Age, sex, and social environmental effects on immune cell composition in a free-ranging non-human primate

Mitchell R. Sanchez Rosado[1], Nicole Marzan-Rivera[1], Marina M. Watowich[2], Andrea D. Negron-Del Valle[3] Petraleigh Pantoja[1,4], Melissa A. Pavez-Fox[5], Erin R. Siracusa[5], Eve B. Cooper[6], Josue E. Negron-Del Valle[7,8], Daniel Phillips[7,8], Angelina Ruiz-Lambides[4], Cayo Biobank Research Unit, Melween I. Martinez[4], Michael J. Montague[9], Michael L. Platt[9,10,11], James P. Higham[6], Lauren J. N. Brent[5], Carlos A. Sariol[1,4], Noah Snyder-Mackler[7,8,12]

Affiliations:

[1]Department of Microbiology & Medical Zoology, University of Puerto Rico-Medical Sciences, San Juan, PR

[2]Department of Biological Sciences, Vanderbilt University, Nashville, TN, USA

[3]Department of Biology, University of Puerto Rico-Humacao, San Juan, PR

[4]Unit of Comparative Medicine, Caribbean Primate Research Center and Animal Resources Center, University of Puerto Rico-Medical Sciences Campus, San Juan, PR, USA

[5]Centre for Research in Animal Behaviour, University of Exeter, Exeter EX4 4QG, UK

[6]Department of Anthropology, New York University, New York, NY 10003, USA

[7]School of Life Sciences, Arizona State University, Tempe, AZ, USA

[8]Center for Evolution and Medicine, School of Life Sciences, Arizona State University, Tempe, AZ, USA

[9]Department of Neuroscience, University of Pennsylvania, Philadelphia, PA 19104, USA

[10]Department of Psychology, University of Pennsylvania, Philadelphia, PA 19104, USA

[11]Department of Marketing, Wharton School, University of Pennsylvania, Philadelphia, PA 19104, USA

[12]School for Human Evolution and Social Change, Arizona State University, Tempe, AZ, USA

Abstract

Increasing age is associated with dysregulated immune function and increased inflammation–patterns that are also observed in individuals exposed to chronic social adversity. Yet we still know little about how social adversity impacts the immune system and how it might promote age-related diseases. Here, we investigated how immune cell diversity varied with age, sex and social adversity (operationalized as low social status) in free-ranging rhesus macaques. We found age-related signatures of immunosenescence, including lower proportions of CD20+ B cells, CD20+/CD3+ ratio, and CD4+/CD8+ T cell ratio – all signs of diminished antibody production. Age was associated 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, and lower levels of CD14+/CD16-/HLA-DR+ classical monocytes, indicating greater amounts of inflammation and immune dysregulation. We also found an effect of exposure to social adversity (i.e., low social status) that was sex-dependent. High-status males, relative to females, had higher CD20+/CD3+ ratios and CD16+/CD3 Natural Killer cell proportions, and lower proportions of CD8+ Cytotoxic T cells. Further, low status females had higher proportions of cytotoxic T cells than high status females, while the opposite was observed in males. High status males had higher CD20+/CD3+ ratios than low status males. Together, our study identifies immune cell types that differ by age in a human-relevant primate model animal, and demonstrates a novel link between sex-dependent immunity and social adversity.

Introduction

The average human lifespan has almost doubled over the past century [1], accompanied by an increase in the prevalence of many age-related diseases, including cardiovascular disease, autoimmune disease, diabetes, arthritis, and cognitive decline [2-5]. 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 alterations, namely increased inflammation ("inflammaging") and a decline in adaptive immune function ("immunosenescence") [6-7]. Both alterations disrupt the balance between pro- and anti-inflammatory mediators that characterize a healthy immune system. For example, with increasing age, innate immune cells such as monocytes become more active and release drivers of inflammation that include proinflammatory cytokines (e.g., TNF-α) [8]. Adaptive immune cells, such as B cells and helper T cells, show age-related declines that directly impact long-term immunity, as exemplified by the lower effectiveness of vaccination in older individuals [9].

Yet these age-related alterations in immunity are not universal in their trajectories across individuals. There is substantial heterogeneity with age; 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 is due to sex differences in the immune system, which alter the prevalence and onset of age-related diseases. For example, in many species, females mount a stronger immune response with increasing age compared to males [10-11]. Females also have a stronger age-related increase in inflammatory cells compared to males [12]. Further, in humans, older men are more susceptible to infections, such as leptospirosis, tuberculosis, and hepatitis A, than are women [13].

Heterogeneity in aging can also arise from life experiences, such as exposure to social adversity, which can influence the onset and progression of disease and, ultimately, mortality [14]. Social adversity, which is often associated with low social status, and other social stressors [15-16], has been linked to accelerated aging as indexed by biomarkers like epigenetic age and telomere attrition [17-19]. There are also broad similarities between the effects of age and social adversity on peripheral immune function [20]. For instance, early life social adversity in humans has been linked to increases in proinflammatory T cells [21] – a characteristic usually seen with increasing

age. Further, various adversities and social stressors are associated with a decrease in naïve CD4 T helper cells and an increase in naïve CD8 T cells [22], pointing to how the social environment can shape immunity. However, the extent to which social adversity may be associated with immunity across the lifecourse remains unknown. Social adversity might lead to accelerated aging-related disease onset and death and/or social advantage may confer protection from the effects of aging.

Social structures in human populations are complex and multifaceted, including structural inequities and discrimination, and these factors can vary across cultures. Thus, it can be difficult to measure how social adversity "gets under the skin" in humans to affect immune and overall health. Rhesus macaques (*Macaca mulatta*), a non-human primate, are an established animal model that exhibits aging trajectories similar to humans, such as decreases in mobility, but compressed into a lifespan 3-4x times shorter [23]. Aging parallels are also manifested at the molecular level: rhesus macaques and humans show similar age-related alterations in immune cell DNA methylation and gene expression [24]. Rhesus macaques also share many social factors with humans including the expression of affiliative behaviors, despotism, among other behaviors [25], making them an ideal animal model for translational aging research.

In rhesus macaques, exposure to social adversity can be captured by measures of social status (i.e., dominance rank). Social status is acquired differently for males and females [26-27]: females inherit status from their mothers, while males typically disperse from their natal group and enter a new group where they acquire status through a combination of "queuing" and physical contests [28-29]. Similar to humans, social status in macaques patterns access to resources, and can impact health and lifespan [30-32]. For instance, low status macaques experience more conspecific aggression and are therefore more likely to be injured [33], and high status female macaques can live longer than those with lower status [34]. In addition, social status affects immunity in rhesus macaques; one experimental study showed that social status predicts gene expression patterns in peripheral blood mononuclear cells [35].

Here, we characterized age-related variation in immune cell types, as well as the influence of social adversity and sex on immune cell composition. To do so, we studied a free-ranging population of rhesus macaques living on the island of Cayo Santiago, Puerto Rico where we were able to

simultaneously document age, sex, and social status in a semi-naturalistic social setting with minimal human intervention [36].

Methods

Study population:

Cayo Santiago is an island located off the southeastern coast of Puerto Rico inhabited by approximately 1,600 rhesus macaques. The population is managed by the Caribbean Primate Research Center (CPRC) and is the oldest primate field station in the world [37]. Cayo Santiago provides unique research opportunities for behavioral, physiological, demographic, morphological and genomic studies. The Cayo Santiago Field Station has a minimal intervention policy, which means that the animals are not managed medically or reproductively. There are no predators on the island, and senescent phenotypes are commonly observed in this population [24,38-41]. The animals are direct descendants of rhesus macaques introduced from India in 1938; since 1957 these animals have been continuously monitored [42]. The animals are identified with tattoos and ear notches, and demographic data such as age, sex and pedigree have been collected for decades. During the annual capture and release period, researchers have the opportunity to collect biological and morphological samples with the assistance of CPRC veterinary staff. For the past 15 years, the Cayo Biobank Research Unit has collected detailed behavioral data to combine with the biological samples collected each year. In combination, these data provide the opportunity to test the relationships between the social environment, immune function, and aging.

Blood sampling:

We collected whole blood from sedated rhesus macaques over three capture and release periods (n=96 in October - December 2019, n=153 in October 2020 - February 2021 and n=120 in October 2021 - February 2022). Samples were collected in 6ml K2 EDTA tubes (Beckton, Dickson and Company, cat #367899). We collected a total of 369 samples (200 from males, 169 from females) from 230 unique individuals (113 males, 117 females; i.e., some animals were sampled across multiple years of the study), spanning the natural lifespan of macaques on Cayo Santiago (mean age = 11.8 years, range 0-28 years; **Figure 1A and 1B**). Fresh blood samples were transported at 4°C to the University of Puerto Rico Medical Sciences campus where flow cytometric analysis was performed within 6 hours of sample collection.

157

158 *Antibodies and flow cytometric analysis:*

159 An 8-panel antibody cocktail previously validated in rhesus macaques [43-46], consisting of the
160 following antibodies, was used: CD20-PacBlue/Clone 2H7 (Biolegend), CD3-PerCP/Clone SP34-
161 2 (BD), CD4-APC/Clone L200 (BD), CD8-Viogreen/Clone BW135/80 (Miltenyi), CD25-
162 PE/Clone 4E3 (Miltenyi), CD14-FITC/Clone M5E2 (BD), CD16-PEVio770/Clone REA423
163 (Miltenyi), HLA-DR-APCVio770/Clone REA805 (Miltenyi).

164

165 We performed phenotypic characterization of rhesus macaque peripheral blood mononuclear cells
166 (PBMCs) using multicolor flow cytometry with direct immunofluorescence (View **S. Figure 1** for
167 gating strategy and **Table S1** for Ab panel) on all 369 animals. Aliquots of 150 µl of heparinized
168 whole blood were incubated with a mix of the antibodies described for 30 minutes at 25°C (room
169 temperature). After incubation, the red blood cells were fixed and lysed with BD FACS fix and
170 lyse solution (Cat #349202). Cells were washed twice using PBS containing 0.05% BSA at 1,700
171 RPM for 5 minutes and processed in a MACSQuant Analyzer 10 flow cytometer (Miltenyi Biotec,
172 CA).

173

174 Lymphocytes and monocytes were gated based on their characteristic forward scatter (measures
175 cells based on their size) and side scatter (measures cells based on their granularity) patterns.
176 Lymphocytes were then further subdivided according to their cell surface markers. Natural killer
177 (NK) cells were defined as the CD3- and CD16+ population; B cells were defined as CD20+
178 population and T cells as the CD3+ population. We further subdivided T cells from the CD3+ gate
179 into CD4+ and CD8+ populations. CD4+CD25+ and CD8+CD25+ T regulatory cells were further
180 gated from the CD4+ and CD8+ gates. Monocytes were gated based on the combined expression
181 of the HLA-DR/CD14 markers for classical monocytes, HLA-DR/CD16 markers for non-classical
182 monocytes, and HLA-DR/CD14/CD16 for intermediate monocytes (**S. Figure 2**). Flow cytometry
183 gating was performed using Flowjo version 10.7.1 (FlowJo LLC Ashland, OR).

184

185 To obtain an accurate representation of the proportions of cell types, we counted only the stained
186 events of the cells of interest and calculated their proportions based on the subsets of lymphocytes
187 and monocytes. To calculate cell ratios, such as CD20 + B cell to CD3+ T cell ratio (CD20+/CD3+

188 ratio) and CD4+ T cell to CD8+ T cell ratio (CD4+/CD8+ ratio), we divided the calculated
189 proportion of these cell types in each individual sample (e.g., CD20+ B cell/ CD3+ T cell and
190 CD4+/CD8+ respectively). (Calculations detailed in **Table S2**).
191
192 *Quantification of social adversity (social status):*
193 We quantified the social status of a subset of animals for which we had behavioral data (**Figure**
194 **1C,** n = 250 total samples, 134 from males and 116 from females & n = 145 unique individuals,
195 73 males and 72 females). We calculated social status (i.e., dominance rank) using the outcomes
196 of win-loss dominance interactions between pairs of same sex adult groupmates. Because of the
197 different routes through which males and females acquire their status, we quantified social status
198 separately for males and females within each group for each year of the study [47-48].
199 Observations of adult animals (older than 6 years) were collected from two different social groups,
200 groups V and F, in the year prior to sample collection. In 2019 and 2021, behavioral data were
201 collected using a previously established 10-minute focal sampling protocol [26]. Briefly, the
202 protocol consisted of recording state behaviors (e.g., resting, feeding) and agonistic encounters,
203 which included recording the identity of the focal animal and their social partner. Win-loss
204 agonistic interactions included threat and submissive behaviors, along with contact and non-
205 contact aggression. In 2019 and 2021 we also collected additional agonistic interactions *ad-*
206 *libitum*. In 2020, all agonistic interactions were collected *ad-libitum* due to restrictions imposed
207 on behavioral data collection due to the COVID-19 pandemic. In all years, we used known
208 maternal relatedness to settle behavioral gaps in the female hierarchy [49]. To control for variation
209 in group size, social status (i.e., dominance rank) was quantified as the percentage of same-sex
210 adults that an animal outranked in their group. For all analyses, we grouped animals into one of
211 two social status categories: high-rank (>80% of same-sex adults outranked) and low-rank (< 79%
212 of same-sex adults outranked)[50].

213 *Statistical analysis:*
214 All statistical analyses were performed using R statistical software R version 4.2.3 [51].
215 First, we performed a principal component analysis of the cell composition data for all samples (n
216 = 369, 230 unique individuals) using the *prcomp* function of the *stats* package. Next, we employed
217 a linear additive mixed-effects approach, using the *lmer* function in the *lmerTest* package to run

sample projections on principal components as a function of age (in years), sex, and sample period - to control for the technical variation in the flow cytometer lasers, which changed over the sampling years (*model 1* - **Table S3**) and individual ID as a random effect. We also modeled sample projections on principal components as a function of the interaction between age and sex (age*sex) and sampling period - which will ultimately allow us to identify possible sex-dependent associations with age - and individual ID as a random effect (*model 2* - **Table S3**).

To evaluate each cell type at a more granular level, we employed the same additive linear mixed-effects to test the proportion of each cell type and certain cell type ratios (e.g., CD4+/CD8+) as a function of age, sex, and sample period with individual ID as a random effect (*model 3* - **Table S3**). Finally, we tested the proportion of each cell type and certain cell type ratios (e.g., CD4+/CD8+) as a function of the interaction between age and sex (age*sex) and sampling period with individual ID as a random effect (*model 4* - **Table S3**).

For the subset of samples in which information on social status was available (n = 250 total samples, 145 unique individuals), we tested for the additive effect of principal component projections as a function of social status, age, sex and sample period, with individual ID and social group as a random effect (*model 5* - **Table S3**). We also tested for the principal component interaction between status and age (status*age) and between status and sex (status*sex), with individual ID and social group as a random effect (*model 6* - **Table S3**). We then additively tested the proportion of each cell type and certain cell type ratios as a function of social status, age, sex, and sample period (*model 7* - **Table S3***)*. To test whether the relationship between the proportion of cell types and social status depended on sociodemographic variables, we tested the interaction between: social status and age (status*age) and for social status and sex (status*sex, *model 8* - **Table S3**). Furthermore, since we identified interactions between social status and sex, and since social status is acquired differently for male and females in rhesus macaques, we fitted a separate model for males and females to test if there was a main effect of social status within each sex on the proportions of different cell types. Age and sample period were also included in the model to control for these covariates (*model 9* - **Table S3**).

The linear models and sample sizes for each are summarized in **Table S3.** For every predictor variable in the full (n = 369) and status (n = 250) datasets, we corrected for multiple hypothesis tests using the Benjamini Hochberg FDR approach and considered significant associates at FDR < 0.10. Because Model 7 was only performed in cell types that showed a significant interaction between sex and social status (and not all the cell types measured), we did not correct for multiple testing in this model.

Results

Macaques exhibit age-related variation in immune cell composition and inflammation

Age was significantly positively associated with the first principal component (PC) of immune cell composition (*model 1* - $\beta_{PC1\ age}$ = 0.14, FDR = 7.2 x 10^{-18}, **Figure 1D)**. This first PC, which explained 32.4% of the variance in cell composition across all samples, was associated with higher proportions of inflammation-associated cell types, such as cytotoxic T cells and regulatory T cells, and lower proportions of cells involved in pathogen clearance, including CD20+ B cells and classical monocytes (**Figure 1E, Table S4).** Thus, older animals exhibited a pattern of greater inflammation and immunosenescence than younger individuals did.

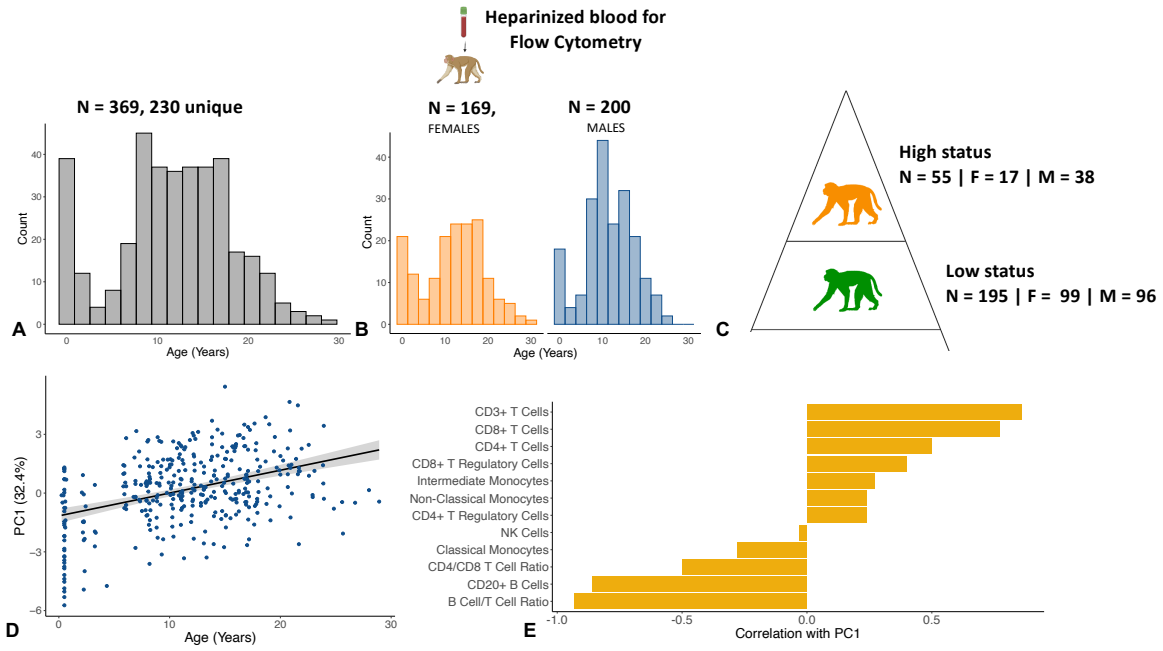


Figure 1: Sample collection and demographics. A) We collected 369 whole blood samples from 230 unique individuals across three years, and quantified immune cell proportions using flow

268 cytometry. **B)** The dataset was roughly balanced between males and females and captured the
269 entire natural lifespan of macaques in this population. **C)** We calculated social status by assigning
270 dominance ranks to 250 samples using observational data collected in the year before each sample
271 was collected. Animals were assigned to one of three dominance ranks: high, medium, and low.
272 The social status dataset is a subset of the original age dataset because behavioral data were not
273 available for all study animals (i.e., it is not collected for infants and juveniles). **D)** PC1 of immune
274 cell compositions is significantly associated with age (β_{PC1} = 0.14, FDR = 7.2 x 10^{-18}). **E)** The T
275 cell compartment is positively associated with PC1 (and thus age), while the B cell compartment
276 is negatively associated with PC1 of immune cell composition.
277
278 We then conducted a more granular analysis of the factors associated with the proportions of
279 individual cell types. Age was significantly associated with signatures of immunosenescence,
280 including a decline in adaptive immune cells. This was largely driven by lower proportions of
281 CD20+ B cells in older individuals (*model 3* - $\beta_{CD20\ age}$ = -0.83 ± 0.09, FDR = 1.3 x 10^{-16}, **Figure**
282 **2A**), which resulted in significantly lower CD20+/CD3+ ratios in older individuals (*model 3* -
283 $\beta_{CD20:CD3\ age}$ = -0.04 ± 0.004, FDR = 4.9 x 10^{-15}, **Figure 2B**). Age was also associated with higher
284 proportions of inflammation-related cells. The proportion of cytotoxic CD8+ Cytotoxic T cells
285 was significantly higher in older animals (*model 3* - $\beta_{CD8\ age}$ = 0.60 ± 0.07, FDR = 3.2 x 10^{-14},
286 **Figure 2C**), resulting in a strong and significant effect of lower CD4+/CD8+ ratios (*model 3* -
287 $\beta_{CD4:CD8\ age}$ = -0.06 ± 0.008, FDR = 4.3 x 10^{-14}, **Figure 2D**) and higher proportions of CD3+ T cells
288 in older individuals (*model 3* - β_{CD3}= 0.67 ± 0.11, FDR = 2.2 x 10^{-8}, **S. Figure 3)**.
289
290 Next, we examined the less abundant but immunologically important regulatory CD8+ and CD4+
291 T cell populations (CD25+), which are involved in immune suppression and maintenance of self-
292 tolerance [52] (i.e., the ability to recognize self-antigens). Both CD4+ and CD8+ T regulatory cells
293 were significantly more abundant in older animals (*model 3* - CD3+CD4+CD25+: β_{age}= 0.16 ±
294 0.02, FDR = 3.6 x 10^{-10}, **Figure 2E**; *model 3* - CD3+CD8+CD25+: β_{age} = 0.03 ± 0.005, FDR =
295 1.8 x 10^{-6}, **Figure 2F**), suggesting a reduced age-related ability to regulate endogenous and
296 exogenous antigens.
297

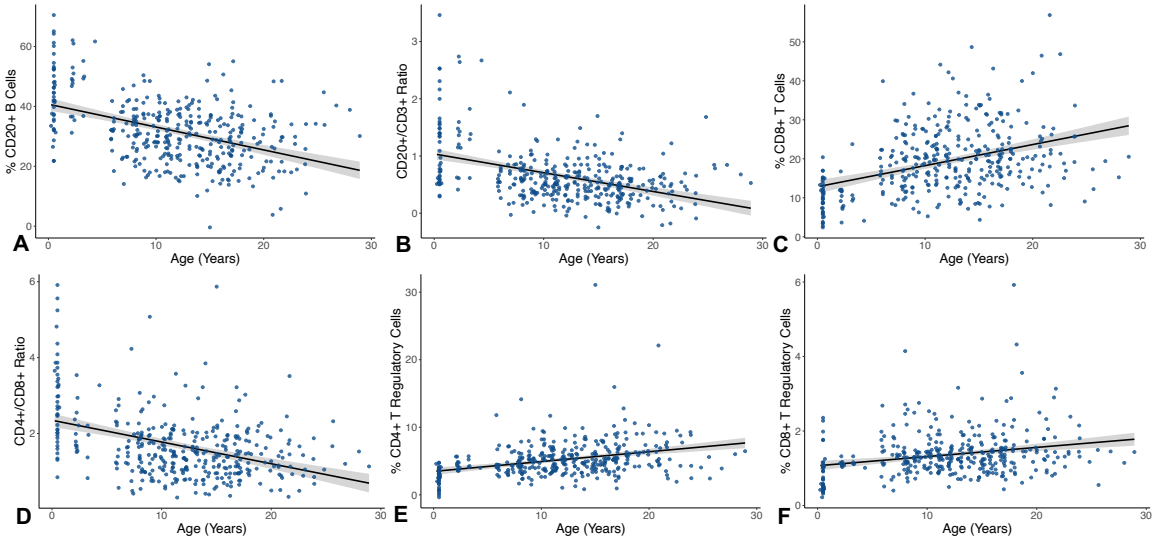


Figure 2: Age-associated differences in adaptive immune cell proportions. A) CD20+ B cells (β_{CD20} = -0.83 ± 0.09, FDR = 1.3 x 10^{-16}) proportions and **B)** CD20+/CD3+ ratio ($\beta_{CD20:CD3}$ = -0.04 ± 0.004, FDR = 4.9 x 10^{-15}) are lower in older individuals. **C)** CD8+ Cytotoxic T cells (β_{CD8} = 0.60 ± 0.07, FDR = 3.2 x 10^{-14}) are higher in older individuals, while the **D)** CD4+/CD8+ T cell ratio ($\beta_{CD4:CD8}$ = -0.06 ± 0.008, FDR = 4.3 x 10^{-14}) is lower in older individuals. **E)** CD4+ T regulatory cells (β = 0.16 ± 0.02, FDR = 3.6 x 10^{-10}) and **F)** CD8+ T regulatory cells are higher in older individuals compared to younger individuals (β = 0.03 ± 0.005, FDR = 1.8 x 10^{-6}), possibly because of higher baseline levels of inflammation (i.e., inflammaging).

Innate immune cells also showed significant associations with age. Classical monocytes (HLA-DR+/CD14+/CD16-), which are involved in phagocytosis and extracellular pathogen clearance [53], were lower in older individuals (*model 3* - $\beta_{CD14++\ age}$ = -0.31 ± 0.16, FDR = 0.07, **Figure 3A**), while intermediate monocytes (HLA-DR+/CD14+/CD16+), involved in immune cell recruitment and proinflammatory cytokine secretion [53], were higher in older individuals (*model 3* - $\beta_{CD14+CD16+\ age}$ = 0.21 ± 0.09, FDR = 0.04, **Figure 3B)**. The proportion of CD16+CD3- NK cells – which have a similar role to CD8+ Cytotoxic T cells presenting natural cytotoxicity but are not antigen specific – was also significantly higher in older individuals (*model 3* - $\beta_{NK\ age}$ = 0.17 ± 0.07, FDR = 0.03, **Figure 3C**). Together, these results indicate that older individuals show a decrease in adaptive immunity along with an increase in inflammation-related innate immune cells compared to younger individuals, potentially disrupting a "healthy" homeostatic immune system.

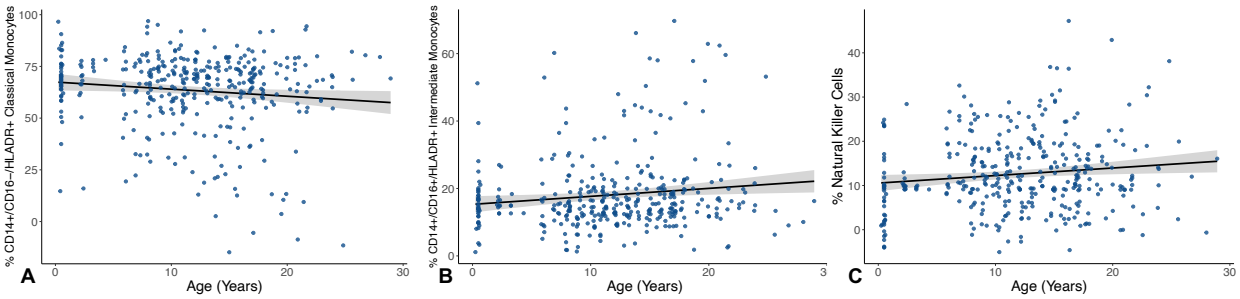


Figure 3: Age is associated with variation in innate immune cell proportions. A) CD14+/CD16-/HLADR+ Classical monocytes ($\beta_{CD14++} = -0.31 \pm 0.16$, FDR = 0.07) are lower and **B)** CD14+/CD16+/HLADR+ intermediate monocytes ($\beta_{CD14+CD16+} = 0.21 \pm 0.09$, FDR = 0.04) are higher in older individuals, while **C)** CD16+ NK cells ($\beta_{NK} = 0.17 \pm 0.07$, FDR = 0.03) are higher in older individuals.

We did not observe statistically significant main effects of sex (*model 3* in *Methods*) or a sex-age interaction (*model 4* in *Methods*) on immune cell proportions. Nevertheless, a trend toward sex differences was observed in both the proportions of CD8+ Cytotoxic T cells (*model 3* - $\beta_{CD8\ sex} = 2.19 \pm 0.95$, FDR = 0.14) and in the CD4+/CD8+ ratio (*model 3* - $\beta_{CD4:CD8\ sex} = -0.24 \pm 0.09$, FDR = 0.14, **S. Figure 4**), with males having a higher proportion of CD8+ Cytotoxic T cells compared to females, and females having a higher CD4+/CD8+ ratio compared to males, suggesting a stronger adaptive immune response in females, which, in part, is generated by CD4+ T helper cells.

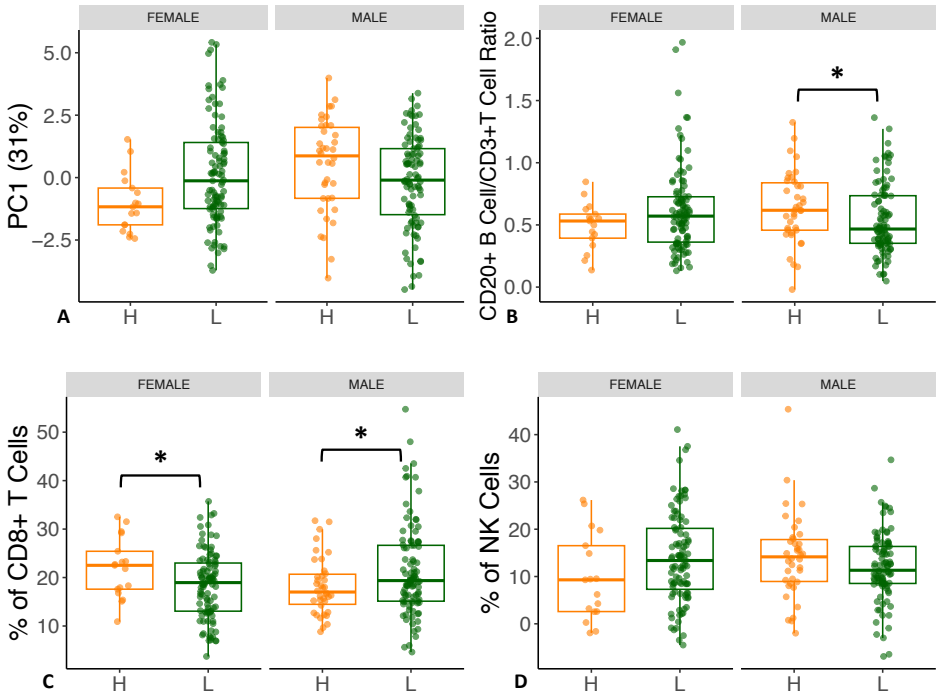
Social status and immune cell composition

There was a significant interaction between social status and sex on PC1 (31% of the variance in cell composition across all samples) of immune cell composition (*model 6* - $\beta_{PC1\text{-}sex*status} = -1.7 \pm 0.63$, FDR = 0.04, **Figure 4A)**, documenting the sex-dependent impact of social status on immunity.

When modeling males and females together in an additive modeling framework, we found no significant effects of social status on immune cell proportions (all FDR > 0.10, *model 7* in *Methods*), or between the interaction between social status and age (*model 8* in *Methods*). However, we found many significant interactions between social status and sex on immune cell

346 composition (*model 8* in *Methods*). Because social status is acquired differently for male and
347 female rhesus macaques, we also carried out post-hoc analyses of the social status effects within
348 each sex separately (*model 9* in *Methods*).
349
350 The CD20+/CD3+ ratio interacted between social status and sex such that it was higher in males
351 of high social status, but lower in females with high social status (*model 8* - $\beta_{CD20/CD3\ ratio\text{-}sex*status}$ =
352 -0.26 ± 0.11, FDR = 0.06, **Figure 4B)**, both part of the adaptive immune system. This difference
353 in the CD20+/CD3+ ratio seems to be partially driven by interaction between sex and social status
354 on the proportion of CD3+ T cells (*model 8* - $\beta_{CD3\text{-}sex*status}$ = 11.8 ± 4.42, FDR = 0.04, **S. Figure**
355 **5)**, such that CD3+ T cells were higher in low social status males, but this pattern was flipped
356 in females. There was a within-sex main effect of social status in males in the CD20+/CD3+ ratio,
357 with high social status males having a significantly higher ratio than low social status males did
358 (*model 9* - $\beta_{CD20/CD3\ ratio\text{-}males/status}$ = -1.3 ± 0.06, p = 0.04, **Figure 4B**). No significant effect of social
359 status on the CD20+/CD3+ ratio was found in females (*model 9* - $\beta_{CD20/CD3\ ratio\text{-}females/status}$ = 0.10 ±
360 0.13, p = 0.19).
361
362 Additionally, there was a significant interaction between social status and sex in the proportion of
363 CD8+ Cytotoxic T cells (*model 8* - $\beta_{CD8\text{-}sex*status}$ = 8.3 ± 2.94, FDR = 0.04, **Figure 4C)**, which were
364 higher in high social status males, but this relationship again flipped females. Our within-sex
365 analysis revealed a significant main effect of CD8+ Cytotoxic T cells, in which low social status
366 males had significantly higher proportions of this cell type than did high social status males (*model*
367 *9* - $\beta_{CD8\text{-}males/status}$ = 4 ± 1.8, p = 0.03, **Figure 4C**). The opposite main effect was observed in females,
368 in which high status females had significantly higher proportions of CD8+ Cytotoxic T cells than
369 did low social status females (*model 9* - $\beta_{CD8\text{-}females/status}$ = -4.8 ± 2.1, p = 0.03, **Figure 4C**).
370
371 In the innate arm of the immune system, we detected an interaction between social status and sex
372 on the proportion of CD3-CD16+ NK cells (*model 8* - $\beta_{NK\text{-}sex*status}$ = -7.9 ± 3.2, FDR = 0.05, **Figure**
373 **4D)**, where the proportion was lower in males of low status, but higher in females of low status.
374 Social status approached significance in the proportions of CD3-CD16+ NK cells in females
375 (*model 9* - $\beta_{NK\text{-}females/status}$ = 5.6 ± 3, p = 0.06, **Figure 4D**), in which low social status females
376 displayed higher proportions of this cell type compared to high social status females. We found no

377 significant main effect of social status on CD3-CD16+ NK cells in males (*model 9* - $\beta_{NK\text{-}males/status}$
378 = -2.27 ± 3.4, p = 0.18).
379



380

381 **Figure 4: Sex and social status interact to impact immune cell composition. A)** PC1 (31% of
382 the variation in the dataset, $\beta_{PC1\text{-}sex*status}$ = -1.7, FDR = 0.03) recapitulates the interaction between
383 sex and social status. **B)** Interaction in the CD20+/CD3+ ratio ($\beta_{CD20/CD3\ ratio\text{-}sex*status}$ = -0.25 ± 0.05,
384 FDR = 0.06) shows that this ratio is higher with higher social status in males while it is lower with
385 higher social status in females; within-sex analysis show that high social status males have a
386 significantly higher ratio than low social status males ($\beta_{CD20/CD3\ ratio\text{-}males/status}$ = -1.3 ± 0.06, p =
387 0.04). **C)** CD8+ Cytotoxic T cells show an interaction ($\beta_{CD8\text{-}sex*status}$ = 8.3 ± 2.94, FDR = 0.04)
388 where this cell type is higher in lower social status in males, while it is higher in high social status
389 females. Within-sex analysis of CD8+ T cells showed low social status males had significantly
390 higher proportions of this cell type than did high social status males ($\beta_{CD8\text{-}males/status}$ = 4 ± 1.8, p =
391 0.03), while the opposite effect was observed in females ($\beta_{CD8\text{-}females/status}$ = -4.8 ± 2.1, p = 0.03).
392 **D)** Interaction in the proportion of NK Cells ($\beta_{NK\text{-}sex*status}$ = -7.9 ± 3.2, FDR = 0.05), with high
393 social status males showing higher proportions, while high social status females show lower
394 proportions.
395

Discussion

We examined how social status, age, and sex were related to immune cell distributions in a large sample of adult rhesus macaques living in semi-natural conditions. Overall, we found strong and consistent signatures of age-related immune cell dysregulation. We also identified significant links between social status and sex in cells of innate and adaptive arms of the immune system. Together, this variation is likely to influence immune responses to pathogenic challenges as well as the development of inflammation-related diseases.

Overall, macaques exhibited age-related differences in immune cells similar to those observed in humans, including declines in lymphocytes [54]. Here, we also identified more specific cell types with age-related differences. We detected lower proportions of CD20+ B cells at older ages, which may reflect immunosenescence, as these cells are responsible for antibody production, pathogen clearance, and are key cells in the generation of immune memory. Further, a key factor underlying the limited efficacy of vaccines in older individuals is a weakened B cell response [55]; B cells have also been associated with protection against certain types of cancer, such as lung cancer [56].

Similar to two other studies in captive macaques, we found higher CD8+ T cell proportions at older ages [67-58]. Notably, this differs from findings in humans, where both CD8+ Cytotoxic T cells and their effector responses (i.e., stimulus responsiveness) exhibit lower proportions at older ages [59]. It is possible that this discrepancy is only reflected in the overall CD8+ cytotoxic T cell pool, as it has been reported that certain CD8+ T cell subsets – such as memory subsets – increase in proportion and efficacy with age [60]. Alternatively, given that CD8+ T cell subsets have been associated with inflammation and 'inflammaging' [61], there is a possibility that higher overall CD8+ Cytotoxic T cell pool in rhesus macaques is indicative of higher levels of inflammation. The age-related reduction in CD4+/CD8+ ratio corroborates this hypothesis. In support of increased inflammation with age, we found that older animals had significantly higher CD3-CD16+ NK cell proportions in our dataset. Similar to CD8+ Cytotoxic T cells, CD3-CD16+ NK cells respond to intracellular pathogens, secrete multiple proinflammatory mediators, and are crucial during tumor surveillance and signaling [62]. The higher proportions of CD3-CD16+ NK cells predict a higher incidence of inflammation and/or tissue injury in the older population, which is commonly observed in the Cayo Santiago macaque population [33]. As expected, CD4+CD25+ T regulatory

427 cells as well as CD8+CD25+ T regulatory cells were associated with age, indicating higher levels
428 of inflammation in older individuals [63]. These results, together with lower levels of CD20+ B
429 cell proportions and higher levels of CD8+ T cell and CD3-CD16+ NK cell proportions, further
430 support the hypothesis that the adaptive immune response in rhesus macaques decreases with age
431 and inflammation-related cell types increase (i.e., 'inflammaging'). Taken together, these
432 alterations may drive biological and physiological decline that likely increases the risk of
433 morbidity and mortality in macaques, as it does in humans.
434
435 Monocyte proportions also varied with age. Specifically, we found fewer CD14+ classical
436 monocytes in older animals. These cells are phagocytic cells that ingest pathogens that they
437 encounter [64]. This age-related reduction may indicate a reduction in phagocytosis (ingestion of
438 pathogens by classical monocytes) and thus can possibly increase infections in older individuals.
439 In addition, older individuals had higher proportions of CD14+/CD16+ intermediate monocytes,
440 which are strongly associated with inflammation [65]. For instance, an increase in this cell type
441 has been linked to disorders such as chronic kidney disease [66]. The decrease in classical
442 monocytes, together with an increase in intermediate monocytes, represents yet another signature
443 of immunosenescence and inflammaging.
444
445 One of the strengths of our study system was the ability to quantify social adversity,
446 operationalized as social status, and test if and how social status influenced immune variation and
447 whether the effects of status varied with age and/or sex. We found no main effect of social status
448 on the proportion of immune cell types. Also, there was no interaction between social status and
449 age on the proportions of immune cell types. This result was contrary to our expectations because
450 we expected low social status individuals to experience more variation in immune cell types with
451 increasing age. Nevertheless, we found several interactions between social status and sex, as well
452 as a within-sex main effect of social status on immune cell composition, possibly reflecting the
453 different pathways through which social status is acquired in males and females and thus
454 highlighting the fact that different sexes experience social adversity differently across the
455 hierarchy.
456

457 The interaction of social status and sex influenced adaptive and innate immune cell types such as
458 CD20+/CD3+ ratio and CD8+ T cells and CD3-CD16+ NK cells, where the proportions of these
459 cell types associated with status depended on the sex of the individual. In humans, social stressors,
460 such as lower socioeconomic status and lower subjective social status, can affect cytokine release
461 and inflammatory responses in peripheral blood mononuclear cells in a sex-dependent manner [67-
462 68]. However, studies in humans that have looked at the interaction between social stressors (such
463 as socioeconomic status) and sex on immune cell proportions have found no significant interaction
464 between these covariates [22], thus making our study unique in reporting sex-dependent effects of
465 social status.
466
467 We also found a significant main effect of social status in the within-sex analysis on the CD20+ B
468 cell/CD3+ ratio, with high social status males having significantly higher ratios than low status
469 males. The decrease in the CD20+/CD3+ ratio seems to be driven by a decrease in the proportions
470 of CD3+ T cells in low social status males (**S. Figure 5**) compared to high status males. Decreases
471 in this cell type have been associated with decreases in cell-mediated immunity to bacteria and
472 viruses [69-70], potentially showing that the T cell response in macaques is negatively affected by
473 low social status. In addition, CD8+ T cell proportions were higher in low social status males
474 compared to high social status macaques. Few prior studies have assessed sociality-related immune
475 cell differences in male rhesus [71], likely because of ethical and husbandry constraints, such as
476 aggressive behavior between males. One study in male Barbary macaques (*Macaca sylvanus*)
477 reported that males with strong social bonds had lower levels of fecal glucocorticoids [72], which
478 is typically associated with reduced inflammation [73]. Additionally, studies in cynomolgus
479 macaques (*Macaca fascicularis*) have shown that low social status males had a higher probability
480 of being infected with a virus than did high social status macaques [74]. These findings should be
481 taken with caution, however, as other studies of macaques (rhesus and other species) found no
482 differences in infection rate or immune responses between high and low status males [75-76].
483 Although there are currently no data associating social status (or other social stressors) with CD8+
484 T cells in rhesus macaques, there are reports in other species that CD8+ T cells can mediate the
485 release of proinflammatory cytokines during stressful conditions [77]. Our finding of higher
486 proportions of CD8+ T cells in low social status macaques might indicate higher levels of baseline

487 cytotoxic T cell activation, potentially affecting the CD4+ T cell response. Testing this idea will
488 require methods such as cytokine analysis or next generation sequencing.
489
490 There was also a main effect of social status on the proportion of CD8+ T cells in females, but in
491 contrast to males, high social status females had significantly higher proportions of this cell type
492 compared to low social status females. One study also reported lower proportions of CD8+ T cells
493 in low social status in non-free-ranging female rhesus macaques [34]. Given that females tend to
494 have lower proportions of CD8+ T cells than males regardless of age [78-80], a lower proportion
495 of this cell type in low social status females might indicate lower cytotoxic immunity at baseline.
496 Female social status also had a main effect on the proportion of CD3-CD16+ NK cells (associated
497 with immune surveillance, inflammation and innate responses), with low social status females
498 having significantly higher proportions of this cell type than high social status females. Although
499 a prior study found that the proportion of CD3-CD16+ NK cells did not vary with social status in
500 female rhesus macaques, it did find that this cell type was the most sensitive to social status.
501 Specifically, low social status females showed patterns of gene expression consistent with a
502 proinflammatory phenotype in this cell type in response to lipopolysaccharide [81]. These results
503 highlight that low status female rhesus macaque may experience higher levels of basal
504 inflammation, consistent with other studies in this species [81-83].
505
506 In conclusion, our results demonstrate that, at the level of circulating immune cell proportions,
507 macaques and humans show similar age-related variation in immune cell types. Although we did
508 not detect any significant main effects of sex or sex-age interaction, it is possible that more specific,
509 but unmeasured adaptive immune cells, such as the effector and memory subsets of B cells and T
510 cells, could differ between males and females. In future studies, it will be important to measure
511 other innate immune cell types, such as dendritic cells and granulocytes, since these cell types are
512 critical for antigen presentation and the development of adaptive immune response. We found that
513 the effects of social status differed between males and females, which is likely due to sex-
514 differences in how rhesus macaques obtain social status. Specifically, females inherit their social
515 status, which remains relatively stable throughout their lives, while males queue and occasionally
516 fight to establish and maintain their social status, which may lead to stronger effects of status on
517 immune cell distribution and function. Overall, our study provides detailed insights into the

impacts of social and demographic variation on immune cell status in a non-human primate model with unparalleled translatability to humans. Future research should quantify the proportions of these cell types as a function of age using a longitudinal approach, which will require sampling individuals over the course of years. Immune stimulation tests would also be informative by testing whether both the age-associated and status-associated differences in immune cell types translate to immune function.

Author contributions

M.R.S.R., 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., A.D.N.-D, P.P., M.A.P.-F., E.R.S., E.B.C., J.E.N.-D., D.P., A.R.L., M.J.M. and CBRU performed research; M.R.S.R and N.S.-M. analyzed data; and M.S.R. and N.S.-M. wrote the manuscript. All authors reviewed and revised the manuscript. CBRU members: Lauren J.N. Brent, Jampes P. Higham, Noah Snyder-Mackler, Michael J. Montague, Michael L. Platt, Melween I. Martinez, Susan C. Antón, Amanda D. Melin, Jérôme Sallet

The authors declare no competing interest.

Acknowledgements:

Part of Figure 1, panel A, was created with BioRender.com.

We thank the Caribbean Primate Research Center for their help in collecting data, especially Giselle Carabllo Cruz, and Nahiri Rivera Barreto; Crisanta Serrano and Stephanie Dorta for laboratory advice; Corbin Johnson, Laura Newman, Alice Baniel, India Schneider-Crease, Kenneth Chiou, Trisha Zintel, and other members of the Snyder-Mackler, Sariol, Higham, Platt, and Brent labs for helpful discussions during the course of this work.

Funding:

This work was supported by the National Institutes of Health (R01-AG060931; R01AG060931-S1 R00-AG051764; R01-MH118203; R01-MH096875; R56-AG071023; C06-OD026690; P40-OD012217; NSF-1800558; ERC-864461).

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).

The authors declare no competing interest.

References

1. Max Roser, Esteban Ortiz-Ospina and Hannah Ritchie (2013) - "Life Expectancy". Published online at OurWorldInData.org. Retrieved from: 'https://ourworldindata.org/life-expectancy' [Online Resource]

2. Strait, J. B., & Lakatta, E. G. (2012). Aging-associated cardiovascular changes and their relationship to heart. https://doi.org/10.1016/j.hfc.2011.08.011

3. Yung, R. L., & Julius, A. (2008). Epigenetics, aging, and autoimmunity. *Autoimmunity*, *41*(4), 329-335. https://doi.org/10.1080/08916930802024889

4. Halim, M., & Halim, A. (2019). The effects of inflammation, aging and oxidative stress on the pathogenesis of diabetes mellitus (type 2 diabetes). *Diabetes & metabolic syndrome: clinical research & reviews*, *13*(2), 1165-1172. https://doi.org/10.1016/j.dsx.2019.01.040

5. Sacitharan, P. K. (2019). Ageing and osteoarthritis. *Biochemistry and cell biology of ageing: part II clinical science*, 123-159. DOI: 10.1007/978-981-13-3681-2_6

6. Koh, S. H., Choi, S. H., Jeong, J. H., Jang, J. W., Park, K. W., Kim, E. J., ... & Kang, S. (2020). Telomere shortening reflecting physical aging is associated with cognitive decline and dementia conversion in mild cognitive impairment due to Alzheimer's disease. *Aging (Albany NY)*, *12*(5), 4407. https://doi.org/10.18632/aging.102893

7. Ferrucci, L., & Fabbri, E. (2018). Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. *Nature Reviews Cardiology*, *15*(9), 505-522. https://doi.org/10.1038/s41569-018-0064-2

8. Aw, D., Silva, A. B., & Palmer, D. B. (2007). Immunosenescence: emerging challenges for an ageing population. *Immunology*, *120*(4), 435-446. 10.1111/j.1365-2567.2007.02555.x

9. Hearps, A. C., Martin, G. E., Angelovich, T. A., Cheng, W. J., Maisa, A., Landay, A. L., ... & Crowe, S. M. (2012). Aging is associated with chronic innate immune activation and dysregulation of monocyte phenotype and function. *Aging cell*, *11*(5), 867-875. https://doi.org/10.1111/j.1474-9726.2012.00851.x

10. Giefing-Kröll, C., Berger, P., Lepperdinger, G., & Grubeck-Loebenstein, B. (2015). How sex and age affect immune responses, susceptibility to infections, and response to vaccination. *Aging cell*, *14*(3), 309-321. https://doi.org/10.1111/acel.12326

608 11. Gubbels Bupp, M. R., Potluri, T., Fink, A. L., & Klein, S. L. (2018). The confluence of sex
609 hormones and aging on immunity. *Frontiers in immunology*, *9*, 1269.
610 https://doi.org/10.3389/fimmu.2018.01269
611 12. Klein, S. L., & Flanagan, K. L. (2016). Sex differences in immune responses. *Nature Reviews*
612 *Immunology*, *16*(10), 626-638. https://doi.org/10.1038/nri.2016.90
613 13. Guerra-Silveira, F., & Abad-Franch, F. (2013). Sex bias in infectious disease epidemiology:
614 patterns and processes. *PloS one*, *8*(4), e62390. https://doi.org/10.1371/journal.pone.0062390
615 14. Pantell, M., Rehkopf, D., Jutte, D., Syme, S. L., Balmes, J., & Adler, N. (2013). Social
616 isolation: a predictor of mortality comparable to traditional clinical risk factors. American journal
617 of public health, 103(11), 2056-2062. https://doi.org/10.2105/AJPH.2013.301261
618 15. Cole, S. W. (2014). Human social genomics. *PLoS genetics*, *10*(8), e1004601.
619 https://doi.org/10.1371/journal.pgen.1004601
620 16. L. F. Berkman, T. Glass, I. Brissette, T. E. Seeman, From social integration to health:
621 Durkheim in the new millennium. Soc. Sci. Med. 51, 843–857 (2000). doi: 10.1016/S0277-9536
622 (00)00065-4; pmid: 10972429
623 17. Anderson, J. A., Johnston, R. A., Lea, A. J., Campos, F. A., Voyles, T. N., Akinyi, M. Y., ...
624 & Tung, J. (2021). High social status males experience accelerated epigenetic aging in wild
625 baboons. *Elife*, *10*, e66128. https://doi.org/10.7554/eLife.66128
626 18. Fiorito, G., Polidoro, S., Dugué, P. A., Kivimaki, M., Ponzi, E., Matullo, G., ... & Vineis, P.
627 (2017). Social adversity and epigenetic aging: a multi-cohort study on socioeconomic differences
628 in peripheral blood DNA methylation. *Scientific reports*, *7*(1), 1-12.
629 https://doi.org/10.1038/s41598-017-16391-5
630 19. Ridout, K. K., Levandowski, M., Ridout, S. J., Gantz, L., Goonan, K., Palermo, D., ... & Tyrka,
631 A. R. (2018). Early life adversity and telomere length: a meta-analysis. *Molecular psychiatry*,
632 *23*(4), 858-871. https://doi.org/10.1038/mp.2017.26
633 20. Snyder-Mackler, N., Somel, M., & Tung, J. (2014). Shared signatures of social stress and aging
634 in peripheral blood mononuclear cell gene expression profiles. *Aging Cell*, *13*(5), 954-957.
635 https://doi.org/10.1111/acel.12239
636 21. Elwenspoek, M. M., Hengesch, X., Leenen, F. A., Schritz, A., Sias, K., Schaan, V. K., ... &
637 Muller, C. P. (2017). Proinflammatory T cell status associated with early life adversity. *The*
638 *Journal of Immunology*, *199*(12), 4046-4055. https://doi.org/10.4049/jimmunol.1701082

22. Klopack, Eric T., Eileen M. Crimmins, Steve W. Cole, Teresa E. Seeman, and Judith E. Carroll. 2022. "Social Stressors Associated with Age-Related T Lymphocyte Percentages in Older US Adults: Evidence from the US Health and Retirement Study." *Proceedings of the National Academy of Sciences of the United States of America* 119 (25): e2202780119. https://doi.org/10.1073/pnas.2202780119

23. Roth, G. S., Mattison, J. A., Ottinger, M. A., Chachich, M. E., Lane, M. A., & Ingram, D. K. (2004). Aging in rhesus monkeys: relevance to human health interventions. *Science*, *305*(5689), 1423-1426. https://doi.org/10.1126/science.1102541

24. Chiou, K. L., Montague, M. J., Goldman, E. A., Watowich, M. M., Sams, S. N., Song, J., ... & Snyder-Mackler, N. (2020). Rhesus macaques as a tractable physiological model of human ageing. *Philosophical Transactions of the Royal Society B*, *375*(1811), 20190612. https://doi.org/10.1098/rstb.2019.0612

25. Thierry, B., Singh, M., & Kaumanns, W. (Eds.). (2004). *Macaque societies: a model for the study of social organization* (Vol. 41). Cambridge University Press.

26. Brent, L. J., MacLarnon, A., Platt, M. L., & Semple, S. (2013). Seasonal changes in the structure of rhesus macaque social networks. *Behavioral Ecology and Sociobiology*, *67*(3), 349-359. https://doi.org/10.1007/s00265-012-1455-8

27. Kulik, L., Amici, F., Langos, D., & Widdig, A. (2015). Sex differences in the development of social relationships in rhesus macaques (Macaca mulatta). *International journal of primatology*, *36*(2), 353-376. https://doi.org/10.1007/s10764-015-9826-4

28. Vandeleest, J. J., Winkler, S. L., Beisner, B. A., Hannibal, D. L., Atwill, E. R., & McCowan, B. (2020). Sex differences in the impact of social status on hair cortisol concentrations in rhesus monkeys (Macaca mulatta). *American journal of primatology*, *82*(1), e23086. https://doi.org/10.1002/ajp.23086

29. Datta, S. (1988). The acquisition of dominance among free-ranging rhesus monkey siblings. *Animal Behaviour*, *36*(3), 754-772. https://doi.org/10.1016/S0003-3472(88)80159-3

30. Snyder-Mackler, N., Burger, J. R., Gaydosh, L., Belsky, D. W., Noppert, G. A., Campos, F. A., ... & Tung, J. (2020). Social determinants of health and survival in humans and other animals. *Science*, *368*(6493). https://doi.org/10.1126/science.aax9553

31. Kohn, J. N., Snyder-Mackler, N., Barreiro, L. B., Johnson, Z. P., Tung, J., & Wilson, M. E. (2016). Dominance rank causally affects personality and glucocorticoid regulation in female

670 rhesus macaques. *Psychoneuroendocrinology*, *74*, 179-188.
671 https://doi.org/10.1016/j.psyneuen.2016.09.005
672 32. Debray, R., Snyder-Mackler, N., Kohn, J. N., Wilson, M. E., Barreiro, L. B., & Tung, J. (2019).
673 Social affiliation predicts mitochondrial DNA copy number in female rhesus macaques. *Biology*
674 *letters*, *15*(1), 20180643. https://doi.org/10.1098/rsbl.2018.0643
675 33. Pavez-Fox, M. A., Kimock, C. M., Rivera-Barreto, N., Negron-Del Valle, J. E., Phillips, D.,
676 Ruiz-Lambides, A., ... & Brent, L. J. (2022). Reduced injury risk links sociality to survival in a
677 group-living primate. *Iscience*, *25*(11), 105454. https://doi.org/10.1016/j.isci.2022.105454
678 34. Blomquist, G. E., Sade, D. S., & Berard, J. D. (2011). Rank-related fitness differences and
679 their demographic pathways in semi-free-ranging rhesus macaques (Macaca mulatta).
680 *International Journal of Primatology*, *32*, 193-208. https://doi.org/10.1007/s10764-010-9461-z
681 35. Tung, J., Barreiro, L. B., Johnson, Z. P., Hansen, K. D., Michopoulos, V., Toufexis, D., ... &
682 Gilad, Y. (2012). Social environment is associated with gene regulatory variation in the rhesus
683 macaque immune system. *Proceedings of the National Academy of Sciences*, *109*(17), 6490-6495.
684 https://doi.org/10.1073/pnas.1202734109
685 36. Kessler, M. J., & Rawlins, R. G. (2016). A 75-year pictorial history of the Cayo Santiago
686 rhesus monkey colony. *American Journal of Primatology*, *78*(1), 6-43. doi: 10.1002/ajp.22381
687 37. Clutton-Brock, T. (2016). *Mammal societies*. John Wiley & Sons.
688 https://doi.org/10.1093/jmammal/gyx078
689 38. Cerroni, A. M., Tomlinson, G. A., Turnquist, J. E., & Grynpas, M. D. (2000). Bone mineral
690 density, osteopenia, and osteoporosis in the rhesus macaques of Cayo Santiago. *American Journal*
691 *of Physical Anthropology: The Official Publication of the American Association of Physical*
692 *Anthropologists*, *113*(3), 389-410. https://doi.org/10.1002/1096-8644(200011)113:3<389::AID-
693 AJPA9>3.0.CO;2-I
694 39. Lee, D. S., Kang, Y. H., Ruiz-Lambides, A. V., & Higham, J. P. (2021). The observed pattern
695 and hidden process of female reproductive trajectories across the life span in a non-human primate.
696 *Journal of Animal Ecology*, *90*(12), 2901-2914. https://doi.org/10.1111/1365-2656.13590
697 40. Watowich, M. M., Chiou, K. L., Montague, M. J., Cayo Biobank Research Unit, Simons, N.
698 D., Horvath, J. E., ... & Snyder-Mackler, N. (2022). Natural disaster and immunological aging in
699 a nonhuman primate. *Proceedings of the National Academy of Sciences*, *119*(8), e2121663119.
700 https://doi.org/10.1073/pnas.2121663119

41. Cooper, E. B., Brent, L. J., Snyder-Mackler, N., Singh, M., Sengupta, A., Khatiwada, S., ... & Higham, J. P. (2022). The Natural History of Model Organisms: The rhesus macaque as a success story of the Anthropocene. *Elife*, *11*, e78169-e78169. 10.7554/elife.78169

42. Rawlins, R. G., & Kessler, M. J. (Eds.). (1986). *The Cayo Santiago macaques: History, behavior, and biology*. SUnY Press.

43. Pérez-Guzmán, E. X., Pantoja, P., Serrano-Collazo, C., Hassert, M. A., Ortiz-Rosa, A., Rodríguez, I. V., ... & Sariol, C. A. (2019). Time elapsed between Zika and dengue virus infections affects antibody and T cell responses. *Nature communications*, *10*(1), 1-14. https://doi.org/10.1038/s41467-019-12295-2

44. Marzan-Rivera, N., Serrano-Collazo, C., Cruz, L., Pantoja, P., Ortiz-Rosa, A., Arana, T., ... & Sariol, C. A. (2022). Infection order outweighs the role of CD4+ T cells in tertiary flavivirus exposure. *Iscience*, *25*(8), 104764. https://doi.org/10.1016/j.isci.2022.104764

45. Asiedu, C. K., Goodwin, K. J., Balgansuren, G., Jenkins, S. M., Le Bas-Bernardet, S., Jargal, U., ... & Thomas, J. M. (2005). Elevated T regulatory cells in long-term stable transplant tolerance in rhesus macaques induced by anti-CD3 immunotoxin and deoxyspergualin. *The Journal of Immunology*, *175*(12), 8060-8068. DOI: https://doi.org/10.4049/jimmunol.175.12.8060

46. Morita, D., Hattori, Y., Nakamura, T., Igarashi, T., Harashima, H., & Sugita, M. (2013). Major T cell response to a mycolyl glycolipid is mediated by CD1c molecules in rhesus macaques. *Infection and immunity*, *81*(1), 311-316. https://doi.org/10.1128/IAI.00871-12

47. Van Noordwijk, M. A., & van Schaik, C. P. (2004). Sexual selection and the careers of primate males: paternity concentration, dominance–acquisition tactics and transfer decision. *Sexual selection in primates: New and comparative perspectives*, 208-229. https://doi.org/10.1017/CBO9780511542459.014

48. Kimock, C. M., Dubuc, C., Brent, L. J., & Higham, J. P. (2019). Male morphological traits are heritable but do not predict reproductive success in a sexually-dimorphic primate. *Scientific reports*, *9*(1), 1-11. https://doi.org/10.1038/s41598-019-52633-4

49. Brent, L. J. N., Semple, S., Dubuc, C., Heistermann, M., & MacLarnon, A. (2011). Social capital and physiological stress levels in free-ranging adult female rhesus macaques. *Physiology & behavior*, *102*(1), 76-83. https://doi.org/10.1016/j.physbeh.2010.09.022

730 50. Madlon-Kay, S., Brent, L., Montague, M., Heller, K., & Platt, M. (2017). Using machine
731 learning to discover latent social phenotypes in free-ranging macaques. *Brain sciences*, *7*(7), 91.
732 https://doi.org/10.3390/brainsci7070091
733 51. R Core Team (2021). R: A language and environment for statistical computing. R Foundation
734 for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
735 52. Vignali, D. A., Collison, L. W., & Workman, C. J. (2008). How regulatory T cells work. *Nature*
736 *reviews immunology*, *8*(7), 523-532. https://doi.org/10.1038/nri2343
737 53. Idzkowska, E., Eljaszewicz, A., Miklasz, P., Musial, W. J., Tycinska, A. M., & Moniuszko,
738 M. (2015). The role of different monocyte subsets in the pathogenesis of atherosclerosis and acute
739 coronary syndromes. *Scandinavian journal of immunology*, *82*(3), 163-173.
740 https://doi.org/10.1111/sji.12314
741 54. Erkeller-Yuksel, F. M., Deneys, V., Yuksel, B., Hannet, I., Hulstaert, F., Hamilton, C., ... &
742 Lydyard, P. M. (1992). Age-related changes in human blood lymphocyte subpopulations. *The*
743 *Journal of pediatrics*, *120*(2), 216-222. https://doi.org/10.1016/S0022-3476(05)80430-5
744 55. Frasca, D., Blomberg, B. B., Garcia, D., Keilich, S. R., & Haynes, L. (2020). Age-related
745 factors that affect B cell responses to vaccination in mice and humans. *Immunological reviews*,
746 *296*(1), 142-154. https://doi.org/10.1111/imr.12864
747 56. Germain, C., Gnjatic, S., Tamzalit, F., Knockaert, S., Remark, R., Goc, J., ... & Dieu-Nosjean,
748 M. C. (2014). Presence of B cells in tertiary lymphoid structures is associated with a protective
749 immunity in patients with lung cancer. *American journal of respiratory and critical care medicine*,
750 *189*(7), 832-844. https://doi.org/10.1164/rccm.201309-1611OC
751 57. Asquith, M., Haberthur, K., Brown, M., Engelmann, F., Murphy, A., Al-Mahdi, Z., &
752 Messaoudi, I. (2012). Age-dependent changes in innate immune phenotype and function in rhesus
753 macaques (Macaca mulatta). *Pathobiology of Aging & Age-related Diseases*, *2*(1), 18052.
754 https://doi.org/10.3402/pba.v2i0.18052
755 58. Zheng, H. Y., Zhang, M. X., Pang, W., & Zheng, Y. T. (2014). Aged Chinese rhesus macaques
756 suffer severe phenotypic T-and B-cell aging accompanied with sex differences. *Experimental*
757 *gerontology*, *55*, 113-119. https://doi.org/10.1016/j.exger.2014.04.004
758 59. Quinn, K. M., Fox, A., Harland, K. L., Russ, B. E., Li, J., Nguyen, T. H., ... & La Gruta, N. L.
759 (2018). Age-related decline in primary CD8+ T cell responses is associated with the development

760 of senescence in virtual memory CD8+ T cells. *Cell reports*, *23*(12), 3512-3524.
761 https://doi.org/10.1016/j.celrep.2018.05.057
762 60. Li, M., Yao, D., Zeng, X., Kasakovski, D., Zhang, Y., Chen, S., ... & Xu, L. (2019). Age related
763 human T cell subset evolution and senescence. *Immunity & Ageing*, *16*(1), 1-7.
764 https://doi.org/10.1186/s12979-019-0165-8
765 61. Mogilenko, D. A., Shpynov, O., Andhey, P. S., Arthur, L., Swain, A., Esaulova, E., ... &
766 Artyomov, M. N. (2021). Comprehensive profiling of an aging immune system reveals clonal
767 GZMK+ CD8+ T cells as conserved hallmark of inflammaging. *Immunity*, *54*(1), 99-115.
768 https://doi.org/10.1016/j.immuni.2020.11.005
769 62. Meza Guzman, L. G., Keating, N., & Nicholson, S. E. (2020). Natural killer cells: tumor
770 surveillance and signaling. *Cancers*, *12*(4), 952. doi:10.3390/cancers12040952
771 63. Rocamora-Reverte, L., Melzer, F. L., Würzner, R., & Weinberger, B. (2020). The complex
772 role of regulatory T cells in immunity and aging. *Frontiers in Immunology*, *11*.
773 https://doi.org/10.3389/fimmu.2020.616949
774 64. Kapellos, T. S., Bonaguro, L., Gemünd, I., Reusch, N., Saglam, A., Hinkley, E. R., & Schultze,
775 J. L. (2019). Human monocyte subsets and phenotypes in major chronic inflammatory diseases.
776 *Frontiers in immunology*, *10*, 2035. https://doi.org/10.3389/fimmu.2019.02035
777 65. Italiani, P., & Boraschi, D. (2014). From monocytes to M1/M2 macrophages: phenotypical vs.
778 functional differentiation. *Frontiers in immunology*, *5*, 514.
779 https://doi.org/10.3389/fimmu.2014.00514
780 66. Naicker, S. D., Cormican, S., Griffin, T. P., Maretto, S., Martin, W. P., Ferguson, J. P., ... &
781 Griffin, M. D. (2018). Chronic kidney disease severity is associated with selective expansion of a
782 distinctive intermediate monocyte subpopulation. *Frontiers in immunology*, *9*, 2845.
783 https://doi.org/10.3389/fimmu.2018.02845
784 67. Moieni, M., Muscatell, K. A., Jevtic, I., Breen, E. C., Irwin, M. R., & Eisenberger, N. I. (2019).
785 Sex differences in the effect of inflammation on subjective social status: a randomized controlled
786 trial of endotoxin in healthy young adults. *Frontiers in psychology*, *10*, 2167.
787 https://doi.org/10.3389/fpsyg.2019.02167
788 68. Gassen, J., White, J. D., Peterman, J. L., Mengelkoch, S., Proffitt Leyva, R. P., Prokosch, M.
789 L., ... & Hill, S. E. (2021). Sex differences in the impact of childhood socioeconomic status on
790 immune function. *Scientific reports*, *11*(1), 9827. https://doi.org/10.17605/OSF.IO/DXPZU

69. Monserrat, J., de Pablo, R., Reyes, E., Díaz, D., Barcenilla, H., Zapata, M. R., ... & Álvarez-Mon, M. (2009). Clinical relevance of the severe abnormalities of the T cell compartment in septic shock patients. *Critical care*, *13*(1), 1-8. https://doi.org/10.1186/cc7731

70. Chinen, J., Easley, K. A., Mendez, H., & Shearer, W. T. (2001). Decline of CD3-positive T-cell counts by 6 months of age is associated with rapid disease progression in HIV-1–infected infants. *Journal of allergy and clinical immunology*, *108*(2), 265-268. 10.1067/mai.2001.116573

71. Pavez-Fox, M. A., Negron-Del Valle, J. E., Thompson, I. J., Walker, C. S., Bauman, S. E., Gonzalez, O., ... & Brent, L. J. (2021). Sociality predicts individual variation in the immunity of free-ranging rhesus macaques. *Physiology & behavior*, *241*, 113560. https://doi.org/10.1016/j.physbeh.2021.113560

72. Young, C., Majolo, B., Heistermann, M., Schülke, O., & Ostner, J. (2014). Responses to social and environmental stress are attenuated by strong male bonds in wild macaques. *Proceedings of the National Academy of Sciences*, *111*(51), 18195-18200. https://doi.org/10.1073/pnas.1411450111

73. Adcock, I.M., Mumby, S. (2016). Glucocorticoids. In: Page, C., Barnes, P. (eds) Pharmacology and Therapeutics of Asthma and COPD. Handbook of Experimental Pharmacology, vol 237. Springer, Cham. https://doi.org/10.1007/164_2016_98

74. Cohen, S., Line, S., Manuck, S. B., Rabin, B. S., Heise, E. R., & Kaplan, J. R. (1997). Chronic social stress, social status, and susceptibility to upper respiratory infections in nonhuman primates. *Psychosomatic medicine*, *59*(3), 213-221. 10.1097/00006842-199705000-00001

75. McAuliffe, J., Vogel, L., Roberts, A., Fahle, G., Fischer, S., Shieh, W. J., ... & Subbarao, K. (2004). Replication of SARS coronavirus administered into the respiratory tract of African Green, rhesus and cynomolgus monkeys. *Virology*, *330*(1), 8-15. https://doi.org/10.1016/j.virol.2004.09.030

76. Skinner, J. M., Caro-Aguilar, I. C., Payne, A. M., Indrawati, L., Fontenot, J., & Heinrichs, J. H. (2011). Comparison of rhesus and cynomolgus macaques in a Streptococcus pyogenes infection model for vaccine evaluation. *Microbial pathogenesis*, *50*(1), 39-47. 10.1016/j.micpath.2010.10.004

77. Clark, S. M., Song, C., Li, X., Keegan, A. D., & Tonelli, L. H. (2019). CD8+ T cells promote cytokine responses to stress. *Cytokine*, *113*, 256-264. 10.1016/j.cyto.2018.07.015

78. Lee, B. W., Yap, H. K., Chew, F. T., Quah, T. C., Prabhakaran, K., Chan, G. S., ... & Seah, C. C. (1996). Age-and sex-related changes in lymphocyte subpopulations of healthy Asian subjects: From birth to adulthood. *Cytometry: The Journal of the International Society for Analytical Cytology*, *26*(1), 8-15. https://doi.org/10.1002/(SICI)1097-0320(19960315)26:1<8::AID-CYTO2>3.0.CO;2-E

79. Lisse, I. M., Aaby, P., Whittle, H., Jensen, H., Engelmann, M., & Christensen, L. B. (1997). T-lymphocyte subsets in West African children: impact of age, sex, and season. *The Journal of pediatrics*, *130*(1), 77-85. https://doi.org/10.1016/S0022-3476(97)70313-5

80. Uppal, S. S., Verma, S., & Dhot, P. S. (2003). Normal values of CD4 and CD8 lymphocyte subsets in healthy Indian adults and the effects of sex, age, ethnicity, and smoking. *Cytometry Part B: Clinical Cytometry: The Journal of the International Society for Analytical Cytology*, *52*(1), 32-36. https://doi.org/10.1002/cyto.b.10011

81. Snyder-Mackler, N., Sanz, J., Kohn, J. N., Brinkworth, J. F., Morrow, S., Shaver, A. O., ... & Barreiro, L. B. (2016). Social status alters immune regulation and response to infection in macaques. Science, 354(6315), 10411045. https://doi.org/10.1126/science.aah3580

82. Sanz, J., Maurizio, P. L., Snyder-Mackler, N., Simons, N. D., Voyles, T., Kohn, J., ... & Barreiro, L. B. (2020). Social history and exposure to pathogen signals modulate social status effects on gene regulation in rhesus macaques. *Proceedings of the National Academy of Sciences*, *117*(38), 23317-23322. https://doi.org/10.1073/pnas.1820846116

83. Snyder-Mackler, N., Sanz, J., Kohn, J. N., Voyles, T., Pique-Regi, R., Wilson, M. E., ... & Tung, J. (2019). Social status alters chromatin accessibility and the gene regulatory response to glucocorticoid stimulation in rhesus macaques. *Proceedings of the National Academy of Sciences*, *116*(4), 1219-1228. https://doi.org/10.1073/pnas.1811758115