SPECIAL FEATURE

Social history and exposure to pathogen signals modulate social status effects on gene regulation in rhesus macaques

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Edited by W. Thomas Boyce, University of California, San Francisco, CA, and accepted by Editorial Board Member Gene E. Robinson September 19, 2019 (received for review February 20, 2019)

Social experience is an important predictor of disease susceptibility and survival in humans and other social mammals. Chronic social stress is thought to generate a proinflammatory state characterized by elevated antibacterial defenses and reduced investment in antiviral defense. Here we manipulated long-term social status in female rhesus macaques to show that social subordination alters the gene expression response to ex vivo bacterial and viral challenge. As predicted by current models, bacterial lipopolysaccharide polarizes the immune response such that low status corresponds to higher expression of genes in NF-κB–dependent proinflammatory pathways and lower expression of genes involved in the antiviral response and type I IFN signaling. Counter to predictions, however, low status drives more exaggerated expression of both NF-κB– and IFN-associated genes after cells are exposed to the viral mimic Gardiquimod. Status-driven gene expression patterns are linked not only to social status at the time of sampling, but also to social history (i.e., past social status), especially in unstimulated cells. However, for a subset of genes, we observed interaction effects in which females who fell in rank were more strongly affected by current social status than those who climbed the social hierarchy. Taken together, our results indicate that the effects of social status on immune cell gene expression depend on pathogen exposure, pathogen type, and social history—in support of social experience-mediated biological embedding in adulthood, even in the conventionally memoryless innate immune system.

dominance rank | social adversity | immune response | biological embedding | gene expression

The social environment, both in early life and in adulthood, has a profound and often long-lasting impact on health and life expectancy in humans and other social mammals (1–4). This relationship is thought to arise in part through changes in gene regulation, which mediate the genomic response to physiological signals of social stress (e.g., glucocorticoids, catecholamines) (5, 6). Gene expression signatures of social status and social adversity have now been reported in multiple studies, including clinical and population-based samples in humans, and studies of both experimental and natural populations in other social animals (5, 7–16) (see also refs. 17–19 for evidence in social insects and other social vertebrates). Because this work has concentrated most extensively on peripheral white blood cells, it provides a direct window into how social experiences are reflected in the regulation of the immune system (20–22).

Several broad patterns have emerged from these studies. First, high social adversity, including social isolation, early life insults, and low social status, tends to predict higher expression of genes in proinflammatory pathways. This observation dovetails with associations between chronic social stress and elevated levels of protein biomarkers of inflammation (e.g., IL-6) (12, 23, 24). Second, high social adversity tends to predict lower expression of genes that function in the innate immune defense against virus, especially genes involved in type I IFN signaling. In most cases, this pattern has been shown based on data in unstimulated cells (5); however, in bacterial lipopolysaccharide (LPS)-stimulated cells, induction of type I IFN-associated gene expression responses is also attenuated in low-status rhesus macaques (15). Third, these findings are explained in part by socially patterned differences in the use of immune defense-modulating transcription factors (TFs). For example, genes that are more highly

Significance

Social adversity is strongly linked to health and fitness outcomes in humans and other social mammals. This observation arises in part through "biological embedding": persistent, social environment-induced biological changes that may affect immune function. Here we show that low social status in female rhesus macaques leads to a highly proinflammatory response to both bacterial and viral challenge. In addition, we show that past social status also affects gene expression, and that past low status leads to reduced sensitivity to current social conditions. Thus, the first line of defense in the macaque immune system is altered by both current social conditions and a biological memory of past events. Our results provide insight into how social adversity gets under the skin over long time spans.

Author contributions: M.W., J.T., and L.B.B. designed research; J.S., P.L.M., N.S.-M., N.D.S., T.V., J.K., V.M., M.W., J.T., and L.B.B. performed research; J.S. and P.L.M. analyzed data; and J.S., J.T., and L.B.B. wrote the paper with contributions from all authors.

The authors declare no competing interest.

This article is a PNAS Direct Submission. W.T.B. is a guest editor invited by the Editorial Board.

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Data deposition: Data generated in this study have been submitted to the National Center for Biotechnology Information's Gene Expression Omnibus and Short Read Archive (accession no. GSE136124). Relevant code and materials needed to reproduce the main results are available in Zenodo (https://zenodo.org/record/3367713).

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1820846116/-/DCSupplemental.

First published October 14, 2019.

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expressed in low-status rhesus macaques are enriched near binding sites for the TF complex NF-κB, a master regulator of inflammation. In contrast, genes that are more highly expressed in high-status females fall near binding sites for IFN regulatory factors, which coordinate type I IFN-mediated responses (15, 25).

These observations have led some authors to propose social environment-mediated trade-offs between antibacterial defense, associated with NF-κB–driven proinflammatory signaling, and antiviral defense, associated with the type I IFN response (5, 26). Such a model argues that high social adversity shifts investment in the immune system toward resistance against bacterial pathogens and wound healing (possibly in anticipation of physical insults), at the cost of increased susceptibility to viral pathogens. This in turn accounts for both social gradients in conditions linked to chronic inflammation, such as cardiovascular disease, and social gradients in viral infections. Consistent with this hypothesis, psychosocial stress predicts reactivation rates of latent herpesvirus in mice and humans (27, 28) and rates of respiratory virus infection in experimentally exposed human subjects (29, 30). Similarly, low-status cynomolgus macaques males housed in a controlled environment have both increased susceptibility to experimentally administered adenovirus and elevated rates of coronary artery stenosis (29, 31).

Nevertheless, it remains unclear whether social patterning of viral susceptibility is directly related to social patterning of gene expression in peripheral blood cells. In particular, increased susceptibility to virus has been suggested to occur because the trade-offs induced by social adversity lead to insufficient production of antiviral gene transcripts (26). This logic is largely based on lower expression levels for key antiviral genes (e.g., *OAS* family genes, IFN regulatory factors) measured at baseline. However, exposure to immune stimulants can radically change the transcriptional landscape of immune cells; for example, in rhesus macaque females, social status has more pronounced effects on gene expression after exposure to LPS (15, 25). Because no study to date has evaluated the effects of social adversity on gene expression after both bacterial and viral challenge, it is unclear whether chronic social stress in fact attenuates the gene regulatory response to virus, consistent with a trade-offs model. In addition, although increased expression of inflammation-related genes and decreased expression of type I IFN-related genes have been associated with social adversity in both adulthood and early life (8, 12, 15, 32; but see ref. 33), we do not yet understand how the timing of social experiences affects the response to either pathogen type.

To address these gaps, we turned to an animal model for social subordination-induced chronic stress: dominance rank in female rhesus macaques. This model takes advantage of the highly hierarchical social structure of female macaques, in which low rank predicts increased harassment and reduced social control, and combines it with the ability to manipulate rank via controlled introduction into newly formed social groups (earlier-introduced females are higher-ranking) (15, 34). Using this study design, we previously showed that social status effects on blood cell gene expression are pervasive, cell type-specific, and altered by bacterial stimulation (15). Importantly, by rearranging group composition a second time and placing females of previously similar rank into the same social group, the same individuals can be observed occupying 2 distinct positions in the social status hierarchy. This approach provides an ideal setting to investigate whether, and for what genes, immune gene regulation in adulthood is influenced by biological embedding—when social experience leads to systematic, stable biological changes with the potential to influence health (35).

Results

Pathogen Exposure- and Type-Dependent Effects of Social Status on Gene Expression. We experimentally manipulated the dominance ranks of 45 adult female rhesus macaques by sequentially introducing them into newly constructed social groups of 5 females each (n = 9 social groups; Dataset S1), as described previously (15). We maintained these groups for approximately 1 y (February 2013 to March 2014; phase I). We then rearranged group composition by performing a second series of sequential introductions, designed so that females from the same or adjacent ranks in phase I were cohoused in the new groups (*SI Appendix*, Fig. S1). We followed the rearranged groups for another 10 mo (April 2014 to February 2015; phase II). As expected (36), earlier introduction predicted higher social status (Elo rating) in both phases (Pearson's r between order of introduction and Elo score: phase I, −0.57, $P = 4.3 \times 10^{-5}$; phase II, −0.71, $P = 4.3 \times 10^{-8}$). Dominance ranks remained highly stable throughout each study phase, and individual Elo scores were uncorrelated between phases ($r = 0.06$, $P = 0.68$) (*SI Appendix*, Fig. S2).

To characterize the effects of social status on the immune response, including the contribution of social history, we obtained blood samples from each study subject in phase II of the study. We generated 3 gene expression profiles per animal, from (*i*) a control sample cultured in media only (negative control [NC]); (*ii*) a sample cultured in media spiked with LPS, a Toll-like receptor (TLR) 4 agonist that mimics infection by gram-negative bacteria; and (*iii*) a sample cultured in media spiked with Gardiquimod (Gard), a TLR7 agonist that mimics infection by a single-stranded RNA virus (37). We also generated flow cytometry-based immunophenotyping data to estimate cell type proportions for 11 major cell types from the same draw (Dataset S1). We incubated samples from each individual for 4 h in parallel and generated RNA-sequencing data from each sample. To confirm successful immune stimulation, we performed a principal component analysis (PCA) on the correlation matrix of normalized gene expression levels for all conditions, after controlling for other biological and technical effects (Fig. 1*A* and *SI Appendix*, *Material and Methods*). We observed distinct clusters corresponding to the control, LPS-stimulated, and Gard-stimulated samples, with treatment effects most clearly reflected along PC1 ($r = 0.61$, $P = 6.5 \times 10^{-10}$ for LPS vs. control) and PC4 (r = 0.76, P = 1.7 × 10^{-17} for Gard vs. control). As expected, genes up-regulated after stimulation were enriched for immune-related and inflammatory processes associated with bacterial and viral defense (Dataset S2).

Consistent with previous findings (15, 16), we also observed a strong signature of dominance rank. Dominance rank (Elo score) was correlated with PC3 of the full gene expression matrix within all 3 conditions (Pearson's r = 0.75 [NC]; r = 0.75 (LPS), r = 0.69 (Gard), $P < 6.0 \times 10^{-7}$ for all conditions) (*SI Appendix*, Fig. S3). This observation translated to gene-level analyses, where dominance rank drove the expression of 3,675, 5,322, and 2,694 genes

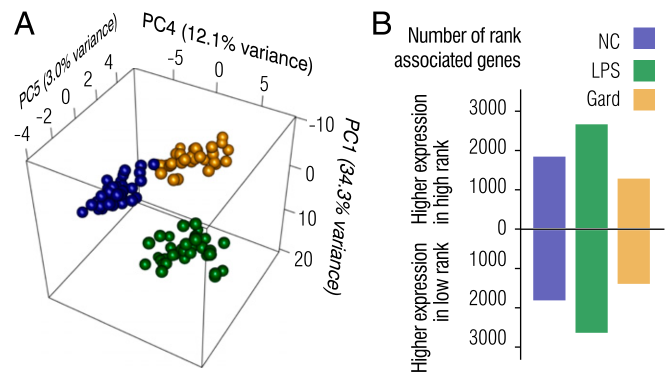

Fig. 1. Social status effects on gene expression within and across conditions. (*A*) PCA of gene expression data across all 3 conditions. PC1, PC4, and PC5 separate negative controls (NC, blue) from LPS (green) and Gard (yellow)-stimulated samples. (*B*) Number of rank-associated genes (FDR <5%) that are more highly expressed in high-ranking females (top bars) or low-ranking females (bottom bars), within condition.

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(false discovery rate [FDR] <5%) in the control, LPS, and Gard conditions, respectively, controlling for cell type composition, age, and batch (Fig. 1*B* and Dataset S3). Strikingly, the number of rank-associated genes in the LPS condition was 1.5- to 2-fold higher than in the Gard or control conditions. Thus, although rank effects are amplified after activation of the bacterial-sensing TLR4 pathway, this pattern does not appear to be a universal feature of immune activation. Indeed, the number of genes for which the intensity of the response (i.e., gene expression in LPS/Gard conditions, relative to paired control samples) depended on dominance rank was almost 5-fold lower in the Gard condition than for the LPS-stimulated samples (851 vs. 4,111; FDR <0.05). Furthermore, while 73% of rank effects on the LPS response were directionally biased toward larger responses in low-status females, rank effects on the response to Gard were almost perfectly balanced (49.5% were stronger in low-status females and 51.5% were stronger in high-status females; Fig. 2*A*).

To focus our analysis on the hypothesis that social status-induced stress mediates trade-offs between antibacterial and antiviral responses, we investigated the Gene Ontology categories that were most enriched among genes up-regulated by LPS, Gard, or both. As we have shown previously (15), genes involved in immune defense, inflammation, and cytokine signaling are biased toward higher expression in low-ranking females, in the LPS condition (Fig. 2*B*). In contrast, genes involved in viral defense-associated type I IFN signaling are biased toward higher expression in high-ranking females in the same condition (Fig. 2*B*). A trade-offs model predicts a similar dichotomy after viral challenge. This is not what we observed; in the Gard condition, low status predicted increased expression of both proinflammatory/cytokine signaling-associated and type I IFN-associated genes. For example, for rank-associated genes involved in type I IFN signaling, 57% of genes in the LPS condition, but only 25% in the Gard condition, were more highly expressed in high status females. Consequently, status-related effects for type I IFN genes differ significantly between LPS and Gard conditions (Wilcoxon test, $P = 6.4 \times 10^{-4}$). This pattern holds for key viral defense genes, such as *OAS2* and *OAS3*; the IFN-inducible genes *IFIT2*, *IFIT3*, *MX1*, and *MX2*; and *STAT1*, a master regulator of IFN-mediated defense (Fig. 2*C*). It also extends to measures of the response to immune challenge; genes involved in type I IFN signaling tend to be more strongly up-regulated in high-status females after LPS challenge but more strongly up-regulated in low-status females after Gard challenge (*SI Appendix*, Fig. S4)

Previous studies suggest that social environmental effects on gene expression arise through socially structured differences in immune defense-associated TF binding (9, 12, 15). Therefore, we investigated the TFs that might account for rank effects on gene expression during the immune response to LPS and Gard. To do so, we identified predicted TF-binding sites in gene promoter regions that are also accessible to TF binding (i.e., in open chromatin regions identified using ATAC-seq data from rhesus macaque peripheral blood mononuclear cells) (15). As reported previously (15), genes that were more highly expressed in low-status animals were enriched for NF-κB–binding sites in the LPS condition, while genes that were more highly expressed in high-status animals were enriched for IFN regulatory factor (IRF1, IRF2, and IRF7) and STAT1 binding sites (Fig. 3). Strikingly, that dichotomy disappeared when samples were stimulated with a viral mimic. In the

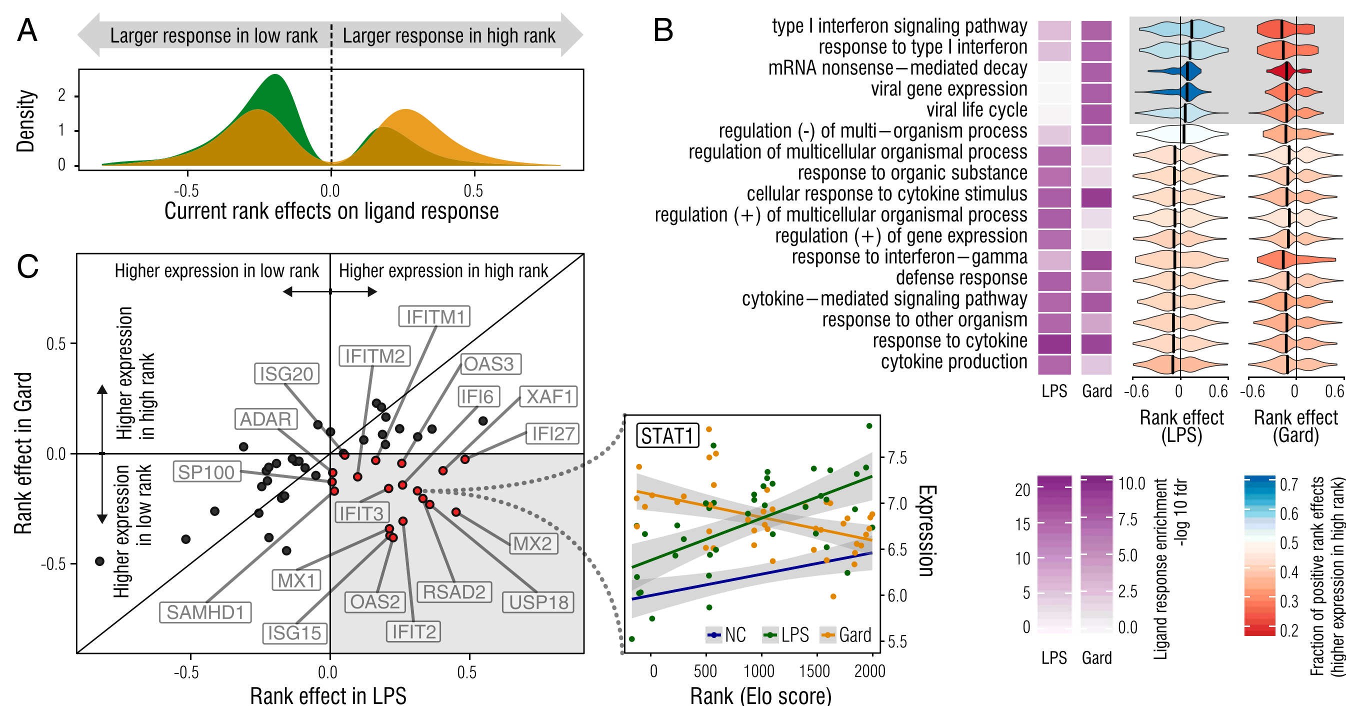

Fig. 2. Contrasting effects of dominance rank in cells challenged with a bacterial mimic vs. a viral mimic. (*A*) Distribution of effect sizes among genes for which the magnitude of the response to LPS (green) and Gard (yellow) depends on dominance rank. After LPS stimulation, but not after Gard stimulation, most genes respond more strongly in low-status females. (*B*) Polarization of rank effects in the union of the top 10 GO categories, per condition, that were most enriched for up-regulation by LPS/Gard. Darker squares correspond to stronger statistical support for enrichment. Violin plots are colored based on the proportion of rank-associated genes that are more highly expressed in high-ranking individuals, separately by condition. Positive *x*-axis values: more highly expressed in high-ranking females; negative *x*-axis values: more highly expressed in low-ranking females. The gray-shaded box contains gene categories for which the distributions of rank effects differ significantly between LPS and Gard conditions (Wilcoxon test: Benjamini–Hochberg FDR-corrected $P < 9 \times 10^{-3}$). (*C*) Rank effects in the LPS (*x*-axis) vs. Gard (*y*-axis) conditions, for genes in the GO category "type I interferon signaling pathway" that were rank-associated in the LPS condition, Gard condition, or both. Labeled genes show cases in which rank effects are directionally reversed in LPS- vs. Gard-stimulated samples, including key master regulators of the response to virus, such as *STAT1*. In *B* and *C*, all genes affected by rank at an FDR of ≤20% are plotted; the overall pattern is qualitatively unchanged at a more stringent FDR threshold.

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Gard condition, the promoter regions of genes that were more highly expressed in low-status animals were enriched for TF-binding sites for virtually all immune-associated TFs, including NF-κB, several IRFs, and STAT1 (Fig. 3). These results corroborate our analyses of the gene expression data alone (Fig. 2*B*) and suggest that differences in TF activity account for the distinct patterns of social status-associated gene expression after LPS stimulation vs. after Gard stimulation.

Social History Effects on Immune Gene Regulation. Although the gene expression data were generated in phase II, we also collected behavioral data that allowed us to quantify rank in phase I (samples were collected at a mean of 9.01 ± 0.60 months after phase I groups were dissolved and 7.65 ± 0.50 months after phase II group introductions). We took advantage of this study design to investigate whether past social status affected immune cell gene expression independent from social status at the time of sampling ("current rank"), in support of biological embedding. Indeed, we identified a strong global signature of past social status on gene expression levels; past Elo score was correlated with PC2 within each condition (control: Pearson's $r = -0.76$, $P = 2.4 \times 10^{-9}$; LPS: $r = -0.58$, $P = 8.0 \times 10^{-5}$; Gard: $r = -0.44$, $P = 3.8 \times 10^{-3}$) (*SI Appendix*, Fig. S3).

For individual genes, social history effects were detectable in all 3 conditions but were far more common in the control condition (Fig. 4*A*). At an FDR of 10%, we identified 3,735 past rank-associated genes in the control condition, compared with 1,712 and 141 in the LPS and Gard conditions, respectively (Fig. 4*A* and *SI Appendix*, Fig. S5). Genes associated with past rank were also enriched for different biological functions (Dataset S2); for example, past rank-associated genes were significantly enriched for epigenetic regulatory processes, such as chromatin organization (FDR-corrected $P = 4.8 \times 10^{-11}$) and histone modification (FDR-corrected $P = 3.6 \times 10^{-7}$) (*SI Appendix*, Fig. S6 and Dataset S2), neither of which was enriched among current rank-associated genes. Conversely, current rank-associated genes were strongly enriched for viral transcription (FDR-corrected $P = 2.8 \times 10^{-11}$) and viral gene expression (FDR-corrected $P = 5.5 \times 10^{-11}$), but genes associated with past rank were not overrepresented in either category. This difference, as well as the increased variance explained by current status in the LPS-stimulated condition, may drive the reduced signal of social history in immune-challenged conditions.

To investigate the relative contribution of social history vs. current rank at a more granular scale, we developed a gene-specific measure of plasticity, Θ. We defined Θ as the square root of the ratio between the variance in gene expression explained by current rank and the total variance explained by both past and current rank (*SI Appendix*, *Materials and Methods*). Θ values range from 0 to 1, with values close to 1 implying a high degree of plasticity and little evidence of memory and values close to 0 implying a high degree of memory and little evidence of plasticity. Overall, we identified a much greater contribution of social history to gene expression levels in the control samples than in either of the stimulated conditions (control: median Θ for the top 1,000 rank-associated genes in NC = 0.52; LPS = 0.87; Gard = 0.74; Wilcoxon test, $P < 2.2 \times 10^{-16}$ for all pairwise comparisons) (Fig. 4*B*; *SI Appendix*, Fig. S7). Social status effects on the response to LPS and Gard were also dominated by the effects of current rank. We identified 4,111 and 851 genes for which the magnitude of the response to LPS and Gard, respectively, depended on current dominance rank (FDR <5%), but no cases in which these responses depended on past rank.

Finally, we tested whether past and current rank combined nonadditively to influence gene expression. In the control condition, where the effects of social history are most pronounced, we identified 1,079 genes in which past and current rank interacted to influence gene expression (FDR <5%). Interaction effects were strongly directionally biased; for 88% of these genes, a history of past low status predicted reduced sensitivity to current dominance rank (Fig. 4 *C* and *D*). Thus, females who fell in rank were strongly affected by their current, lower rank in phase II, whereas females who achieved higher status in phase II were proportionally more affected by their lower status in phase I.

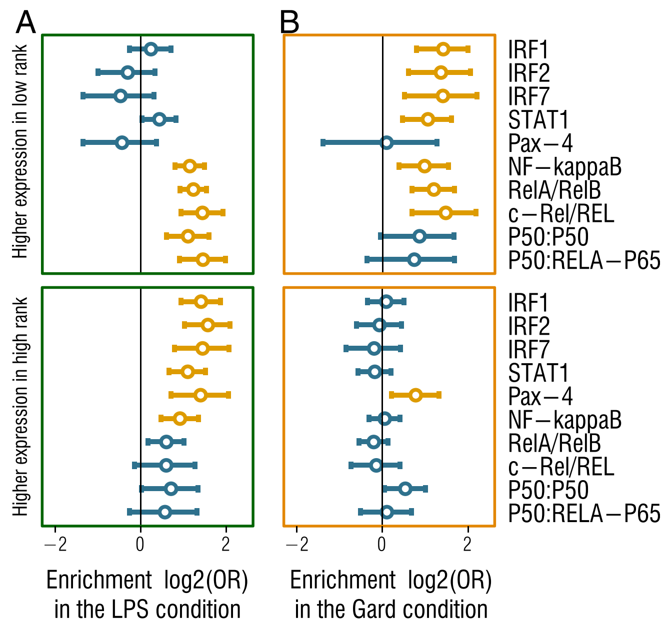

Fig. 3. TF-binding sites enriched near rank-associated genes differ between LPS- and Gard-challenged cells. (*A*) In the LPS condition, predicted NF-κB and NF-κB subunit (RelA/RelB/p50/p65) binding sites are enriched in the promoter regions (5 kb upstream of gene transcription start sites) of genes that are up-regulated by LPS and more highly expressed in low-ranking females (*Top*), while predicted IRF and STAT1 binding sites dominate the enriched categories for genes that are up-regulated by LPS but more highly expressed in high-ranking females (*Bottom*). (*B*) This polarization disappears in the Gard condition, where predicted NF-κB, NF-κB subunit, IRF, and STAT1 binding sites are all enriched in the promoter regions of genes that are up-regulated by Gard and more highly expressed in low-ranking individuals (*Top*), with no clear signature of immune-related TF binding site enrichment among genes that are up-regulated by Gard but more highly expressed in high-ranking individuals (*Bottom*). Enrichment analyses shown here include all rank-associated genes at an FDR <20%. Error bars represent 95% confidence interval; TFs in yellow indicate enrichment at an FDR <10% ($P < 1.3 \times 10^{-3}$).

Discussion

Convergent evidence from humans, wild animal populations, and experimental animal models indicates that social interactions are reflected in the regulation and activity of the immune system (5, 6, 10, 20–22). Our findings join those of others to suggest that social adversity is particularly relevant to the inflammatory response. Because biomarkers of inflammation in turn predict disease and mortality outcomes (38), these findings suggest that social regulation of immune gene expression may partly mediate social gradients in health. By investigating multiple types of immune challenge and by testing for social history, as well as current social environmental effects, our analysis also extends previous work in 3 ways.

First, our results call into question a simple trade-offs hypothesis between bacterial defense and viral defense, at least at the transcriptional level (5, 26). Contrary to predictions, low-status females did not mount an attenuated gene regulatory response to viral challenge. Instead, they up-regulated both antibacterial and antiviral pathways more strongly than high-status females, showing that increased investment in the former does not necessarily signify decreased investment in the latter. This observation argues that

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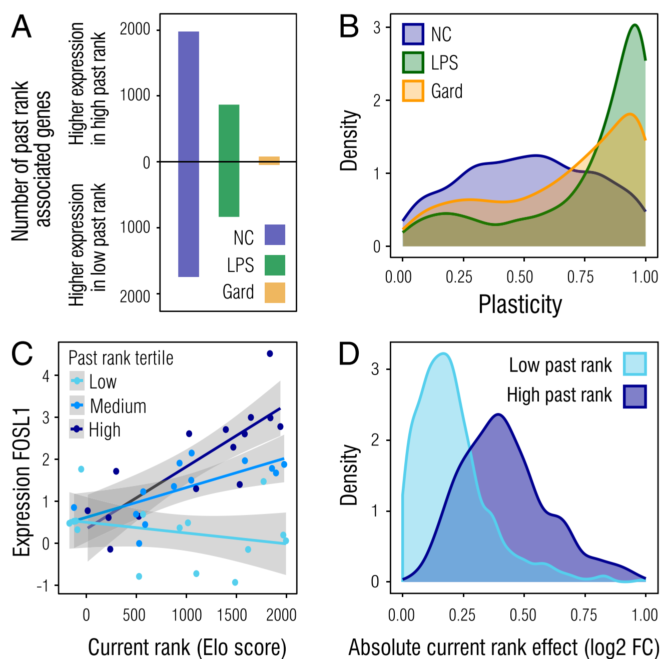

Fig. 4. Social history effects on immune gene regulation. (*A*) Number of past rank-associated genes (FDR <10%) more highly expressed in previously high-ranking females (top bars) or low-ranking females (bottom bars), within condition. (*B*) Distribution of plasticity scores (Θ) for each condition for the top 1,000 rank-associated genes (*SI Appendix, Materials and Methods* and Fig. S7). (*C*) Current rank effects (*x*-axis) on expression of the TF *FOSL1*, which is involved in the type I IFN response (49), are strongest in females who were previously of high rank and weakest in females who were previously of low rank ($\beta_{interaction}$ = 0.54, FDR-corrected P = 0.01). (*D*) Predicted current rank effects (*SI Appendix, Materials and Methods*) for low-past rank and high-past rank females (based on the mean Elo scores for the lowest-ranking and highest-ranking females in phase I groups). Distributions show estimated effect sizes across 1,079 genes for which we identified a significant current rank–past rank interaction effect in the control condition. Current rank effects were systematically larger in females who were previously high-ranking than in females who were previously low-ranking (Wilcoxon test, $P < 2.2 \times 10^{-16}$).

there is no simple map between social environmental effects on baseline gene expression and social environmental effects following pathogen exposure. We note, however, that although the type I IFN response is often discussed in opposition to proinflammatory, NF-κB–mediated antibacterial responses, type I IFN signaling itself can also be proinflammatory (39). Therefore, high reactivity to both viral and bacterial ligands may represent 2 distinct sources of elevated inflammation in low-status individuals.

Second, our findings support the idea that while the gene expression signature of social stress may be somewhat conserved across different types of social adversity, it is also quantitatively and qualitatively context-dependent. Our observations build on previous reports noting that social status interacts with LPS and glucocorticoid exposure to affect immune gene expression (15, 25). However, while previous work has primarily shown differences in the presence or magnitude of effects, here we observed an even more striking pattern: directional shifts in the effects of social status, specifically for genes involved in the antiviral response. In addition, we identified a second, novel form of context dependence: more than 1,000 genes for which social status effects on gene expression levels interact with social history, such that females with a history of low status were more affected by social history compared with females with a history of high status. These observations are consistent with several possibilities. First, a history of high social status could confer increased plasticity in response to environmental change. Second, historical exposure to social subordination-induced stress could blunt responses to future high-quality environments. Finally, social history effects may dissipate over time, but at a faster pace for formerly high-status females.

Third, our observations extend the concept of biological embedding to adulthood. Although biological embedding is generally discussed in relation to early life, the criteria for biological embedding—environmental exposures that "get under the skin" to alter biological processes, remain stable over the long-term, and have the capacity to influence health over the life course (35) —do not restrict it to early life. Indeed, the molecular mechanisms thought to mediate the embedding process early in life (e.g., DNA methylation, histone marks) remain environmentally sensitive across the life course. In this light, it is intriguing that several of the pathways enriched among social history-associated genes are themselves involved in the epigenetic regulation of gene expression. This observation raises the possibility that social history effects arise in part through an epigenetic mechanism, consistent with evidence showing that even short-lived cells in the innate immune system can be stably epigenetically altered by environmental experience (40). Alternatively, slow cellular turnover could be responsible for some of the social history effects that we detected; memory T cells, for example, can represent up to 40% of the total circulating T cells in adulthood (41) and can survive in the body for years. Notably, these explanations are not mutually exclusive, and could help explain why some loci retain more of a signature of social history than others.

Finally, our findings raise a number of questions. First, with respect to understanding biological embedding, this study is limited by its focus on a single time point and cannot assess locus-specific stability or rates of change. Future studies that collect longitudinal, prospective, and repeated samples will be essential to address this gap. Second, our results suggest that social status-sensitive regulatory elements involved in the response to LPS are, at least to some degree, distinct from social status-sensitive regulatory elements involved in the response to Gard. Quantifying social status-dependent enhancer usage in control vs. stimulated conditions would help test this possibility. Third, given the increasing availability of data on social status, social stress, and gene regulation across species (e.g., ref. 18), our findings suggest that comparative studies will help clarify the conditions in which social subordinacy is costly. For example, status hierarchies that are stable, as is the case for female rhesus macaques and many human societies, may have different effects on gene regulation than dynamic hierarchies that are determined by physical condition (10). Finally, to understand how our findings connect to organism-level outcomes, future studies should collect additional biomarker data (especially for cytokines involved in immune signaling, which we did not generate in the present study) to investigate how changes in gene expression translate into downstream consequences. When combined, such studies promise to reveal how past experience, timing, and social context combine to shape social environmental effects on gene regulation, including their role in social gradients in fitness and health.

Materials and Methods

Study subjects were 45 adult female rhesus macaques housed in 9 specific pathogen-free social groups at the YNPRC Field Station, which are regularly tested for multiple viral exposures/infections (*SI Appendix*). We manipulated the dominance ranks of the animals via sequential introduction, such that earlier introduction predicts higher status (phase I: $P = 6.2 \times 10^{-5}$; phase II: $P = 1.1 \times 10^{-7}$ in a mixed-effects model controlling for social group; Pearson's $r = -0.57$, $P = 4.3 \times 10^{-5}$ and $r = -0.71$, $P = 4.3 \times 10^{-8}$, respectively). Dominance rank values were assigned using Elo ratings (42, 43), across 2 approximately 1-y-long phases (*SI Appendix*, Fig. S1).

In phase II, we drew 1 mL of whole blood from each female into each of 3 TruCulture blood collection tubes (Myriad RBM) containing cell culture media (NC), media plus 1 μg/mL of LPS (LPS condition), or media plus 1 μg/mL of Gardiquimod (Gard condition). Samples were incubated for 4 h at 37 °C, and extracted RNA was used to generate RNA-seq libraries (NEBNext Ultra RNA Library Prep Kit; New England Biolabs).

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RNA-seq reads were mapped to the rhesus macaque genome (*MacaM* v7) using the software package STAR (44). Gene expression levels were normalized using the TMM algorithm in *edgeR* (45), log-transformed, and corrected for potential batch effects. To investigate past and current rank effects, we modeled batch-corrected gene expression levels using a nested mixed model that takes into account variation in cell type composition, age, and kinship (46). We considered only linear effects of Elo rating here; however, we note that nonlinear effects of rank have been reported in other contexts (e.g., ref. 47), motivating future work in larger samples and/or hierarchies. FDRs were calculated based on comparisons to permutation-derived empirical null distributions (*SI Appendix*, Fig. S8). GO term enrichment analyses were performed using the Cytoscape module ClueGO (48) (Dataset S2). TF enrichment analyses were performed as described previously (15).

ACKNOWLEDGMENTS. We thank J. Whitley, A. Tripp, N. Brutto, and J. Johnson for maintaining the study subjects; A. Dumaine, V. Yotova, A. Bailey, and A. J. Ericsen for experimental support; M. Gutierrez for help with figures; O. Tastet and S. Gona for help with data submission to the National Center for Biotechnology Information; members of the L.B.B. and J.T. laboratories for helpful discussions; and 3 anonymous reviewers for constructive comments on an earlier version of the manuscript. This work was supported by NIH Grants R01 GM102562, R01 AG057235, P51-OD011132, K99/R00-AG051764, F32-AG062120, and T32-AG000139; NSF Grant SMA-1306134; Canada Research Chairs Program 950-228993; Natural Sciences and Engineering Research Council of Canada Grant RGPIN/435917-2013; and North Carolina Biotechnology Center Grant 2016-IDC-1013. J.S. was supported by the Canadian Institute of Health Research (CIHR) Banting Fellowship and by the Spanish Ministry of Science and Innovation through the Ramon y Cajal Research Grant RYC-2017-23560.

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