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Effect of Single Housing on Innate Immune Activation in Immunodeficiency Virus–Infected Pigtail Macaques (*Macaca nemestrina*) as a Model of Psychosocial Stress in Acute HIV Infection

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ABSTRACT

Objective: Simian immunodeficiency virus (SIV) infection of macaques recapitulates many aspects of HIV pathogenesis and is similarly affected by both genetic and environmental factors. Psychosocial stress is associated with immune system dysregulation and worse clinical outcomes in people with HIV. This study assessed the impact of single housing, as a model of psychosocial stress, on innate immune responses of pigtailed macaques (*Macaca nemestrina*) during acute SIV infection.

Methods: A retrospective analysis of acute SIV infection of 2- to si6-year-old male pigtailed macaques was performed to compare the innate immune responses of socially ($n = 41$) and singly ($n = 35$) housed animals. Measures included absolute monocyte count and subsets, and in a subset ($n \leq 18$) platelet counts and activation data.

Results: SIV infection resulted in the expected innate immune parameter changes with a modulating effect from housing condition. Monocyte number increased after infection for both groups, driven by classical monocytes ($CD14^+CD16^-$), with a greater increase in socially housed animals (227%, $p < .001$, by day 14 compared with preinoculation time points). Platelet numbers recovered more quickly in the socially housed animals. Platelet activation (P-selectin) increased by 65% ($p = .004$) and major histocompatibility complex class I surface expression by 40% ($p = .009$) from preinoculation only in socially housed animals, whereas no change in these measures occurred in singly housed animals.

Conclusions: Chronic psychosocial stress produced by single housing may play an immunomodulatory role in the innate immune response to acute retroviral infection. Dysregulated innate immunity could be one of the pathways by which psychosocial stress contributes to immune suppression and increased disease severity in people with HIV.

Key words: simian immunodeficiency virus, psychosocial stress, innate immunity, infectious disease, animal models, human immunodeficiency virus.

INTRODUCTION

Human immunodeficiency virus (HIV), the agent responsible for AIDS, affects an estimated 38 million people worldwide (1). Although about two-thirds of people with HIV (PWH) have access to antiretroviral therapy (ART), many remain untreated or experience interruption in access to medication, and approximately 690,000 deaths were attributed to AIDS-related illness in 2019 (1,2). In addition, clinical comorbidities still develop during ART. For example, central nervous system (CNS) complications remain highly prevalent in ART-treated individuals and are associated

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ART = antiretroviral therapy, ***B*** = unstandardized β coefficient, **CBC** = complete blood count, **CSF** = cerebrospinal fluid, **CNS** = central nervous system, **HIV** = human immunodeficiency virus, **PWH** = people with HIV, **SE** = standard error, **SIV** = simian immunodeficiency virus

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with lower quality of life and increased mortality (3). A major barrier to curing HIV infection is the establishment of latent viral reservoirs within resting cells and tissues inaccessible to ART (4,5). These reservoirs may play a role in predisposition to the comorbidities seen in PWH by serving as an ongoing inflammatory stimulus (6,7).

For these reasons, it is imperative that the immunomodulatory response to HIV infection is better understood. Clinical outcomes from HIV infection vary widely between individuals; these variations are modulated by both genetic and environmental factors that alter immune response (8–10). Among these, PWH are at greater risk of external stress from social factors, such as social isolation and loss of support due to the stigmatized nature of HIV infection. These psychosocial stressors (11) are associated with worse mental and physical quality of life in PWH compared with other chronic conditions and are associated with increased incidence of comorbidities such as cognitive impairment (12–17). Establishment of increased social support improves personal resilience and has been shown to mitigate the negative impacts of social stressors (14,17–19).

Chronic stressors and HIV infection independently induce persistent immune activation, and many studies have demonstrated the importance of immune dysregulation, rather than viral load, in the development of adverse outcomes (20,21). Monocytes in particular have been identified as a pivotal cell type in mediating damage in HIV infection secondary to inflammation. Activation of monocytes and macrophages, as indicated by plasma level of sCD14 and sCD163, is associated with a greater risk of cardiovascular disease, cognitive impairment, and other comorbidities in PWH (22,23). In particular, monocyte subsets expressing CD16 ($CD14^+CD16^+$ intermediate monocytes and $CD14^{low}$ $CD16^+$ nonclassical monocytes) have been found to be permissive to HIV and simian immunodeficiency virus (SIV) infection. These monocytes may play an important role in transporting virus into the brain, contributing to the establishment of latent viral reservoirs in this sanctuary organ (23,24). Platelets also play an important role in modulating immune responses to HIV infection, demonstrated both in the formation of activated platelet-monocyte aggregates that drive the adoption of the $CD16^+$ monocyte phenotype during acute infection and by the persistent platelet activation seen in PWH receiving ART (25–27).

Direct study of the impact of psychosocial stress in HIV infection is confounded by the complex nature of sociobehavioral factors and their potential to affect access to care (18,19). However, psychosocial stress can be recapitulated with animal models to allow for its investigation in a controlled setting (28–30). The pigtail macaque (*Macaca nemestrina*) model of SIV infection pathophysiologically resembles HIV infection in humans, and disease progression is similarly characterized by CD4 T-cell depletion and a persistent viral reservoir during ART (31,32). Previous work by this group demonstrates that the psychosocial stress associated with single housing results in elevated viral load and dysregulation of both CD4 and CD8 T-cell activation during acute SIV infection in this social species (33). Because these changes in viral dynamics and adaptive immunity occur acutely after infection, these findings raised the question of whether these differences may be driven by alterations in innate immune function. In this investigation, we seek to expand on previous findings by characterizing the innate immune response to inoculation with SIV in singly and socially housed macaques. A retrospective analysis of monocyte and platelet hematology and flow cytometry data was performed. We hypothesized that psychosocial stress would

result in a less robust innate immune response during acute infection in the SIV-infected pigtail macaque model.

METHODS

Animals
A retrospective analysis of acute infection data from 76 two- to six-year-old male pigtailed macaques (*M. nemestrina*) dual-inoculated with a single stock of the neurovirulent clone SIV/17E-Fr and immunosuppressive swarm SIV/DeltaB670, as previously described (32,33), was performed. All were specific pathogen free for SIV, simian T-cell leukemia virus, and simian type D retrovirus before inoculation and negative for the Mane-A1*08401 major histocompatibility complex class I allele, which confers resistance to SIV (34). Three baseline time points of sample collection were performed before inoculation, each at least 2 weeks apart. Postinoculation samples were collected on days 7, 10, and 14. Macaques were sedated with ketamine (10 mg/kg intramuscular) at each time point for cerebrospinal fluid collection from the cisterna magna followed by collection of 13 ml of blood via femoral venipuncture. Samples were collected with a 21-gauge 1.5-inch needle directly into vacutainer tubes containing 3.2% sodium citrate at a 9:1 ratio of blood to anticoagulant for platelet samples or into syringes with acid citrate dextrose solution at a 5:1 ratio of blood to anticoagulant for monocyte samples. Socially housed macaques were always inoculated and sampled on the same day at the same time as the other animals in their pair or trio, and sedation order remained constant throughout the study. Most macaques initiated ART on day 12; those that did not were excluded from day 14 analysis (Supplemental Digital Content, Table S1, http://links.lww.com/PSYMED/A873).

All animals were housed in the same animal facility and had ad libitum food (Purina 5038) and water throughout the study, as well as daily enrichment from behavior staff. All macaques used before 2013 were kept in single housing ($n = 35$): animals were able to visualize and interact with conspecifics in the room, but had no direct, full contact. After 2013, incoming animals were grouped in compatible pairs or trios ($n = 41$) by an animal behaviorist and observed over time to ensure pair stability. Socially housed animals were all introduced at least 2 months before initiation of the study, and groupings remained stable with full contact for the duration of this study.

All procedures were approved by the Johns Hopkins University Institutional Animal Care and Use Committee and were conducted in accordance with guidelines set forth in the Animal Welfare Regulations and the Guide for the Care and Use of Laboratory Animals. These studies were all conducted within a fully AAALAC-accredited facility.

Sample Processing
Complete blood counts (CBCs) were performed on citrated whole blood samples using either a Hemavet (Drew Scientific) hematology analyzer or a Procyte Dx Hematology Analyzer (IDEXX Laboratories; Supplemental Digital Content, Tables S1 and S2, http://links.lww.com/PSYMED/A873); both had been calibrated for use in pigtailed macaques. To assess monocyte phenotype, citrated whole blood was stained with antibodies for CD14 and $CD16^{+/-}$TLR2 (Supplemental Digital Content, Table S3, http://links.lww. com/PSYMED/A873) for 20 minutes at room temperature, followed by a 10-minute fixation with BD FACS Lysing Solution (BD Biosciences) (35). To assess platelet activation, citrated whole blood was stained within 1 hour of collection with antibodies for CD42a, P-selectin, CD40L, and HLA-ABC (Supplemental Digital Content, Table S3, http://links.lww.com/ PSYMED/A873) for 15 minutes at room temperature followed by fixation with 2% neutral-buffered formalin. The samples were analyzed by a single technician using a FACSCalibur (BD Biosciences) or a BD LSRFortessa (BD Biosciences) flow cytometer (Supplemental Digital Content, Tables S1 and S2, http://links.lww.com/PSYMED/A873).

Data Analysis
For this investigation, flow cytometry data files were reanalyzed in FlowJo (version 10) by a single blinded researcher. Results were compared between

animals that were singly housed (2007–2012) versus those who were housed socially long term with a compatible conspecific (2013–2017). Because of the retrospective nature of this study, sample size for each parameter varies by the data available for each animal (Supplemental Digital Content, Table S4, http://links.lww.com/PSYMED/A873); day 14 samples were excluded from monkeys that did not initiate ART on day 12. Gating was performed as shown in Supplemental Digital Content, Figure S3, http://links.lww.com/PSYMED/A873.

To calculate absolute cell numbers for monocyte subtypes, the percent gated cells for each parameter were multiplied by the total monocyte number from the corresponding CBC for that animal and time point. A series of linear mixed-effects regression models were conducted to accommodate animals being nested in different studies. Post hoc analyses of hematology analyzer, animal age, and animal sedation order were conducted when possible and showed no effect on the outcomes under investigation. Flow cytometry machine used did show an effect on percentage of monocytes classified as $CD14^+CD16^+$ intermediate monocytes (post hoc data not shown). For this reason, only data from a single FACS machine (BD LSRFortessa) was used for subsequent analyses ($n = 41$ socially housed, $n = 17$ singly housed). Analysis assessed the influence of housing status from baseline (before infection) up to 14 days after inoculation and the relationship with previously published infection data (33). Statistical analyses were performed in SAS PROC MIXED (version 9.4) and GraphPad Prism (version 9.1.2), and graphs were generated using GraphPad Prism (version 9.1.2). Results with a $p < .05$ were considered significant after applying a false discovery rate correction to adjust for multiple comparisons.

RESULTS

Psychosocial Stress Associated With Transient Decline in Total Monocytes During Acute SIV Infection

Monocytes are innate immune effector cells that play a critical role in the initial response to viral challenge and thus are an important determinant in the outcome of acute HIV and SIV infection (36–38). To assess the changes in circulating monocyte numbers over the course of acute infection with SIV, CBCs were performed at three baseline time points and at 7, 10, and 14 days after inoculation (Figure 1A). Before inoculation, a single preinoculation time point showed a higher monocyte count in singly housed animals than in those that were socially housed (unstandardized β coefficient [B] = −0.13, standard error [SE] = 0.05, $p = .012$). Monocyte numbers increased over the first 2 weeks after infection with SIV in all animals, reflective of typical innate immune activation in response to viral inoculation (39–41). However, at day 7 after inoculation, the singly housed animals experienced a transient 40% decline in monocyte number that was not observed in socially housed animals ($B = 0.29, 0.28, 0.29$; $SE = 0.06, 0.05, 0.06$; $p < .001$ for all comparisons with preinoculation time points).

Increase in Classical and Intermediate Monocytes During Acute Infection Delayed With Psychosocial Stress

Circulating monocytes can be classified by their transcriptional profiles into three major subtypes. Acute inflammatory processes stimulate release of $CD14^+$ classical monocytes from the bone marrow, contributing to peripheral monocytosis during early infection (39–41). Monocytes expressing CD16 exhibit a more mature phenotype associated with patrolling the vasculature but have also been shown to be more susceptible to infection with HIV and SIV (23,42). Monocyte subsets were classified at each time point by their level of CD14 and CD16 expression. Classical monocytes were gated as monocytes negative for CD16 ($CD14^+CD16^-$), whereas

intermediate ($CD14^+CD16^+$) and nonclassical ($CD14^-CD16^+$) monocytes were distinguished by the relative level of surface CD14 (Figures 1B–D). All macaques demonstrated the same pattern of change in classical ($CD14^+CD16^-$) monocyte number over acute infection (Figure 1B), consisting of transiently decreased classical monocytes at day 7 after inoculation followed by increased numbers by day 14. However, singly housed macaques experienced a greater decline in classical monocytes at day 7 ($B = 0.32, 0.32, 0.23$; $SE = 0.05, 0.04, 0.05$; $p < .001$ for all preinoculation time points) and numbers did not increase until day 14 (127% increase from baseline; $B = −0.42, −0.42, −0.51$; $SE = 0.12$ and $p < .001$ for all preinoculation time points), whereas socially housed macaques first demonstrated a 43% increase in classical monocytes on day 10 after inoculation ($B = −0.13, −0.16, −0.15$; $SE = 0.04$ and $p \leq .001$ for all preinoculation time points), which increased to 227% by day 14 ($B = −0.76, −0.79, −0.78$; $SE = 0.06$ and $p < .001$ for all preinoculation time points). Thus, classical monocyte counts were lower in singly housed macaques at all acute infection time points, and the magnitude of this difference increased over time.

Before inoculation, all macaques had similar numbers of each monocyte subset, with the exception of intermediate monocytes at a single time point. In singly housed macaques, neither $CD16^+$ monocyte subset count increased significantly after infection. Although classical monocytes represented the primary driver of change in total monocyte number in all macaques because of the large proportion of circulating monocytes with this phenotype, intermediate monocytes ($CD14^+CD16^+$; Figure 1C) also increased after SIV inoculation in socially housed macaques only (effect of housing: $F(1,312) = 9.87$, $p = .002$). There were no differences in nonclassical monocytes ($CD14^-CD16^+$; Figure 1D) between housing groups or over time.

Prior work has demonstrated differences in both CD4+ T-cell count and viral load in socially housed compared with singly housed macaques (33). To determine if there may be an association between the dynamics in circulating monocyte subsets and the progression of infection, previously published viral load and CD4 T-cell data (33) at day 7 after inoculation were compared with monocyte subsets in the context of housing groups (Figure 3). This time point demonstrated the largest differences in monocytes subsets between singly and socially housed animals and occurred before peak viral load. Numbers of the canonical target for SIV infection, the CD4+ T cell (Figure 3A), did not correlate with plasma or cerebrospinal fluid (CSF) viral load overall, or in the singly or socially housed subsets. Similarly, classical monocytes ($CD14^+CD16^-$; Figure 3B) showed no direct correlation with viral load in either the plasma or the CSF despite distinct patterns of association with viral load between socially and singly housed animals. Intermediate monocytes ($CD14^+CD16^+$; Figure 3C) also showed no correlation with viral load in the plasma for all macaques. However, CSF viral load showed an association with the number of intermediate monocytes ($p = .03$, Spearman $r = 0.33$) in socially housed macaques only, although no relationship was present in singly housed macaques. There was no relationship between either housing group or viral loads and nonclassical monocytes (Figure 3D).

SIV-Associated Thrombocytopenia Prolonged With Psychosocial Stress

Transient thrombocytopenia is a typical manifestation of early retroviral infection, which resolves without observable clinical signs (21,25–27).
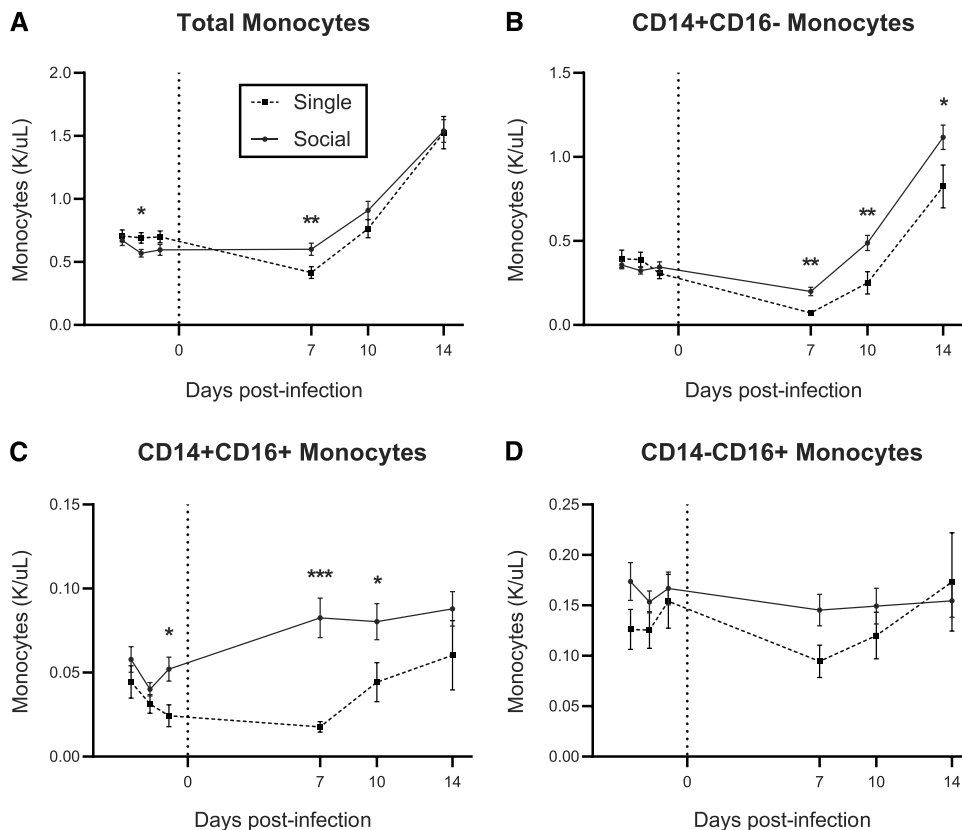
FIGURE 1. Monocyte response to acute SIV infection in singly compared with socially housed macaques. Housing groups were compared from baseline (three time points) to 7, 10, and 14 days after inoculation with SIV (for n for each time point, see Supplemental Digital Content, Table S4, http://links.lww.com/PSYMED/A873). ART was started on day 12 for all animals. A, White blood cell counts in whole blood CBCs were compared. Using a linear mixed-effects regression model, differences between housing groups over time were significant ($F(5,406) = 4.27$, $p < .001$). B–D, CD14 and CD16 monocytes expression profiles were compared using flow cytometry, and total monocyte count was used to calculate absolute numbers of each subtype. Error bars display the standard error of estimated least squares means generated by a linear mixed-effects regression model. * $p < .05$; ** $p < .01$; *** $p < .001$. SIV = simian immunodeficiency virus; ART = antiretroviral therapy; CBC = complete blood count.

Data from CBC also allowed for comparison of circulating platelet counts between singly and socially housed animals over the course of acute SIV infection (Figure 2A). Both groups developed thrombocytopenia after inoculation, consistent with previous reports of response to acute infection in SIV (25). Although the onset and magnitude of thrombocytopenia were similar between housing groups, platelet numbers in singly housed macaques did not return to baseline by day 14 after inoculation, leading to a divergence between housing groups at this time point ($B = 130.46$, SE = 29.79, $p < .001$). The nadir in platelet count occurred on day 10 for both housing groups, with a 58% decline from baseline in singly housed macaques and a 43% decline in socially housed macaques (Supplemental Digital Content, Figure S1A, http://links.lww.com/PSYMED/A873).

Platelet Activation During Acute Infection Is Suppressed With Psychosocial Stress

Platelets are increasingly recognized for their role as immune effector cells upon activation, and both signal to other immune cells and directly interact with SIV and HIV in vivo (25,43–46). Platelet activation in whole blood samples was assessed using flow cytometry based on surface expression of P-selectin, CD40L, and MHC-I at two baseline time points and at day 10 after inoculation (Figures 2B–D;

Supplemental Digital Content, Figures S1B–D, http://links.lww.com/PSYMED/A873). At day 10, singly housed macaques showed no change in expression of P-selectin and MHC-I from their baseline levels, although socially housed macaques exhibited an increase in platelet activation as shown by both increased P-selectin (65% increase from baseline, $B = -4.59$, -8.50; SE = 1.52, 0.92; $p = .004$, $p < .001$; Figure 2B) and MHC-I (40% increase, $B = -29.76$, -23.18; SE = 10.85, 11.16; $p = .009$, $p = .044$; Figure 2C) expression. P-selectin expression was 70% to 83% lower in singly housed than socially housed macaques before and after infection ($B = 9.38$, 5.46, 13.00; SE = 1.27, 1.32, 2.15; $p < .001$ at each time point), whereas MHC-I expression diverged only after infection (35% lower in singly housed animals, $B = 32.72$, SE = 7.28, $p < .001$). Conversely, no increase with infection or difference between groups was observed in CD40L expression (Figure 2D) other than a single elevated baseline time point in singly housed macaques ($B = -8.02$, SE = 2.55, $p = .003$), although the effect of housing over time was significant ($F(2,41) = 10.03$, $p < .001$).

DISCUSSION

The immune response of pigtail macaques to SIV infection was impacted by housing status, with singly housed macaques showing
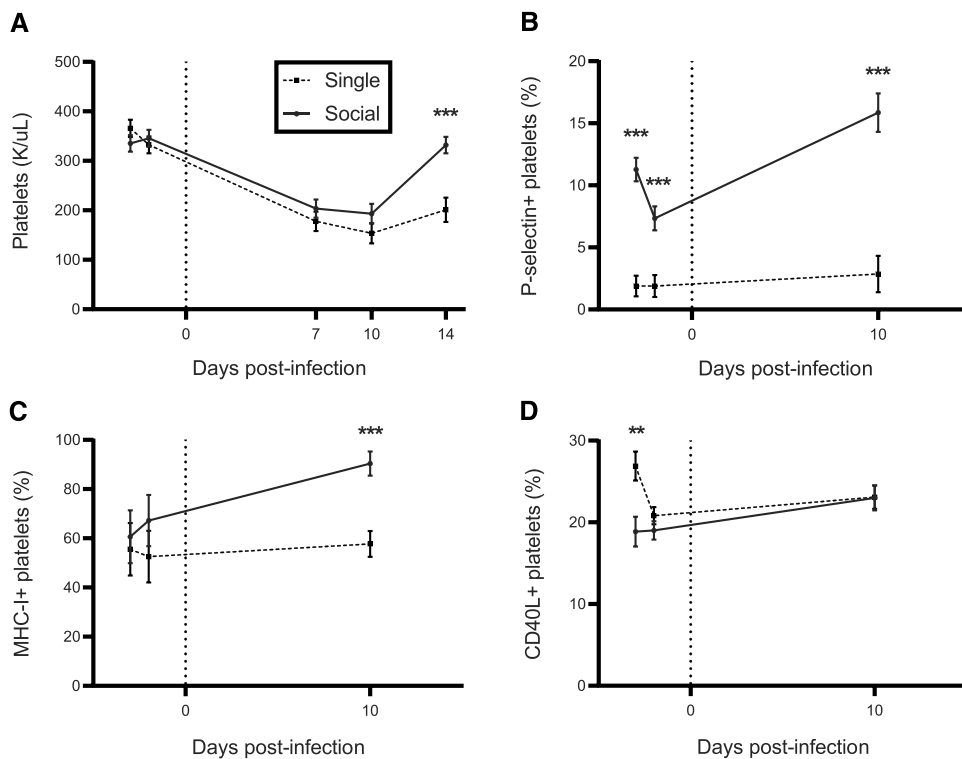
FIGURE 2. Platelet response to acute SIV infection in singly compared with socially housed macaques. Housing groups were compared at baseline (two time points) against day 10 after inoculation with SIV (for n for each time point, see Supplemental Digital Content, Table S4, http://links.lww.com/PSYMED/A873). ART was started on day 12 for all animals. A, Platelet counts in whole blood CBCs were compared. Singly housed animals showed a delayed recovery from thrombocytopenia during acute infection, failing to return to baseline by day 14. Differences between housing groups over time were significant ($F(4,64) = 8.26$, $p < .001$). B, Platelet P-selectin expression in singly housed macaques was less than in socially housed both at baseline and at day 10. C, The effects of housing ($F(2,41) = 47.03$, $p < .001$) and housing over time ($F(2,41) = 53.29$, $p = .009$) were significant. Platelet CD40L expression was not different between groups at day 10; however, a difference was present in a single baseline time point. In addition, the effect of housing over time was significant ($F(2,41) = 10.03$, $p < .001$). D, Platelet MHC-I expression was not as upregulated at day 10 in singly housed animals. In addition, the effect of housing over time was significant ($F(2,39) = 4.18$, $p = .023$). Error bars display the standard error of estimated least squares means generated by a linear mixed-effects regression model. * $p < .05$; ** $p < .01$; *** $p < .001$. SIV = simian immunodeficiency virus; ART = antiretroviral therapy; CBC = complete blood count.

reduced expansion of classical and intermediate monocytes, prolonged thrombocytopenia, and a suppression of platelet activation during the first 2 weeks after inoculation. Circulating monocytes and platelets are two effector cells critical to a rapid response during immune challenge, and our findings indicate that both populations may exhibit a blunted antiviral response under a condition modeling psychosocial stress. These data reflect that psychosocial stress may induce clinically significant immunomodulatory effects in the innate immune system during acute SIV infection, and are consistent with previous work in this model showing alterations in T-cell phenotype before infection and higher viral loads after infection in singly housed macaques (33).

Psychosocial stressors, such as social adversity, disruption, or exclusion, can induce broad physiological changes through their effects on the hypothalamic-pituitary-adrenal axis and autonomic nervous system. In people, these effects are ubiquitously confounded with socioeconomic status, which is associated with health outcomes through additional factors such as access to health resources and risk behaviors. Because of the complexity of these relationships, it is difficult to demonstrate their direct influence

on regulation of the immune system, especially in the context of HIV infection (47,48).

Macaques are naturally social animals that require interaction with conspecifics to maintain their psychological welfare (49,50). This trait makes them a natural model to recapitulate psychosocial stress in a controlled setting, and multiple experimental designs have been used to validate the physiological and behavioral changes that occur in macaques under different social conditions (29,51–53). Because this was a retrospective investigation, no stress biomarkers or behavioral assessments are available for the animals in this study. We acknowledge that this is a significant limitation of this study given that the complexity of social interactions in macaques can sometimes lead to individuals who experience greater stress from their social status than they would without contact with conspecifics (54–57). However, numerous publications have demonstrated the overall positive impact of social housing in comparison to single housing in diverse populations of macaques as assessed by both behavioral outcomes and immune function (53,58–60). One of these positive impacts is the phenomenon of social buffering. Social buffering, or the ability of a compatible social partner to improve coping,
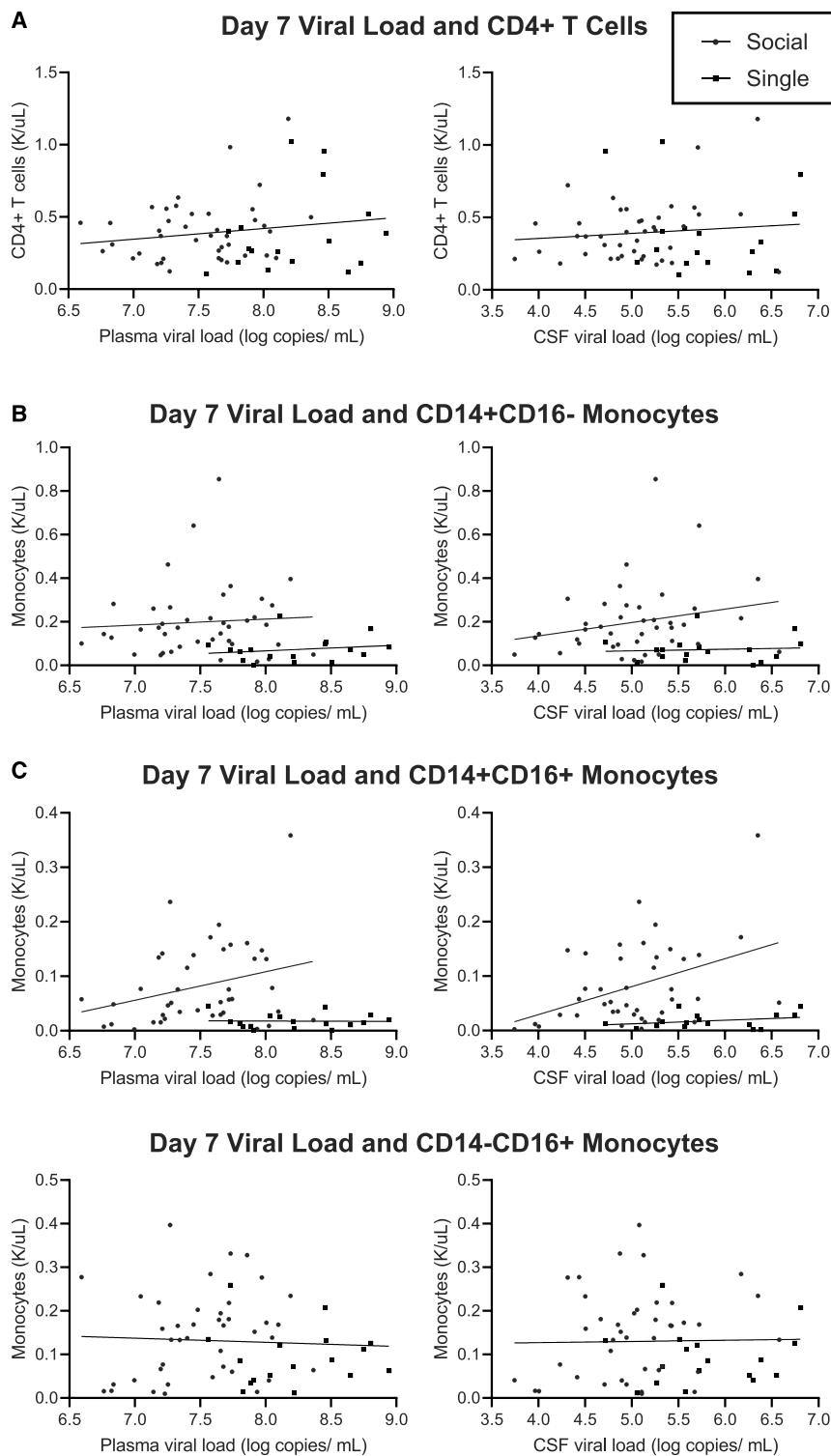
FIGURE 3. Correlation of housing status and immune parameters with viral load at day 7 after inoculation. Housing group and monocyte subsets at day 7 were correlated with previously reported data on CD4 T-cell count and plasma and CSF viral load (33). A least squares regression model was generated by using a sum-of-squares F test to determine if one line or individual lines best fit each data set. A, When directly compared, CD4 T-cell count did not correlate with plasma or CSF viral load overall, or in each housing subset. B, The line of best fit comparing $CD14^+CD16^-$ monocytes and viral load was different between housing groups ($p = .03$ plasma, $p = .004$ CSF), but showed no correlation with viral load. C, The line of best fit for $CD14^+CD16^+$ monocytes was different between housing groups ($p < .001$), and socially housed animals only showed a positive correlation with CSF viral load ($p = .03$, Spearman $r = 0.33$). D, Plasma and CSF viral load showed no relationship with $CD14^-CD16^+$ monocytes. CSF = cerebrospinal fluid.

has been associated with fewer abnormal behaviors and reduced cortisol release in response to stressors (61). In particular, full contact housing with conspecifics has been shown to increase the amount of species-typical behavior, reduce maladaptive behaviors such as stereotypies, increase coping ability, and reduce the need for veterinary care (62). It is important to note that, although numerous studies report positive behavioral outcomes from social housing of macaques, which is currently the standard for assessment of animal welfare, measurement of cortisol as a biomarker of stress has yielded highly inconsistent results (60,63–66). There are a number of factors that may contribute to this variability, including method and timing of measurement as well as the numerous physiological factors that affect cortisol levels (67–69).

Previous studies investigating psychosocial stressors in SIV-infected macaques have demonstrated an adverse effect on disease outcomes, including altered glucocorticoid regulation, greater viral load, and decreased survival time (28,29). Although the binary system of psychosocial stress used by our study cannot fully recapitulate the complexity of psychosocial and socioeconomic effects experienced by PWH, this model demonstrates a relationship between reduced social contact and immunomodulation. The retrospective nature of this investigation limits the scope of longitudinal data because macaques were involved in different studies and, at the final time point, had received 2 days of ART intervention. In addition, the chronological separation between housing groups allows for the possibility that, despite rigorous efforts to control experimental procedures, unidentified variables may have contributed to the effects found in this investigation (Supplemental Digital Content, Table S5, http://links.lww.com/PSYMED/A873). Because of the limited availability of SIV infection data in female macaques, only males were included in this study. Future work will be needed to elucidate whether sex, age, diet, or the microbiota-immune axis also drive differences in these immune alterations. In addition, more investigation is needed to uncover potential mechanisms for how psychosocial stress may initiate these immunological changes.

Monocytes play a complex role in the response to and persistence of retroviral infections. As innate immune effector cells, they are important for mounting effective antiviral responses before the adaptive immune response develops. However, monocytes and tissue macrophages are also susceptible to infection with HIV or SIV and are a major contributor in the establishment of tissue reservoirs (22–24,36,42). In addition to being an integral component of the viral reservoir in the CNS, neuroinflammation leading to cognitive impairment is mediated by monocyte activation (7). Monocytes are similarly critical effectors in the systemic inflammatory response that persists during chronic HIV and SIV infection and contribute to the clinical symptoms seen in PWH, with tissue factor expression on persistently activated monocytes in PWH on ART specifically associated with coagulopathies (20,70). Inappropriate monocyte activation can also lead to excess production of proinflammatory cytokines, such as tumor necrosis factor, which can impair mucosal integrity and contribute to lipopolysaccharide and gut luminal microbe translocation (71).

Our findings indicate that the number of classical and intermediate monocytes is persistently lowered with psychosocial stress over the course of acute SIV infection. All macaques had a similar baseline before infection, but only singly housed animals showed a transient drop in classical monocytes on day 7, and although numbers increased in all animals, differences between housing groups persisted over time. Singly housed animals similarly did not show increased intermediate monocytes after infection; these differences

were also present in the percentage of gated cells from immunophenotyping data (Supplemental Digital Content, Figure S2, http://links.lww.com/PSYMED/A873). Classical monocytes are the most numerous monocyte subset in circulation, representing cells that are newly released from bone marrow and predisposed to an inflammatory, antiviral phenotype (24,37,40). Classical monocytes are relatively resistant to infection and may play an important role in limiting the viral peak during acute infection through mechanisms involving increased interleukin 18 and inflammasome activation (37,38,41). Conversely, intermediate and nonclassical monocytes ($CD14^+CD16^+$ and $CD14^{low}CD16^+$, respectively) are more permissive to HIV infection, and intermediate monocytes are associated with transport of virus across the blood-brain barrier and induction of CNS inflammation that persists with ART. These subsets of monocytes have been shown to increase in HIV and correlate with viral load in SIV infection (23,24). Despite the importance of nonclassical monocytes in retroviral pathogenesis, intermediate monocyte counts were lower in singly housed compared with socially housed animals. In addition, a transient decrease in total monocyte number was observed in singly housed animals on day 7 after infection. Peripheral monocyte counts are affected both by rate of monocyte extravasation into tissues and by the rate of release of new monocytes into circulation from hematopoietic bone marrow (40). Both of these factors can be impacted by stressors because they are directly affected by signaling from the sympathetic nervous system and hypothalamic-pituitary-adrenal axis (72,73). In addition to an effect on circulating cell numbers, models of psychosocial stress have demonstrated an altered transcriptome in classical monocytes that result in blunted antiviral responses and reduced glucocorticoid sensitivity (74). This monocytopenia in singly housed macaques could therefore be caused by increased migration into tissues, a lack of upregulation of monocyte release in response to infection, or a combination of these factors (39,41).

Although platelet activation has been associated with physiological stress in both people and animal models, it is not a reliable measure of stress because it is variable based on the chronicity of the stressor and is directly affected by multiple other parameters (75,76). Increased surface expression of P-selectin and MHC-I on platelets consistent with previous observations during acute infection (25) was observed in socially housed macaques, although no change in platelet activation parameters was seen in the singly housed animals. Platelets are another innate immune cell that play a role in responding to HIV infection and in symptoms of PWH. Because of the complexity of their functions, it remains unclear if platelets play a permissive or protective role during HIV infection (21,27,43). Platelets are able to uptake HIV virions and productively infect other cells *in vitro* (45,46). However, platelets also express TLRs, and stimulation with TLR7 upregulates P-selectin and CD154 as a direct antiviral response. Activated platelets contribute to antiviral responses through multiple mechanisms including interleukin 1β–mediated inflammasome activation and an array of immunomodulatory signals released by α-granules (43,77). For example, CXCL4 from α-granules has been demonstrated to inhibit host cell entry of HIV-1 (44). Release of α-granules also enhances antigen presentation by increasing surface MHC-I, and platelets have been demonstrated to increase T-cell activation in response to viral inoculation (78). Neutrophil (via P-selectin and CCL5) and monocyte (via P-selectin, CD40L, PGE2,

and PF4) migration, activation, and survival are modulated both by direct interaction with activated platelets and by secreted signals (77). Persistent platelet activation, as indicated by increased CD40L and P-selectin, is one element of the chronic inflammatory state induced by HIV and has been reported to persist even during successful ART (27).

Psychosocial stress as modeled by single housing was associated with a failure to return to baseline platelet counts by 2 weeks after infection, although the magnitude of thrombocytopenia during acute infection was similar across all animals. Thrombocytopenia is a common finding in PWH and is associated with viral load and disease progression. Reduction in circulating platelet count can be caused by sequestration through activation and binding, increased destruction of platelets, or decreased production by megakaryocytes (79). Sequestration of platelets through the formation of platelet-monocyte aggregates has been associated with thrombocytopenia during acute SIV infection (25). However, thrombopoiesis may be affected by glucocorticoid resistance (80), and the concomitant lack of platelet activation seen with psychosocial stress suggests that persistence of thrombocytopenia may be related to a failure to appropriately upregulate platelet production rather than increased rate of sequestration. Additional investigation could clarify the mechanism driving thrombocytopenia in the context of psychosocial stress.

Activated platelets can signal to and directly bind with circulating immune cells (21,43), including monocytes (25), and the changes we observed in monocyte and platelet phenotype in psychosocial stress may be mechanistically connected. The formation of platelet-monocyte aggregates has been shown to increase monocyte adhesion to the endothelium and drive them toward a CD16$^+$ phenotype (81,82), and both monocytosis and platelet activation during acute SIV infection may increase these interactions (25). Psychosocial stress was associated with reductions in intermediate monocytes, but no differences in the nadir of platelet count during acute infection in this study. Unfortunately, we did not have the opportunity to examine platelet-monocyte aggregates in this retrospective study. More work is needed to understand if decreased platelet-monocyte aggregation occurs during psychosocial stress and how this affects immune cell extravasation into tissues and susceptibility to retroviral infection.

The changes in monocyte and platelet phenotype associated with psychosocial stress in this study may reflect multiple functional deficits through which increased viral load can be established during acute SIV infection. Because these innate effector cells are critical components of the immune response before the development of adaptive immunity (37,43), lower monocyte and platelet numbers and activation in singly housed macaques may allow greater viral replication during early infection. Peak viral load during acute infection is associated with worse clinical outcomes in PWH (83) and is also associated with the extent the viral reservoir is established before the adaptive immune response develops (84). Thus, the loss of classical and intermediate monocytes and activated platelets with psychosocial stress may represent an immunosuppressive state that is permissive to SIV infection. These findings are consistent with previous work from our laboratory, which demonstrated higher viral loads in the blood and CSF of singly housed macaques during acute SIV infection. Activation of CD4 and CD8 T cells was greater in singly housed animals, which may represent compensation of the adaptive immune system to an inadequate innate response during acute infection (33). The interplay

of different components of the immune system with virus and with each other is mechanistically complex and represents an area for future work. These findings emphasize the importance of mitigating psychosocial stressors in PWH, such as through provision of strong social support systems.

Of note, the associations between classical and intermediate monocyte numbers with plasma and CSF viral load differed significantly between the socially and singly housed animals. A positive correlation between intermediate monocyte number and CSF viral load was revealed only in animals that were socially housed. Intermediate monocytes have previously been implicated in the establishment of latent reservoirs in the brain and other tissues, and such an association could be interpreted as further support for such a hypothesis. Psychosocial stress may have masked detection of this relationship in singly housed animals and should be considered as a variable in future investigations.

Single housing, as a model of psychosocial stress, modulates the innate immune response to SIV infection as shown by reduced numbers of circulating classical and intermediate monocytes and platelets during acute infection. Platelet activation is similarly suppressed under conditions of limited social contact. These changes are suggestive of a reduced antiviral innate immune response and may represent an inadequate response to inoculation with SIV. This may allow for the establishment of a greater viral burden (33) and long-term clinical consequences of retroviral infection. These findings not only highlight the need for interventions to address chronic stressors such as social isolation in PWH but also illustrate the importance of cohabitation as the standard for housing of social species such as macaques. These data supply evidence that single housing can directly impact study outcomes in addition to compromising animal welfare. Robust animal models for infectious disease research must reflect a normal immunological state to best translate findings into successful treatments. Further work is needed to validate the translatability of these findings to PWH and identify the mechanisms by which psychosocial stress alters antiviral responses.

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