05, 2/4/05, 2/10/05 and 2/21/05 (weeks 13 – 16). This animal went through a second round of treatments, this time with seven procedures occurring on 6/22/05, 6/24/05, 6/27/05, 7/1/05, 7/6/05, 7/11/05, and 7/15/05 (weeks 34 – 37). Upon physical exam, enlarged inguinal and axillary lymph nodes and increased bilateral lung sounds were noted on 1/28/05 (week 13). During the procedure occurring 1/31/05 (week 13), approximately 27 ml of blood was lost from the metal chamber due to the chamber not sitting properly in the administration kit. On 2/24/05 (week 17) very minimal discharge out of right nostril was noted and a nasal swab was taken. On 3/4/05 (week 18) the veterinarians repaired a right inguinal hernia, which had been present since the animal was assigned to us but surgery was put off until the project work slowed down. The surgery went well and the incision healed as normal. Physical examinations on 4/6/05 and 4/26/05 (weeks 23 and 26, respectively) noted the axillary lymph nodes enlarged. Bilateral discharge was noted on 6/8/05 (week 32); auscultation was normal and a swab was taken. On 7/1/05 (week 35), approximately 10 ml of blood leaked out from the return catheter (right cephalic vein) sub-cutaneously. The animal was started on Cefazolin for 10 days as a prophylaxis. On 7/29/05 (week 39) mild enlargement of the left inguinal lymph node was noted. The animal care staff made note of this animal coughing/sneezing and/or runny nose many times in May and June 2005 (approximately weeks 27 to 35) and again on 7/12/05 (week 36). Soft/fluid stools were often noted from 7/15/05 to 8/25/05 (approximately week 37 to 43). This animal has maintained his weight between 9.85 and 11 kg since assignment to this project. As of 8/16/05 (week 42) he weighed 10.35 kg. His body condition has remained on average 3 out of 5 since assignment to this project.

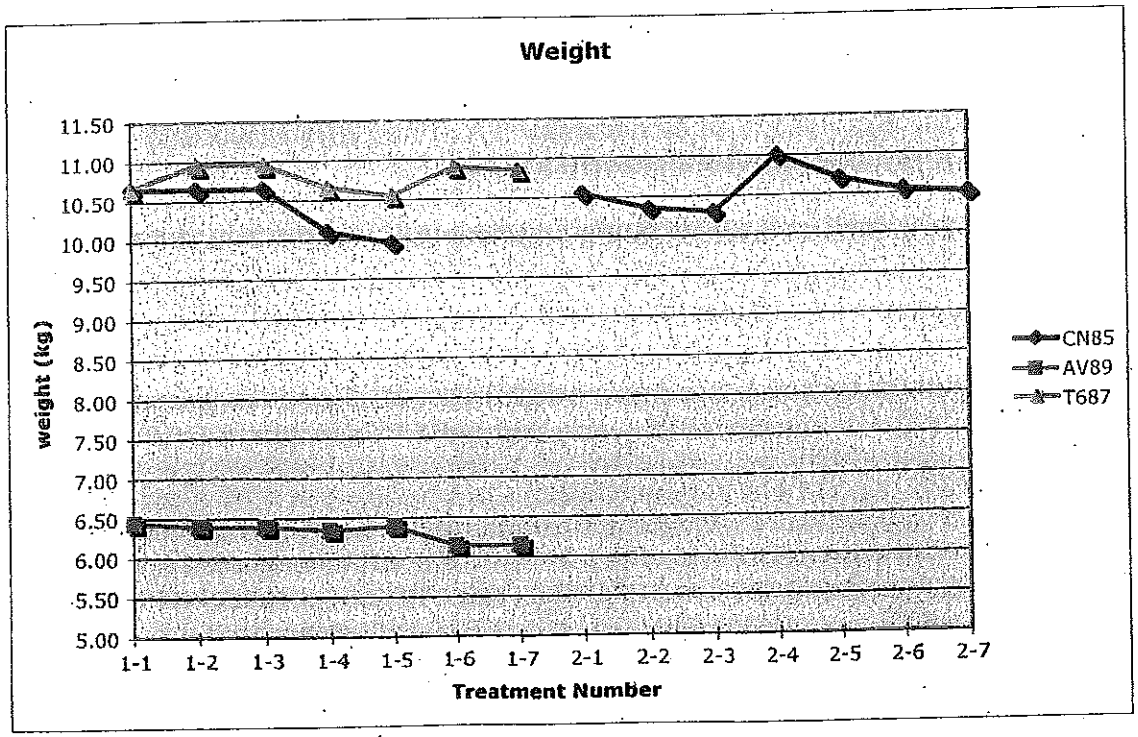

Figure A.

Hemo-Modulator procedure: 1.5ml whole blood/lb body weight was withdrawn and mixed with 4% sodium citrate (7ml blood: 1 ml Na citrate) an 18 gauge Venocath (Venisystem, Inc.) was placed in one saphenous vein for blood withdrawal. A 22 gauge Jelco catheter (Johnson & Johnson, Inc.) was placed in the other for fluid administration. The blood was processed through the Hemo-Modulator machine then returned to the animal. A CBC was taken pre and post-procedure; 35ml of blood was drawn for the procedure.

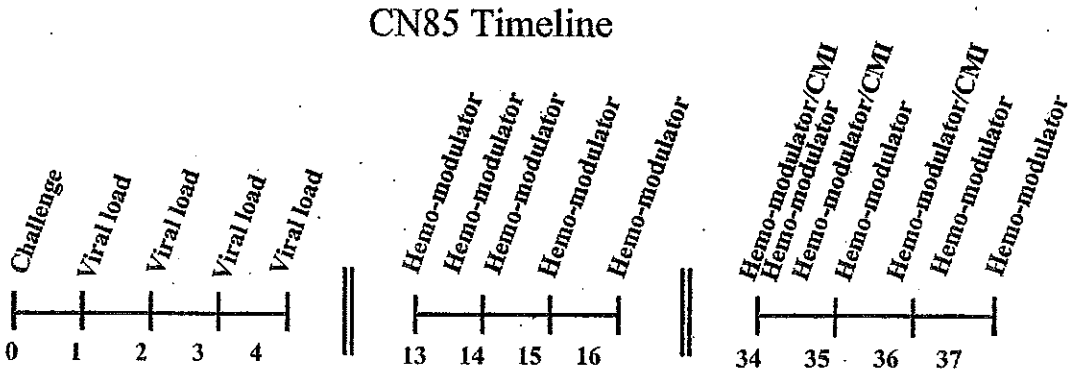

Figure 1A.

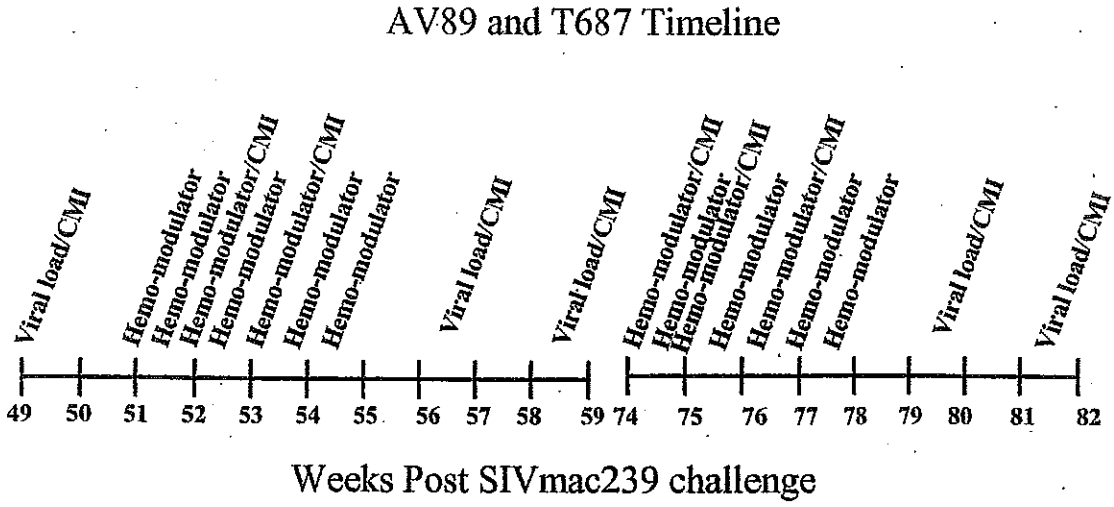

Figure 1B

Figure 1A & B. Time Lines of Hemo-Modulator project

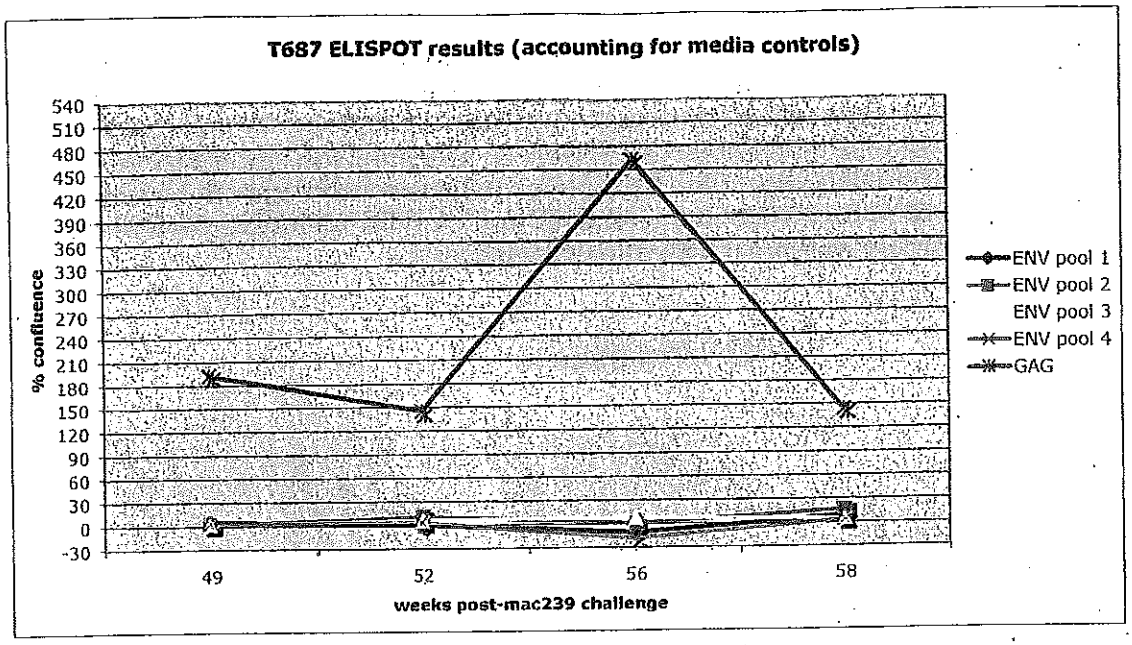

Figure 2A.

ELISPOT were performed on peripheral blood mononuclear cells collected two weeks before (day 49) HM treatment, during treatment (week 52) and two weeks and four weeks post treatment (weeks 56 and 58 respectively). The results show a significant boost in Cell mediated immunity at two weeks after treatment and a decrease to pre-treatment levels by one month post-treatment (see GAG plot). The major response was directed to the viral proteins coded by the SIV gag gene. CMI directed against the gag gene have been reported to control the infection and to be capable of lowering virus load in the blood. Responses to the env gene were weak to negative. Similar results were obtained for AV89 as seen in Figure 2B below except that responses to env were stronger.

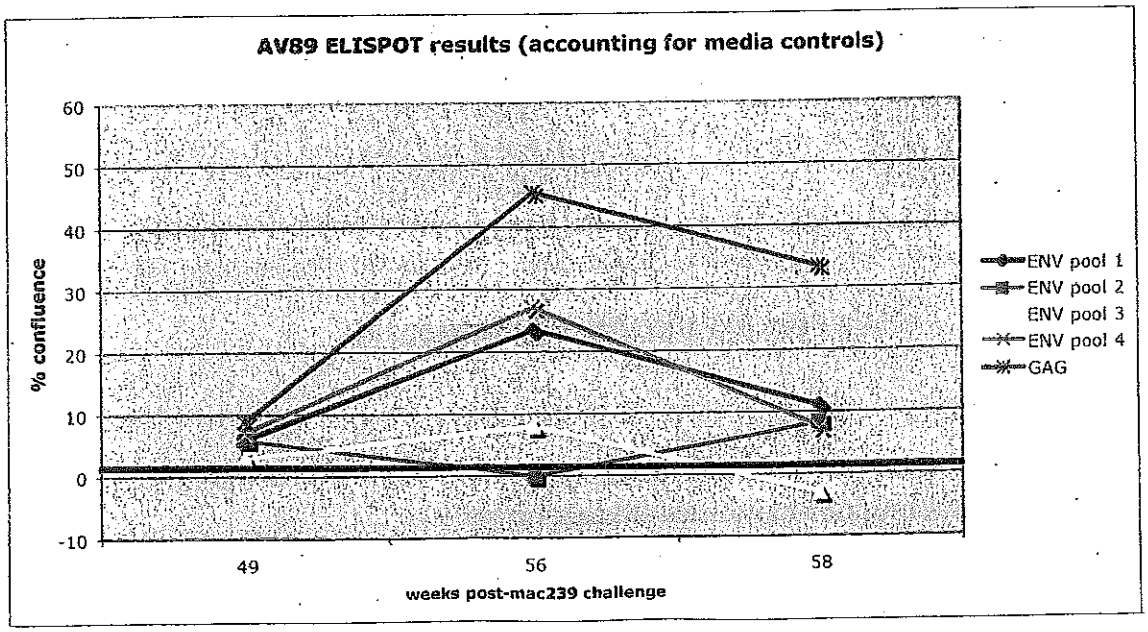

Figure 2B.

Figure 3A below shows a decline in virus load that occurred during the HM treatment series. The samples were taken immediately before each HM treatment, so that they reflect the steady state plasma virus load achieved during the treatment period. Virus loads for AV89 were reduced as much as five fold during the treatment. T687 plasma virus load declined more than four fold (4.1) during the treatment period.

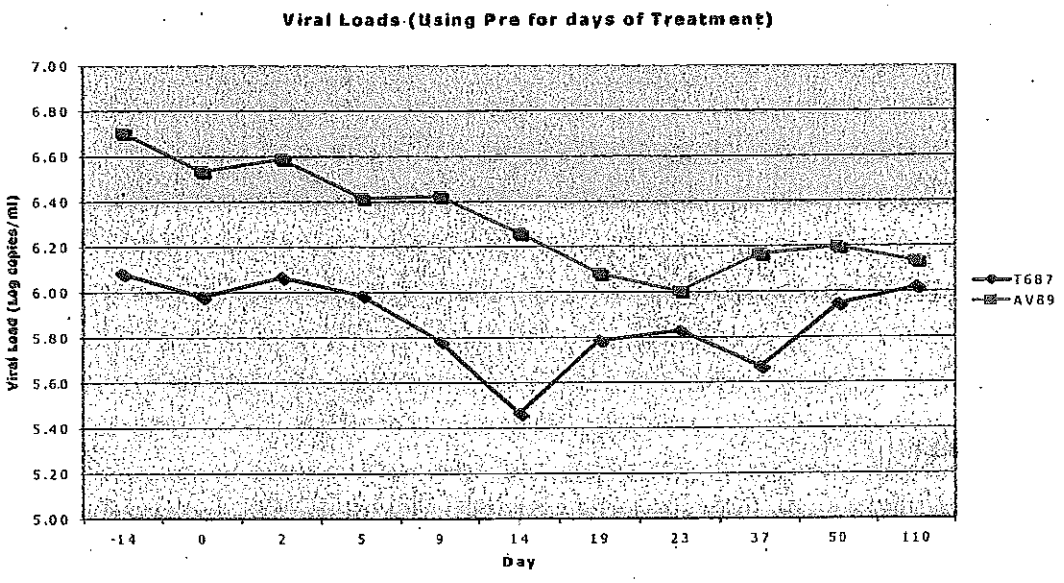

Figure 3A

Figure 3B below shows the plasma virus load results for CN85. In contrast to AV89 and T687, plasma virus loads did not decline significantly. CMI ELISPOT studies are in progress. It remains to be determined whether or not the immune system of CN85 was stimulated by the treatment.

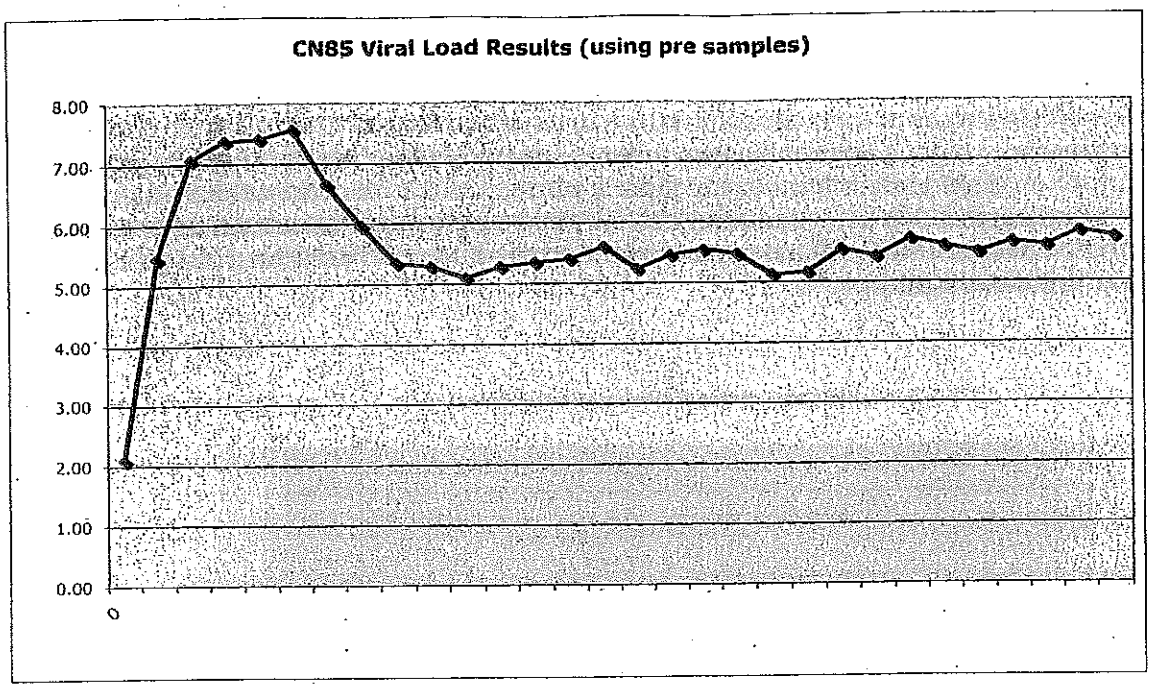

Figure 3B.

Laboratory Methods:

bDNA: Plasma virus loads were determined by bDNA. Samples obtained before and after treatments were tested for SIVmac viral RNA by the branched DNA method (Bayer Diagnostics, Emeryville, CA). The lower limit of detection was 120 SIV RNA copies per ml of plasma.

ELISPOT: Cryopreserved PBMC were tested in duplicate or triplicate using a SIVmac peptide based ELISPOT assay. Briefly, PBMC ($2x10^5$/well) were placed in a 96-well sterile culture plate and exposed to SIVmac peptides or control tissue culture (source for peptides was the AIDS Reagent Repository, Bethesda, MD). Phytohemagglutinin (PHA-m) was used as a positive control. Following overnight incubation at 37°C, cells were transferred to a 96-well ELISPOT plate (Millepore # MAIP45419)) previously coated with monoclonal antibody to macaque IFN-?. ELISPOTs were incubated for 18h, then developed. Spot-forming cells (SFC) were quantified using an automated ELISPOT reader (Zellnet Ft. Lee, NJ). Results were calculated as spot-forming cells per $1x10^6$ PBMC after subtracting background SFC for TK- control vaccinia virus. Based upon the responses to SIV antigens observed in four unexposed rhesus macaques, a negative cutoff

Determination of TCID50

Tissue culture fluids from the transport control and HM Passages 1-3 were each diluted in a total volume of 10 ml of RPMI 1640. Each of the virus containing fluids was diluted 18 times in 2 fold (1:2) serial dilutions in RPMI 1640 tissue culture medium, and then 1 ml of each of the 18 dilutions was added to 1 ml of $5x10^6$ CEMx174 cells. The cell/virus solutions were incubated at 37 degrees

Celsius for 2 hours. The cells were then washed once and re-suspended in individual wells of a 24 well plate (1 plate/passage or control). The cultures were incubated at 37 degrees Celsius until they became positive for SIV p27 antigen by the RETROtek SIV-1 p27 Antigen ELISA (Zeptometrix Corporation, Catalog# 0801169). Wells were scored positive and negative according to the manufacturer's recommendation.

Appendix Results: Clinical laboratory chemistry and blood work are shown in the appendix.