Sociodemographic effects on immune cell composition in a free-ranging non-human primate.

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Abstract

Aging results in declines in immune function and increases in inflammation, which underlie many age-related diseases. These immunosenescent signatures are similar to those seen in individuals exposed to social adversity, who may age more rapidly than those unexposed. Yet, it is unclear how social adversity alters immunity across demographic factors - data that are essential to identify how it might increase aging-related diseases. Here, we investigated how age, sex, and social adversity predicted immune cell proportions in 250 rhesus macaques living in a semi-naturalistic colony. As macaques aged, they exhibited signatures of immunosenescence. Older individuals had signatures of diminished antibody production and adaptive immunity, with declines in CD20+ B cells, CD20+/CD3+ cell ratio, and the CD4+/CD8+ T cell ratio. At all ages, females had higher CD20+/CD3+ and CD4+/CD8+ ratios, indicative of a stronger antibody and adaptive immune response that may facilitate pathogen clearance even with increasing age. Older individuals had signatures of inflammation, with higher proportions of CD3+/CD8+ Cytotoxic T cells, CD16+/CD3- Natural Killer cells, CD3+/CD4+/CD25+ and CD3+/CD8+/CD25+ T regulatory cells, and CD14+/CD16+/HLA-DR+ intermediate monocytes, combined with lower levels of CD14+/CD16-/HLA-DR+ classical monocytes. Notably, we found an interaction between age and social adversity, where low-status individuals had higher proportions of CD3+/CD4+/CD25+ T regulatory cells for their age, compared to higher-status individuals. Together, our study identifies immune cell types that are affected by age and sex in the premier nonhuman primate model of human biology and behavior, and demonstrate a novel link between inflammatory CD4+ T regulatory cells and social adversity.

Introduction

The average human lifespan has almost doubled over the past century, leading to an increase in the prevalence of diseases of aging, like cardiovascular disease (1), autoimmune disease (2), diabetes, arthritis, and cognitive decline (3). As individuals age, there is a disruption in the homeostatic balance between innate and adaptive immunity linked to both increases in age-related disease and susceptibility to infection. This imbalance is reflective of two age-related changes, namely increased inflammation

("inflammaging") and decline in adaptive immune function ("immunosenescence"). Both changes disrupt the balance between pro-and anti-inflammatory mediators that are indicative of a healthy immune system. For example, monocytes become more proinflammatory with increasing age, releasing drivers of inflammation that include proinflammatory cytokines (4) (e.g., TNF-a). Other immune cells, such as B cells and helper T cells, decline in abundance across the lifespan, which has a direct impact on long-term immunity, as evidenced by the fact that vaccination is less effective in older individuals (5).

There is substantial heterogeneity in aging, and not all individuals age at the same rate or fall victim to the same age-related diseases. For instance, some people become hypertensive in their 30s, while a 60-year-old may never suffer from this condition. Part of this heterogeneity in aging is due to sex differences in the immune system, which alter the prevalence and onset of age-associated diseases in males and females. For example, females mount a stronger immune response compared to males (6), while males have a dampened age-related increase in inflammatory cells (7). Although these studies have provided some insight into sex differences in immunological aging, there is still a lack of data demonstrating how these differences affect individuals across their lifespan and the specific cell types that contribute to these differences.

Heterogeneity in aging can also be attributed to life experiences, such as exposure to social adversity, which can influence the onset and progression of disease and, ultimately, mortality (8). Social adversity has been linked to accelerated aging as measured using biomarkers like epigenetic age and telomere attrition (9, 10). There are also broad similarities between the effect of age and social adversity on peripheral immune function (11). For instance, early life adversity in humans has been linked to increases in proinflammatory T cells (12) – a characteristic usually seen with increasing age. However, it is still unclear if and how social adversity alters immune function across the life course - data that are essential to identifying how social adversity might lead to increased aging-related disease and death, or, conversely, how social advantage can help protect an individual from the effects of aging. Taken together, there is mounting evidence that social

93 adversity accelerates the pace of biological aging and is a possible driver of interindividual
94 variability in morbidity and mortality.
95
96 Because social structures in human populations are affected by characteristics such as
97 economic advantages/disadvantages, discrimination, and sociocultural differences, it is
98 difficult to measure how social adversity "gets under the skin" to affect immune and overall
99 health, as these may have an impact on how an individual perceives and responds to
100 their social environment. Rhesus macaques (*Macaca mulatta*), a non-human primate, are
101 an established animal model that exhibits aging trajectories similar to humans - such as
102 decreases in mobility with increasing age and physical changes like coat greying and skin
103 atrophies - but compressed into a lifespan 3-4x times shorter (13). This is also reflected
104 at the molecular level: rhesus macaques and humans show similar age-related changes
105 in immune cell DNA methylation and gene expression (14). Additionally, rhesus
106 macaques are an ideal model to study the effects of social adversity because of their
107 complex social structure. Differences in social status result in differences in the extent to
108 which individuals can access resources (15), which is a feature also commonly seen in
109 human populations. Like humans, social status in macaques affects health and survival
110 (16), with individuals that have more social connections presenting lower white blood cells
111 (17)–possibly affecting inflammatory responses.
112
113 Here, we characterized the immunological effects of aging and quantified whether social
114 adversity recapitulates or interacts with these effects. We studied a free-ranging
115 population of rhesus macaques living on the island of Cayo Santiago, Puerto Rico where
116 we could simultaneously measure the effects of aging, sex, and social adversity in a semi-
117 naturalistic social setting with minimal human intervention. Using objective measures of
118 social adversity, we were able to avoid some of the biases that sometimes affect studies
119 of humans that rely on self-reporting and questionnaires (18, 19). We identified which
120 immune cell proportions exhibit age-associated changes, how these proportions are
121 affected by sex and social adversity, and if/how the social adversity-induced changes
122 recapitulate the immunological changes seen during aging.
123

Methods

Study population:

Cayo Santiago, an island located off the southeast coast of Puerto Rico, is inhabited by 1,800 rhesus macaques living in an easily accessible and semi-natural setting. The monkeys are direct descendants of rhesus macaques introduced from India in 1938. The population has a minimal intervention policy managed by the Caribbean Primate Research Center (CPRC), and is the longest-running primate field station in the world (20). It provides unique research opportunities for studies of behavior, physiology, demography, morphology, and genomics. There are no predators on the island, and the most common causes of death are due to senescence. During the annual trap-and-release period, researchers have the unique opportunity to collect biological and morphological samples with the assistance of the CPRC veterinary staff. Over the last 10 years, the Cayo Biobank Research Unit (CBRU), has established the ability to collect behavioral data; access to these data provides a unique opportunity to probe the relationships between the social environment, immune function, and aging.

Blood sampling:

In October - December 2019 and October 2020 - February 2021, we collected whole blood from sedated rhesus macaques (n=97 in October - December 2019 and n=153 in October 2020 - February 2021) into 6ml K2 EDTA tubes (Beckton, Dickson and Company, cat #367899). We sampled a total of 250 animals (115 females; 135 males) spanning their natural lifespan (mean age = 11.05 years, range 0-28 years; **Figure 1A**). Fresh blood samples were transported at 4C to the UPR-Medical Sciences campus where flow cytometric analysis was performed within 6 hours of sample collection.

Antibodies and flow cytometric analysis:

We used an 8-panel antibody cocktail consisting of the following antibodies: CD20-PacBlue/Clone 2H7 (Biolegend), CD3-PerCP/Clone SP34-2 (BD), CD4-APC/Clone L200 (BD), CD8-Viogreen/Clone BW135/80 (Miltenyi), CD25-PE/Clone 4E3 (Miltenyi), CD14-FITC/Clone M5E2 (BD), CD16-PEVio770/Clone REA423 (Miltenyi), HLA-DR-APCVio770/Clone REA805 (Miltenyi).

We performed phenotypic characterization of rhesus macaque peripheral blood mononuclear cells (PBMCs) using multicolor flow cytometry with direct immunofluorescence (View **S. Figure 3** for gating strategy and **Table S1** for Ab panel) on all 250 animals. Aliquots of 150 µl of heparinized whole blood were incubated with a mix of the antibodies described for 30 minutes at 25C (room temperature). After incubation, red blood cells were fixed and lysed with the BD FACS fix and lyse solution (Cat #349202). Cells were washed twice using PBS containing 0.05% BSA at 1,700 RPM for 5 minutes and processed in a MACSQuant Analyzer 10 flow cytometer (Miltenyi Biotec, CA).

Lymphocytes (B cells, T cells, NK cells) and monocytes (classical and non-classical) were gated based on their characteristic forward and side scatter patterns. Lymphocytes were then further subdivided based on their cell surface markers. NK cells were defined as the CD3- and CD16+ population; B cells were defined as CD20+ population, ; and T cells as the CD3+ population. We further subdivided T cells from the CD3+ gate into CD4+ and CD8+ populations. CD4+CD25+ and CD8+CD25+ T regulatory cells were further gated from the CD4+ and CD8+ gates. Monocytes were gated based on the combined expression of the HLA-DR/CD14 markers for classical monocytes, HLA-DR/CD16 markers for non-classical monocytes, and HLA-DR/CD14/CD16 for intermediate monocytes (**S. Figure 4**). Data analysis was performed using Flowjo version 10.7.1 (FlowJo LLC Ashland, OR).

To get an accurate representation of cell-type proportions, we counted only stained events from the cells of interest and calculated their proportions based on lymphocytes and monocytes subsets. To calculate cell ratios, such as CD20 + B cell to CD3+ T cell ratio and CD4+ T cell to CD8+ T cell ratio, we divided the calculated proportion of these cell types across each individual sample (e.g., CD20+ B cell/ CD3+ T cell and CD4+/CD8+ respectively). (Calculations detailed in **Table S2**).

Quantification of social adversity (social status):

Social status of individuals was determined using the outcome of win-loss interactions between pairs of adult groupmates. Observations of behaviors of adults (6+ years old) were collected from two different social groups, groups V and F, in the year leading up to sample collection. In 2019, behavioral data were collected using a previously established 10-minute focal sampling protocol (21). Briefly, the protocol consisted of recording state behaviors (e.g. resting, feeding) and agonistic encounters, which included recording the identity of the focal animal's social partner. Win-loss agonistic interactions included threat and submissive behaviors, along with contact and non-contact aggression. In 2020, agonistic interactions were collected *ad-libitum* due to COVID-19 pandemic restrictions A total of 370 hours of behavioral data were collected for group V in 2019. In 2020 in group F we collected a total of 407 female-female agonistic interactions and 292 male-male interactions, while in group V we collected 170 female-female agonistic interactions and 153 male-male interactions.

Social status was computed by group for each year and independently for males and females (22, 23). In rhesus macaques, social status is obtained differently for males and females (24), with females forming maternally inherited stable linear hierarchies, while males typically disperse from their natal group and enter a new group where they attain rank by physical contest and queuing (25, 26). Hierarchies were built using the win-loss outcomes of agonistic encounters from focal sampling and *ad-libitum* observations, and known maternal relatedness was used to settle behavioral gaps in the female hierarchy (27). To control for group size variation, dominance rank was calculated as the percentage of same-sex group peers that a subject outranked; a score above 80% corresponded to high-ranking animals, scores from 50-79% to medium ranking animals and scores below 49% to low ranking animals (28). Overall, we were able to quantify circulating immune cell proportions in 140 macaques (71 F and 69 M) for which we had behavioral data.

Statistical analysis:

Statistical analyses were performed using R Studio version 4.0.1. software (RStudio, PBC, Boston, MA, USA).

Using all 250 samples, we performed principal component analysis of all the measured cell types using the *prcomp* function in RStudio. We then employed a linear mixed-effects model using the *lmerTest* R package to model sample projections onto principal components as a function of age, sex, and sample period (to control for any technical factors that might vary across the two sampling periods), while including individual ID as a random effect to account for repeated samples from the same individual.

To evaluate each cell type at a more granular level, we employed the same linear mixed-effects to model the proportion of each cell type and certain cell-type ratios (e.g., CD4+/CD8+) as a function of age, sex, and sample period. We also included an interaction between age and sex to identify sex-dependent age-related associations. For the subset of samples where social status information was available (n = 140), we also modeled cell proportions as a function of social status (rank), age, sex, and sample period, again with individual ID as a random effect. We also included an interaction between rank and age as well as for rank and sex to test if changes in the relationship between cell type proportion and social status were dependent on either of these variables.

Results

Free-ranging macaques exhibit immunosenescence and inflammaging

Age was the strongest predictor of immune cell composition in our dataset, and was significantly associated with the first principal component of immune cell composition (β_{PC1} = 0.13, p = 4.3 x 10^{-15}, **Figure 1B)**. Individuals with more positive PC1 values had higher proportions of cytotoxic T cells and NK cells and lower proportions of cells involved in pathogen clearance, including CD20+ B cells and classical monocytes (**Figure 1C).** Overall, this suggests a pattern of increased inflammation and immunosenescence with increasing age.

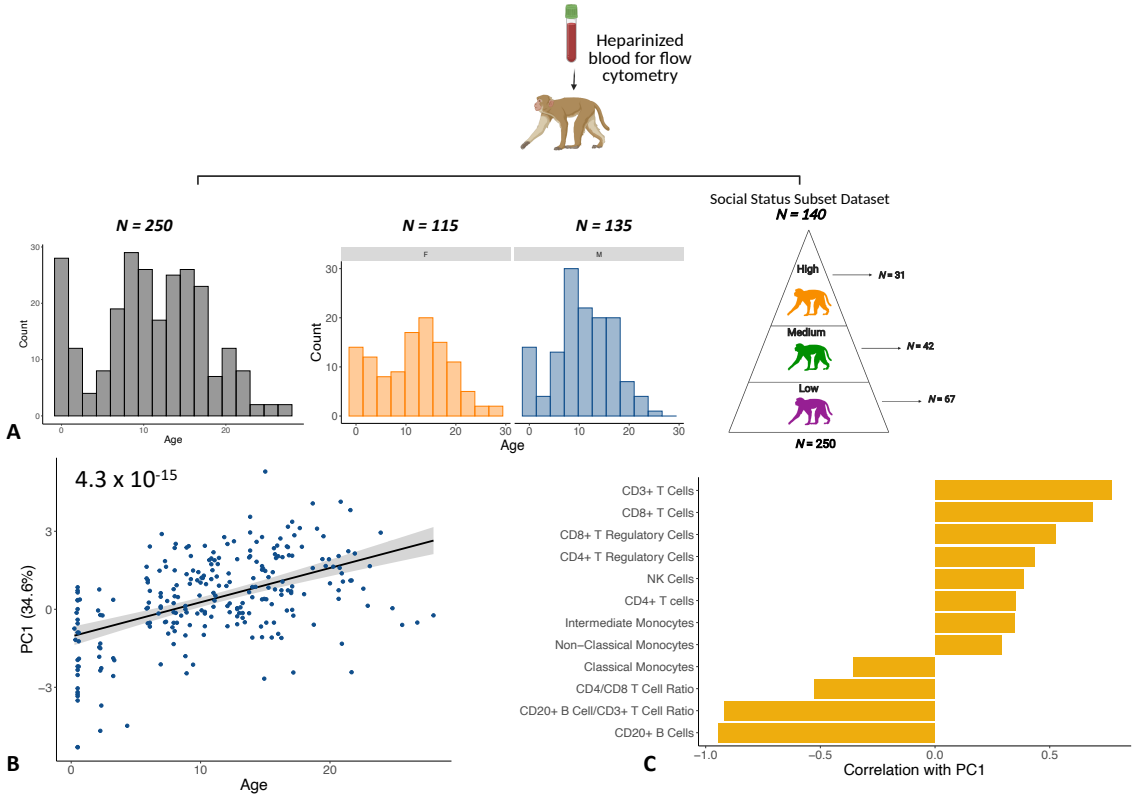

Figure 1: Sample collection and demographics. A) We collected whole blood samples from 250 animals and quantified immune cell proportions using flow cytometry. The dataset was roughly balanced between males and females and captured the entire natural lifespan of macaques in this population. We calculated social status by assigning dominance ranks to 140 animals using observational data collected in the year before each sample was collected. Animals were assigned to one of three dominance ranks: high (n = 31), medium (n = 42) and low (n = 67). The social status dataset is a subset of the original age dataset because behavioral data were not available for all study animals. **B)** PC1 of immune cell compositions is significantly associated with age (β_{PC1} = 0.13, p = 4.3 x 10^{-15}). **C)** The T cell compartment is positively associated with PC1 (and thus age), while the B cell compartment is negatively associated with PC1 of immune cell composition.

We then conducted a more granular analysis of what factors predicted individual cell proportions (**Table S3**). Age was significantly associated with signatures of

immunosenescence in adaptive immune cells, characterized by a significant decrease in the CD20+ B cell to CD3+ T cell ratio ($\beta_{CD20:CD3}$ = -0.03 ± 0.004, p = 2.3 x 10^{-9}, **Figure 2A**). This change was largely driven by age-related declines in the proportion of CD20+ B cells (β_{CD20} = -0.87 ± 0.11, p = 1.8 x 10^{-12}, **Figure 2B**). Age was also associated with a slight decrease in the relative proportion of CD4+ T helper cells (**S.Figure 1**), and when paired with an age-associated increase in cytotoxic CD8+ T cells (β_{CD8} = 0.60 ± 0.09, p = 1.4 x 10^{-10}, **Figure 2C**), there was a strong and significant age-associated decline in the CD4+/CD8+ ratio ($\beta_{CD4:CD8}$ = -0.06 ± 0.008, p = 1.9 x 10^{-13}, **Figure 2D**).

We then examined less common, but extremely immunologically important, regulatory T cell populations (CD25+) that are involved in immunity suppression and maintenance of self-tolerance (29). Proportions of both CD4+ and CD8+ T regulatory cells increased significantly with age (CD3+CD4+CD25+: β = 0.11 ± 0.02, p = 2.4 x 10^{-9}, **Figure 2E**; CD3+CD8+CD25+: β = 0.01 ± 0.005, p = 5.8 x 10^{-3}, **Figure 2F**). Innate immune system cells also showed significant changes with age, with two monocyte populations presenting weak, but differing age-related changes in abundance.

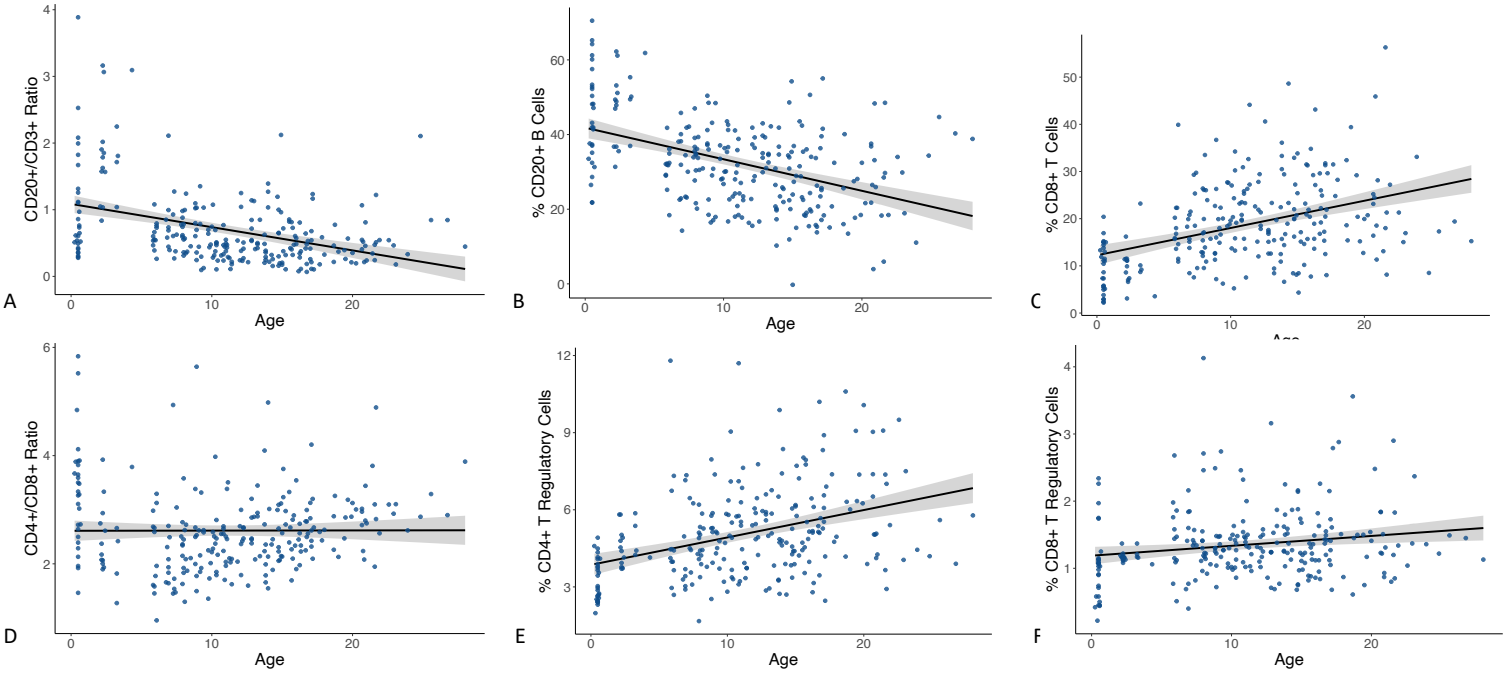

Figure 2: Adaptive immune cell proportions are associated with age. A) CD20+ B cell to CD3+ T cell ratio decreases across the life-course ($\beta_{CD20:CD3}$ = -0.03 ± 0.004, p =

2.3 x 10^{-9}), and **B)** CD20+ B cells decrease across the life-course (β_{CD20} = -0.87 ± 0.11, p = 1.8 x 10^{-12}). **C)** CD8+ T cells increase with age (β_{CD8} = 0.60 ± 0.09, p = 1.4 x 10^{-10}). **D)** CD4+/CD8+ T cell ratio decreases with age ($\beta_{CD4:CD8}$ = -0.06 ± 0.008, p = 1.9 x 10^{-13}). **E)** CD4+ T regulatory cells (β = 0.11 ± 0.02, p = 2.4 x 10^{-9}) and **F)** CD8+ T regulatory cells increase with age (β = 0.01 ± 0.005, p = 5.8 x 10^{-3}), possibly because of higher baseline levels of inflammation in the older population (i.e., inflammaging).

Classical monocytes (HLA-DR+/CD14+/CD16-), which are involved in phagocytosis and extracellular pathogen clearance (30), decreased with age (β = -0.49 ± 0.22, p = 0.03, **figure 3A**), while intermediate monocytes (HLA-DR+/CD14+/CD16+), involved in immune cell recruitment and secretion of pro-inflammatory cytokines (30), increased with increasing age (β = 0.31 ± 0.13, p = 0.02, **Figure 3B)**. The proportion of CD16+CD3- NK cells – which have a similar role to CD8+ T cells presenting natural cytotoxicity but are non-antigen specific – also increased significantly with age (β = 0.35 ± 0.10, p = 3.2 x 10^{-4}, **Figure 3C**). Together, these changes indicate an age-related decline in adaptive immunity paired with an increase in inflammation profile by innate immune cells, potentially disrupting a "healthy" homeostatic immune system.

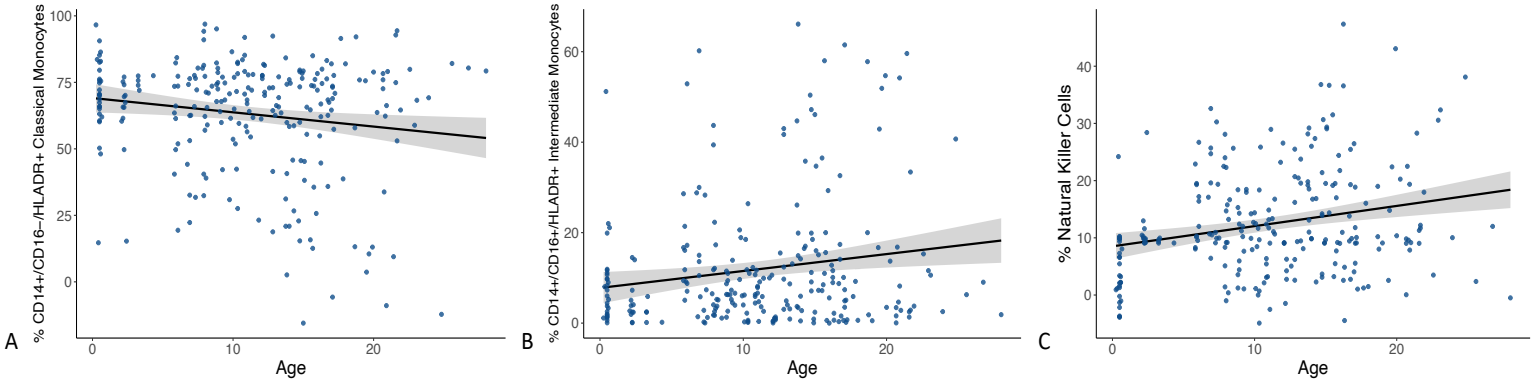

Figure 3: Age is associated with changes in innate immune cell proportions. A) CD16+ NK cell increase with age ($\beta_{CD3\text{-}CD16+}$ = 0.35 ± 0.10, p = 3.2 x 10^{-4}) **B)** CD14+/CD16-/HLADR+ Classical monocytes (β_{CD14++}= -0.49 ± 0.22, p = 0.03) decrease and **C)** CD14+/CD16+/HLADR+ intermediate monocytes increase with age

($\beta_{CD14+CD16+}$ = 0.31 ± 0.13, p = 0.02), suggesting decreases in phagocytosis, pathogen clearance and increases in inflammation with increasing age.

Sex differences in immunity

We found sex differences in CD4+ T cells (β_{CD4} = -2.11 ± 1, p = 0.04, **Figure 4A**), with females having higher proportions than males. Additionally, there was a significant sex difference in the CD4+/CD8+ ratio ($\beta_{CD4:CD8}$ = -0.23 ± 0.10, p = 0.03, **Figure 4B)**, with males having a lower CD4+ to CD8+ ratio. These differences between the sexes are representative of a stronger adaptive immune response in females, which, in part, is generated by CD4+ T helper cells. There were no significant interactions between age and sex on any immune cell proportion.

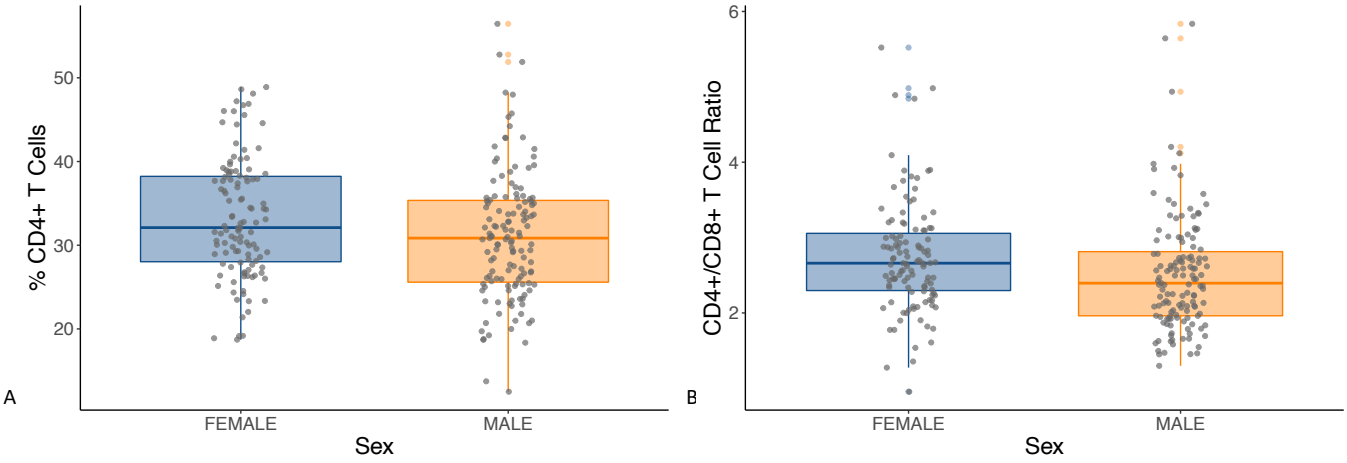

Figure 5: Sex differences in T cell proportions. A) percent of CD4+ T cells and **B)** the CD4+/CD8+ T cells ratio show significant sex differences (β_{CD4} = -2.11 ± 1, p = 0.04 and $\beta_{CD4:CD8}$ = -0.23 ± 0.10, p = 0.03 respectively). Females have, on average, had higher proportions of both measurements.

Social adversity is associated with lymphocyte proportions

Contrary to our expectations–where we expected that low social status would exhibit similar effects on immune cell proportions as aging–we found no significant main effects

of social status on lymphocyte proportions. However there was a significant interaction between age and social status on the proportion of CD4+CD25+ T regulatory cells, where the age-associated increase in CD4+ Tregs became stronger in lower status animals ($\beta_{status*age}$ = 0.096 ± 0.045, p = 0.035; **Figure 5**).

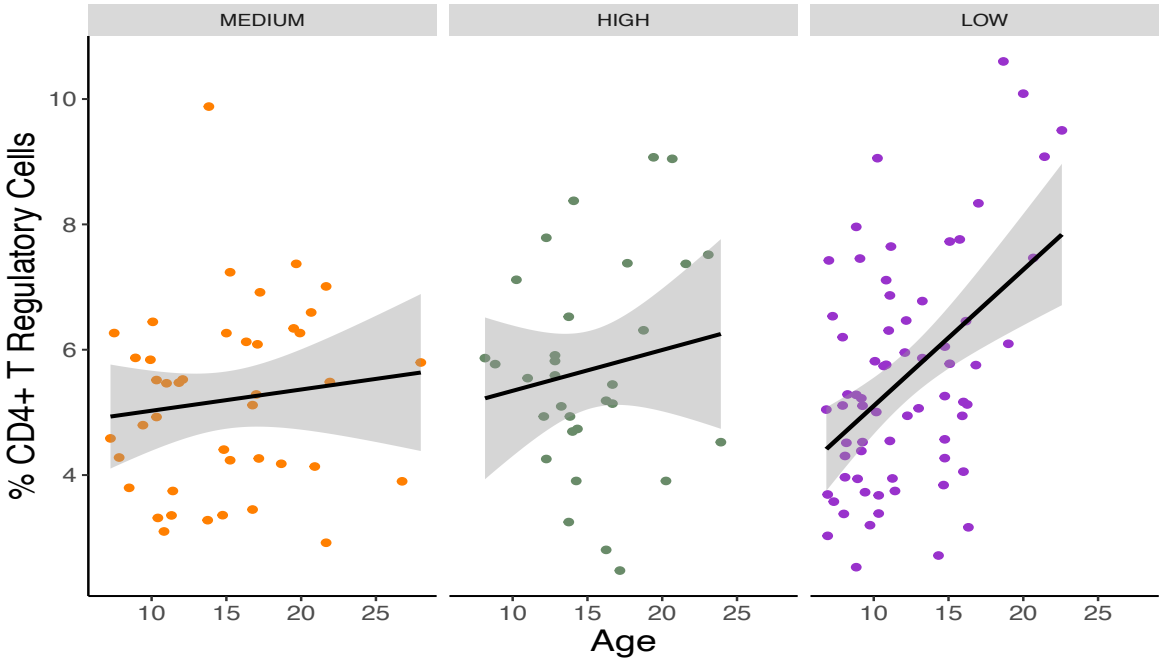

Figure 5: Low social status individuals exhibit accelerated inflammaging. Interaction between social status and age, such that the effect of age on CD4+ T regulatory cell proportions is strongest in lower status individuals ($\beta_{status*age}$ = 0.096 ± 0.045, p = 0.035). Status has been split into three groups (low, medium, and high) for visualization purposes.

Discussion

We found that age was associated with a range of changes in immune cell populations in a naturalistic colony of nonhuman primates–an important animal model for human aging. Intriguingly, we also identified social status-dependent age-related changes in T regulatory cells. Together these changes likely shape immune responses to future pathogenic challenges as well as the development of inflammatory-related diseases. Generally, macaques exhibited age-related changes similar to those seen in human

372 populations, including declines in lymphocytes (31). Here, we identified more specific cell
373 types that changed in frequency with age. We detected age-associated declines in
374 CD20+ B cells, which are responsible for antibody production and pathogen clearance
375 and are a key cell in the generation of immune memory, reflecting immunosenescence.
376 A weakened B cell response is one of the main factors why vaccination is less effective
377 in older populations (32). B cells have also been associated with protection against certain
378 types of cancers, such as lung cancer (33). Together, decreases in B cells, decreased B
379 cell to T cell ratios, and increases in T cells in older individuals (**S. Figure 3)** reflect
380 changes in the adaptive immune response that likely reduces the ability to effectively clear
381 pathogens.
382
383 Similar to two other studies in captive macaques, we found that CD8+ T cell proportions
384 increased with age (34, 35). Notably, this differs from findings in humans where both
385 CD8+ T cells and their effector (i.e., response to stimulus) responses decrease
386 significantly with age (36). This discrepancy may be due to the fact that CD8+ T cells tend
387 to have significantly more rounds of division than CD4+ T cells (37), possibly resulting in
388 higher proportions of these cells in individuals of older age – when usually general cell
389 division rates decrease, providing CD8+ T cells with an opportunity to present higher
390 proportions than cells that do not have fast division cycles. Alternatively, since CD8+ T
391 cell subsets have been associated with inflammation and 'inflammaging' (38), there is a
392 possibility that the increase in the general pool of CD8+ T cells in rhesus macaques is
393 indicative of higher levels of inflammation. This is further supported by the decrease with
394 age of the CD4+/CD8+ T cell ratio, which reflects the increase in CD8+ T cells with age
395 along with possible increases in inflammation. Furthermore, proportions of NK cells
396 significantly increased across the life-course in our dataset. Similar to CD8+ T cells, NK
397 cells respond to intracellular pathogens, secrete multiple pro-inflammatory mediators and
398 are crucial during tumor surveillance and injury repair. Increases in NK cells suggest a
399 higher incidence of inflammation and/or tissue injury in the older population. As expected,
400 CD4+CD25+ T regulatory cells as well as CD8+CD25+ T regulatory cells increased with
401 age, indicating higher levels of inflammation in older individuals (39). These results, along
402 with decreases in B cell and increases in CD8+ T cell and NK cell proportions, further

support the hypothesis that the adaptive immune response in rhesus macaques declines with age and inflammation related factors may increase due to 'inflammaging'. Together, these changes may drive biological and physiological decline that leads to higher risk of morbidity and mortality, as in humans.

Monocyte proportions also changed with age. Specifically, we found a decrease in the proportions of CD14+ classical monocytes, which are phagocytic cells that ingest pathogens that they encounter (40). This age-associated decrease may indicate a dampened phagocytic response, and thus an increased likelihood of infection in older individuals. This decrease in classical monocytes was accompanied by an increase in the proportion of CD14+/CD16+ intermediate monocytes, which are strongly associated with inflammation (41). For instance, increases in this cell type have been linked to disorders such as chronic kidney disease (42). The decrease in classical monocytes paired with an increase in intermediate monocytes represent yet another signature of immunosenescence and inflammaging.

We also identified important sex-differences in cells linked to adaptive immune cell populations. Females had more CD4+ T cells across the life-course compared to males, which recapitulates changes seen in humans (43). Furthermore, we found sex specific differences in the CD4+/CD8+ T cell ratio, with males having a lower ratio across the life-course than females. CD4+ T cells aid in the affinity maturation process that results in the production of antibodies in the lymph nodes (44). Thus, their higher abundance in females across the life-course, which is also reflected in a lower CD4+/CD8+ ratio for males, may be linked to a better and more effective antibody response and thus adaptive immune protection in females against invading pathogens (45).

A strength of our study was the ability to quantify measures of social adversity (i.e., dominance rank), and to test if and how social status altered age-related immune changes. Contrary to our expectations, we found no main effect of social status on the abundance of any immune cell population when controlling for age and sex. This may be due to the fact that we only had behavioral data, and thus social status measures, for a

subset of adults in our dataset (n = 140). Nevertheless, we did detect a strong interaction between age and status on the proportion of the inflammatory CD4+ T regulatory cells. CD4+ T regulatory cells increased with age, but this increase was strongest in those individuals on the bottom of the social hierarchy, which typically experience the most social adversity. Thus, older individuals exhibited the strongest increase in these inflammatory cells if they were also low social status. This finding points to the age-dependence of some effects of social adversity. In other words, as individuals get older, the adversity associated with low status disproportionally increases inflammation, which may accelerate the onset of aging-related diseases and early mortality, which might help to explain why low status is associated with shorter lifespan in this population (46, 47). This also provides a novel addition to previous work that established links between social adversity and inflammation in humans and rhesus macaques (48, 49).

In conclusion, our results demonstrate that, at the level of circulating immune cell proportions, macaques and humans exhibit very similar age-related changes indicating both immunosenescence and inflammaging. Sex differences in these patterns indicate that the immune system has evolved similarly in different species and that responses are conserved between them, with females typically presenting a better and more effective adaptive immune response. Furthermore, we report that low social status is associated with a stronger increase in inflammation with aging. Although we found age-related changes in both adaptive and innate immune cells, we did not measure specific adaptive immune cells, such as the effector and memory subsets of B cells and T cells, which can change with age. In future studies, it will be important to measure other innate immune cell types, such as dendritic cells and granulocytes, because these cell types are key to the antigen presentation process and adaptive immune response development. While we found an interaction between age and social status, we were limited by our sample size \ and it would be interesting to evaluate what other interactions or additive effects are present when the number of individuals in the status dataset is increased. Overall, our study provides further evidence that rhesus macaques are an ideal non-human primate model in which to study chronological aging, and are also an excellent system in which to study the effects that social adversity can have on biological aging. Future research

should seek to evaluate the performance of the immune cell types reported here on an infection, and how both age and social adversity mediate that response.

Ethical note:

This work was approved by the Institutional Animal Care and Use Committees of the University of Puerto Rico, Medical Sciences Campus (IACUC Number: A400117).

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M.R.S.R., M.L.P., J.P.H., L.J.N.B., C.A.S., M.J.M, M.L.P., and N.S.-M designed research; M.R.S.R., N.M.R., M.M.W., P.P., M.A.P.-F., E.R.S., E.B.C., J.E.N.-D., D.P., A.R.L., and M.J.M. performed research; M.R.S.R and N.S.-M. analyzed data; and M.S.R. and N.S.-M. wrote the paper with contributions from all authors.

The authors declare no competing interest.

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