Keep your macrophages fit for healthy aging

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As age is the greatest risk factor for the development of most prevalent chronic diseases, there is an enormous interest in understanding the process of aging, with the hope of delaying or preventing age-related comorbidities. Along these lines, a recent study by Minhas et al. (2021) describes how aged macrophages downregulate glycolysis and mitochondrial oxidative phosphorylation (OXPHOS), inducing an energy-deficient state that compromises macrophage function and supports maladaptive inflammation that together cause brain dysfunction.

Aging is a natural process that affects us all. Chronic low-grade inflammation is a common characteristic of aging that has been defined as inflammaging and is thought to have different sources. The appearance of macrophages with aberrant activation profiles is such a factor that is thought to drive inflammaging, but the causal mechanisms remain ill defined (van Beek et al., 2019). Macrophages reside in virtually every tissue of our body and are crucial for securing tissue homeostasis by monitoring their tissue environment and responding to stressors. Fighting intruders and clearing tissue debris are energy-demanding processes, so when macrophages are activated, they ramp up and rewire their metabolism to fuel the immune functions that maintain organ health. Recent work in the immunometabolism field highlights the critical role of cellular metabolism in regulating macrophage functions and disease progression (Russell et al., 2019).

Minhas et al. started their study with the observation that synthesis of the lipid mediator prostaglandin E2 (PGE_2) by cyclooxygenase-2 (COX-2) was increased in macrophages of both aged humans and mice (Minhas et al., 2021). Related to this, they found a dose-dependent suppression of glycolysis and OXPHOS in aged human macrophages as measured by extracellular acidification rate and oxygen consumption rate, respectively. They hypothesized that increases in inflammatory PGE_2 signaling might explain the progression of age-associated maladaptive inflammation and cognitive decline. While PGE_2 can signal via four different receptors, the metabolic suppression was specifically mediated by the EP2 receptor. As these data suggested that inhibition of the PGE_2-EP2 axis might improve the metabolic fitness of aging macrophages, the authors next generated myeloid-specific EP2 knockout mice. Peritoneal macrophages from aged EP2-deficient mice had a "young phenotype" as shown by healthy mitochondria and increased metabolic activity, reduced expression of inflammatory markers, and restored phagocytic capabilities compared to their wild-type controls. In parallel, aged myeloid-specific EP2-deficient mice performed better in spatial memory tasks and showed improved hippocampal synaptic plasticity (Figure 1).

To understand the mechanisms by which EP2 deficiency prevented metabolic and cognitive decline, the authors examined signaling downstream of this PGE_2 receptor. In wild-type cells, EP2 signaling activated AKT, leading to phosphorylation and inhibition of GSK3β and subsequent induction of glycogen synthase (GYS1) and glycogen synthesis. Genetic or pharmacological inhibition of EP2 in mouse and human macrophages reduced glycogen synthesis and promoted the flux of glucose into glycolysis and TCA cycle to fuel mitochondrial respiration. This beneficial effect of EP2 blockade on bioenergetics was GYS1 dependent and increased phagocytic activity of macrophages. Also, it functionally reversed the cognitive decline in mice during aging. Interestingly, while young macrophages could bypass increased GYS1 activity and glycogen synthesis by using alternative carbon sources, such as lactate or glutamine, aged cells lost this metabolic flexibility and became dependent on glucose as a single carbon source to fuel mitochondrial respiration. Why macrophages from aged individuals seem unable to utilize alternative energy sources beyond glucose remains an intriguing question for future research. Another striking and unresolved observation was that peripheral EP2 inhibition with a brain-impermeant EP2 antagonist indirectly improved age-associated hippocampal function and downregulated the levels of inflammatory markers within the brain. Future studies should clarify which immune factors outside the brain are responsible for the observed effects of peripheral EP2 blockade on microglia and cognitive function.

This new study makes it tempting to speculate that inflammaging-associated macrophage dysfunction and cognitive decline are not necessarily permanent and might be reversed by targeting inflammatory signaling downstream of the myeloid receptor EP2. This notion might provide a more focused approach than the use of non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, that have been proposed as anti-aging drugs, but have the drawback of suppressing beneficial prostaglandin effects downstream of COX-2. For example, PGE_2 signaling appears to improve muscle function during aging, as was recently published in *Science* (Palla et al., 2021). In the latter study, stimulating PGE_2 signaling via the EP4 receptor rejuvenated mitochondrial biogenesis and function, and therewith improved muscle strength. Macrophages also exhibit a nuanced response to PGE_2 as it has been shown to induce "M2-like" macrophages (Luan et al., 2015), which are generally considered to be

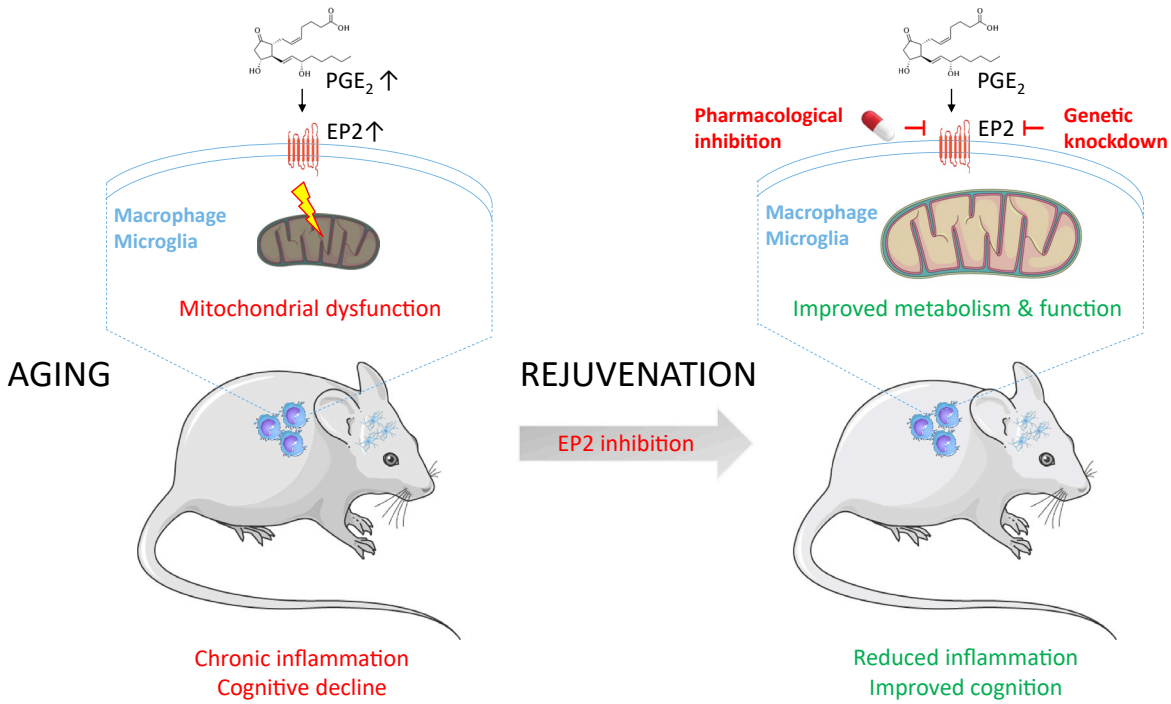

Figure 1. Inhibiting PGE_2 receptor EP2 signaling in aged myeloid cells restores their metabolism and function, and reverses cognitive decline during aging

Enhanced PGE_2 signaling via the EP2 receptor is associated with metabolic and functional defects in aged macrophages, and with chronic inflammation underlying cognitive decline in aging. Targeting the EP2 receptor in myeloid cells by pharmacological inhibition or genetic knockdown restores the metabolism and function of macrophages and microglia. This dampens inflammation and improves cognitive function in aged mice.

pro-resolving. Also, PGE_2 can inhibit the pro-inflammatory functions of the inflammasome when signaling through the EP4 receptor (Sokolowska et al., 2015). Therefore, PGE_2 should not be simply marked a pro-inflammatory mediator, but rather an immune modulator (Kalinski, 2012; Scher and Pillinger, 2009). Depending on the presence of additional stimuli and the (co)expression of its distinct receptors, PGE_2 can elicit a range of macrophage activation states (Xue et al., 2014).

In the recent study by Minhas et al., PGE_2 signaling via EP2 is presented as a pro-inflammatory factor, but numerous cell-intrinsic and -extrinsic stimuli have been shown to contribute to inflammaging (van Beek et al., 2019). Whether those factors all converge in enhanced EP2 signaling remains to be shown. The ability to reverse age-related metabolic changes and cognitive decline with pharmacological inhibition of EP2, or perhaps via stimulation of EP4, raises very interesting therapeutic possibilities. Yet the multifaceted role of PGE_2 signaling in other processes requires proper biomarkers to monitor adequate application. Meanwhile, it is probably wiser to promote regular exercise as a natural way to improve cognitive functions during aging and Alzheimer disease. These beneficial effects of exercise might very well be also regulated by improved bioenergetics in macrophages. Endurance exercise promotes mitochondrial biogenesis not only via activation of the transcriptional co-activator peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α) in skeletal muscle, but also in the hippocampus (Wrann et al., 2013). Therefore, it would be interesting to investigate whether myeloid cells also respond to exercise by increasing their cellular metabolic fitness, thereby suppressing the deleterious effects of inflammaging in a similar way to EP2 blockade.

While the link between PGE_2 and EP2 signaling, downregulation of macrophage metabolism, and cognitive decline is well supported by the data in mice, this pathway still needs to be validated in humans. If so, such results would give impetus to finding new ways to restore the metabolism of macrophages. This could potentially improve immune cell function in any context of metabolic dysfunction with consequences far beyond the context of inflammaging.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Mitochondrial dysfunction defines T cell exhaustion

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When T cells are exposed to continuous antigen stimulation, they become exhausted. Here, we preview findings from Scharping et al. (2021), who have illuminated the molecular mechanism by which the persistent antigen stimulation and severe hypoxic conditions in the intratumoral environment drive T cell exhaustion, losing their cytotoxic function and anticancer effects.

$CD8^+$ T cells recognize and eliminate infected or malignant cells. However, after long exposure to the stimulus, $CD8^+$ T cells stop proliferating and lose their effector capacity, compromising the protective immune response. Persistent antigen stimulation give rise to different types of dysfunctional T cells including exhausted and senescent T cells. Both exhausted and senescent T cells present similar features, including impaired TCR-driven proliferation and expression of terminal differentiation markers. However, whereas exhausted T cells are characterized by reduced secretion of effector cytokines, such as IFN-γ and TNF-α, senescent T cells are characterized by persistent secretion of these inflammatory cytokines. Understanding the molecular mechanism that drives the acquisition of these dysfunctional, and sometimes even detrimental, states is essential to invigorate immunity.

Three different articles recently published in *Nature Immunology* from Thompson, Ho, and Delgoffe have dissected how intratumoral conditions, including hypoxia and persistent antigen exposure, induce the exhaustion of tumor infiltrating lymphocytes (TILs) (Scharping et al., 2021; Vardhana et al., 2020; Yu et al., 2020). In particular, Scharping et al. found that exhausted TILs presented a higher hypoxic state and mitochondrial stress than effector $CD8^+$ T cells (Scharping et al., 2021). Upon exhaustion conditions, T cells progressively acquire inhibitory molecule expression and their secretion properties are reduced, becoming progenitor exhausted cells. When the exhaustion environment is maintained, these progenitor exhausted T cells finally differentiate into terminally exhausted T cells, which present higher levels of inhibitory molecules and null response. To understand the causality between mitochondrial dysfunction and exhaustion, the authors developed an *in vitro* system to mimic T cell exhaustion by combining hypoxia and persistent TCR stimulation. Under these conditions, $CD8^+$ T cells recapitulated *in vivo* exhaustion features like expression of the transcriptional regulator Tox, increased expression of inhibitory receptors (PD-1, TIM-3, and LAG-3), and impaired cytokine production (IFN-γ and TNF-α) and cytotoxic activity.

Remarkably, hypoxia-inducible factor 1α was dispensable for the exhaustion phenotype. Instead, Blimp1, a transcriptional repressor and a critical regulator of T cell homeostasis, was required. Removing Blimp1 in tumor terminally exhausted T cells was sufficient to restore TIL functional roles *in vivo* and *in vitro*. Blimp1 overexpression reduced PGC1-α transcription, a transcriptional coactivator controlling mitochondrial biogenesis and antioxidant response. Previously, the authors had shown that overexpression of PGC1-α mitigates the exhaustion phenotype by boosting mitochondrial biogenesis (Scharping et al., 2016). Now, they extend these observations describing the contribution of PGC1-α as regulator of mitochondrial reactive oxygen species (mtROS) during T cell exhaustion. The researchers used low doses of chemical inhibitors targeting the electron transport chain (ETC) complexes I (rotenone) and

