work for making discoveries. They defined previously uncharacterized cell subtypes by comparing expression and chromatin patterns across organs, and they used this multi-organ approach for cell-lineage analysis.

Domcke *et al.* compared cell-lineage diversity across organs and revealed that circulating blood cell subtypes are almost identical, irrespective of the organ from which they were isolated. Conversely, they found that endothelial cells (key components of blood vessel walls) are regulated by many tissue-specific factors and differ by organ. Therefore, in trying to understand functional relationships between these subtypes and other cells during development, tissue context might figure more prominently in endothelial cell variability than it does in other lineages. Cao *et al.* precisely annotated three subtypes of red blood cell (erythroid) progenitor, each representing a different stage of maturation, and measured their presence across organs. They identified early erythroid progenitors in the adrenal gland, a previously unknown site of erythroid development, which might bridge the developmental switch in the site of production of these cells from fetal liver to bone marrow.

Domcke and colleagues also used the paired data sets to assess the relationship between gene expression and its regulation. They identified previously unknown transcription factors specific to discrete developmental stages by analysing transcription-factor binding sites in accessible chromatin regions. Then, on the basis of the relationships between expression of cell-type-specific transcription factors and binding-site availability, they assigned putative functions to these transcription factors as activators or repressors (Fig. 1).

These studies represent the next generation of atlas papers. Currently, standard cell atlases characterize a single organ on a molecular level using one data modality. The new work provides a road map for unifying these disparate data sets.

A limitation of the work is that the current atlases correspond only to a specific developmental window, and not every organ is included. However, the atlas can be expanded with the integration of new data, and the growing resource will be an asset to biologists in standardizing cell types across development. Unidentified cells are typically characterized in relation to the other cell types of that organ, but these definitions might vary between data sets. Multi-organ analysis creates a consistent framework of characteristic cell types against which to compare new data. It can match unidentified cells with corresponding cell types in another organ on the basis of shared expression patterns or, conversely, it can highlight tissue-specific differences between previously grouped cells.

Standardization in the field will yield a more refined, uniform and valuable resource that will facilitate exploration of new questions. For example, it will help researchers to investigate how cells of a given lineage differ depending on tissue-intrinsic properties or dynamic lineage changes. Immune cell expression and function might differ, for instance, depending on the organs they target or on changes in their site of production.

Although the data presented capture healthy development, characterization of these tissues also has implications for the study of disease. For example, this resource will help to enhance our basic understanding of stem-cell differentiation by identifying key regulators of cell fate and development. This, in turn, will aid in the analysis of lineage dysregulation in developmental disorders. Its utility will also extend to investigating adult diseases characterized by changes in cell state and differentiation, such as cancer, degenerative disease and ageing. Ultimately, these comparisons, both to disordered development and to diseased adult tissue, might reveal targets for therapeutic intervention as well as fundamental principles of human physiology and development.

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Ageing

Immune-cell shutdown harms old brains

Jonas J. Neher

Immune cells called macrophages have been found to shut down major metabolic pathways during ageing. Restoring metabolism in these cells is sufficient to alleviate age-associated cognitive decline in mice. **See p.122**

Immune cells called macrophages are found in almost every tissue, and are crucial for maintaining organ health and providing a first line of defence against disease-causing organisms. The energy demands of macrophages increase drastically when they are activated, and so they rebalance or enhance their two main energy-producing metabolic pathways (glycolysis and oxidative phosphorylation) to quickly fuel an effective immune response[1]. Minhas *et al.*[2] report on page 122 that macrophages shut down these metabolic pathways during ageing, severely compromising macrophage function and, in turn, brain health. This work has implications not only for the preservation of brain health during ageing, but also for conditions such as Alzheimer's disease or sepsis, in which similar maladaptive macrophage states could be common.

As we age, chronic, low-grade inflammation develops in most people[3]. One inflammatory signalling protein whose levels rise not only during ageing[4] but also during neurodegenerative disease[5] is prostaglandin E_2 (PGE_2). Minhas *et al.* set out to investigate whether PGE_2 might cause age-associated changes in macrophages. Interestingly, the authors found increased production of PGE_2 in human and mouse macrophages themselves — both in the brain and elsewhere in the body (the periphery). This led to the activation of PGE_2's receptor protein EP2 in the cells, which in turn resulted in suppression of oxidative phosphorylation and glycolysis. The resulting energy-deficient state both limited the beneficial functions of macrophages and increased inflammation.

To determine whether these changes could cause age-associated cognitive dysfunction, the authors examined a mouse strain in which EP2 receptor levels were reduced exclusively in macrophages in the body and brain, and treated mice with an EP2 inhibitor. Strikingly, EP2 inhibition restored macrophage metabolism to youthful levels in both settings, reducing inflammation in the periphery and brain, and alleviating cognitive decline (Fig. 1). These results indicate that (at least in mice) macrophage dysfunction during ageing affects brain health, and that normal cell function can be restored by reversing metabolic shutdown in the cells.

Minhas and colleagues went on to dive deeper into the metabolic rewiring of aged

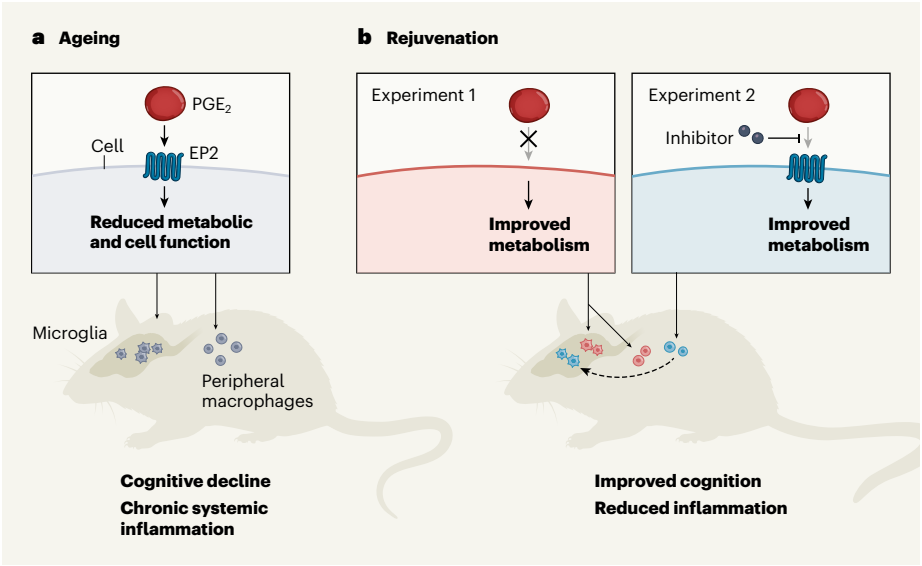

Figure 1 | Reversing metabolic shutdown in aged macrophages. Immune cells called macrophages are found throughout the body (the periphery) and in the brain, where they are called microglia. **a**, Minhas *et al.*[2] report, that during ageing, peripheral macrophages and microglia produce more of the protein prostaglandin E2 (PGE_2), which binds to EP2 receptors on the cells' membranes. They demonstrate that activation of this signalling pathway leads to metabolic dysfunction in the cells, and so to systemic chronic inflammation and cognitive decline. **b**, The authors inhibited the EP2 receptor in two ways. First, they used a genetic approach to reduce levels of EP2 in both macrophages and microglia. Second, they inhibited the receptor pharmacologically – but only in the periphery. Under both conditions, EP2 inhibition improved metabolic function in peripheral macrophages and microglia, reducing inflammation and restoring cognitive ability. The mechanism by which peripheral inhibition of EP2 leads to changes in microglia is unknown (dashed arrow).

macrophages. They found that such macrophages favoured energy storage in the form of glycogen (a large glucose polymer) over the use of glucose for energy production through glycolysis or oxidative phosphorylation. Although glycogen normally serves as a fuel reserve, aged macrophages did not seem to use this reserve, despite their energy-deficient state.

It is unclear why aged macrophages store extra glycogen, but dendritic cells, a related cell type, use their glycogen stock to fuel their earliest inflammatory responses[6]. Therefore, it is conceivable that aged macrophages increase glycogen storage so that they can mount a stronger immune response during acute inflammatory activation. In line with this idea, aged microglia (brain macrophages) are well known to be primed – that is, to respond more strongly to inflammatory insults than do young microglia[7]. Minhas and co-workers did not directly analyse whether microglial priming is enabled by increased glycogen stores. However, this possibility would certainly be worth investigating, because some evidence suggests that exacerbated immune responses in the aged brain contribute to neurodegenerative disease[7].

Notably, there is also evidence for a role of microglial metabolic dysfunction in brain disease, particularly in Alzheimer's disease. The risk of developing Alzheimer's increases several-fold in people who carry mutations in the microglial receptor protein TREM2. In mice, TREM2 deficiency causes breakdown of microglial metabolism and exacerbation of Alzheimer's pathology[8]. Furthermore, chronic exposure of microglia to aggregated amyloid-β protein, a hallmark of Alzheimer's disease, leads to the breakdown of oxidative phosphorylation and glycolysis in these cells in mice[9]. In both cases, enhancing microglial metabolism leads to less-severe Alzheimer's pathology in the mouse models.

In sepsis (a condition that results from excessive inflammation in response to infection), PGE_2 levels also increase[9] and long-term cognitive deficits often develop[7]. Here, macrophages enter a state called immune paralysis, which is also characterized by suppression of both oxidative phosphorylation and glycolysis[1,10]. Thus, the cellular shutdown of macrophages during sepsis or during ageing and neurodegenerative disease might be a response to excessive or chronic immune stimulation, respectively. This adaptation would be beneficial from an evolutionary perspective because it would protect an organism from a hyperactive immune response that could cause tissue damage. However, in the context of an ageing organism, it seems to predispose the brain to dysfunction or even degeneration. Whether macrophage immune states are indeed similar across these different conditions remains to be investigated.

"These results indicate that (at least in mice) macrophage dysfunction during ageing affects brain health."

Another intriguing aspect of Minhas and colleagues' study is the finding that, even when EP2 inhibition was limited to the periphery in aged mice (using a substance that cannot enter the brain), brain inflammation was reversed and cognitive function restored (Fig. 1). This corroborates previous findings that immune signals generated outside the brain can affect microglia[11], and that stimulation of immune cells outside the brain can partially restore the metabolism and function of peripheral macrophages after sepsis[10] and of microglia in mouse models of Alzheimer's disease[9]. Thus, a growing body of evidence indicates that, in mice, macrophages remain responsive to immune stimulation even during disease and ageing.

The next challenges will be to demonstrate that this macrophage plasticity is also retained towards the end of the considerably longer human lifespan, and that the PGE_2–EP2 pathway is relevant for human brain ageing and disease. Moreover, the immune signals that induce initial microglial shutdown or restore microglia to a youthful state in aged animals remain unknown. Their identification could lead to therapeutic approaches to combat a range of diseases.

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