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Comparison of predictors for terminal disease progression in SIV/SHIV-infected rhesus macaques

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Abstract

Objectives: CD4+ T-cell decline and increasing virus levels are considered hallmarks of HIV/AIDS pathogenesis but we previously demonstrated in rhesus macaques that tissue macrophage

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Author contributions

NT, WKK, ESD, and MJK designed and coordinated the studies. NT, YC, CS and MA conducted experiments. NT, YC, CS, MA, AA, ZH, KM, GEH, and WKK acquired and/or analyzed the data. NT, ESD, AA, and MJK wrote the manuscript. All authors reviewed, edited, and approved the final submitted version of the manuscript.

Conflict of interest

There is no conflict of interest.

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destruction by SIV infection associated with increased monocyte turnover also appear to impact pathogenesis. It remains unclear, however, which factors best predict onset of terminal disease progression and survival time. The objective of this study therefore, was to directly compare these co-variates of infection for predicting survival times in retrospective studies of SIV/SHIV-infected adult rhesus macaques

Methods: Rhesus macaques were infected with various strains of SIV/SHIV and evaluated longitudinally for monocyte turnover, CD4+ T cell loss, plasma viral load, and SIV/SHIV strain. Correlation analyses and machine learning algorithm modeling were applied to compare relative contributions of each of the co-variates to survival time.

Findings: All animals with AIDS-related clinical signs requiring euthanasia exhibited increased monocyte turnover regardless of CD4+ T-cell level, viral strain, or plasma viral load. Regression analyses and machine learning algorithms indicated a stronger correlation and contribution between increased monocyte turnover and reduced survival time than between CD4+ T-cell decline, plasma viral load, or virus strain and reduced survival time. Decision tree modeling categorized monocyte turnover of 13.2% as the initial significant threshold that best predicted decreased survival time.

Conclusions: These results demonstrate that monocytes/macrophages significantly affect HIV/SIV pathogenesis outcomes. Monocyte turnover analyses are not currently feasible in humans, so there is a need to identify surrogate biomarkers reflecting tissue macrophage damage that predict HIV infection disease progression.

Keywords

Macrophages; monocytes; pathogenesis; HIV; infectious disease; biomarker; bromodeoxyuridine; cell proliferation

Introduction

Lentiviruses typically infect macrophages, but HIV and SIV evolved to preferentially target and infect CD4+ T cells [1–3]. After destruction and systematic decline in blood and tissue CD4+ T cells during the acute stage of HIV infection, there follows a substantial recovery in CD4+ T cells and virus control. During the later chronic infection phase, however, CD4+ T-cell numbers decline and plasma virus levels increase in untreated hosts. Thus, these biomarkers often are considered indicators of HIV/AIDS disease progression and increased risk for opportunistic infections and cancers [1–4].

HIV and SIV primarily infect CD4+ T cells but remain capable of infecting monocytes/macrophages that also express CD4, albeit at lower levels [5, 6]. As CD4+ T cells decline, macrophages remain accessible for infection [7–9]. Due to the more obvious loss of CD4+ T cells during HIV infection, the roles of monocytes/macrophages in pathogenesis have been relatively less characterized, particularly since the numbers of circulating monocytes remained stable over the course of rhesus macaque SIV infection and AIDS [10]. While monocyte numbers remained level, however, there was a dramatic increase in monocyte turnover during terminal disease progression to AIDS [10, 11]. This increase in monocyte proliferation and trafficking from bone marrow to blood and then tissues appeared to be an

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attempt by bone marrow to produce monocytes at a greater rate to replace the tissue macrophages destroyed by SIV (e.g. lung, intestine) [12–14].

It remains unclear, however, which biomarker(s) best predict onset of AIDS and terminal disease progression. Here, we assessed results from animals infected with SIV/SHIV in previous studies to directly compare relative contributions of currently-applied measures of disease progression such as CD4+ T cell loss, plasma viral loads, and virus strain, as well as monocyte turnover, to time until death from AIDS-associated signs of disease. Statistical correlation analyses and machine learning algorithm modeling were applied to prioritize co-variates predictive for survival times in SIV/SHIV-infected rhesus macaques.

Materials and Methods

Animals and ethics statement

Twenty adult male Indian rhesus macaques were socially paired and housed at the Tulane National Primate Research Center (TNPRC). Animals were specific pathogen-free for SIV, Type D Simian Retrovirus, and Simian T-cell Leukemia Virus type 1 at the time of assignment. All procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Tulane University Institutional Animal Care and Use Committee [15]. Animals were euthanized at times determined by veterinarians based on AIDS-related and clinically-defined endpoint indicators (e.g. weight loss, body score, lethargy, loss of appetite) or experimental endpoint in accordance with IACUC approval and national guidelines [15]. Demographic information about the animals used in this study are provided in Supplemental Table 1.

Animal inoculations

Animals were inoculated intravenously (iv) with SIV_{mac251}, SIV_{mac239}, SIV_{0302-2}, $SIV_{mac239/316ENV}$ or $SHIV_{89.6P}$ as described previously [12, 16–20], in the figure legends of each applicable experiment, and on Supplemental Table 1. Three SIV_{mac251}-infected animals received anti-CD8 antibody (Supplemental Table 2) subcutaneously (10mg/kg) on day 6 post infection and iv (5mg/kg) on days 8 and 12 as previously described [16]. Two of three anti-CD8 antibody-treated animals also received antiretroviral therapy (ART) drugs, PMPA (20mg/kg/day) and FTC (50mg/kg/day) subcutaneously 65-116 days after SIV infection [16].

Plasma viral load measurement

SIV_{mac251} plasma viral loads were measured by branched-DNA amplification as previously described [16]. The remaining SIV strains and SHIV were measured by RTqPCR targeting the SIV_{mac239} gag sequence as described [21].

Monocyte turnover (proliferation) measurement

The thymidine analog, 5-bromo-2'-deoxyuridine (BrdU, Sigma-Aldrich, St. Louis, MO) was injected iv at 60 mg/kg per animal and the percent of BrdU-incorporated monocytes and macrophages were counted in blood or tissue biopsies 24 hr later, respectively, as described

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[22]. Inguinal lymph nodes and duodenum biopsies were collected 24 hr after BrdU injections as described [14, 22].

Immunostaining and flow cytometry

Blood and tissue biopsy processing, cellular immuno-phenotyping, and staining for BrdU incorporation were performed as described [14, 23, 24]. Briefly, 200 μl of EDTA–anticoagulated whole blood or suspension of tissue cells were washed with PBS containing 2% FBS and incubated with surface monoclonal antibodies at room temperature for 20 minutes (Table S1). RBCs were lysed with FACS lysing solution (BD Bioscience, San Jose, CA). To analyze BrdU incorporation, cells were permeabilized using Cytofix/Cytoperm per manufacturer's instructions (BD Biosciences), incubated with DNase I (Sigma-Aldrich) at 37°C for one hour and then stained with anti-BrdU antibody for 20 min at room temperature. After washing, cells were fixed in 250 μl of 1% paraformaldehyde in PBS. Stained samples were acquired using LSRII, LSRFortessa, FACSAria or FACSymphoney (BD Biosciences). Acquired data were analyzed using FlowJo software (FLOWJO, LLC). For quantification of blood CD4+ T cell count, the numbers of lymphocytes were analyzed on the ADVIA 120 Hematology System (Bayer Diagnostics).

Statistical analyses and machine learning algorithms

JMP Pro 15 software (SAS Institute) was used to apply linear and non-linear regression prediction models. Results were applied to Boosted Forest and Decision Tree machine learning algorithms to assess contributions of co-variates to time until euthanasia. Goodness of fit (i.e. R^2) and the Root Average Squared Error (RASE) were used to evaluate the fit of training and validation models. *P* values for bivariate fits of time until necropsy and monocyte turnover were calculated using polynomial fit (degree=2; parabola). The *P* values for bivariate fit of time until necropsy and plasma viral load ($\log_{10}$) and CD4+ T-cell loss were calculated using linear fit. Comparison of high vs low monocyte turnover groups was conducted using Wilcoxon / Kruskal-Wallis tests (Rank Sums) and $P < 0.05$ was considered statistically significant. Data applied to statistical analyses were extracted at repeated sample collection time points during the chronic phase of the infection (>90 days post infection) from animals that developed clinical signs of terminal disease and without having received ART (i.e. animals GK40, HA52, IR99, IT27, ER17, HD43, HD42, HF01 and HE94). Graphpad Prism version 8.0 was used to plot graphs in figures (GraphPad Software, San Diego, California USA, www.graphpad.com). The data set (Takahashi_dataset.csv) and script (Takahashi_JSL_Scripts.jsl) used for the machine learning algorithms are provided in the supplemental digital content.

Results

Monocyte turnover increased prior to death in rhesus macaques infected with SIV_{mac251}.

Following SIV_{mac251} infection in adult rhesus macaques, plasma viral loads fluctuated between 10^5 and 10^8 copies/ml during the chronic phase regardless of transient anti-CD8 antibody orART (Fig. 1A). All animals exhibited declining peripheral CD4+ T-cell numbers during the chronic infection stage or after discontinuation of ART (Fig. 1B). Blood monocyte turnover was defined as the frequency of BrdU-labeled CD14+ monocytes in

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blood 24 hours after each BrdU administration and results are shown Fig. 1C. The overall results indicated that animals succumbed soon after monocyte turnover increased. The mean pre-SIV infection monocyte turnover was 3.55 % (± 2.66). Just prior to necropsy, the mean monocyte turnover was 32.5% ranging from approximately 15 – 50% or about nine-fold over pre-infection levels. One of the animals, GN17 survived 70 weeks after infection when it carried a plasma viral load of 10^5 copies / ml and low level of 221 CD4+ T cells / μl blood, but exhibited monocyte turnover that remained at baseline levels throughout the chronic infection stage.

Regardless of SIV strain, infected rhesus macaques exhibited increased monocyte turnover prior to death from AIDS.

We next followed animals infected with SIV_{0302-2}, SIV_{mac239}, or $SIV_{mac239/316ENV}$ (Fig. 2). Among the animals inoculated with SIV_{0302-2}, two underwent rapid disease progression (HD43 and HE19; plotted in red) and exhibited increased monocyte turnover prior to death. The other two animals (HE29 and HF40; plotted in black) remained free of AIDS-associated clinical signs, survived the 43-week infection experimental timeline, and did not exhibit increased monocyte turnover during this time (Fig. 2C). Plasma viral loads were above 10^5 copies / ml in three of the animals, including the two that succumbed (Fig. 2A). Interestingly, both animals that survived this 43-week infection timespan exhibited loss of CD4+ T cells to below 500 / μl blood, while one animal that developed AIDS-related clinical signs (HD43) retained over 1,000 CD4+ T cells / μl blood at time of euthanasia (Fig. 2B). Animal HD42 that was infected with SIV_{mac239} exhibited high plasma viral load (Fig. 2D), low CD4+ T cell numbers (Fig. 2E) and increased monocyte turnover at time of death (Fig. 2F). Conversely, animals with $SIV_{mac239/316ENV}$ infection expressed variable plasma viral levels (Fig. 2G), somewhat variable but declining CD4+ T cell levels (Fig. 2H), low monocyte turnover (Fig. 2I), and survived the 43 weeks of this infection study period. One animal, HA75, began to express an increase in monocyte turnover to nearly 20% with relatively higher plasma viral load and approximately 700 CD4+ T cells / μl blood 34 weeks after infection. Conversely, monkey HB42 expressed higher plasma viral load, lower CD4+ T cell numbers, and low monocyte turnover at the same time point, but the experiment finished before these animals exhibited AIDS-associated clinical signs of disease.

Despite loss of CD4+ T cells after SHIV infection, only rhesus macaques with increased monocyte turnover soon progressed to AIDS.

We next examined four animals infected with SHIV89.6P that causes massive depletion of CD4+ T cells [25] as also shown here (Fig. 3B). Two animals (HF01 and HE94; graphed in red) expressed high plasma viral loads (Fig. 3A) and increased monocyte turnover just prior to euthanasia 17 and 19 weeks after infection, respectively (Fig. 3C). Despite low CD4+ T cell levels and plasma viral loads near or above 10^5 copies/ml, the other two animals (HF07 and EN33; graphed in black) survived over 30 weeks after infection and with monocyte turnover at pre-infection baseline levels.

We had also examined inguinal lymph node and duodenum biopsies prior to SHIV89.6P inoculation as well as during the course of infection 24 hrs after each BrdU injection. There was a decline in frequency of CD4+ T cells in both tissues (Fig. 3D, E) similar to the loss in

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peripheral blood CD4+ T cells (Fig. 3B). Increased turnover of CD163+ monocytes/ macrophages was detected in both tissues (Fig. 3F, G) from one animal that succumbed (HE94) and in lymph node of HF01 (data were unavailable for duodenum from HF01 at later time points) and this also was consistent with the higher blood monocyte turnover (Fig. 3C). Despite low CD4+ T cell numbers, the percent of BrdU+CD163+ macrophages remained low in the two animals that survived.

Monocyte turnover correlated better to survival time than CD4+ T-cell loss, plasma viral load, or SIV/SHIV virus strain.

Using regression mathematical models, we analyzed the relationships between co-variates of CD4+ T-cell loss, plasma viral loads, and monocyte turnover with time until AIDS-related clinical signs requiring euthanasia in the SIV/SHIV-infected animals. To find the proper fit for each curve, we used linear and polynomial fits for each co-variate (linear, quadratic, cubic, quartic, and quantic) and the best fit was selected based on the Akaike information criterion (AIC) as well as Bayesian information criterion (BIC). Results for monocyte turnover differed from the other co-variates (Supplemental Fig. 1 & Supplemental Table 3) and the quadratic equation was used to correlate monocyte turnover to time until necropsy (Fig. 4A). The linear equation produced the best-fit models for percent CD4+ T-cell loss and plasma viral loads to predict time until necropsy (Fig. 4B and C). The coefficient of determination (R^2) value between monocyte turnover and survival time was 0.575 or 57.5% correlation probability (Fig. 4A; $P < 0.0001$) that was greater than 0.101 for CD4+ T-cell loss (Fig. 4B; $P = 0.0669$) and 0.101 for plasma viral levels (Fig. 4C; $P = 0.0671$) in relation to time until necropsy.

Machine learning algorithms categorized monocyte turnover as the better predictor of survival time.

We applied bootstrap forests (a random-forest technique) to measure the contribution of the covariates in predicting the days to necropsy. This model was built upon 100,000 trees in the forest and tuned using other parameters (Supplemental Fig. 2A). Training and validation datasets were randomly generated using 75% and 25% of the data points, respectively. The training portion of the data was used to build the models and the validation portion was used to validate the models in order to choose the most parsimonious model and minimize overfitting (Supplemental Fig. 2B). a random variable (i.e. RANDOM NORMAL [RN]; generated randomly using Gaussian distribution) was included in the models as a new covariate to control for modeling background noise. The RN variable established the threshold and contributions falling below this threshold were considered insignificant in context of the model. Monocyte turnover generated the highest contribution to survival time at 47.5% (red bar; Supplemental Fig. 2C) followed by CD4+ T-cell loss at 21.6% and plasma viral load at 18.69%.

Next, we applied decision tree algorithm using longitudinal data for the main covariates as also used in the bootstrap modeling. To ensure model validation, we considered that data points were independent within each individual subject. This resulted in the prediction that animals with monocyte turnover equal to or higher than 13.2% exhibited a significantly lower chance of surviving longer than an average of 49 days. Animals with monocyte

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turnover less than 13.2% had a greater likelihood for living longer by an average of 232 days. Viral strains and plasma viral levels were contributing co-variates in the random forest algorithm results (Supplemental Fig. 2C), but these were not statistically meaningful in the decision tree algorithm (Supplemental Fig. 2F). Here, monocyte turnover showed the highest contribution (0.9487 or 94.87%) for predicting time until necropsy (Supplemental Fig. 2 D–G). To further validate results of the machine learning models, the category of days until necropsy was dichotomized to compare data below (Low) and above (High) the 13.2% monocyte turnover (MTO) branch. As predicted by the model, there was a statistically significantly shorter survival time ($P < 0.0001$) in the High MTO versus the Low MTO group (Fig. S2H).

Discussion

CD4+ T cell numbers and plasma viral levels during SIV/HIV infection often are used as metrics of disease progression to AIDS, (as well as for evaluating efficacy of antiretroviral therapies [2, 26]. We previously reported that increasing monocyte turnover also directly accompanied the onset of terminal disease progression to AIDS in SIV-infected rhesus macaques [10, 12, 13] while another report linked increased monocyte turnover to SIV encephalitis in rhesus macaques [11]. Furthermore, very young human and rhesus macaque infants are especially vulnerable to faster disease progression after HIV/SIV infection that we reported coincided with a higher physiological baseline monocyte turnover in pediatric macaques younger than 2-3 months of age [22, 27]. Debate remains however, about which factor(s) best predicts onset of terminal disease or survival time. Here, we applied unbiased machine learning and statistical analyses to directly compare plasma virus levels, CD4+ T cell numbers in blood, and monocyte turnover in groups of adult rhesus macaques infected with different strains of SIV in relation to AIDS-related survival time. Only animals reaching the chronic phase of infection or at least 90 days after virus inoculation and recovery from acute-stage peak virus levels were evaluated. Also, data points in the machine-based modeling were only from animals that developed clinical signs of SIV/AIDS requiring euthanasia based on pre-defined endpoint criteria established at the TNPRC with IACUC approval so that no imputed data were applied.

Impending onset of clinical signs requiring euthanasia occurred soon after monocyte turnover increased. Conversely, some animals that maintained lower monocyte turnover near baseline levels after infection, despite loss of CD4+ T cells, survived. This was especially prominent after $SHIV_{89.6P}$, a virus that is highly destructive for CD4+ T cells. All four animals in this group exhibited sharp declines in CD4+ T cells that remained low after the acute phase and through the chronic phase. However, only the two animals with increased monocyte turnover succumbed during the experiment time course while the other two with lower monocyte turnover survived. While CD4+ T cell numbers declined in blood after infection with SIV/SHIV in these animals, there did not appear to be a specific level or rate of decline that clearly or imminently predicted onset of terminal disease progression. Similarly, plasma virus levels trended higher in animals nearing terminal disease, but with some exceptions. For example, there were higher plasma virus levels in animals that survived, such as observed for HF29 infected with SIV_{0302-2} or in animals HA75 and HB42 with $SIV_{mac239/316ENV}$ that carried nearly 1 million virus copies/ml. Conversely, GK40 that

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was infected with SIV_{mac251} succumbed earlier with high monocyte turnover but had lower plasma viral loads at approximately 10^5 copies / ml several weeks prior to death.

Statistical models were applied to better prioritize the relationships between these covariates and survival. To achieve this, best-fit models were defined to correlate each co-variate to time until AIDS-required euthanasia. Monocyte turnover produced the best fit ($R^2 = 57\%$, $P < 0.0001$) compared to CD4+ T cell loss or plasma viral load (Fig. 4) meaning that nearly 60% of the variation in survival time could be explained by the monocyte turnover during the chronic phase of the infection. This prediction was considerably lower for CD4+ T cell loss or plasma viral load (both at approximately 10%).

Machine learning algorithms then were used to assess which co-variate (or combination) best predicted time until death. To use a threshold for modeling the noise in the data and to provide a more robust predictive analytic, we included a randomly-generated and normally-distributed continuous variable into the model named Random Normal. Our boosted forest and decision tree models suggested that the contribution of monocyte turnover offered the best prediction of time until necropsy. Modeling of the chronic phase infection data using decision tree categorized monocyte turnover 13.2% as the initial significant threshold in relation to decreased survival time. This monocyte turnover rate may offer a practical biomarker for monitoring and predicting health status in nonhuman primates infected with SIV/SHIV.

The biological effect of increased monocyte turnover in blood appears to reflect macrophage tissue destruction by the virus [12, 13]. Monocytes are continuously produced in bone marrow, circulate in blood, and then traffic to tissues. Using BrdU labeling, we observed that the half-life of monocytes is approximately 1 day [23]. In lung and gut, short-lived macrophages in tissue become readily infected and destroyed by SIV while longer-lived macrophages become infected but appear resistant to apoptosis and survive, thereby likely contributing to the virus reservoir [10, 12–14]. An escalating need to replace short-lived tissue macrophages destroyed by virus infection appears to drive an increase in production of monocytes from bone marrow, as detected by increased proliferation of monocytes and trafficking to the tissues. This suggests that eventually, the level of tissue macrophage damage overrides the ability of bone marrow to produce sufficient numbers of monocytes that would differentiate into functional mature macrophages in tissues. This could result in insufficient numbers of mature tissue macrophages available for protection.

Several experiments utilizing SIV/SHIV-infected rhesus macaques, however, were completed prior to animals reaching endpoints with AIDS-related clinical signs requiring euthanasia. Additional longitudinal studies with matched parallel data sets would enable more refined quantitative thresholds. Since monitoring cell turnover or proliferation rate is less feasible in humans than in experimental laboratory animals, longitudinal studies to assess parameters reflecting tissue macrophage damage directly associated to blood monocyte turnover also may identify the best predictive biomarkers of HIV disease progression.

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Additional biomarkers that may be indicative of or predictive for disease progression include sex, MHC type I alleles (i.e. Mamu), and CD4+ T-cell subsets and levels in tissues. For example, there are several reports about the importance of memory CD4+ T cell homeostasis in affecting disease progression in NHP models of SIV infection and disease [28, 29]. In rhesus macaques infected with the CCR5-tropic SIV_{mac} strain, failure of CD4+ memory cells to restore homeostasis, including insufficient proliferation of CD4+ central memory T (TCM) cells, deficient levels of CD4+ effector memory T (TEM) cells in chronic phase, or inability to deliver memory cells into the tissues, affected the rapid rate of disease progression and onset of terminal disease. Conversely, sooty mangabeys expressed lower levels of CCR5 molecules on CD4+ T cells, carried a smaller population or fraction of CD4+ TCM cells during SIV infection, and exhibited lower susceptibility to SIV-associated disease compared to rhesus macaques [30, 31]. This retrospective study described herein comprised all male animals and there were no consistent associations between Mamu alleles with disease progression. For example, EN33 survived with baseline monocyte turnover and did not express Mamu-A*01, B*08, or B*17, whereas HF07 that also survived with normal monocyte turnover did express Mamu-B*08. Conversely, both HF01 and HE94 received SHIV89.6P and underwent disease progression despite expressing Mamu-A*01. Thus, additional studies will better help prioritize relative contributions of such biomarkers for predicting onset of terminal disease progression that would also help define mechanisms of pathogenesis.

In conclusion, the overall results demonstrated that among the covariates tested here, increasing monocyte turnover was a stronger predictive biomarker than CD4+ T-cell numbers, plasma viral levels, or virus strain for impending terminal disease progression in the rhesus macaque model of HIV/AIDS [12–14, 22, 27, 32]. While CD4+ T cells are early targets of HIV/SIV infection and their decline is associated with adaptive immune response deficiency [2, 3], declining innate immunity affected by monocyte/macrophage turnover, also appears relevant. ART extended longevity and lifespan in persons with HIV by effectively reducing plasma viral levels and retaining CD4+ T-cell numbers, and this was accompanied by maintained baseline monocyte turnover in the SIV-infected and ART-treated rhesus macaque model. HIV/SIV, however, persists in latently-infected memory CD4+ T cells [33] and we observed that long-lived tissue macrophages also become infected with SIV, do not undergo apoptosis, and thus may contribute to the virus reservoir [12, 14]. Future studies also may need to focus on the role of infected longer-lived tissue macrophages that likely contribute to the antigenic stimulus promoting chronic inflammation, immune activation, and onset of comorbidities in persons living with HIV [26].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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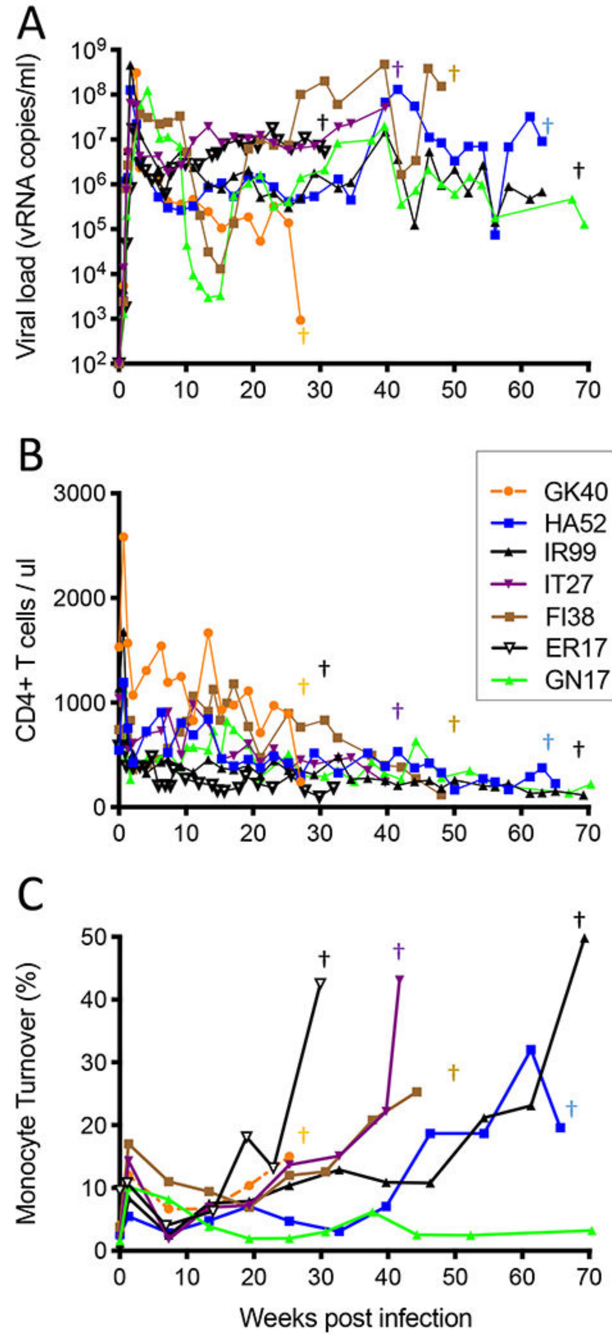

Fig. 1. Increased monocyte turnover occurred prior to death in rhesus macaques infected with SIV_{mac251}.

Adult rhesus macaques (n=7; ranging from 5.4 – 8.3 years of age) were inoculated intravenously with 1000 $TCID_{50}$ SIVmac251. Results are shown to compare **(A)** plasma viral loads (PVL) for SIV and **(B)** absolute CD4+ T cell numbers per μl blood. PVLs from all animals and CD4+ T cell numbers from GK40, HA52, IR99 and IT27 were reported previously [16]. **(C)** Monocyte turnover in blood was defined as frequency (percent) of BrdU+ cells in the CD14+HLA-DR+ population of PBMC that were negative for CD3, CD8

and CD20 24 hr after BrdU injection. Animals ER17, FI38 and GN17 received anti-CD8 antibody in the acute phase, and FI38 and GN17 also received ART on days 65-116 post SIV infection. † is displayed at time of medically-required euthanasia. Plasma viral levels and CD4+ T cells for the animals that had been reported previously were presented here with permission by the publisher, Mary Ann Liebert, Inc. [16], and herein include additional later time-point data to allow for direct comparison of monocyte turnover values over time after SIV infection.

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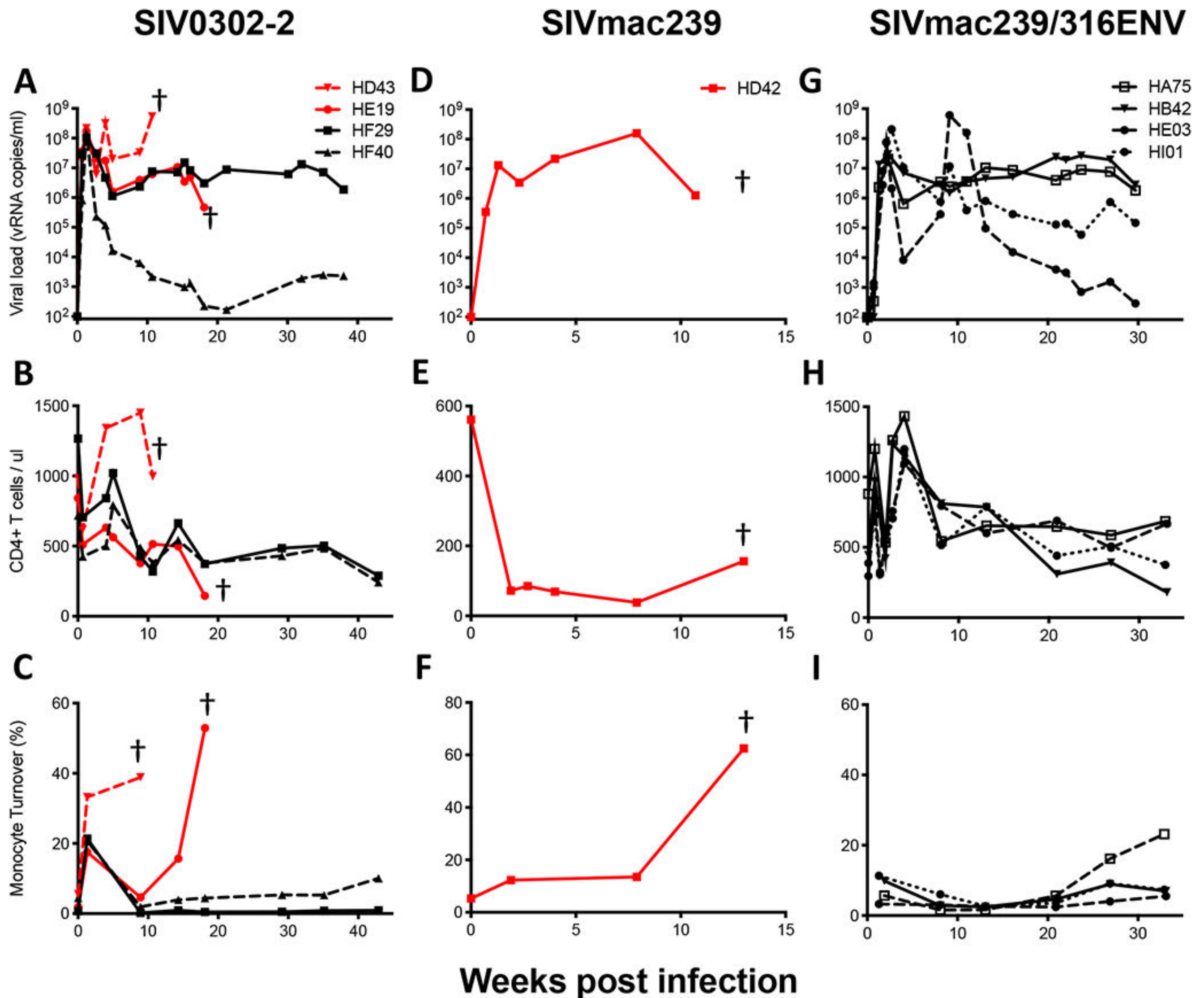

Fig. 2. Regardless of SIV strain, infected rhesus macaques exhibited increased monocyte turnover prior to death from AIDS.

Rhesus macaques were inoculated IV with 1000 $TCID_{50}$ of **(A-C)** SIV_{0302-2} (n=4), (**D-F**) SIV_{mac239} (n=1), or (**G-I**) $SIV_{mac239/316ENV}$ (n=4). Animals exhibiting disease progression were indicated in red. PVL are shown in the upper panels **(A, D, G)**, CD4+ T-cell numbers are shown in the middle panels **(B, E, H)**, and monocyte turnover is shown on the bottom panels **(C, F, I)**. Pre-infection monocyte turnover in the $SIV_{mac239/316ENV}$-infected animals were not available. † indicates time of euthanasia required due to AIDS-defining clinical signs of disease.

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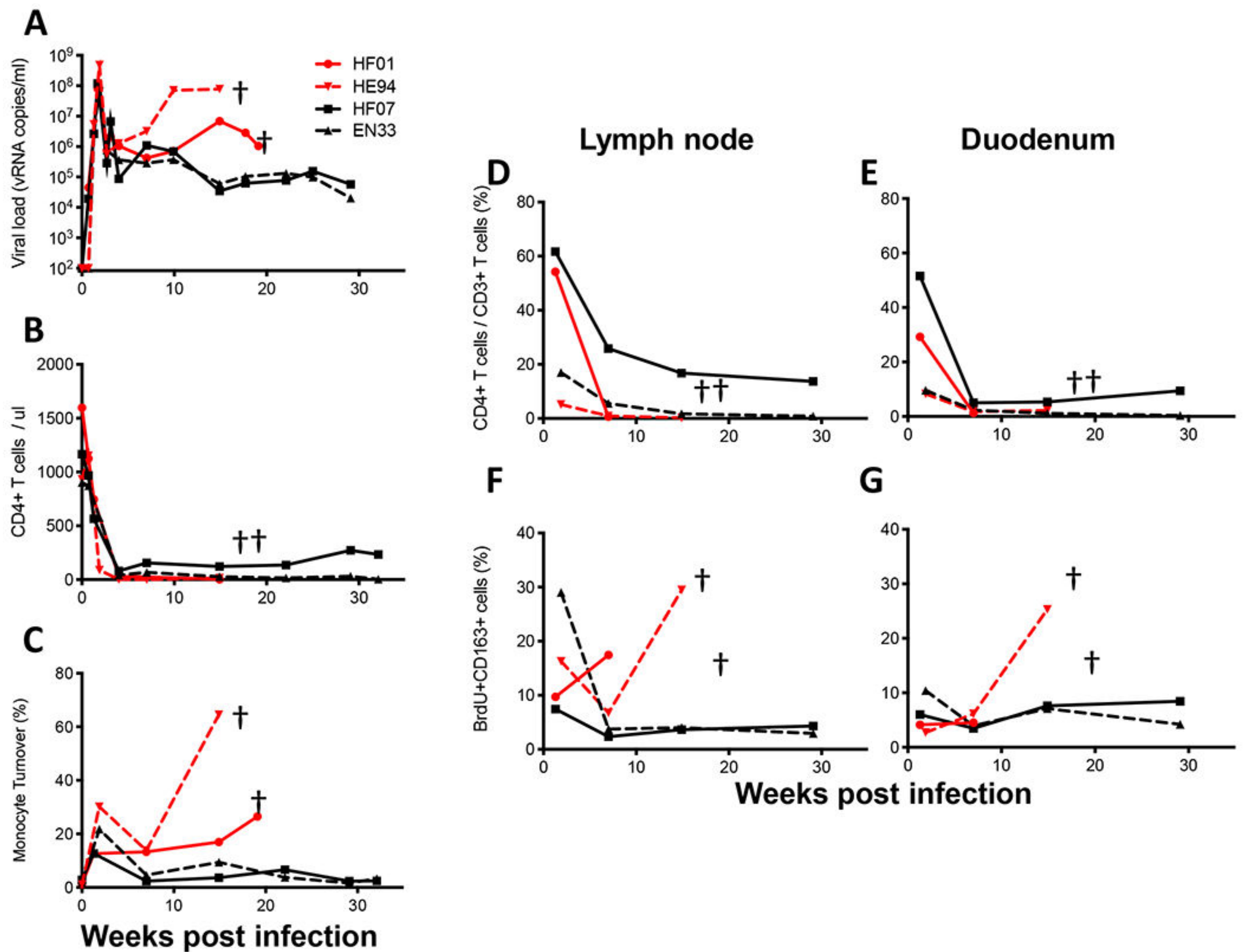

Fig. 3. Despite loss of CD4+ T cells after $SHIV_{89.6P}$ infection, only rhesus macaques with increased monocyte turnover soon progressed to AIDS.

(A) Plasma viral loads**, (B)** CD4+ T cells and **(C)** monocyte turnover were plotted over time after inoculation of rhesus macaques with 400 $TCID_{50}$ of $SHIV_{89.6P}$ (n = 4). Flow cytometery was used to measure the percent of CD4+ T cells in total CD3+ T cells in inguinal lymph node **(D)** and duodenum **(E)** as well as of BrdU+ CD163+ macrophages in lymph node **(F)** and duodenum **(G)**. † designates time of necropsy due to AIDS-defining clinical signs after $SHIV_{89.6P}$ infection.

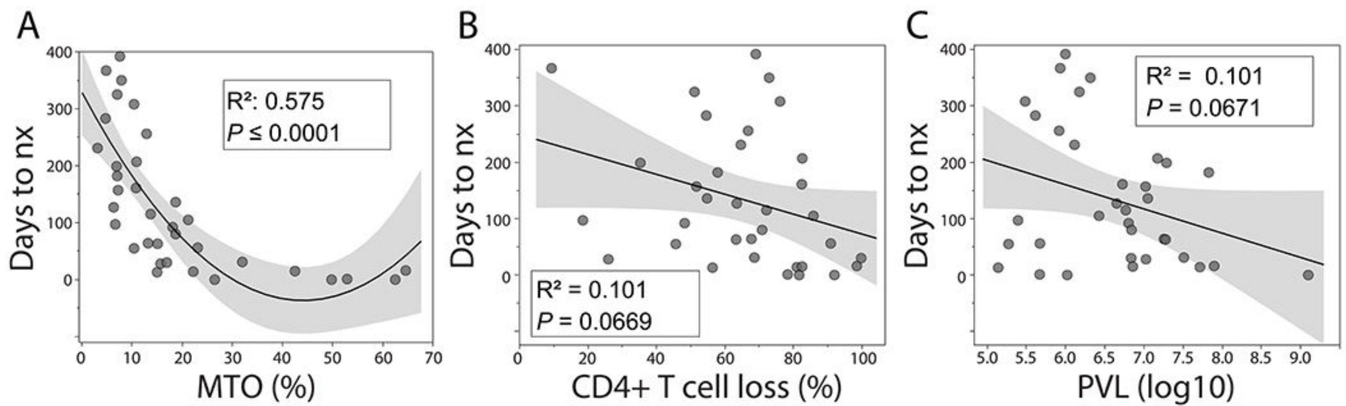

Fig. 4. Monocyte turnover better correlated with survival time than CD4+ T-cell loss, plasma viral load, or SIV/SHIV virus strain in infected rhesus macaques.

The relationships between the remaining survival time (indicated as days to nx) and monocyte turnover were fitted (n=9 animals, 35 time points) using 2nd degree (quadratic) polynomial curves using least squares regression – also see Fig. S1 and Table S2 **(A)**, and least squares regression models for percent CD4+ T cell loss (n=9 animals, 34 time points) **(B)**, and log_{10} / ml plasma viral load (PVL log_{10}; n=9 animals, 34 time points) (**C**). Repeated sampling data were extracted from results during the chronic phase of the infection (after 91 days post infection) from animals requiring euthanasia due to disease progression. The percentage of CD4+ T-cell loss (CD4 loss) and monocyte turnover (MTO) were calculated from the average of pre-infection baseline values. Only matched data points available for each co-variate were used in the modeling and no data points were imputed.

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