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Letter to the Editor

Inflammatory signatures of microglia in hypercortisolemia-related depression

Dear Editor,

Major depressive disorder (MDD) severely disrupts patients' daily life (Otte et al., 2016). Microglia-mediated inflammation represents a key feature of MDD and potentially contributes to the disease's onset and progression (Wang et al., 2022). Meanwhile, antidepressants such as ketamine can exert therapeutic effects partially by modulating microglial inflammatory responses (Mariani et al., 2022). Notably, it has long been documented that depressed patients often have higher plasma levels of cortisol (Keller et al., 2017). This phenomenon of hypercortisolemia may be a consequence of chronic dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, and higher cortisol levels are predictive of more severe or treatment-resistant conditions. However, how chronic exposure to this stress hormone may influence microglia-mediated inflammation remains incompletely charted.

As the entry point of this study, we exploited the standard mouse model recapitulating hypercortisolemia-related depression through the daily administration of corticosterone (Zhao et al., 2008). Microglia were isolated by fluorescence-activated cell sorting (FACS) from the prefrontal cortex, a central brain region involved in depressive-like behaviors of mice, for bulk RNA sequencing (RNA-seq). Approximately 500 differentially expressed genes (DEGs) could be identified between the vehicle- *vs.* corticosterone-treated microglia (Fig. S1a and Table S1). Classic inflammatory markers, e.g., *Tnf*, *Il1a*, and *Il1b*, and signature genes of microglial activation, e.g., *Cd68* and *Apoe*, were among 173 up-regulated DEGs (Fig. 1a). On the other hand, several markers of resting "homeostatic" microglia, e.g., *P2ry12*, *Fcrls*, and *Sall1*, significantly decreased in the corticosterone-treated condition (Fig. 1a). We further compared the transcriptomics of vehicle- *vs.* corticosterone-treated microglia by the Gene Ontology (GO) enrichment (Fig. S1b). In support of microglia-mediated inflammation, immune pathways were highly enriched in the corticosterone-treated condition. Also, pathways of several metabolic processes, e.g., protein synthesis and lipid storage, became up-regulated. Moreover, the GO network of enriched processes revealed a collection of specific genes, e.g., *Il1b*, *Gapdh*, *Gba1*, and *Slc11a1*, that orchestrated such microglial responses (Fig. S1c).

For a more in-depth characterization, we pursued the single-cell RNA sequencing (scRNA-seq) of microglia from the prefrontal cortices of vehicle- *vs.* corticosterone-treated mice. Six microglial clusters could be defined in the pooled scRNA-seq datasets (Fig. 1b and c), consistent with the functional heterogeneity of microglia that has been well documented in the research field. The corticosterone-treated condition showed an apparent increase in clusters #3 and #4 (Fig. 1d). In line with the bulk RNA-seq data, classic inflammatory markers *Tnf* and *Il1b* both increased in clusters #0 and #1 of corticosterone-treated microglia, and the *Il1b* up-regulation additionally occurred in clusters #2, #3, and #4 (Fig. S1d). Also, signature genes of microglial activation, e.g., *Cd68* (clusters #0, #1, #2, and #4), *Cd86* (clusters #0 and #1), and *Apoe* (clusters #0, #1, #2, #3, #4, and #5), became more prominent in the corticosterone-treated condition (Fig. S1d). These results suggested the differential capacity of microglial subpopulations in expressing inflammatory factors or phagocytosis.

Previous studies by colleagues and us have established that the transcription factors Pu.1 and Irf8, which are almost exclusively expressed by microglia but not other cell types in the brain, act cooperatively to target the expression of a repertoire of genes essential for microglial inflammatory responses (Masuda et al., 2012; Yoshida et al., 2014; Zhou et al., 2019, 2020). We observed the increased expression of *Pu.1* (clusters #0, #1, #2, and #3) and *Irf8* (clusters #0, #1, #2, #3, and #4) in the corticosterone-treated microglia by scRNA-seq (Fig. 1e). To verify this microglial up-regulation of Pu.1 and Irf8, brain tissues of the vehicle- *vs.* corticosterone-treated mice were processed for immunofluorescence staining. Expression levels of Pu.1 or Irf8 in $Iba1^+$ microglia in the prefrontal cortex regions of corticosterone-treated mice were significantly elevated compared to the vehicle-treated condition (Fig. 1f–i). In parallel, a similar increase of Pu.1 or Irf8 expression in microglia was observed in the hippocampus (Fig. 1j–m), another key brain region implicated in depressive-like behaviors. On the other hand, there was no significant change in microglial densities in the prefrontal cortex ($162.0 \pm 16.3/mm^2$ in vehicle-treated *vs.* $143.8 \pm 5.6/mm^2$ in corticosterone-treated) or hippocampus ($104.2 \pm 5.7/mm^2$ in vehicle-treated *vs.* $127.3 \pm 12.5/mm^2$ in corticosterone-treated).

We explored the disease relevance of our characterization of microglia-mediated inflammation, taking advantage of the databases PsyGeNET and DisGeNET. 39 DEGs in the microglia of corticosterone-treated mice showed a linkage to mental illness in PsyGeNET. Among a total of 135 associations, the highest number (61 associations) was identified for several categories of depressive disorders (Fig. 1n). In further support of this notion, a collection of gene signatures, including several inflammatory cytokines or chemokines, of corticosterone-treated microglia exhibited strong associations with MDD-related terms in DisGeNET (Fig. S2a). We further examined the involvement of PU.1 and IRF8 in microglial responses of MDD patients by the published single-nucleus RNA sequencing (snRNA-seq) dataset (GSE213982) of total cells in the prefrontal cortices of 20 female MDD patients (Maitra et al., 2023). As a caveat, whether those patients had hypercortisolemia was undocumented in the original study. Neuronal and non-neuronal cell types could be clearly defined in this snRNA-seq dataset (Fig. 1o and Fig. S2b). Of importance, the enriched expression of *PU.1* and *IRF8* was specifically observed in the microglial population (Fig. 1p).

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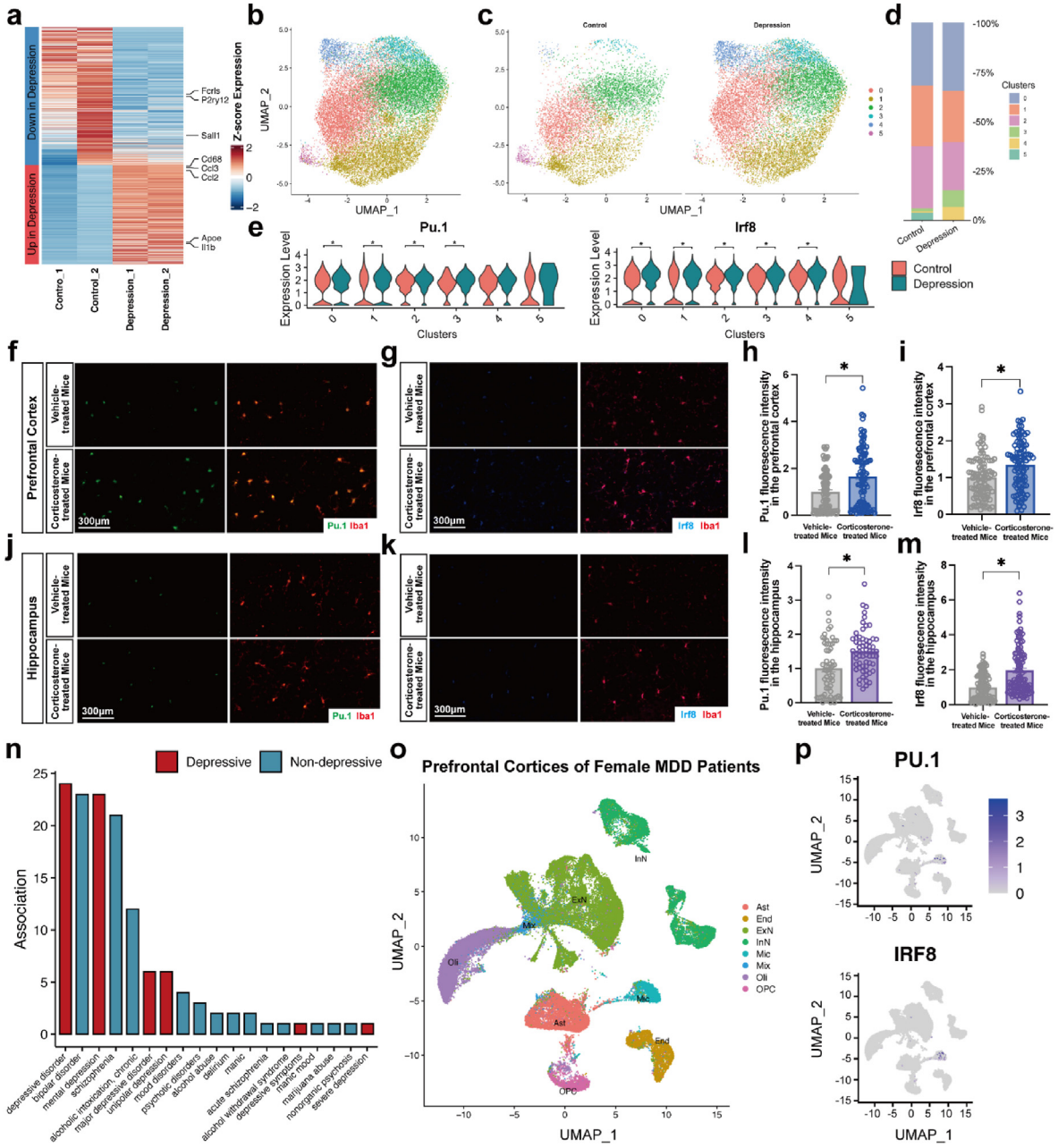

Fig. 1. Inflammatory signatures of microglia in hypercortisolemia-related depression. (a) Microglia were FACS-sorted from the prefrontal cortices of vehicle-treated (control condition) or corticosterone-treated mice (depression condition) and subjected to bulk RNA-seq. Heatmap plot of DEGs in the two replicate preparations of vehicle-treated or corticosterone-treated microglia. **(b–e)** Microglia were FACS-sorted from the prefrontal cortices of vehicle-treated (control condition) or corticosterone-treated mice (depression condition) and subjected to scRNA-seq. **(b)** UMAP plot of microglial clusters (#0 ~ #6) defined in the pooled scRNA-seq datasets. **(c)** UMAP plot of microglial clusters defined in the vehicle-treated or corticosterone-treated condition. **(d)** Percentage of each cluster in vehicle- *vs.* corticosterone-treated microglia. **(e)** Violin plots of *Pu.1* or *Irf8* expression in vehicle- *vs.* corticosterone-treated microglia. $*p < 0.05$ (Student's *t*-test). **(f–m)** Brain tissues of vehicle-treated (control condition) or corticosterone-treated mice (depression condition) were subjected to the immunofluorescence co-staining of Pu.1 and Iba1 or Irf8 and Iba1. **(f, g)** Representative images of the prefrontal cortex regions are shown. **(h, i)** Immunofluorescence intensities of Pu.1 or Irf8 in microglia were quantified. mean ± SEM, $*p < 0.05$ (Student's *t*-test). **(j, k)** Representative images of the hippocampus regions are shown. **(l, m)** Immunofluorescence intensities of Pu.1 or Irf8 in microglia were quantified. mean ± SEM, $*p < 0.05$ (Student's *t*-test). **(n)** Bar graph of the associations of DEGs in corticosterone-treated microglia with mental illness in PsyGeNET. **(o, p)** The published snRNA-seq dataset (GSE213982) of total cells in the prefrontal cortices of female MDD patients. **(o)** UMAP plot of defined cell types. Ast, astrocytes; End, endothelial cells; ExN, excitatory neurons; InN, inhibitory neurons; Mic, microglia; Mix, mixed expression of neuronal and glial markers; Oli, oligodendrocytes; OPC, oligodendrocyte progenitor cells. **(p)** Feature plots of *PU.1* and *IRF8* in defined cell types are shown.

In summary, transcriptomic analyses of this study have identified the distinct inflammatory signatures of microglia in the mouse model of hypercortisolemia-related depression, providing a valuable reference for the examination of microglial functions in MDD patients. Notably, our observation that such chronic exposure to corticosterone in mice was sufficient to trigger microglia-mediated inflammation has implicated a causative role of hypercortisolemia in this disease context. Future studies exploiting the microglia-specific deletion of glucocorticoid receptors will help clarify whether cortisol may directly act on microglia or via other indirect pathways to induce depressive-like behaviors. In addition, whether the condition of hypercortisolemia-related depression may share any feature of microglial inflammatory responses to other

psychiatric disorders, e.g., autism spectrum disorder or schizophrenia, warrants research attention.

Previous studies have demonstrated the central role of transcription factors Pu.1 and Irf8 in microglia-mediated inflammation (Masuda et al., 2012; Yoshida et al., 2014; Zhou et al., 2019, 2020). As a significant expansion, we report here that PU.1 and IRF8 participate in microglial responses in both the mouse model and patients of depression. Whether PU.1 and IRF8 protein expression may be elevated in the brain samples of MDD patients similar to that occurring in the corticosterone-treated mice awaits more clinical studies. Also, it will be essential to determine whether the microglia-specific deletion of Pu.1 or Irf8 in mouse models will mitigate such inflammatory responses as well as depressive-like behaviors. Moreover, whether this PU.1-IRF8 signal may similarly act in other psychiatric diseases appears a tempting possibility to explore. The identification of the PU.1-IRF8 transcriptional module of microglia-mediated inflammation has offered a new diagnostic and therapeutic target for MDD.

CRediT authorship contribution statement

Yanxiang Zhao: Writing – original draft, Formal analysis, Data curation, Conceptualization. **Yingying Huang:** Writing – original draft, Formal analysis, Data curation. **Zhangyuzi Deng:** Formal analysis. **Ying Cao:** Writing – original draft, Formal analysis, Data curation. **Jing Yang:** Writing – original draft, Conceptualization.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cellin.2024.100222.

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