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Stressed-out T cells fragment the mind.

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Abstract

The immune system is increasingly recognized to play an integral role in regulating stress responses. In a recent issue of *Cell*, Fan *et al* demonstrate a novel mechanism through which stress drives mitochondrial fragmentation-induced xanthine accumulation in mouse CD4⁺ T cells, subsequently acting on oligodendrocytes to induce anxiety-like behaviors.

Exposure to physical or mental stress increases the risk of depression or anxiety. The majority of current drugs for treating anxiety or depression directly target neurotransmitters but have high proportions of non-responsive patients or severe side effects [1]. As such, alternative treatment targets are urgently needed. Increasing evidence implicates the immune system as a key mediator of these stress-induced responses, and an underexplored target for therapeutic intervention. In *Cell*, Fan *et al* [2] present evidence linking the peripheral adaptive immune system to stress-induced anxiety-like behaviors (AB) in mice.

The authors first demonstrated that *Rag1*^{-/-} mice lacking T and B cells [3] do not exhibit increased AB following stressors such as electronic foot shock (ES) or acute restraint stress (RS) that are typical of wild type (WT) mice. Treatment of WT mice with blocking antibodies illustrated that depletion of CD4⁺ T cells, but not CD8⁺ T cells, prevented stress-induced AB. Of relevance, adoptive transfer of CD4⁺ T cells from stressed WT mice into *Rag1*^{-/-} mice resulted in AB, demonstrating that stressed CD4⁺ T cells were sufficient to induce AB. That the peripheral adaptive immune system plays an integral role in the induction of stress-induced AB fits well in the context of a previous study demonstrating that microglial-dependent recruitment of inflammatory peripheral monocytes following social defeat stress in mice resulted in AB [4]. Further studies into the role of peripheral monocytes/CD4⁺ T cells in the induction of AB in different stress models may prove beneficial.

RNA sequencing of mouse splenic T cells revealed 128 differentially expressed genes between CD4⁺ T cells from stressed and unstressed mice, a large portion of which encoded mitochondrial proteins [2]. Mitochondrial functional analyses of these CD4⁺ T cells using Seahorse-based extracellular acidification and oxygen consumption assays demonstrated increased glycolytic capacity and decreased basal respiration (physiological energy demand) and ATP-linked respiration (oxygen being used to drive ATP production) in cells from stress-exposed mice relative to cells from control mice. Moreover, CD4⁺ T cell mitochondria from ES-exposed mice were significantly shorter than those from controls, with more

punctate mitochondria. Furthermore, in the same study, CD4⁺ T cells from human patients exhibiting anxiety symptoms displayed fragmented mitochondria compared to those from healthy donors [2]. In addition, Fan and colleagues observed decreased amounts of two proteins intimately involved in mitochondrial fusion, MFN2 and MIGA2, in CD4⁺ T cells from stressed mice relative to those from control mice, suggesting that fragmented mitochondrial morphology stemmed from decreased fusion. Further assessment of the proteins that could actively drive the process of mitochondria fragmentation, such as Drp1, was not performed, but may certainly prove illuminating in the future. Of note, the mitochondrial morphology experiments performed in this study utilized MitoSPY Orange CMTMRos, a probe that localizes to mitochondria in a membrane potential-dependent manner [5]. That is, mitochondria with a depolarized membrane will lose the fluorescence signal, and may have a different shape than what is observed by MitoSpy fluorescence. Considering the alterations in mitochondrial respiration, it is probable that MitoSpy fluorescence is representing both changes in mitochondrial health and morphology in the study by Fan and co-workers [2]. These findings are relevant as mitochondrial fusion proteins such as Mfn1/2 have been implicated in the elimination of dysfunctional mitochondria by mitochondrial autophagy [6]. Stress-induced mitochondrial alterations may induce changes in mitochondrial quality control mechanisms such as autophagy within CD4⁺ T cells. Thus, further study into these mitochondrial mechanisms under stress conditions and how they relate to AB is vital.

Fan et al. further established the importance of CD4⁺ T cell mitochondrial changes in AB through the creation of mitochondria fusion-deficient mouse models. Relative to WT, *Miga2*^{-/-} and T cell-conditional *Miga2* knockout (*Miga*^{TKO}) mice showed fragmented T cell mitochondria and increased AB independent of stress, both of which were reversed by depletion of CD4⁺ T cells [2]. *Rag1*^{-/-} mice that received adoptively transferred *Miga2*-deficient CD4⁺ T cells presented with increased AB on the 'elevated plus maze' and 'open field test', revealing the sufficiency of CD4⁺ T cell mitochondrial fragmentation in leading to AB. Similar to stressed WT mice, CD4⁺ T cells from *Miga2*^{TKO} mice or *Mfn1/2*^{TKO} mice exhibited impaired glycolysis and oxygen consumption independent of stress. *Miga2*^{TKO} CD4⁺ T cells also shifted glucose flow to the pentose phosphate pathway, a process important in the synthesis of purine nucleotides. Liquid chromatography-mass spectrometry analyses of *Miga2*^{TKO} and WT mice found that serum from *Miga2*^{TKO} mice was enriched in purine metabolites such as xanthine, an increase that was mimicked in stress-exposed mice, as well as in the serum of human patients diagnosed with anxiety in the same study. Pharmacological inhibition of purine metabolism decreased serum xanthine concentrations and ameliorated AB in *Miga2*^{TKO} and in ES-exposed stressed mice. Furthermore, xanthine alterations were abrogated by treatment with CD4 blocking antibodies. How was xanthine production from CD4⁺ T cells with fragmented mitochondria leading to AB? To answer this question, the authors examined the amygdala, a region connected to stress and anxiety behaviors. Single-cell RNA sequencing of bilateral amygdala uncovered that *Miga2* deficiency increased oligodendrocyte expression of adenosine receptors (through which adenosine/xanthine primarily function), particularly Adora1. Moreover, this phenomenon was CD4⁺ T cell-dependent. shRNA-mediated knockdown of Adora1 in the amygdala prevented AB in *Miga2*^{-/-} mice, suggesting that CD4⁺ T cell-produced xanthine induced

AB through action on oligodendrocyte Adora1. A final link between mitochondrial fragmentation and purine metabolism was demonstrated as *Miga2*^{TKO} mice presented augmented accumulation of IRF-1, a transcription factor for xanthine dehydrogenase, and removal of IRF-1 from *Miga2*^{TKO} mice prevented AB, relative to WT mice [2].

Given the importance of peripheral adaptive immune cells in the modulation of central nervous system-derived AB, the authors also explored the hypothesis that microglia were involved in *Miga2*^{-/-}-induced behaviors [2]. Indeed, microglia, innate immune cells of the central nervous system, have been linked to AB [4,7]. Microglial depletion however, did not prevent *Miga2*^{-/-}-induced AB, and in fact, the Csf1r antagonist PLX3397 worsened several anxiety-related symptoms relative to *Miga2*^{-/-} mice [2], suggesting that microglia are necessary for protection against mitochondrial fission-induced AB. Nevertheless, that microglial signaling may be important for brain protection is in line with data revealing that relative to WT, microglia-specific knockout of MyD88-related inflammatory signaling in *Cx3cr1-CreBT:MyD88^{f/f}* mice can negatively impact responses to opioid exposures [8]. This is consistent with the fact that microglial inflammatory signaling is evidently not always deleterious.

In sum, the findings described by Jin's group demonstrate a novel role of peripheral adaptive immune CD4⁺ T cells in inducing AB through their actions on oligodendrocytes (Figure 1) [2]. These experiments also set the stage for ideally targeting CD4⁺ T cells or oligodendrocyte purine metabolism in the context of potential treatments for anxiety and/or depression --a refreshing avenue following years of neurotransmitter-focused approaches that have failed to achieve high proportions of patient responses.

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Figure 1. Stress-induced mitochondrial dysfunction in CD4⁺ T cells induces anxiety through oligodendrocyte adenosine receptors in mice.

Stress induces mitochondrial fragmentation and respiratory dysfunction in CD4⁺ T cells, leading to increased xanthine accumulation. This CD4⁺ T cell-produced xanthine functions on oligodendrocyte adenosine A1 receptors to promote anxiety-like behavior in stressed mice (depicted as a cross (elevated plus maze) and a grid (open field test), in mouse behavioral tests).

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