

# CD4+ T Cell Depletion Does Not Affect the Level of Viremia in Chronically SHIV<sub>SF162P3N</sub>-Infected Chinese Cynomolgus Monkeys

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## Research

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# Abstract

**Background:** Peripheral blood CD4<sup>+</sup> T cell counts are a clinical marker for assessing disease progression in HIV-infected patients as well as in SIV-/SHIV-infected macaques. However, it is clinically debatable about whether this marker is a sole indicator for assessing HIV disease progression and need for ART in HIV-infected individuals. Studies with SIV-or SHIV-infected Indian rhesus macaques and sooty mangabeys have generated the conflicting data regarding the association of CD4<sup>+</sup> T cell depletion with viral loads and disease progression.

**Results:** In the present study, we used cynomolgus monkeys, a suitable non-human primate model for chronic HIV infection studies, to determine the effects of the antibody-mediated acute CD4<sup>+</sup> cell depletion on viral load as well as on the immunological factors associated with disease progression. We found that as compared with the control animals, CD4<sup>+</sup> T cell-depleted animals with chronic SHIV infection showed (i) little alteration in plasma viral load over the period of 22 weeks after the depletion; (ii) increased CD4<sup>+</sup> T cell proliferation and turnover of macrophages at the early phase of the depletion, but subsequent decline to the basal levels; and (iii) little impact on the expression of the inflammatory cytokines and CC chemokines associated with disease progression.

**Conclusions:** These findings indicate that the antibody-mediated acute CD4<sup>+</sup> T cell depletion had minimal impact on plasma viral load and disease progression in chronically SHIV<sub>SF162P3N</sub>-infected cynomolgus monkeys. Future investigations are necessary to identify novel and more reliable clinical markers correlated with viremia and disease progression in HIV-infected individuals.

## Background

CD4<sup>+</sup> T cells are the primary target cells for the virus [1, 2] and an immunologic marker of human immunodeficiency virus (HIV) disease progression. CD4<sup>+</sup> T cells are the central mediators for both cellular and humoral immune responses against viral infections including HIV, as they provide antigen information to B cells and CD8<sup>+</sup> T cells for the induction of humoral and cytotoxic response to HIV [3, 4]. In general, the number of circulating CD4<sup>+</sup> T cells gradually decreases to very low level in HIV-infected individuals without antiretroviral therapy (ART), which is the primary cause for disease progression to AIDS. Clinically, the percentage or absolute numbers of circulating CD4<sup>+</sup> T cells are regarded as a key indicator for assessing HIV disease progression and need for ART in HIV-infected individuals. However, several lines of evidence have shown that HIV-infected patients with similar levels of viral loads and CD4<sup>+</sup> T cell progress to AIDS at different rates, especially those on ART [5–9]. This clinical observation in humans has been confirmed in the studies with SIV- or SHIV-infected monkey models, showing that while CD4<sup>+</sup> T cells can be depleted for years before the animals develop AIDS [10–13]. These findings indicate that other immune components, in addition to CD4<sup>+</sup> T cells, are involved in HIV disease progression to AIDS [8, 14].

Because of the complexity and many compounding factors involved in human studies with HIV-infected individuals, the researchers from different laboratories used non-human primate models, particularly the simian immunodeficiency virus (SIV)- or simian-human immunodeficiency virus (SHIV)- infected macaques to determine factors associated with disease progression to AIDS. Several groups showed that virally or artificially induced CD4<sup>+</sup> T cell depletion could increase virus replication and facilitate disease progression, with the emergence of CD4 receptor-independent viral envelopes in the SIV or SHIV-infected rhesus macaques (RMs), particularly Indian RM [15–18]. However, other studies using sooty mangabey (SM) model, a natural host for chronic SIV infection, and RM elite controller reported that CD4<sup>+</sup> T cell depletion had a limited effects on the immune control of SIV replication and predicting of development of AIDS [10, 12, 19]. Because SIV-infected Indian RMs usually have rapid disease progression to AIDS in a median of 9 weeks after CD4<sup>+</sup> T cell depletion [15–17], it is crucial to seek a better animal model that mimic chronic HIV infection in humans. In the present study, we used cynomolgus monkeys (CyM), a non-human primate model that closely resembles chronic HIV infection in humans with regard to set point of viral load, CD4<sup>+</sup> T cell depletion, rates of progression and survival time [20–22]. We examined whether the systemic CD4<sup>+</sup> T cell depletion is associated with virus reactivation and disease progression. We also studied whether CD4<sup>+</sup> T cell depletion induces the activation of the inflammatory components (macrophage, cytokines and chemokines) which are implicated in HIV disease progression.

## Results

### 1□ Treatment of chronically SHIV<sub>SF162P3N</sub>-infected CyMs with anti-CD4 antibody (CD4R1) results in CD4<sup>+</sup>T cell depletion in the peripheral blood, BM and LNs

To determine effect of CD4<sup>+</sup> T cell depletion on the viral load in chronic SHIV infection, three infected Chinese-origin CyMs were administered (i.v. twice at one-week interval) with the humanized monoclonal antibodies (mAb) CD4R1 (50mg/kg) against CD4 receptor. Three untreated animals were used as the control. All 6 animals had been infected with SHIV<sub>SF162P3N</sub> for 38 weeks prior to the CD4R1 injection. As shown in Figure 1a, peripheral blood (PB), bone marrow (BM) and inguinal lymph node (Ing LN) specimens were collected at multiple time points throughout the study.

Prior to CD4<sup>+</sup> T cell depletion, the baseline % of CD3<sup>+</sup>CD4<sup>+</sup> T cells in the animals of both groups was about 55% ± 5%, and the absolute numbers of CD4<sup>+</sup> T cells were 1,500 ± 600 cells/ul blood (mean ± SD). As shown in Figure 1b, c, immediately after the anti-CD4 antibody treatment, the 3 treated animals had a significant decrease (70%–99% relative to baseline) in both percentage and absolute number of circulating CD4<sup>+</sup> T cells, which persisted till the end of this study. Among the anti-CD4 antibody-treated animals, animal WCE03 exhibited severer CD4<sup>+</sup> T cell depletion (>98%) than other two (WEC02 and WEC05), as it had only a nadir CD4<sup>+</sup> T-cell percentage of 1.45% and counts of 5.8 cells/ml (Figure 1b). The same degree of CD4<sup>+</sup> T cell depletion was observed in BM, with mean decline of 80% ± 15% (Figure 1d). In the Ing LN, severe depletion (>80%) of CD4<sup>+</sup> T cell, however, was not observed till 4<sup>th</sup> week after the

anti-CD4 antibody injection (Figure 1d). In addition, unlike CD4<sup>+</sup> T cells in blood and BM, cell numbers in Ing LN at the time of necropsy rebounded to the levels comparable to those at 2nd week after the antibody injection (Figure 1d).

We next examined CD4<sup>+</sup> T cell depletion efficiency in the different tissues collected at the necropsy, including peripheral LNs (pLNs), LNs in the gastrointestinal (GI) tract (GI LNs) and spleens. As shown in Figure 1e, CD4<sup>+</sup> T cell depletion in PB, BM and spleen (>70%) were severer than that (25-35%) in pLNs and GI LNs. To determine specificity of CD4R1 antibody, we next examined the number of CD8<sup>+</sup> T cells in peripheral blood. As shown in Supplemental Figure 1, the number of peripheral blood CD8<sup>+</sup> T cells was not affected in the animals treated with CD4R1 antibody.

## **2) CD4<sup>+</sup> T cell depletion has little effect on plasma viral load and LN tissues of chronically SHIVSF162P3N-infected CyMs**

To determine the impact of CD4<sup>+</sup> T cell depletion on the viremia in chronically SHIV-infected CyMs, we examined the kinetics of plasma viral loads of the study animals. As shown in Figure 2, the animal in both groups became infected after SHIV inoculation and had a typical exponential elevation of virus load at acute infection stage. The peak levels of viral load were observed at week 2 post infection (p.i.) or week -36 post depletion (p.d), with the median levels of  $7.0 \times 10^8$  ( $1.1 \times 10^8 \sim 1.8 \times 10^9$ ) copies/ml and  $8.3 \times 10^8$  ( $1.7 \times 10^8 \sim 1.49 \times 10^9$ ) copies/ml in control group and CD4<sup>+</sup> T cell-depleted group, respectively. Following peak levels of the viremia, the plasma viral loads of SHIV-infected animals gradually decrease and fluctuated between undetectable to  $10^5$  copies/ml. At the 38<sup>th</sup> week p.i. and the 0-week p.d., three animals with chronic SHIV infection and at asymptomatic stage were administered with the anti-CD4 antibody. While all antibody-treated animals showed a significant decrease in both percentage and absolute number of CD4<sup>+</sup> T cells, there was little change of plasma viral loads (Figure 2).

## **3) CD4<sup>+</sup> T cell depletion results in increased expression of ki67 on CD4<sup>+</sup>T cells**

To determine the impact of CD4<sup>+</sup> T cell depletion on the expression of ki67, a T cell proliferation marker correlated with plasma viral load, we measured the number of CD4<sup>+</sup> ki67<sup>+</sup> T cells in the PB, BM and Ing LNs of the study animals prior to and after anti-CD4 antibody injection. As shown in Figure 3a, there was a significant increase (3.9- to 6.8-fold) in the expression of Ki67 on PB CD4<sup>+</sup> T cells in the anti-CD4 antibody-treated animals, which started from the 2<sup>nd</sup> week and peaked at the 8<sup>th</sup> week post the antibody injection (Figure 3a). However, the number of CD4<sup>+</sup> ki67<sup>+</sup> T cells declined to the basal levels at the late stage of the depletion. There was no significant difference in ki67 expression on BM CD4<sup>+</sup> T cells between the two groups (Figure 3b). Among three animals with CD4<sup>+</sup> T cell depletion, WCE05 had the highest level of ki67 expression in both PB and BM throughout the study. When we examined the correlation between the plasma viral loads and the levels of Ki67 on CD4<sup>+</sup> T cells (Figure 3c), although animal WCE05 presented a positive correlation ( $r=0.7$  and  $P=0.04$ ), no correlation was found in other two animals (WCE02 and WCE03). We also determined the levels of CD4<sup>+</sup> Ki67<sup>+</sup> T cells in LNs, showing a

significant increase of Ki67 expression in inguinal LNs of the depleted animals in the week 2 to week 4 p.d. (Figure 3d). However, there was no difference in all LNs, including inguinal, mesenteric and colon LNs at the time of necropsy (Figure 3e).

#### **4) CD4<sup>+</sup>T cell depletion has little effect on SHIV specific antibody response.**

To determine whether the persistence of low levels of viremia in chronically SHIV infected animals was due to an effective antiviral humoral immune response, we first examined the impact of CD4<sup>+</sup> T cell depletion on SHIV-specific antibody response. We found that there was no significant difference in plasma levels of SHIV binding antibody between CD4<sup>+</sup> T cell-depleted animals and the those in control group (Table S1). We next measured the *in vitro* neutralization activity of plasma specimens from two animals (one from depleted group and one from control group). As shown in Figure 4, the plasma neutralization activity to SHIV was absent or very low in both animals.

#### **5) CD4<sup>+</sup> T cell depletion results in increased turnover and proliferation (Ki67<sup>+</sup>) of monocytes**

Increased turnover and proliferation of monocytes, particularly the cells with CD14<sup>+</sup>CD16<sup>+</sup> phenotype, predict disease progression to AIDS in SIV-infected Indian RMs [23]. We thus determined the levels of monocyte turnover and Ki67 expression in CD4<sup>+</sup> T cell-depleted and control animals. Based on the expression of CD14 and/or CD16, we defined monocytes as either classical (CD14<sup>+</sup>CD16<sup>-</sup>), or pro-inflammatory (CD14<sup>+</sup>CD16<sup>+</sup>), and or non-classical (CD14<sup>-</sup>CD16<sup>+</sup>). As shown in Figure 5, comparing with monocytes from the control animals, the animals with CD4<sup>+</sup> T cell depletion had significant higher numbers of pro-inflammatory and non-classical monocytes. There were an average 1.9-fold increase in turnover (1.5~2.2) for pro-inflammatory monocytes and an average 2.1-fold increase (1.7~2.7) for non-classical monocytes relative to baseline levels in the week 2 to week 8 p.d. Of note, pro-inflammatory CD14<sup>+</sup>CD16<sup>+</sup> monocytes exhibited an average 10-fold increase (7.1 to 13.2) of Ki67 expression in the week 2 to week 5 p.d, with a maximum increase of 13-fold at week 4 p.d. However, Ki67 expression on pro-inflammatory monocytes gradually declined to the basal levels at the time of necropsy. No difference was found between the animals in two groups regarding the expression of classical monocytes (CD14<sup>+</sup>CD16<sup>-</sup>).

#### **6) CD4<sup>+</sup> T cell depletion has little effect on inflammatory cytokines and CC chemokines**

Since the inflammatory cytokines and CC chemokines play an important role in systemic immune activation and HIV infection progression, it is of importance to determine whether these immunologic factors are affected by CD4<sup>+</sup> T cell depletion. In examining mRNA expression of the cytokines (IL-1 $\beta$ , IL-10, TNF- $\alpha$ ) and chemokines (MCP-1/CCL-2, MIP-1 $\alpha$ /CCL3, MIP-1 $\beta$ /CCL4, CXCL-9, CXCL-10) in peripheral blood mononuclear cells (PBMCs), spleen and LNs, we found a significant increase of the levels of IL-10, MCP-1(CCL-2) and MIP-1 $\alpha$  (CCL3) in colon LNs of CD4<sup>+</sup> T cell depleted animals as compared to those in the control animals (Figure 6). However, no significant differences in the expression of the cytokines and

chemokines were observed in PBMC, spleen and ileum LNs of the animals regardless of CD4<sup>+</sup> T cell depletion (Figure 6).

## Discussion

It has been documented that CD4<sup>+</sup> T cell depletion does not consistently correlate with disease progression to AIDS in HIV-infected individuals [14, 24–26] as well as in SIV-or SHIV-infected non-human primates [10–13]. Several studies found that a severe depletion of CD4<sup>+</sup> T cells of SIV-infected Indian rhesus macaques resulted in increased plasma viral load, emergence of macrophage-tropism variant and rapid progression to AIDS [15–17]. In contrast, CD4<sup>+</sup> T cell depletion in the SIV natural host model (sooty mangabey) was not associated with high levels of virus replication, robust infection of macrophages and rapid onset of AIDS [10, 19]. These conflicting findings argue for further investigation on this issue with appropriate animal models. In this study, we for the first time examined the impact of CD4<sup>+</sup> T cell depletion on plasma viral load and the immunological factors associated with disease progression in chronically SHIV<sub>SF162P3N</sub>-infected CyMs, a suitable non-human primate model for studying chronic HIV infection. We demonstrated that while CD4<sup>+</sup> T cells could be successfully depleted in the animals administered with CD4R1 antibody, there was little change in plasma viral loads over the period of 22 weeks after the systemic CD4<sup>+</sup> T cell depletion.

The early studies demonstrated that increase of T cell proliferation marker Ki67<sup>+</sup> expression is an indicator of rapid propagation of AIDS virus [10, 27]. We found that although there was an initial increase in the expression of the proliferation marker on peripheral CD4<sup>+</sup> T cell after systemic CD4<sup>+</sup> T cell depletion, the levels of CD4<sup>+</sup> T cell proliferation in BM and LNs remained little change. Among 3 animals with CD4<sup>+</sup> T cell depletion, WCE05 had the highest number of CD4<sup>+</sup> Ki67<sup>+</sup> T cells in blood and BM, but the cell numbers declined after they peaked at week 8 p.d. The animal had undetectable viral load and no sign of AIDS at the late stage of the depletion (Fig. 3). In addition to CD4<sup>+</sup> T cell proliferation marker, we also examined the effect of CD4<sup>+</sup> T cell depletion on the expression of the inflammatory cytokines and CC chemokines which are implicated in the systemic immune activation, a hallmark of HIV disease progression [28–32]. We found that there was no significant difference in mRNA expression of these cytokines and chemokines in PBMC, spleen and ileum-LNs between the animals in the two groups. A significant increase of some (IL-10, MCP-1/CCL-2 and MIP-1 $\alpha$ /CCL3) of these factors was only observed in the colon LNs of the animals with CD4<sup>+</sup> T cell depletion.

Several studies showed that increased monocyte turnover better predicts the tempo of progression to AIDS in HIV-infected patients and SIV/SHIV-infected animals than declining level of CD4<sup>+</sup> T cells alone [33, 34]. Although CD4<sup>+</sup> T cells are the major source of virus production in HIV-infected individuals, monocytes/macrophages contribute substantially to plasma viral load during late-stage of HIV disease when the levels of circulating CD4<sup>+</sup> T cell are very low [35], even is associated with the gradual loss of nonprogressor status in the long-term nonprogressors (LTNPs) [36]. Our previous study showed that

SHIV<sub>KU-1</sub>-infected Chinese RMs had the persistence of high virus loads ( $10^6$  - $10^8$  viral RNA copies per ml) with a 1.5 years survival time, although their circulating CD4<sup>+</sup> T cell levels were very low (10–250 cells/ul) over the course of infection. The pathological analyses at the necropsy showed that SHIV<sub>KU-1</sub>-infected macaques had macrophage activation and virus infection in brain and LNs, suggesting that macrophage is the main target cells for sustaining the high production of virus for several months after CD4<sup>+</sup> T cell depletion [13]. In this study, we monitored the frequency and proliferation of monocytes (classical, pro-inflammatory and nonclassical) in blood of SHIV-infected CyMs. Consistent with the studies with SIV natural host monkey [10, 37, 38], we showed that while there was little change of number of monocytes with classical phenotype (CD14<sup>+</sup> CD16<sup>-</sup>) throughout the depletion experiment, the number of monocytes with pro-inflammatory (CD14<sup>+</sup> CD16<sup>+</sup>) and non-classical (CD14<sup>-</sup> CD16<sup>+</sup>) phenotype increased at the early stage of the depletion (Fig. 5). It has been reported that increased activation and rapid turnover of monocytes in SIV- infected RMs, in particular pro-inflammatory CD14<sup>+</sup>CD16<sup>+</sup> monocytes, account for the robust replication of virus and the higher death rate of tissue macrophages [23, 39]. We observed an initial increase and then decline of turnover and proliferation of monocytes in the animals with CD4<sup>+</sup> T cell depletion. There was little correlation between CD4<sup>+</sup> T cell number and the monocyte turnover and proliferation. These findings suggest the possibility that SHIV<sub>SF162P3N</sub>-infected CyMs with severe CD4 depletion have the immunological ability to control the net turnover of monocytes and proliferation of CD4<sup>+</sup> T cells and maintain a stable levels of chemokines and cytokines that are associated with systemic immune activation and macrophages infiltration. To confirm this speculation, we examined the macrophage infiltration and virus infection in the tissues (mesenteric LNs and brain) of the infected animals. We observed that there was little macrophage infiltration and SHIV infection in the tissues of the animals with severe CD4<sup>+</sup> T cell depletion (data not shown). These data provide the justification for lacking impact of CD4<sup>+</sup> T cell depletion on viral propagation and disease progression in SHIV-infected animals treated with anti-CD4 antibody.

## Conclusion

With the limited number of the study animals, our study for the proof of concept demonstrates that the antibody-mediated acute CD4<sup>+</sup> T cell depletion per se has little effect on viral replication and is not a facilitator of development of AIDS in SHIV<sub>SF162P3N</sub>-infected CyMs. We also show that the CD4<sup>+</sup> T cell depletion has minimal impact on the activation of the immune system, as evidenced by lacking proliferation of CD4<sup>+</sup> T cells and turnover of monocytes. These findings indicate that other immunological and cellular factors, in addition to CD4<sup>+</sup> T cells, are involved in controlling viral replication and disease progression. It is likely that the cumulative results of multiple aberrant immunologic factors contribute to the immunopathogenesis of HIV infection and development of AIDS. Future investigations with larger number of animals and longer observation time after CD4 T cell depletion are necessary in order to identify novel and more reliable clinical markers correlated with viremia and disease progression in HIV-infected individuals, which is crucial for development of multifaceted therapeutic approaches.

# Methods

## Ethics Statement

All study protocols were approved by the Institutional Animal Care and Use Committee of the Wuhan University (Wuhan, China) in accordance with the regulations of the National Institute of Health “Guide for the Care and Use of Laboratory Animals” and details of animal welfare and steps taken to ameliorate suffering were in accordance with the recommendations of the Weatherall report “The use of nonhuman primates in research”. The animals were housed at the Animal Bio-Safety Level-III (ABSL-III) laboratory of Wuhan University. The ABSL-III Laboratory is certified by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International). All procedures were performed under anesthesia with intramuscular injection of ketamine hydrochloride (10 mg/kg). All efforts were made to minimize suffering of the study monkeys.

## Animals, SHIV Challenge and CD4 T cell depletion

Six Chinese-origin CyMs (females, 4–5 years of age, 4–6 kg of weight) were purchased from Hainan Non-human Primate Development of Laboratory Animal Co., Ltd. (Hainan, China). All animals were screened and negative for the pathogens (simian retrovirus D, simian immunodeficiency virus, simian T leukemia virus type I, and herpes virus B). All 6 study animals were inoculated intravenously with the R5-tropic and pathogenic SHIV<sub>SF163P3N</sub> strain. At 38th week post SHIV<sub>SF163P3N</sub> infection, three of the animals were intravenously administrated with the anti-CD4 antibody (CD4R1, 50 mg/kg) twice at a one-week interval, as recommended by the “NIH Nonhuman Primate Reagent Resource” protocol. Three untreated animals were used as the controls.

## Sample collection and processing

Collections and processing of PB, BM aspirate, and Inguinal LN biopsies were performed longitudinally and at necropsy (Figure 1). Briefly, whole blood samples were collected in EDTA tubes for flow cytometry analysis, and plasma was separated by centrifugation. PBMCs were prepared by density gradient centrifugation. BM aspirates (1 ml) were collected with an aspiration kit (Xinchuang precision products company, Shenzhen, China). For LN biopsies, the skin over the inguinal region was prepared for aseptic surgery. An incision was made over the LN, and blunt dissection was used to isolate and excise the nodes which were homogenized and filtered through a 70-mm cell strainer to mechanically isolate lymphocytes for flow cytometry analysis. At necropsy, blood, BM, brain and LNs were collected from the animals after euthanasia. LNs included pLNs (axillary and inguinal LNs), and GI LNs (ileum, colon and mesenteric LNs). All the specimens were cryopreserved or fixed in 10% buffered formalin.

## SHIV-specific antibody detection

SHIV-specific antibody in plasma samples were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s instructions (HIV-1/HIV-2 Antibody EIA; Wantai, Beijing). Optical

density values at a 1:10 serum dilution (3 times above the cutoff value) were considered positive.

### **Flow cytometric analysis**

Six-parameter flow cytometric analysis was performed on whole blood, BM-, LN- and spleen-derived cells according to standard procedures using a panel of mAbs purchased from BD Biosciences (Pharmingen, San Diego, CA) as follows: anti-CD3-APC-Cy7 (clone SP34), anti-CD8-PE (clone RPA-T8), anti-Ki-67- PE-Cy7 (clone B56), anti-CD14-PE (clone M5E2), anti-CD16-FITC (clone 3G8), and anti-HLA-DR-APC (clone G46-6). The anti-CD4-PerCP-Cy5.5 (clone OKT4) was purchased from Biolegend (San Diego, CA, USA). Isotype antibody was used for a negative control of CD4, Ki67, HLA-DR, CD14 and CD16 expression. Intracellular staining for Ki-67 was performed at room temperature for 30 minutes following permeabilization with cytofix/cytoperm (BD Bioscience). Flow cytometric acquisition and analysis were performed on a BD Verse cytometer driven by the FACS Verse software (BD Biosciences). Analysis of the acquired data was performed using FlowJo software (TreeStar, Ashland, OR, USA) and graphs were prepared using Prism version 6.0 (GraphPad).

### **Determination of viral load RNA**

Quantitative real-time RT-PCR assay to determine SHIV viral load was performed as previously described [11].

### **Neutralization assay**

Plasma samples from the study animals were tested for neutralizing Ab activity to neutralize SHIV<sub>SF162P3N</sub>. Briefly, SHIV<sub>SF162P3N</sub> (2ng p24 Gag equivalent) was incubated with serial dilutions (1/4, 1/8, 1/16 and 1/32) of heat-inactivated plasma in duplicate and added to TZM-bl cells. One set of control wells received cells and virus (virus control), and another set received cells only (background control). Cells were incubated for 48h and then lysed for the luciferase activity in each well. The 50% SHIV neutralizing endpoint was calculated as the reciprocal of the highest dilution of sample that resulted in a 50% reduction of the number of TZM-bl cells in comparison with a negative plasma control. Each experiment was performed twice independently with duplicate wells.

### **Real-time PCR**

Total cellular RNA was isolated from PBMC, spleen and LNs (ileum and colon) and using Trizol Reagent (Invitrogen, Carlsbad, CA) and reverse-transcribed to cDNA using a PrimeScript RT reagent kit (TaKaRa, Dalian, China). The mRNA levels of the target genes were quantified with SYBR Green-based real-time PCR analysis (Bio-Rad). The PCR amplification was performed using the CFX96 real-time PCR detection system (Bio-Rad, Richmond, CA, USA) and the following cycling conditions: The cycling was initiated by heating at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 58–61 °C for 30 s depending on the specific set of primers, and extension at 72 °C for 30 s. The expression levels of each transcript were normalized using the  $2^{-\Delta Ct}$  method relative to GAPDH. The primer sequences used

were as follows: TNF- $\alpha$  (sense 5'-TGAGCACTGAAAGCATGATCCG-3' and antisense 5'-AAGGAG AAGAGGCTGAGGAACCAG-3'); MCP-1 (sense 5'- TGTCCCAAAGAAGCTGTGATC T-3' and antisense 5'- GGAATCCTGAACCCACTTCTG-3'), MIP-1 $\alpha$  (sense 5'- GCTG ACTACTTTGAGACGAGC-3' and antisense 5'- CCAGTCCATAGAAGAGGTAGC-3'), MIP-1 $\beta$  (sense 5'- CCAAACCAAAGAAGCAAGC-3' and antisense 5'- AGAAAC AGTGACAGTGGACC-3'), CXCL-9 (sense 5'- TGGGCATCATCTTCCTGGTTCTGA-3' and antisense 5'- TTTCTCGCAGGAAAGGTT TGGAGC-3'), CXCL-10 (sense 5'-TCCACGTGTTGAGATCATTGC-3' and antisense 5'- TCTTGATGGCCTTCGATTCTG-3'), IL-10 (sense 5'-TGCCTTCAGCAGAGTGAAGA-3' and antisense 5'-ATGTCT GGGTCGTGGTTCTC-3'), and GAPDH (sense 5'-AGGTCGGTGTGAACGGATTTG-3' and antisense 5'-TGTAGACCATGTAGTTGAGGTCA-3').

## Statistical analysis

All statistical analyses were performed using GraphPad Prism 6.0. The data were presented as the mean  $\pm$  SD. Statistical analysis was performed by the unpaired Student's t-test.  $P < 0.05$  was considered statistically significant.

## List Of Abbreviations

HIV

Human immunodeficiency virus; SIV:simian immunodeficiency virus; SHIV:simian-human immunodeficiency virus; ART:antiretroviral therapy; RM:rhesus macaques; SM:sooty mangabey; CyM:cynomolgus monkeys; PB:peripheral blood; BM:bone marrow; Ing LN:inguinal lymph node; PBMcs:peripheral blood mononuclear cells; pLNs:peripheral LNs; GI LNs:LN in the gastrointestinal (GI) tract; ELISA:enzyme-linked immunosorbent assay; mAbs:monoclonal antibodies

## Declarations

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### Author's Contributions

Conceived and designed the experiments: KZ, WZH. Performed the experiments: HL, JBL, FZM, XQX, YW, QYX, RHZ, QHX, ZXH, LZ. Analyzed the data: KZ, JLL, XDL, XW, WZH. Wrote the paper: KZ, WZH. All authors read and approved the final manuscript.

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## Availability of data and materials

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

## Ethics approval and consent to participate

This study was carried out at the Animal Biosafety Level-III Laboratory at the Center for Animal Experiment of Wuhan University with AAALAC International accreditation (001274). All the animal protocols were approved by the Institutional Animal Care and Use Committee (approval number: 2015028) of Wuhan University in accordance with the NIH "Guide for the Care and Use of Laboratory Animals".

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing of interests.

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## References

1. Haase AT: **Targeting early infection to prevent HIV-1 mucosal transmission.** *Nature* 2010, **464**:217-223.
2. Kelleher AD, Zaunders JJ: **Decimated or missing in action: CD4+ T cells as targets and effectors in the pathogenesis of primary HIV infection.** *Curr HIV/AIDS Rep* 2006, **3**:5-12.
3. Lackner AA, Lederman MM, Rodriguez B: **HIV pathogenesis: the host.** *Cold Spring Harb Perspect Med* 2012, **2**:a007005.
4. Provine NM, Badamchi-Zadeh A, Bricault CA, Penaloza-MacMaster P, Larocca RA, Borducchi EN, Seaman MS, Barouch DH: **Transient CD4+ T Cell Depletion Results in Delayed Development of Functional Vaccine-Elicited Antibody Responses.** *J Virol* 2016, **90**:4278-4288.
5. Begtrup K, Melbye M, Biggar RJ, Goedert JJ, Knudsen K, Andersen PK: **Progression to acquired immunodeficiency syndrome is influenced by CD4 T-lymphocyte count and time since**

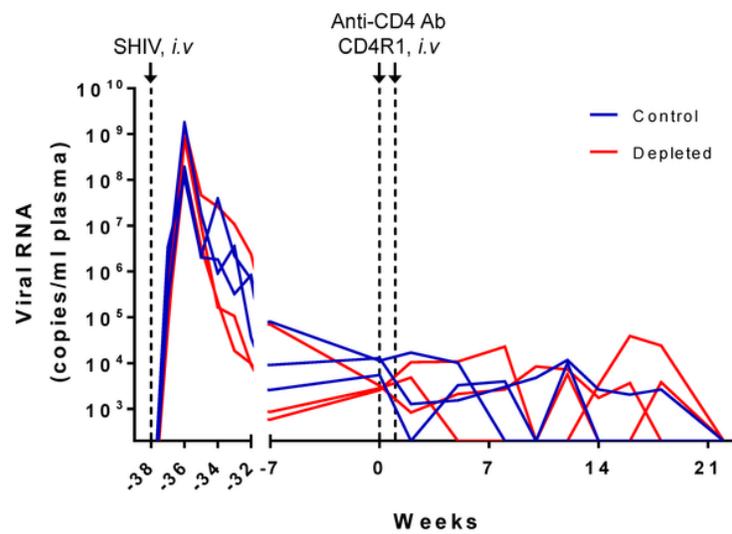
- seroconversion.*Am J Epidemiol* 1997, **145**:629-635.**
6. d'Ettorre G, Paiardini M, Zaffiri L, Andreotti M, Ceccarelli G, Rizza C, Indinnimeo M, Vella S, Mastroianni CM, Silvestri G, Vullo V: **HIV persistence in the gut mucosa of HIV-infected subjects undergoing antiretroviral therapy correlates with immune activation and increased levels of LPS.***Curr HIV Res* 2011, **9**:148-153.
  7. Keet IP, Krol A, Koot M, Roos MT, de Wolf F, Miedema F, Coutinho RA: **Predictors of disease progression in HIV-infected homosexual men with CD4+ cells < 200 x 10<sup>6</sup>/l but free of AIDS-defining clinical disease.***AIDS* 1994, **8**:1577-1583.
  8. Kuroda MJ: **Macrophages: do they impact AIDS progression more than CD4 T cells?***J Leukoc Biol* 2010, **87**:569-573.
  9. Mills EJ, Kelly S, Bradley M, Mollon P, Cooper C, Nachega J: **Antiretroviral effects on HIV-1 RNA, CD4 cell count and progression to AIDS or death: a meta-regression analysis.***HIV Med* 2008, **9**:849-857.
  10. Klatt NR, Villinger F, Bostik P, Gordon SN, Pereira L, Engram JC, Mayne A, Dunham RM, Lawson B, Ratcliffe SJ, et al: **Availability of activated CD4+ T cells dictates the level of viremia in naturally SIV-infected sooty mangabeys.***J Clin Invest* 2008, **118**:2039-2049.
  11. Liu H, Xiao QH, Liu JB, Li JL, Zhou L, Xian QY, Wang Y, Zhang J, Wang X, Ho WZ, Zhuang K: **SIV Infection Impairs the Central Nervous System in Chinese Rhesus Macaques.***J Neuroimmune Pharmacol* 2016, **11**:592-600.
  12. Milush JM, Reeves JD, Gordon SN, Zhou D, Muthukumar A, Kosub DA, Chacko E, Giavedoni LD, Ibegbu CC, Cole KS, et al: **Virally induced CD4+ T cell depletion is not sufficient to induce AIDS in a natural host.***J Immunol* 2007, **179**:3047-3056.
  13. Xiao Q, Li J, Yu Q, Bao R, Liu J, Liu H, Zhou L, Xian Q, Wang Y, Cheng-Mayer C, et al: **Distinct Compartmentalization in the CNS of SHIVKU-1-Infected Chinese Rhesus Macaque Is Associated With Severe Neuropathology.***J Acquir Immune Defic Syndr* 2015, **70**:e168-171.
  14. Giorgi JV, Hultin LE, McKeating JA, Johnson TD, Owens B, Jacobson LP, Shih R, Lewis J, Wiley DJ, Phair JP, et al: **Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage.***J Infect Dis* 1999, **179**:859-870.
  15. Igarashi T, Brown CR, Endo Y, Buckler-White A, Plishka R, Bischofberger N, Hirsch V, Martin MA: **Macrophage are the principal reservoir and sustain high virus loads in rhesus macaques after the depletion of CD4+ T cells by a highly pathogenic simian immunodeficiency virus/HIV type 1 chimera (SHIV): Implications for HIV-1 infections of humans.***Proc Natl Acad Sci U S A* 2001, **98**:658-663.
  16. Micci L, Alvarez X, Iriete RI, Ortiz AM, Ryan ES, McGary CS, Deleage C, McAtee BB, He T, Apetrei C, et al: **CD4 depletion in SIV-infected macaques results in macrophage and microglia infection with rapid turnover of infected cells.***PLoS Pathog* 2014, **10**:e1004467.
  17. Ortiz AM, Klatt NR, Li B, Yi Y, Tabb B, Hao XP, Sternberg L, Lawson B, Carnathan PM, Cramer EM, et al: **Depletion of CD4(+) T cells abrogates post-peak decline of viremia in SIV-infected rhesus macaques.***J Clin Invest* 2011, **121**:4433-4445.

18. Zhuang K, Leda AR, Tsai L, Knight H, Harbison C, Gettie A, Blanchard J, Westmoreland S, Cheng-Mayer C: **Emergence of CD4 independence envelopes and astrocyte infection in R5 simian-human immunodeficiency virus model of encephalitis.***J Virol* 2014, **88**:8407-8420.
19. Mudd PA, Ericson AJ, Price AA, Wilson NA, Reimann KA, Watkins DI: **Reduction of CD4+ T cells in vivo does not affect virus load in macaque elite controllers.***J Virol* 2011, **85**:7454-7459.
20. Bruel T, Hamimi C, Dereuddre-Bosquet N, Cosma A, Shin SY, Corneau A, Versmisse P, Karlsson I, Malleret B, Targat B, et al: **Long-term control of simian immunodeficiency virus (SIV) in cynomolgus macaques not associated with efficient SIV-specific CD8+ T-cell responses.***J Virol* 2015, **89**:3542-3556.
21. Okamura T, Tsujimura Y, Soma S, Takahashi I, Matsuo K, Yasutomi Y: **Simian immunodeficiency virus SIVmac239 infection and simian human immunodeficiency virus SHIV89.6P infection result in progression to AIDS in cynomolgus macaques of Asian origin.***J Gen Virol* 2016, **97**:3413-3426.
22. ten Haaft P, Almond N, Biberfeld G, Cafaro A, Cranage M, Ensoli B, Hunsmann G, Polyanskaya N, Stahl-Hennig C, Thortensson R, et al: **Comparison of early plasma RNA loads in different macaque species and the impact of different routes of exposure on SIV/SHIV infection.***J Med Primatol* 2001, **30**:207-214.
23. Burdo TH, Soulas C, Orzechowski K, Button J, Krishnan A, Sugimoto C, Alvarez X, Kuroda MJ, Williams KC: **Increased monocyte turnover from bone marrow correlates with severity of SIV encephalitis and CD163 levels in plasma.***PLoS Pathog* 2010, **6**:e1000842.
24. Opportunistic Infections Project Team of the Collaboration of Observational HIVERiEiE, Young J, Psychogiou M, Meyer L, Ayayi S, Grabar S, Raffi F, Reiss P, Gazzard B, Sharland M, et al: **CD4 cell count and the risk of AIDS or death in HIV-Infected adults on combination antiretroviral therapy with a suppressed viral load: a longitudinal cohort study from COHERE.***PLoS Med* 2012, **9**:e1001194.
25. Strategies for Management of Antiretroviral Therapy Study G, El-Sadr WM, Lundgren J, Neaton JD, Gordin F, Abrams D, Arduino RC, Babiker A, Burman W, Clumeck N, et al: **CD4+ count-guided interruption of antiretroviral treatment.***N Engl J Med* 2006, **355**:2283-2296.
26. Konrad S, Skinner S, Kazadi GB, Gartner K, Lim HJ: **HIV disease progression to CD4 count <200 cells/ $\mu$ L and death in Saskatoon, Saskatchewan.***Can J Infect Dis Med Microbiol* 2013, **24**:97-101.
27. Sachsenberg N, Perelson AS, Yerly S, Schockmel GA, Leduc D, Hirschel B, Perrin L: **Turnover of CD4+ and CD8+ T lymphocytes in HIV-1 infection as measured by Ki-67 antigen.***J Exp Med* 1998, **187**:1295-1303.
28. Fantuzzi L, Conti L, Gauzzi MC, Eid P, Del Corno M, Varano B, Canini I, Belardelli F, Gessani S: **Regulation of chemokine/cytokine network during in vitro differentiation and HIV-1 infection of human monocytes: possible importance in the pathogenesis of AIDS.***J Leukoc Biol* 2000, **68**:391-399.
29. Cozzi-Lepri A, French MA, Baxter J, Okhuysen P, Plana M, Neuhaus J, Landay A, group IS: **Resumption of HIV replication is associated with monocyte/macrophage derived cytokine and chemokine changes: results from a large international clinical trial.***AIDS* 2011, **25**:1207-1217.

30. French MA, Cozzi-Lepri A, Arduino RC, Johnson M, Achhra AC, Landay A, Group ISS: **Plasma levels of cytokines and chemokines and the risk of mortality in HIV-infected individuals: a case-control analysis nested in a large clinical trial.***AIDS* 2015, **29**:847-851.
31. Muema DM, Akilimali NA, Ndumnego OC, Rasehlo SS, Durgiah R, Ojwach DBA, Ismail N, Dong M, Moodley A, Dong KL, et al: **Association between the cytokine storm, immune cell dynamics, and viral replicative capacity in hyperacute HIV infection.***BMC Med* 2020, **18**:81.
32. Sereti I, Altfeld M: **Immune activation and HIV: an enduring relationship.***Curr Opin HIV AIDS* 2016, **11**:129-130.
33. Hasegawa A, Liu H, Ling B, Borda JT, Alvarez X, Sugimoto C, Vinet-Oliphant H, Kim WK, Williams KC, Ribeiro RM, et al: **The level of monocyte turnover predicts disease progression in the macaque model of AIDS.***Blood* 2009, **114**:2917-2925.
34. Merino KM, Allers C, Didier ES, Kuroda MJ: **Role of Monocyte/Macrophages during HIV/SIV Infection in Adult and Pediatric Acquired Immune Deficiency Syndrome.***Front Immunol* 2017, **8**:1693.
35. Clayton KL, Garcia JV, Clements JE, Walker BD: **HIV Infection of Macrophages: Implications for Pathogenesis and Cure.***Pathog Immun* 2017, **2**:179-192.
36. D'Souza RR, Gopalan BP, Rajnala N, Phetsouphanh C, Shet A: **Increased monocyte activation with age among HIV-infected long term non-progressor children: implications for early treatment initiation.***HIV Med* 2019, **20**:513-522.
37. Raetz K, Pandrea I, Apetrei C: **The well-tempered SIV infection: Pathogenesis of SIV infection in natural hosts in the wild, with emphasis on virus transmission and early events post-infection that may contribute to protection from disease progression.***Infect Genet Evol* 2016, **46**:308-323.
38. Greenwood EJD, Schmidt F, Liegeois F, Kondova I, Herbert A, Ngoubangoye B, Rouet F, Heeney JL: **Loss of memory CD4+ T-cells in semi-wild mandrills (*Mandrillus sphinx*) naturally infected with species-specific simian immunodeficiency virus SIVmnd-1.***J Gen Virol* 2014, **95**:201-212.
39. Swan ZD, Bouwer AL, Wonderlich ER, Barratt-Boyes SM: **Persistent accumulation of gut macrophages with impaired phagocytic function correlates with SIV disease progression in macaques.***Eur J Immunol* 2017, **47**:1925-1935.

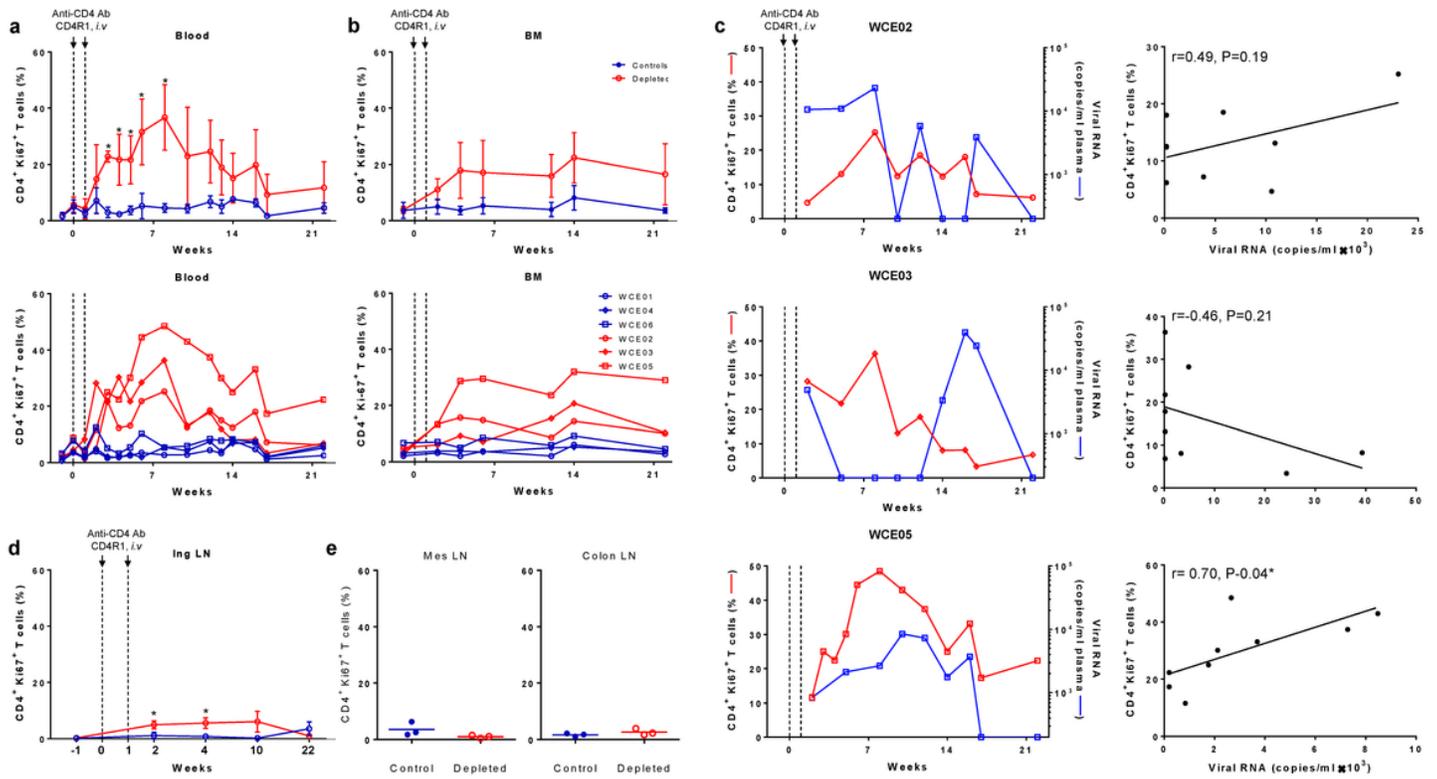
## Figures





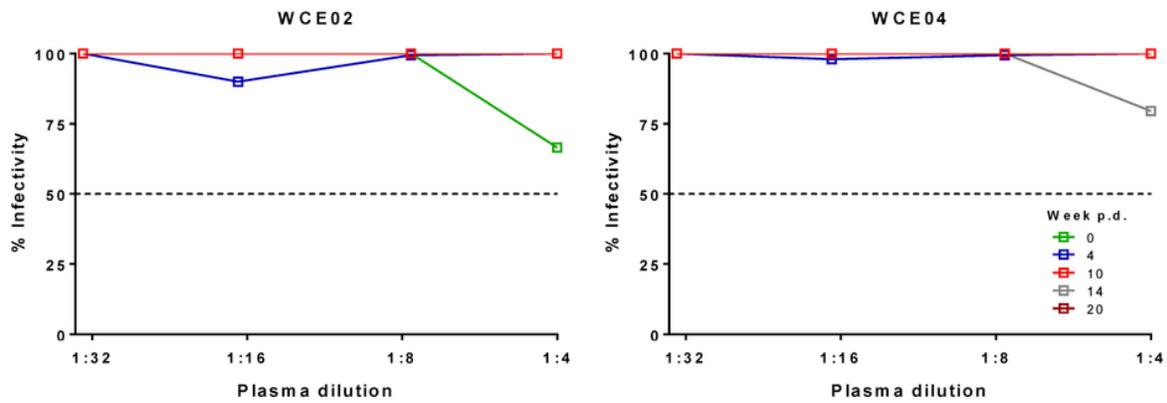
**Figure 2**

CD4+ T cell depletion has little effect on viremia in SHIVSF163P3N-infected CyMs. Longitudinal assessment of SHIV plasma viral loads of the animals in CD4-depleted group (red line; n=3) and control group (blue line; n=3). Dotted lines represent CD4R1 antibody treatment. The plasma viremia fluctuated at similar levels between two groups after SHIV infection.



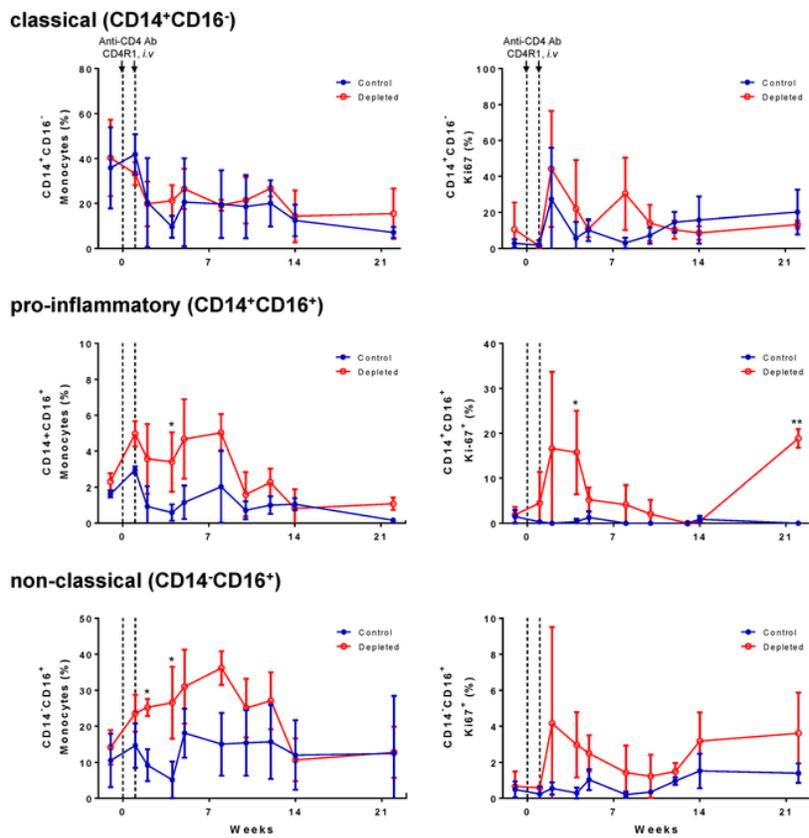
**Figure 3**

Effect of CD4+ T cell depletion on ki67 expression. CD4 T cell proliferation marker Ki67 was measured in PB (a), BM (b) and Inguinal LN (d) prior to and after CD4+ T cell depletion. (a, b) Upper panels represent the longitudinal level of expression of CD4+Ki67+ cells in PB and BM of CD4-depleted (red line; n=3) and undepleted control (blue line; n=3) CyMs, while lower panels represent the level of CD4+Ki67+ for each animal. (c) The correlation between plasma virus replication and the level of CD4+ T cell proliferation in three CD4-depleted SHIV-infected CyMs. Plasma viral load significantly correlates with the level of CD4+Ki67+ T cells in animal WCE05 (p=0.04), but not in WCE02 and WCE03. (e) The levels of the percentage of CD4+Ki67+ cells in colon and mesenteric LNs at necropsy. \*P<0.02.



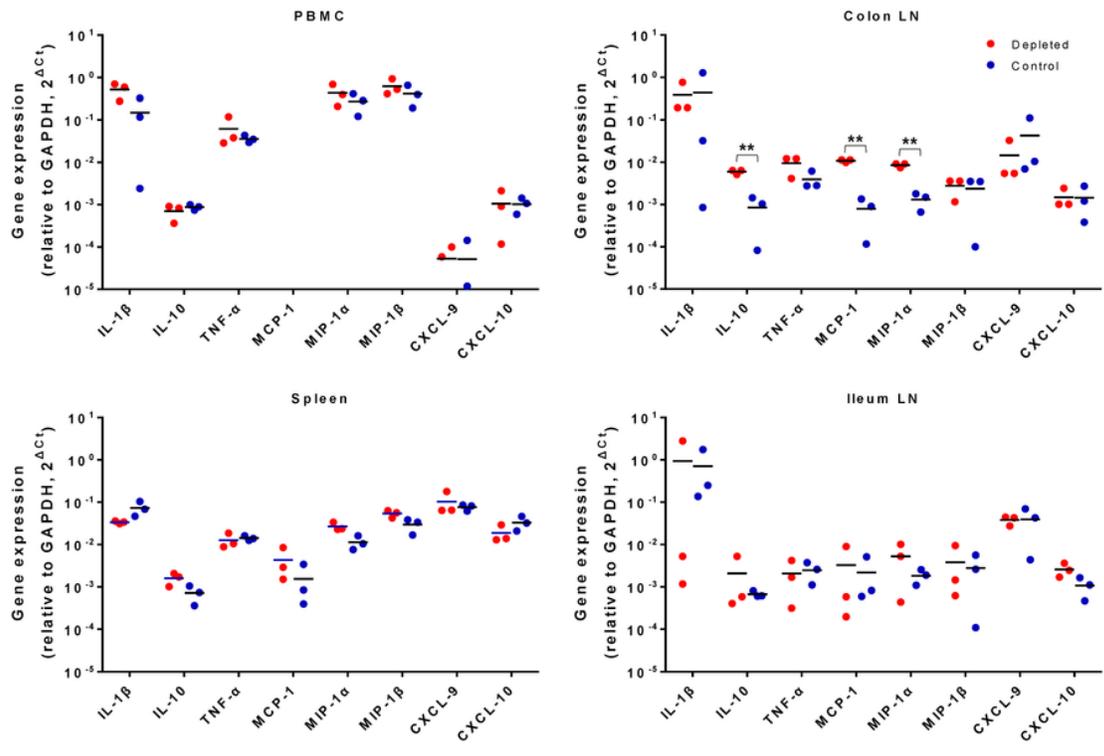
**Figure 4**

The absence or Low levels of plasma neutralizing activity against SHIVSF162P3N in both depleted and control animals. After incubated with serial dilutions of plasma collected at various time points p.d., SHIVSF162P3N infectivity was measured in CD4+ T cell depleted and control animals. Each line represents an individual time point. Dotted lines indicate 50% infectivity.



**Figure 5**

Proliferation of monocytes after depletion. Longitudinal levels (mean±SD) of blood monocyte subsets in depleted (red line; n=3) and control (blue line; n=3) animals. the percentage of turnover and proliferating CD14+CD16+ monocytes was significant higher in the depleted animals than those in control animals at week 4 p.d. \*\*P<0.01 and \*P<0.05.



**Figure 6**

Expression of the cytokines and chemokines. mRNA expression of cytokines and chemokines were measured in PB, spleen and LNs (colon and ileum) collected at necropsy. \*\* $P < 0.001$ .

## Supplementary Files

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