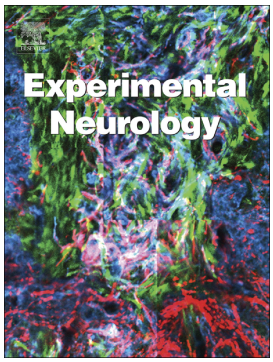

Soluble factors influencing the neural stem cell niche in brain physiology, inflammation, and aging

Cory M. Willis, Alexandra M. Nicaise, Grzegorz Krzak, Rosana-Bristena Ionescu, Vasiliki Pappa, Andrea D'Angelo, Ravi Agarwal, Maria Repollés-de-Dalmau, Luca Peruzzotti-Jametti, Stefano Pluchino

PII: S0014-4886(22)00149-2

DOI: https://doi.org/10.1016/j.expneurol.2022.114124

Reference: YEXNR 114124

To appear in: *Experimental Neurology*

Received date: 3 November 2021

Revised date: 16 May 2022

Accepted date: 21 May 2022

Please cite this article as: C.M. Willis, A.M. Nicaise, G. Krzak, et al., Soluble factors influencing the neural stem cell niche in brain physiology, inflammation, and aging, *Experimental Neurology* (2021), https://doi.org/10.1016/j.expneurol.2022.114124

Soluble Factors Influencing the Neural Stem Cell Niche in Brain Physiology, Inflammation, and Aging

Cory M Willis[1]*, Alexandra M Nicaise[1], Grzegorz Krzak[1], Rosana-Bristena Ionescu[1], Vasiliki Pappa[1], Andrea D'Angelo[1], Ravi Agarwal[1], Maria Repollés-de-Dalmau[2], Luca Peruzzotti-Jametti[1], Stefano Pluchino[1]*

[1]Department of Clinical Neurosciences and National Institute for Health Research (NIHR) Biomedical Research Centre, University of Cambridge, Cambridge, UK

[2]Unitat de Recerca, Hospital Universitari de Tarragona Joan XXIII, Institut d'Investigació Sanitària Pere Virgili, Tarragona, Spain

*Corresponding Authors contact: sp624@cam.ac.uk or cw739@cam.ac.uk

Abstract

Within the adult central nervous system (CNS) of most mammals resides a resident stem cell population, known as neural stem cells (NSCs). NSCs are located within specific niches of the CNS and maintain a self-renewal and proliferative capacity to generate new neurons, astrocytes, and oligodendrocytes throughout adulthood. The NSC niches are dynamic and active environments that are within proximity to the systemic circulation and the cerebrospinal fluid (CSF). Therefore, NSCs respond not only to factors present in the local microenvironment of the niche but also to factors present in the systemic macroenvironment. The factors can be soluble forms such as cytokines and chemokines located in the circulation or directly from local cells, such as microglia and astrocytes. Additionally, recent evidence points towards physiological aging and its association with a progressive loss of function and a decline in the self-renewal and regenerative capacities of CNS NSCs, which can be further exacerbated by changes in the local and systemic milieu.

This review will highlight the main intrinsic and extrinsic regulators of neural stem cell function under homeostatic and inflammatory conditions including those trafficked within extracellular membrane vesicles. Further, discussion will center around how intrinsic and extrinsic factors impact normal homeostatic functions within the adult brain and in aging.

Key Words: neural stem cells, secretome, inflammation, aging

Acknowledgments

We are grateful to Giovanni Pluchino for critically reading the manuscript. The authors acknowledge the contribution of past and present members of the Pluchino laboratories, who have contributed to (or inspired) this review.

Funding

This work has received support from the National Multiple Sclerosis Society (USA; grant RG-1802-30200 to SP), the Italian Multiple Sclerosis Association (AISM, grant 2018/R/14 to SP), the United States Department of Defense (DoD) Congressionally

Directed Medical Research Programs (CDMRP) (grant MS-140019 to SP), and the Bascule Charitable Trust (RG 75149 and RG 98181 to SP). CMW is funded through a National Multiple Sclerosis Society Personal Fellowship (FG-2008-36954). AMN is funded through a European Committee for Treatment and Research in Multiple Sclerosis Postdoctoral Research Fellowship Exchange Program (G104956). RBI is funded through a Medical Research Council Doctoral Training Partnership (MRC DTP) award (RG86932) and a Cambridge Trust scholarship. AD is funded through an Erasmus+ student internship. MR was funded through The Federation of European Biochemical Societies Personal Summer Fellowship.

Conflict of Interest

SP is co-founder and shareholder (>5%) of CITC Ltd.; co-founder and Non-Executive Director (NED) at asitia Therapeutics and iSTEM Therapeutics; and CSO at ReNeuron.

Author Contributions

All authors contributed equally to the research, writing, and editing of this review.

1. Introduction

Neural stem cells (NSCs), which are the resident stem cell population of the central nervous system (CNS), are capable of differentiating into neurons, astrocytes, and oligodendrocytes and reside in specialized niches in the developing and adult mammalian brain. Niches are defined as zones in which stem cells are retained even after embryonic development for the production of new cells during an organisms lifetime (1). Therefore, NSC niches support their self-renewal and differentiation throughout life (2). Three different regions of the mammalian brain possess a NSC niche: the subgranular zone (SGZ) in the hippocampal dentate gyrus (DG), the subventricular zone (SVZ) near the lateral ventricles, and a third niche in the hypothalamus (2-5). Within the distinct niches exists a local microenvironment of not only NSCs but also neurons, glial cells (such as microglia, astrocytes and oligodendrocytes), immune cells, and blood vessels, which we will define as niche constituents. Signaling and communication within the niche microenvironment can be thought of as a feedback interaction system wherein NSCs, their progeny, and other cells dynamically interact with each other via numerous secreted and contact-mediated signals (6, 7). These NSC secreted molecules, factors, and components is defined as the secretome, and their precise contribution is only beginning to be understood.

The majority of studies which have established our understanding of mammalian NSC niches and their behaviors have been primarily in rodent model systems. Here, NSCs are found to exist throughout adulthood, where they are capable of neurogenesis and cell cycling (8). On the one hand, in humans, the presence of bona fide brain stem cell niches, which have the capability to differentiate into neurons, has been subject to debate. Recent work, has found that adult hippocampal neurogenesis is possible in aging as well as neurodegenerative diseases (9). On the other hand, transcriptomic analysis using single cell sequencing technology found no transcriptomic evidence of adult hippocampal neurogenesis (10). These discrepancies may be due to differences in techologies and markers used for early neurons. Nonetheless, there is still evidence in support of the presence of stem cells in niches of the adult human brain (11-13).

NSC behavior is tightly regulated by changes within the niches where they reside, which includes maintenance of quiescence, activation of replication capacity, and differentiation when needed. Alterations to NSC function occur mainly through the uptake and release of soluble factors (i.e., cytokines, extracellular matrix molecules, growth factors, or neurotrophins) that are present in the microenvironment. The majority of the soluble factors derive from the niche constitutents (i.e. neurons and glial cells) and act in an autocrine and paracrine manner to maintain homeostatic function under non-diseased conditions (14). Thus, the functions of NSCs such as self-renewal, activation, or differentiation relies on the finely tuned signaling between resident niche cells (14). However, emerging evidence has indicated widespread dysfunction occurs within NSC niches in neuroinflammation, neurological diseases, and physiological aging (15-17). The increased presence of peripherally activated immune cells, changes in the soluble content of the systemic milieu, and activation of CNS resident microglia all contribute to this dysfunction (18-20). Therefore, studying the bidirectional crosstalk between NSCs and their niche constituents is key to furthering our understanding of the signaling properties of NSCs in brain physiology, aging, and neuroinflammation in which NSC function is altered (14).

In this review we will first highlight how local soluble factors released within the NSC secretome are critical regulators of niche function under physiologic conditions. Then we will discuss how extrinsic soluble factors present in the niche microenvironment and systemic milieu during neuroinflammation and aging alter and disrupt the function of NSCs within the niches. Understanding how soluble factors (and their cellular sources) impact NSC function will provide greater insight into the present challenges of promoting regeneration and rejuvenation of the damaged and aged CNS.

2. Extrinsic regulators of NSC function in neuroinflammation

a. NSC secretome in normal physiology

Under physiological conditions brain NSCs are largely quiescent, maintaining a low metabolic rate, and slowly undergo self-renewal coupled with a very long cell cycle (21). The activity state of NSCs (quiescence, self-renewal, amplification, or

differentiation) is influenced by a complex machinery that regulates their biology and is comprised of both soluble intrinsic and extrinsic factors (22). The diverse molecular repertoire (i.e., cell surface receptors) of NSCs allows them to sense the array of soluble mediators present within the niches. This results in complex and bidirectional signalling to regulate the behavior of neighboring cells of the NSC niches under both physiological and pathological conditions (23). Through the use of single cell transcriptomics using mice it has now been revealed that there are a multitude of NSC states that exist under homeostasis (24). This includes quiescent, activated, and primed NSCs, which can transition between these states based on intrinsic and extrinsic signals (25). Of those, notch is perhaps the most important and most studied intrinsic cue that regulates NSC quiescence. Notch ligands from the delta or jagged families are expressed by activated NSCs and intermediate progenitor cells in the stem cell niches (26, 27). Through these ligands, cells in the NSC lineage provide feedback signals that are essential to prevent further activation of the quiescent NSC population. This feedback loop regulates and mitigates cellular exhaustion of the stem cell niche. Moreover, notch signaling has been shown to lie at the crossroad of intrinsic and extrinsic signalling in the stem cell niche in drosophilia. Here, it was shown that notch signaling regulates quiescence via coordination of the intrinsic temporal programs of NSCs based on the presence of extrinsic nutrient cues (28). Under homeostatic conditions NSCs maintain a quiescent state in order to not overproliferate and exhaust their population (29). However, quiescent NSCs are more inert to proliferative cues, which may make it difficult for them to become activated upon injury (25). Importantly, a subset of cells termed ‘primed for activation NSCs’ has been identified in the neural stem cell niches. The primed state defines a subpopulation of slow-cycling NSCs with a distinctive intermediary molecular signature to the quiescent and activated states. Behaviorally, in contrast to activated NSCs, primed NSCs only exhibit a transient reversible activation without neurogenic production. This intermediary state allows for a fine tuning between activation and return to quiescence, in response to extrinsic signals such as tissue injury (25).

The main route through which the NSC secretome influences cells is via paracrine signaling. Paracrine signaling involves the release of molecules into the extracellular space which then act on cells locally. To date, the most studied and best characterised property of NSCs is their constitutive production and release of specific factors termed

neurotrophins, which are known to regulate the development, maintenance, and function of the vertebrate nervous system (30, 31). The exogenous secretome from NSCs is comprised of neurotrophic factors with known roles in promoting neuronal and glial maturation. These neurotrophins include human brain derived neurotrophic factor (BDNF) (32-34), vascular endothelial growth factor (VEGF) (35-37), nerve growth factor (NGF) (33, 37, 38), neurotrophin-3 (NT3) (39), fibroblast growth factor 8 (FGF8) (40), and glial cell line-derived neurotrophic factor (GDNF) (38).

It is important to note that our current understanding regarding the extrinsic neurotrophic support by NSCs is primarily derived from studying the NSC secretome in either *in vitro* conditions or *in vivo* following transplantation into rodent models of neurodegenerative diseases (e.g., stroke, multiple sclerosis [MS], Parkinson's disease (PD), Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS)) (41). Similarly, until recently much of the scientific endeavour has focused on exploring the influence that the stem cell niche has on NSC function and behavior (6). This has resulted in little insight into the reciprocal signaling properties of NSCs towards other cells within the niche under homeostatic conditions.

Nevertheless, newer studies have revealed more direct evidence of the crucial role the NSC secretome plays in providing neurotrophic support for the maintenance of brain homeostasis under physiological conditions. For example, rodent NSCs within their niches have the capability to differentiate into new-born neurons which integrate into the brain. Specifically, the ablation of NSCs in the adult mouse hippocampus leads to impaired new-born neuron maturation as a result of the loss of the NSC-secreted factor pleiotrophin (PTN) (42). It was also found that murine NSC-derived VEGF was indispensable for induction of proliferation, migration, and phagocytosis of microglia *in vitro* (43).

b. Brain inflammation, soluble factors, and NSC function

Brain inflammation is a complex cellular and molecular response of innate and adaptive immune cells as a consequence of the loss of the homeostatic stuatus of the CNS, such as occurs in disease or tissue injury. Although brain inflammation is mediated by astrocyes, endothelial cells, and peripheral immune cells that cross the blood brain barrier (BBB), brain resident microglia have a key role in maintaining homeostatic function in the CNS. As a consequence of CNS disease or tissue injury,

microglia become activated and drive CNS damage via pro-inflammatory cytokines, chemokines, nitric oxide (NO) production, and reactive oxygen species (ROS). Persistent activation of microglia in the absence of the resolution of inflammation leads to the continued damage of otherwise healthy, unaffected tissue over time. It is this persistent, unresolved inflammation that is thought to have a central role in numerous neurodegenerative conditions of the CNS such as AD, PD, and ALS, autoimmune diseases such as MS, and neuropsychiatric diseases such as major depression disorder (44).

The first reports describing the effects of inflammation on NSC function demonstrated that lipopolysaccharide (LPS) treated microglia led to diminished neurogenesis in the SGZ (45, 46), and that inhibition of the microglial response to LPS led to a restoration of neurogenesis in the inflamed brain (46, 47). It has also been found that the proliferation potential of NSCs is disrupted under inflammatory conditions. In the rodent model of MS, experimental autoimmune encephalomyelitis (EAE), NSC proliferation significantly increases during the acute phase of EAE and then decreases during the chronic phase (16). This was supported by *in vitro* evidence of induction of quiescence by the the cell-cycle inhibitor p21 in murine NSCs treated with a cytokine cocktail (interferon gamma [IFN-γ], interleukin 1 beta [IL1β], and tumor necrosis factor alpha [TNF-α]) that mimics the presence and release of these cytokines by T helper cells in EAE *in vivo* (16). The migratory potential of NSCs is also disrupted in EAE owing to the persistent inflammation present in the chronic phase of the disease. Here, within neurogenic niches the migration of NSCs toward an established anatomical location, such as the olfactory bulb, is disrupted due to the release of chemoattractants from immune cells that creates new migratory paths for NSCs (16).

Brain inflammation can also affect the differentiation potential of NSCs (48). Primary murine microglia stimulated with LPS inhibits neurogenesis from murine NSCs *in vitro* (49), which is thought to be due to the production of pro-inflammatory cytokines (e.g. IL-1β, interleukin 6 [IL-6], TNF-α) and NO by microglia (50-52). Indeed, pro-inflammatory cytokines exert detrimental effects on NSCs such as decreased proliferation and inhibiting the differentiation of NSC progenitors to neurons (**Figure 1**). Specifically, chronic subcutaneous administration of recombinant human IL-1β or genetic overexpression of brain restricted human IL-1 receptor antagonist (IL-1ra) has been shown to significantly reduce neurogenesis in the mouse DG (53, 54), whereas

TNF-α seems to play a detrimental role in neuronal survival/differentiation (47, 55). Interestingly, peripherally induced inflammation was found to regulate NSC activation and play an important role in supporting quiescence via TNF-α signaling (25). Primed NSCs become activated upon TNF receptor 2 (TNFR2) binding, but return to quiescence upon TNFR1 binding (25). This work signifies the importance of extrinsic factors in the regulation of NSC state. NO is also capable of negatively regulating neurogenesis. For example, a significant increase in SVZ neurogenesis was demonstrated in mice lacking neuron-specific NO synthase (nNOS) (56) and following small molecule inhibition of NOS activity in mice (57, 58). Moreover, pathophysiological concentrations of NO *in vitro* directly impact NSC differentiation whereby NSCs preferentially differentiate into astrocytes rather than neurons (59).

Activated microglia can promote neurogenesis under certain conditions. For example, both neurogenesis and oligodendrogenesis are blocked by LPS-activated primary murine microglia *in vitro*, however stimulation with interleukin-4 (IL-4) or low levels of IFN-γ promotes neurogenesis and oligodendrogenesis (49). It has also been found that IFN-γ administered directly to NSCs *in vitro* enhances neuronal proliferation and differentiation (60, 61). Additionally, transgenic mice producing sub-clinical levels of IFN-γ in the brain had increased NSC proliferation and differentiation in the adult DG that was associated with neuroprotection and improved spatial cognitive performance (62). Activated microglia can also positively regulate neurogenesis by increasing the expression of the neuroprotective mediator insulin-like growth factor 1 (IGF-1) (63). It has indeed been demonstrated that microglial cells (activated *in vitro* with IL-4) are able to promote neurogenesis from adult NSC via IGF-1 upregulation (49) (**Figure 1**). The differential effects of inflammation likely depend on the phenotype of the inflammatory cells and their overall cytokine production profile, which dynamically affects NSC functions in the context of disease (45).

c. NSC secretome and immunomodulation

Beyond being influenced by soluble factors released from immune cells, NSCs are also fully capable of modulating both the innate and adaptative immune systems through the production and release of a wide array of signaling molecules (64). Soluble factors released from murine NSCs are known to regulate T lymphocyte mediated adaptative immune responses through the release of factors, such as the cytokines IL-10 (65), transforming growth factor-beta 2 (TGF-β2) (66), leukemia inhibitory factor

(LIF) (67), prostaglandin E2 (PGE2), and NO (68) (**Figure 1**). Of interest, metabolic signaling has recently emerged as a distinct mechanism by which stem cells mediate part of their paracrine immunomodulatory functions. For instance, upon cytokine exposure, murine NSCs have been shown to increase the secretion of extracellular arginase-1, leading to the decreased proliferation of lymph node cells (69).

The other component of the NSC secretome that is relevant and significantly impactful are extracellular vesicles (EVs), which include small, nanosized exosomes up to large apoptotic bodies (70). EVs can communicate both locally on neighboring cells within the microenvironment and globally across the organism as a whole through their release into the systemic circulation (71-75). Under normal, non-diseased conditions, NSC-EVs are thought to contribute to regeneration and, perhaps, in maintaining an anti-inflammatory state (70). Most of the EVs identified thus far are positive for either CD63 and CD81 (76) (**Figure 1**). Whereas CD63 is typically associated with the biogenesis of EVs, CD81 is known to be part of the B-cell receptor signaling pathway and MHC class I-mediated antigen presentation. Most interestingly, CD81 is involved in immune suppression and is a common marker when studying immunodeficiency, and thus may be an active part of the NSC secretome even if the internal contents (i.e., proteins and miRNAs) are not (77). Naturally, further study of this point is required to confirm this possibility, however it remains a tantalizing point of speculation.

EVs contain a variety of bioactive molecules (referred to as EV cargo) such as RNA, DNA, proteins, and lipids that are thought to be the key components of the NSC secretome. EVs are natural carriers of shoty non-coding RNA; microRNAs (miRNAs), protecting their content from RNA degradation and delivering their cargo to other cells to regulate their biological processes. Out of 446 human NSC-derived siRNAs,113 were found the be localized within EVs indicating that NSCs specifically sort these siRNA for extracellular release to regulate biological processes of recipient cells. Specifically, five miRNAs identified within NSC-EVs were found to be the most biologically active and abundant per current studies. These are: *Homo sapiens*(hsa)-miR-1246, hsa-miR-4488, hsa-miR-4508, hsa-miR-4492, and hsa-miR-4516 (76). Further, let-7, which is one of the first miRNAs ever discovered, is readily detected in NSC conditioned media and is thought to have direct immunomodulatory properties, though there are still very few studies that have explored this relationship (78-80).

Additionally, let-7 has been shown to play a role in stem cell division and induction of a less differentiated state, suggesting a role in the maintenance of the NSC pool (81).

Similarly, both human and murine NSC-derived EVs have been shown to harbor functional metabolic enzymes and thus act as independent metabolic units that are able to affect the physiology of the niche microenvironment (82). NSCs have also been reported to display immunomodulatory functions on the innate immune system by decreasing macrophage-mediated secondary CNS damage. Persistently activated macrophages result from a sustained exposure to positive mediators of inflammation (such as cytokines, chemokines, and the endotoxin LPS). Here, persistently activated macrophages exposed to LPS accumulate the tricraboxylic acid cycle intermediate metabolite succinate, which results in a sustained and protracted inflammatory phenotype. To this end, findings from our lab identified the expression of succinate receptor 1 (SUCNR1), the cognate receptor for succinate, on both murine and human NSCs. When SUCNR1-expressing murine NSCs were transplanted into the spinal cord of mice during a period of active neuroinflammation in the mouse model of MS, EAE, they actively scavenged extracellular succinate that had accumulated in the tissue microenvironment. This active scavenging via the succinate-SUCNR1 signaling axis resulted in the activation of an anti-inflammatory transcription program in the transplanted NSCs that lead to the production and secretion of PGE2 reducing the pro-inflammatory phenotype of macrophages (83) (**Figure 1**). We have also discovered that NSC-derived EVs contain whole mitochondria with conserved functional properties. Within this context the lateral transfer of EV encapsulated mitochondria from murine NSCs to pro-inflammatory macrophages modulates the function of the latter (**Figure 1**). Specifically, EV-trafficked mitochondria derived from murine NSCs have been shown to be taken up by LPS-activated murine macrophages and subsequently integrated into their mitochondrial network. This phenomenon results in the metabolic reprogramming of macrophages, which dampens their inflammatory activation (84). Injected murine NSC-derived EVs alone have also been found to lead to a phenotypic ‘switch’ in macrophages from a pro-inflammatory to anti-inflammatory state that leads to a subsequent decrease in the number of Th17 cells with a comcomitant increase in Tregs (85).

In addition to their paracrine functions, murine NSCs exert important anti-inflammatory effects on the adaptive immune system through juxtacrine signaling,

leading to reduced proliferation, decreased activation, and increased apoptosis of pro-inflammatory T lymphocytes (86, 87). Similarly, murine NSCs have demonstrated immunomodulatory properties through their direct engagement with infiltrating macrophages in a mouse model of contusion spinal cord injury (SCI) (88). Specifically, analysis of cell-to-cell interactions at the perivascular niches revealed the presence of tight contacts between transplanted murine NSCs and macrophages via connexin 43 to instruct phagocytic macrophages and reduce secondary tissue damage (89). Further studies investigating the NSC secretome, as well as their role in juxtacrine signaling, and subsequent identification of novel signaling molecules such as small metabolites, would greatly benefit our understanding of the anti-inflammatory properties of NSCs (90).

The niche microenvironments where NSCs reside in the brain are immensely complex and NSC states are constantly changing dependent on signals, which include secreted molecules and EVs (**Figure 1**). However, even though immense progress has been made in our understanding of the different components of the niche and their specific effects, further research should investigate how different niche molecules affect the complex maintenance and regulation of NSC function under neuroinflammatory states. Therefore, targeting specific mechanisms that regulate the stem cell niche under inflammatory conditions may help understand how cells that interact with the niche and NSCs behave during states of disease. This may lead to new treatments of neurological disorders potentially leveraging NSC properties of cell replacement and anti-inflammation.

3. Brain stem cells in aging

a. Altered NSC secretome with natural aging

Physiological aging is associated with a progressive loss of function and decline in the homeostatic capabilities of NSCs. This includes a decline in their ability to undergo neurogenesis, a decline in their cycling abilities, and significant changes to their normal supportive secretome. In turn, changes to the NSC secretome itself with age, which includes changes in growth factors, increased cytokine production, and changes in EV cargo, can impact the function of surrounding cells (91).

It is also important to note that the aged phenotype of the organism as a whole carries a large impact on the NSCs and acts as an extrinsic regulator of their function, thus influencing the NSC niche and the NSC secretome. This is due to the anatomical location of the SVZ niche which is in close proximity to both systemic blood vessels and the ventricles of the brain, leading to exposure of NSCs to circulating cytokines and EVs from throughout the body in addition to factors in the niche (92). Consequently, with advancing age, individuals are more inclined to develop a whole-body pro-inflammatory state, characterized by high levels of circulating inflammatory molecules, known as inflammaging (93) as well as the accumulation of senescent cells, which both increase the risk of developing age-associated neurodegenerative diseases. Senescence is a process in which cells typically stop dividing and undergo alterations that change their normal function and impact surrounding cells via secretion of pro-inflammatory molecules (94).

In the young healthy adult murine brain, tissue damage from injury, disease, or infection stimulates otherwise quiescent NSCs into activity to replenish damaged and dying cells. Here NSCs balance cell replacement (i.e., neurons and astrocytes) with asymmetrical division to maintain a reserve of multipotent NSCs (95). The loss of this ability to balance the two states as a result of aging, chronic systemic or local inflammation, senescence, and tissue damage further enhances changes to the NSC secretome. As a consequence, the NSCs are pushed towards alternative states of increased quiescence and inflammatory senescence, in which their normal functions are either dysregulated or completely absent (95, 96). As such, the function and nature of the NSC secretome is highly dependent on their current cellular state, potentially being in deep quiescence or under inflammatory senescence, and physiological age of the animal.

NSCs from aged animals develop an altered, pro-inflammatory secretome that negatively influences brain health and is thought to contribute, at least partially, to age-releated cognitive declines (96). Within the aged NSC niche there is an increase in factors associated with the senescent associated secretory phenotype (SASP), which is a collection of secreted factors typically associated with senescent cells, including IL-6 and TNF-α (92, 97, 98). Excess secretion of these factors has been found to deplete the SVZ stem cell pool and prevent neurogenesis (98). Further, the presence of the SASP within the NSC niches can directly impact NSC activity. Here, NSCs within

the aged niche become deeply quiescent and fail to respond to cues to become active, which prevents their normal homeostatic functions in the brain, as well as cell replacement through proliferation and differentiation (95, 96). These recent discoveries have prompted the need to further investigate the secretome of NSCs from young and aged organisms and how this secretome is further modified by neurodegenerative disease.

The role of aging on the cargo and function of NSC-EVs cannot be overlooked. For example, NSC-derived EVs containing microRNAs (miRNAs) in the CSF were found to decline during the natural aging process (99). Ablation of NSCs in young mice using genetic targeting was found to advance the aging phenotype, leading to a shortened lifespan and a decline in EVs containing miRNAs within the CSF. Interestingly, there was no specific miRNA identified to cause these changes, instead over 20 miRNA species derived from NSC-EVs were found to substantially decrease in the CSF of aging mice (99). This work suggests that the natural process of physiological aging is partially controlled by the release of exosomal miRNAs from NSC niches, and that with age, the secretome of NSCs is impacted.

Furthermore, induction of cellular senescence in NSCs and the development of the SASP would most likely compound the loss of homeostatic function, including alterations in cell cycling, response to appropriate differentiation, and a pro-inflammatory phenotype. Future studies need to provide a deeper analysis and mechanistic understanding of the NSC secretome as it relates to cellular state, organismal age, and disease status. This will help identify the critical changes that occur with age and disease-status and the impact of the secretome on both the local, and distant, tissue microenvironment.

b. Local soluble factors as regulators of NSC aging

The specialized microenvironment of the NSC niche receives and integrates converging extracellular signals arriving from the larger and more heterogenous macroenvironment (i.e., beyond the boundaries of the niche) that regulates NSC states of quiescence, proliferation, and differentiation (**Figure 2**). Additionally, the aging brain of both humans and rodents (94) accumulates senescent cells that can negatively affect the functioning of the niche microenvironment, which negatively impacts NSC turnover as well as their beneficial role in post-injury regeneration.

Genetic and chemical ablation of senescent cells increases lifespan and provokes a delay in the onset and accumulation of age-associated disorders in mice (100, 101), such as cognitive dysfunction (102). One mechanism through which senescent cells can negatively impact the local microenvironment is through the SASP, which can be triggered by DNA damage and mitochondrial dysfunction (15, 103, 104). Normally, the SASP regulates the removal of senescent cells via local macrophages, which provides beneficial effects (105, 106). However, the chronic exposure of cells to SASP factors reinforces the senescence phenotype and exacerbates cell-intrinsic changes, impacting the surrounding healthy tissue of the NSC niche and inducing further dysfunction. SASP can also lead to a phenomenon known as paracrine senescence, where secreted senescence factors can induce senescence of neighboring cells (107).

Among the many factors secreted by senescent cells, high mobility group box proteins (HMGBs) are under active study due to their known roles in transcriptionally regulating the intiation of the SASP (108). For example, HMGB2 has been found to orchestrate the chromatin organization of specific SASP gene loci thereby promoting their expression as observed in oncogene-induced senescent cells (109). Additionally, senescent human NSCs also secrete the SASP factor HMGB1 which inhibits the differentiation of oligodendrocyte progenitor cells (OPCs) *in vitro* (15, 110) (**Figure 2a**). Moreover, HMGB1 is also an alarmin (111) that alerts the immune system (110) and can promote the activation of microglia by binding to toll-like receptors -2 and -4 (**Figure 2h**) (112). Normally, non-activated microglia in young mice support neurogenesis in the hippocampus and SVZ via secretion of growth factors and cytokines (113, 114). However, the release of pro-inflammatory factors by activated primary microglia *in vitro* has been shown to reduce the proliferation and neurogenic potential of cultured murine NSCs (112). The reduction in proliferation and neurogenic potential of NSCs was further studied *in vivo* in the aged murine SVZ. Here, the inhibitory effect of the activated microglia on NSC function could be due to the expression of TNF-α, IL-1β, and IL-6, which are known to negatively effect NSCs (115) (**Figure 2b**). Furthermore, aging-induced loss of microglial phagocytosis, which is responsible for removing potentially toxic extracellular materials within the hippocampal niche, could contribute to the accumulation of debris and aggregates within the niche, disrupting NSC homeostasis (**Figure 2b**) (116, 117).

Microglia are not the only contributors to inflammaging. When exposed to plasma from aged mice, brain endothelial cells were found to express vascular adhesion molecule (VCAM1) and release TGF-β1, an inflammatory cytokine that triggers NSC apoptosis in the SVZ via TGF-β and SMAD3 signaling, leading to a reduction in NSC numbers, proliferation and differentiation (**Figure 2c**) (115) (19). In addition, astrocytes in the aged brain secrete pro-inflammatory molecules, such as IFN (118) and IL-1β, which increases VCAM1 expression in NSCs and represses them in a quiescent state (119) (**Figure 2d**).

Even the physical properties of the NSC niche are important, such as the stiffness of the ECM (120) (**Figure 2e**). Thus, SASP factors not only include pro-inflammatory molecules but also enzymes involved in ECM remodelling, such as proteinase inhibitors (121), matrix metalloproteinases (MMPs) (103), and tissue inhibitors of metalloproteinases (TIMPs) (122). Since ECM molecules function as an inert scaffold to anchor cells and regulate cell proliferation and differentiation (123) via Rho GTPases, senescent cells can indirectly affect neurogenesis via remodelling of the ECM within the NSC niches. As a matter of fact, *in vitro* studies have shown that neurogenesis is impaired when NSCs are cultured on stiff matrix (124). This has also been demonstrated using soft hydrogels to promote a neural fate of cultured adult NSCs, whereas harder gels promoted their differentiation towards a glial cell fate (125).

Overall, there are many local soluble factors that affect stem cell niches during the course of ageing. This includes factors coming from local microglia and astrocytes, and even from NSCs themselves. Interestingly, the consistent theme seen is that with age there is an increase in the secretion of inflammatory factors, which becomes chronic over time. This chronic exposure of NSCs to inflammatory factors influences their homeostatic behavior, including their ability to cycle and their ability to differentiate. Furthermore, NSCs can also become senescent with age, further promoting the secretion of inflammatory factors in niches. A better understanding on how local factors affect the stem cell niche is warranted to understand how the presence of specific factors may influence NSCs.

c. Systemic soluble factors as regulators of NSC aging

The unique positioning of the SVZ allows for the stem cell niche to be in contact with blood and CSF factors, directly influencing their behavior, including their state of activation and differentiation capabilities (126). NSCs within the niche extend a filum into the CSF and a long basal process which terminates on the leaky niche vasculature, suggesting their function in directly sensing extrinsic factors (126). As aging is associated with the development of inflammaging, the NSC niches are in contact with a multitude of inflammatory systemic factors.

One of the first examples demonstrating the capability of the extrinsic system to influence NSC niches are heterochronic parabiosis studies, where the circulatory system of a young and an aged mouse are joined. This work has unveiled positive effects on neurogenesis and proliferation in the SVZ and DG in aged mice with young blood. However, young mice joined with an aged circulatory system demonstrated decreased neurogenesis and NSC proliferation in the SVZ and DG, as well as decreased synaptic plasticity and impaired memory (17, 127). In profiling the blood and CSF of aged mice a variety of systemic factors have been identified which mediate these detrimental effects on the niches, many related to inflammatory signaling (128). In addition, analysis of human plasma proteins has also found a significant enrichment of proteins associated with diverse inflammatory processes with age, suggestive of the inflammaging hypothesis of aging (129).

The chemokine C-C motif chemokine 11 (CCL11) has been found increased in the blood of aged mice, as well as in aged human plasma and CSF, and promotes age-induced dysfunction, correlating with a decrease in neurogenesis (17). Furthermore, young mice systemically exposed to CCL11 have impaired hippocampal neurogenesis (17) (**Figure 2f**). β2-microglobulin (B2M), which is a component of major histocompatibility complex class 1 (MHC I) molecule, is another circulating factor which was found to negatively regulate the regenerative function of the aged hippocampal niche (130, 131) (**Figure 2f**). Young mice with reduced expression of MHC I (transporter associated with antigen processing 1 deficient mice) are resistant to the negative neurogenic and behavioral effects of parabiosis with aged mice (130).

Parabiosis experiments have also led to the identification of several factors found to have a restorative effect on the NSC niche in aged animals (127, 132, 133). Growth differentiation factor 11 (GDF11), a circulating bone morphogenic protein expressed

higher in young animals, was found to have positive effects on aged SVZ NSCs, including increased proliferation and neuronal differentiation (127). Analysis of young mouse hippocami and plasma identified an enrichment of tissue inhibitor of metalloproteinases 2 (TIMP-2), which decreases with age (132). When injected systemically into aged mice TIMP-2 improves synaptic plasticity and cognition, however there was no change in neurogenesis, suggesting a different mechanism of action in improving behavior of aged mice (132). This could include altering the niche ECM or acting as an anti-inflammatory molecule (134).

In addition to systemic proteins associated with plasma there have been several factors produced by the choroid plexus in the CSF that can convey age-related changes onto the SVZ niche (135) (**Figure 2g**). Aged mice have a reduction in bone morphogenic protein 5 (BMP5) and insulin-like growth factor 1 (IGF1) in the lateral ventricle choroid plexus, which both were found to promote NSC proliferation (135). Furthermore, with age there is increased signaling of type I IFN response from the choroid plexus which was found to inhibit hippocampal neurogenesis and decrease cognitive function in aged mice (136).

The SVZ niche is uniquely positioned to receive signals from the pheriphery, including blood and CSF factors. Additionally, these signals coming from the periphery have been shown to play an important role on the behavior of NSCs in the niche. In aging, inflammation takes center stage, especially in the pheriphery where these inflammatory factors can enter the brain stem cell niches. Early research has shown the detrimental effect of parabiosis in young animals paired with old ones on the NSC niche. Here, aged blood inhibits homeostatic behaviors, such as cell cycling and differentiation. Aged blood has been found to contain a multitude of inflammatory factors, which are just beginning to be uncovered.

d. Brain aging and the immune system

Until recently, the CNS was believed to be an immune-privileged site, due to a lack of inflammatory response upon introduction of antigens (137). However, within the past years peripheral immune cells have been found to have essential roles associated with neuroprotection, brain plasticity, and repair (137). In age and neurodegenerative disease, peripheral immune cells may play a pathological role. Aging and disease are associated with an impaired BBB, which creates a more permissive environment for the entrance of peripheral immune cells, such as T cells. Single cell RNA sequencing

of aged mouse SVZ identified an enrichment of T cells, also identified in the aged human brain (138-140). The infiltrating CD8+ T cells in the aged mouse NSC niche were found to secrete high amounts of IFN-γ, causing impaired NSC proliferation and impairing differentiation, highlighting their role in age-related neurogenic decline (**Figure 2h**) (139). Interestingly, the T cells identified in the aged mouse brain are clonally expanded, compared to T cells found in old blood, suggesting that they may be attracted to specific antigens and chemokines in the aged SVZ (139). Natural killer (NK) cells, a key subset of innate lymphoid cells, were detected in the DG niche in aged humans and mice, associated with senescent NSCs (141). Interestingly, senescent neuroblasts in the DG niche were found to secrete IL-27 reinforcing NK cell expansion and activation (141) (**Figure 2h**). Genetic depletion of the NK cells, using Rag-/- mice, improved neurogenesis and cognitive function in aged animals (141). Furthermore, in aged mice myeloid cell bioenergetics are suppressed in response to increased signaling of prostaglandin E2 (PGE2), which is a major modulator of inlammation. This signaling promotes an energy-deficient state further driving the pro-inflammatory aged phenotype, which in turn was found to promote age-induced cognitive impairments (142). Interestingly, knockdown of the PGE2 receptor, EP2, in myeloid cells prevents age-related decline in the hippocampus. This included improvement in long-term potentiation, a correlate of learning and memory, As well as improved performation in spatial memory tasks (142). Overall, most data suggests that persistent inflammation in NSC niches from extrinsic factors coming from the CSF, blood, or from infiltrating immune cells, is detrimental to the proliferative and differentiation capacity therefore causing impaired behavior associated with aging.

e. Immune senescence and NSC niche

Aging of the immune system, known as immunosenescence, has been found to contribute to whole body aging, including expression of increased aging markers, such as $p16^{Ink4a}$, a cell cycle inhibitor, in the brain (143). Furthermore, immunosenesence leads to increased secretion of inflammatory cytokines in the blood, promoting further “inflammaging” (144). Senescent immune cells, including T cells and macrophages, may infiltrate the niche, secrete pro-inflammatory SASP molcules and promote paracrine senescence on SVZ NSCs, impairing their normal functioning (145, 146). Further work understanding the role of the aged immune system in brain stem cell aging is warranted.

4. Conclusion

Within the last two decades, major scientific interest has led to a groundswell of findings regarding the vitally important roles that NSCs contribute to in the developing brain. Further, we have made significant advances in understanding what happens to this unique cell population in adulthood as well as during neurodegenerative disease and, more recently, organismal aging. What we have gleaned is that the activity and function of NSCs in the adult brain is highly dependent not only on signals from the local microenvironment of the niche and systemic factors coming from the blood and CSF, but also from peripheral immune cells of the systemic circulation and CNS-resident neurons, microglia, and astrocytes. The systemic regulation is even more apparent when discussing NSCs in the context of disease and aging wherein significant changes in the levels of circulating cytokines and chemokines (e.g. inflammaging) can have drastic consequences on the normal, homeostatic functions of NSCs. As new and exciting findings regarding the function of NSCs in the adult brain continue to be investigated, we are on the precipice of entering a new age in NSC research. However, a challenge to the field currently, which is also a matter of intense debate, is to what extent NSCs exist and function in the adult human brain. There is strong evidence for their existence in the adult brain (9), however a consensus must be reached so as to provide a foundation for future work to build from when investigating their contribution to the normal functioning not only of the niche, but rather the brain as a whole. The field is primed to make this next step and continued studies will only strengthen the claims of the necessary importance of NSCs not only during development but also throughtout the life of an organism. Work investigating how natural aging and disease states can affect the NSC niches, as well as the surrounding brain microenvironment, will be important to understanding if these cells can be harnessed to promote brain resilience or if they may be the cause of brain inflammation. Pairing of new sequencing technologies using post-mortem human brain tissue and mechanistic studies will help us understand the signaling modalities of these unique cells.

5. Figure Legends

Figure 1. Extrinsic soluble factors regulate the interaction between NSCs and immune cells during neuroinflammation in the CNS. The impact of myeloid cells on NSC function can have positive or negative effects. (**a**) LPS-stimulated microglia influence NSC behavior through the release of cytokines (IL-1β, TNFα, IL-6, NO) that lead to decreased proliferation, migration, and differentiation of NSCs. Whereas (**b**) alternative (anti-inflammatory) activation of microglia by stimulation with the cytokine IL-4 leads to increased neurogenesis and oligodendrogenesis partly through the release of IGF and other (unknown) factors. Conversely, the NSC secretome is known to modify the phenotype of inflammatory immune cells through various routes. Both human and murine NSCs can reduce the inflammatory phenotype of persistently activated macrophages through (**c**) the paracrine release of PGE_2 or the release of functional mitochondria encapsulated in EVs, which restores aberrant mitochondrial function in macrophages that reduces pro-inflammatory cytokine production and helps to resolve neuroinflammation. (**d**) NSC-derived EVs also contribute to immune suppression by maintaining the anti-inflammatory status of local immune cells through CD63 and CD81 mediated signalling. NSCs have also been found to (**e**) secrete soluble factors (NO, IL-10, PGE_2, TGFβ) that interact with activated T cells to decrease their proliferation capacity and reduce their pro-inflammatory status. Dashed lines indicate extrinsic regulators of NSC function. Solid lines indicate NSC regulation of cellular functions. Green arrow is positive effects and red arrow is negative effects. Abbreviations: neural stem cells (NSCs), unknown factors (???), lipopolysaccharide (LPS), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10), nitric oxide (NO), tumour necrotic factor alpha (TNFα), insulin-like growth factor 1 (IGF-1), extracellular vesicles (EVs), prostaglandin E2 (PGE_2), transforming growth factor beta 2 (TGFβ-2).

Figure 2. Schematic representation of the main described signalling mechanisms leading to cellular dysfunction in the aged stem cell niches. NSCs in the aged niches engage in complex bidirectional signaling processes with the surrounding microenvironment. (**a**) Senescent NSCs secreting the SASP factor HMGB1 which signals to OPCs. Advancing age leads to (**b**) activated microglia that show increased release of cytokines into the microenvironment that negatively regulates NSCs. Microglia also undergo age-induced loss of phagocytosis which can

lead to the accumulation of toxic cellular debris. (**c**) Plasma from aged mice alters the expression of VCAM1 on endothelial cells and increases expression of TGFβ1 which has negative effects on NSCs. Additionally, in the aged brain (**d**) astrocytes secrete the pro-inflammatory molecules IFN and IL-1β that result in increased VCAM1 expression on NSCs. The stiffness of the brain also changes, (**e**) which alters the composition of the extracellular matrix to influence NSC functions. The proximity with both the (**f**) blood and (**g**) cerebrospinal fluid places NSCs at the intersection of systemic signal integration. Lastly, (**h**) increased infiltration of peripheral T cells and NK cells into the aged brain leads to a bidirectional signaling axis with NSCs. NSCs also communicate with microglia via the alarmin HMGB1 to direct their activation. The overall result of these changes is an age-associated decline in NSC self-renewal, proliferation, activation, regenerative and neurogenic potentials. Green arrows indicate an increase and red arrows indicate a decrease. Dashed lines indicate extrinsic regulators of NSC function. Solid lines indicate NSC regulation of cellular functions. Abbreviations: neural stem cells (NSCs), vascular cell adhesion molecule (VCAM1), transforming growth factor β1 (TGFβ1), β2-microglobulin (B2M), chemokine C-C motif chemokine 11 (CCL11), growth factors (GFs), interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor-α (TNFα), oligodendrocyte progenitor cell (OPC), high mobility group box proteins (HMGBs**)**, interleukin-27 (IL-27), interferon γ (IFN-γ), interleukin-15 (IL-15), chemokine C-C motif chemokine 3 (CCL3), extracellular matrix (ECM), insulin-like growth factor 1 (IGF-1), bone morphogenic protein 5 (BMPs), type I interferon (IFN-I), cerebrospinal fluid (CSF).

6. References

1. J. C. Conover, R. Q. Notti, The neural stem cell niche. *Cell Tissue Res* **331**, 211-224 (2008).
2. S. Pfaff, S. Shaham, Development of neurons and glia. *Curr Opin Neurobiol* **23**, 901-902 (2013).
3. R. Lin, L. Iacovitti, Classic and novel stem cell niches in brain homeostasis and repair. *Brain Res* **1628**, 327-342 (2015).
4. A. Paul, Z. Chaker, F. Doetsch, Hypothalamic regulation of regionally distinct adult neural stem cells and neurogenesis. *Science* **356**, 1383-1386 (2017).
5. J. Li, Y. Tang, D. Cai, IKKβ/NF-κB disrupts adult hypothalamic neural stem cells to mediate a neurodegenerative mechanism of dietary obesity and pre-diabetes. *Nat Cell Biol* **14**, 999-1012 (2012).
6. V. Silva-Vargas, E. E. Crouch, F. Doetsch, Adult neural stem cells and their niche: a dynamic duo during homeostasis, regeneration, and aging. *Curr Opin Neurobiol* **23**, 935-942 (2013).
7. M. Kudo, K. Ohta, Regulation of the Brain Neural Niche by Soluble Molecule Akhirin. *J Dev Biol* **9** (2021).
8. D. K. Ma, M. A. Bonaguidi, G. L. Ming, H. Song, Adult neural stem cells in the mammalian central nervous system. *Cell Res* **19**, 672-682 (2009).
9. J. Terreros-Roncal *et al.*, Impact of neurodegenerative diseases on human adult hippocampal neurogenesis. *Science* 10.1126/science.abl5163, eabl5163 (2021).
10. D. Franjic *et al.*, Transcriptomic taxonomy and neurogenic trajectories of adult human, macaque, and pig hippocampal and entorhinal cells. *Neuron* **110**, 452-469.e414 (2022).
11. M. Boldrini *et al.*, Human Hippocampal Neurogenesis Persists throughout Aging. *Cell Stem Cell* **22**, 589-599.e585 (2018).
12. E. P. Moreno-Jiménez *et al.*, Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. *Nat Med* **25**, 554-560 (2019).
13. M. K. Tobin *et al.*, Human Hippocampal Neurogenesis Persists in Aged Adults and Alzheimer's Disease Patients. *Cell Stem Cell* **24**, 974-982.e973 (2019).
14. J. P. Andreotti *et al.*, Neural stem cell niche heterogeneity. *Semin Cell Dev Biol* **95**, 42-53 (2019).
15. A. M. Nicaise *et al.*, Cellular senescence in progenitor cells contributes to diminished remyelination potential in progressive multiple sclerosis. *Proc Natl Acad Sci U S A* **116**, 9030-9039 (2019).
16. S. Pluchino *et al.*, Persistent inflammation alters the function of the endogenous brain stem cell compartment. *Brain* **131**, 2564-2578 (2008).
17. S. A. Villeda *et al.*, The ageing systemic milieu negatively regulates neurogenesis and cognitive function. *Nature* **477**, 90-94 (2011).

18. P. A. Carpentier, T. D. Palmer, Immune influence on adult neural stem cell regulation and function. *Neuron* **64**, 79-92 (2009).

19. H. Yousef *et al.*, Aged blood impairs hippocampal neural precursor activity and activates microglia via brain endothelial cell VCAM1. *Nat Med* **25**, 988-1000 (2019).

20. M. Colonna, O. Butovsky, Microglia Function in the Central Nervous System During Health and Neurodegeneration. *Annu Rev Immunol* **35**, 441-468 (2017).

21. Y. Z. Wang, J. M. Plane, P. Jiang, C. J. Zhou, W. Deng, Concise review: Quiescent and active states of endogenous adult neural stem cells: identification and characterization. *Stem Cells* **29**, 907-912 (2011).

22. I. Decimo, F. Bifari, M. Krampera, G. Fumagalli, Neural stem cell niches in health and diseases. *Curr Pharm Des* **18**, 1755-1783 (2012).

23. S. J. Morrison, A. C. Spradling, Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. *Cell* **132**, 598-611 (2008).

24. E. Llorens-Bobadilla *et al.*, Single-Cell Transcriptomics Reveals a Population of Dormant Neural Stem Cells that Become Activated upon Brain Injury. *Cell stem cell* **17**, 329-340 (2015).

25. G. Belenguer *et al.*, Adult Neural Stem Cells Are Alerted by Systemic Inflammation through TNF-alpha Receptor Signaling. *Cell stem cell* **28**, 285-299 e289 (2021).

26. D. Kawaguchi, S. Furutachi, H. Kawai, K. Hozumi, Y. Gotoh, Dll1 maintains quiescence of adult neural stem cells and segregates asymmetrically during mitosis. *Nat Commun* **4**, 1880 (2013).

27. A. Lavado, G. Oliver, Jagged1 is necessary for postnatal and adult neurogenesis in the dentate gyrus. *Dev Biol* **388**, 11-21 (2014).

28. C. Sood, V. T. Justis, S. E. Doyle, S. E. Siegrist, Notch signaling regulates neural stem cell quiescence entry and exit in Drosophila. *Development* **149** (2022).

29. N. Urban, I. M. Blomfield, F. Guillemot, Quiescence of Adult Mammalian Neural Stem Cells: A Highly Regulated Rest. *Neuron* **104**, 834-848 (2019).

30. J. A. Smith *et al.*, Stem Cell Therapies for Progressive Multiple Sclerosis. *Frontiers in cell and developmental biology* **9**, 1751 (2021).

31. E. J. Huang, L. F. Reichardt, Neurotrophins: roles in neuronal development and function. *Annu Rev Neurosci* **24**, 677-736 (2001).

32. E. J. Huang, L. F. Reichardt, Neurotrophins: roles in neuronal development and function. *Annual review of neuroscience* **24**, 677-736 (2001).

33. D. Park *et al.*, Improvement of cognitive function and physical activity of aging mice by human neural stem cells over-expressing choline acetyltransferase. *Neurobiology of Aging* **34**, 2639-2646 (2013).

34. B. Mendes-Pinheiro *et al.*, Secretome of undifferentiated neural progenitor cells induces histological and motor improvements in a rat model of Parkinson's disease. *Stem cells translational medicine* **7**, 829-838 (2018).

35. T. Kondo *et al.*, Focal transplantation of human iPSC-derived glial-rich neural progenitors improves lifespan of ALS mice. *Stem cell reports* **3**, 242-249 (2014).

36. C. Hicks *et al.*, In vivo and in vitro characterization of the angiogenic effect of CTX0E03 human neural stem cells. *Cell transplantation* **22**, 1541-1552 (2013).

37. I.-S. Lee *et al.*, Human neural stem cells alleviate Alzheimer-like pathology in a mouse model. *Molecular neurodegeneration* **10**, 1-16 (2015).

38. N. Romanyuk *et al.*, Beneficial effect of human induced pluripotent stem cell-derived neural precursors in spinal cord injury repair. *Cell transplantation* **24**, 1781-1797 (2015).

39. T. Kondo *et al.*, Focal transplantation of human iPSC-derived glial-rich neural progenitors improves lifespan of ALS mice. *Stem Cell Reports* **3**, 242-249 (2014).

40. N. Romanyuk *et al.*, Beneficial Effect of Human Induced Pluripotent Stem Cell-Derived Neural Precursors in Spinal Cord Injury Repair. *Cell Transplant* **24**, 1781-1797 (2015).

41. C. M. Willis, A. M. Nicaise, L. Peruzzotti-Jametti, S. Pluchino, The neural stem cell secretome and its role in brain repair. *Brain Res* **1729**, 146615 (2020).

42. C. Tang *et al.*, Neural stem cells behave as a functional niche for the maturation of newborn neurons through the secretion of PTN. *Neuron* **101**, 32-44 (2019).

43. K. I. Mosher *et al.*, Neural progenitor cells regulate microglia functions and activity. *Nature neuroscience* **15**, 1485-1487 (2012).

44. S. Voet, M. Prinz, G. van Loo, Microglia in Central Nervous System Inflammation and Multiple Sclerosis Pathology. *Trends Mol Med* **25**, 112-123 (2019).

45. C. T. Ekdahl, Z. Kokaia, O. Lindvall, Brain inflammation and adult neurogenesis: the dual role of microglia. *Neuroscience* **158**, 1021-1029 (2009).

46. M. L. Monje, S. Mizumatsu, J. R. Fike, T. D. Palmer, Irradiation induces neural precursor-cell dysfunction. *Nature medicine* **8**, 955-962 (2002).

47. M. L. Monje, H. Toda, T. D. Palmer, Inflammatory blockade restores adult hippocampal neurogenesis. *Science* **302**, 1760-1765 (2003).

48. R. Covacu, L. Brundin, Effects of Neuroinflammation on Neural Stem Cells. *Neuroscientist* **23**, 27-39 (2017).

49. O. Butovsky *et al.*, Microglia activated by IL-4 or IFN-gamma differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. *Molecular and cellular neurosciences* **31**, 149-160 (2006).

50. T. Ben-Hur *et al.*, Effects of proinflammatory cytokines on the growth, fate, and motility of multipotential neural precursor cells. *Mol Cell Neurosci* **24**, 623-631 (2003).

51. E. Cacci, M. A. Ajmone-Cat, T. Anelli, S. Biagioni, L. Minghetti, In vitro neuronal and glial differentiation from embryonic or adult neural precursor cells are differently affected by chronic or acute activation of microglia. *Glia* **56**, 412-425 (2008).

52. B. P. Carreira *et al.*, Nitric oxide from inflammatory origin impairs neural stem cell proliferation by inhibiting epidermal growth factor receptor signaling. *Front Cell Neurosci* **8**, 343 (2014).

53. I. Goshen *et al.*, Brain interleukin-1 mediates chronic stress-induced depression in mice via adrenocortical activation and hippocampal neurogenesis suppression. *Mol Psychiatry* **13**, 717-728 (2008).

54. S. Spulber, M. Oprica, T. Bartfai, B. Winblad, M. Schultzberg, Blunted neurogenesis and gliosis due to transgenic overexpression of human soluble IL-1ra in the mouse. *Eur J Neurosci* **27**, 549-558 (2008).

55. Y. P. Liu, H. I. Lin, S. F. Tzeng, Tumor necrosis factor-alpha and interleukin-18 modulate neuronal cell fate in embryonic neural progenitor culture. *Brain Res* **1054**, 152-158 (2005).

56. Y. Sun *et al.*, Neuronal nitric oxide synthase and ischemia-induced neurogenesis. *J Cereb Blood Flow Metab* **25**, 485-492 (2005).

57. A. Cheng, S. Wang, J. Cai, M. S. Rao, M. P. Mattson, Nitric oxide acts in a positive feedback loop with BDNF to regulate neural progenitor cell proliferation and differentiation in the mammalian brain. *Dev Biol* **258**, 319-333 (2003).

58. B. Moreno-López *et al.*, Nitric oxide is a physiological inhibitor of neurogenesis in the adult mouse subventricular zone and olfactory bulb. *J Neurosci* **24**, 85-95 (2004).

59. R. Covacu *et al.*, Nitric oxide exposure diverts neural stem cell fate from neurogenesis towards astrogliogenesis. *Stem Cells* **24**, 2792-2800 (2006).

60. J. H. Song *et al.*, Interferon gamma induces neurite outgrowth by up-regulation of p35 neuron-specific cyclin-dependent kinase 5 activator via activation of ERK1/2 pathway. *J Biol Chem* **280**, 12896-12901 (2005).

61. G. Wong, Y. Goldshmit, A. M. Turnley, Interferon-gamma but not TNF alpha promotes neuronal differentiation and neurite outgrowth of murine adult neural stem cells. *Exp Neurol* **187**, 171-177 (2004).

62. R. Baron *et al.*, IFN-gamma enhances neurogenesis in wild-type mice and in a mouse model of Alzheimer's disease. *Faseb j* **22**, 2843-2852 (2008).

63. P. Thored *et al.*, Long-term accumulation of microglia with proneurogenic phenotype concomitant with persistent neurogenesis in adult subventricular zone after stroke. *Glia* **57**, 835-849 (2009).

64. S. Pluchino, C. Cossetti, How stem cells speak with host immune cells in inflammatory brain diseases. *Glia* **61**, 1379-1401 (2013).

65. J. Yang *et al.*, Adult neural stem cells expressing IL-10 confer potent immunomodulation and remyelination in experimental autoimmune encephalitis. *J Clin Invest* **119**, 3678-3691 (2009).

66. D. De Feo *et al.*, Neural precursor cell–secreted TGF-β2 redirects inflammatory monocyte-derived cells in CNS autoimmunity. *J Clin Invest* **127**, 3937-3953 (2017).

67. W. Cao *et al.*, Leukemia inhibitory factor inhibits T helper 17 cell differentiation and confers treatment effects of neural progenitor cell therapy in autoimmune disease. *Immunity* **35**, 273-284 (2011).

68. L. Wang *et al.*, Neural stem/progenitor cells modulate immune responses by suppressing T lymphocytes with nitric oxide and prostaglandin E2. *Experimental neurology* **216**, 177-183 (2009).

69. D. Drago *et al.*, Metabolic determinants of the immune modulatory function of neural stem cells. *Journal of neuroinflammation* **13**, 1-18 (2016).

70. C. M. Willis, A. M. Nicaise, L. Peruzzotti-Jametti, S. Pluchino, The neural stem cell secretome and its role in brain repair. *Brain research* **1729**, 146615 (2020).

71. A. Vogel, R. Upadhya, A. K. Shetty, Neural stem cell derived extracellular vesicles: Attributes and prospects for treating neurodegenerative disorders. *EBioMedicine* **38**, 273-282 (2018).

72. M. Colombo, G. Raposo, C. Théry, Biogenesis, Secretion, and Intercellular Interactions of Exosomes and Other Extracellular Vesicles. *Annual Review of Cell and Developmental Biology* **30**, 255-289 (2014).

73. H. Valadi *et al.*, Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nature Cell Biology* **9**, 654-659 (2007).

74. C. Frühbeis, S. Helmig, S. Tug, P. Simon, E. M. Krämer-Albers, Physical exercise induces rapid release of small extracellular vesicles into the circulation. *J Extracell Vesicles* **4**, 28239 (2015).

75. C. M. Willis *et al.*, A Refined Bead-Free Method to Identify Astrocytic Exosomes in Primary Glial Cultures and Blood Plasma. *Front Neurosci* **11**, 335 (2017).

76. L. Stevanato, L. Thanabalasundaram, N. Vysokov, J. D. Sinden, Investigation of Content, Stoichiometry and Transfer of miRNA from Human Neural Stem Cell Line Derived Exosomes. *PLoS One* **11**, e0146353 (2016).

77. S. Chettimada *et al.*, Exosome markers associated with immune activation and oxidative stress in HIV patients on antiretroviral therapy. *Scientific Reports* **8**, 7227 (2018).

78. J. Lv *et al.*, MicroRNA let-7c-5p improves neurological outcomes in a murine model of traumatic brain injury by suppressing neuroinflammation and regulating microglial activation. *Brain Res* **1685**, 91-104 (2018).

79. E. D. Ponomarev, T. Veremeyko, N. Barteneva, A. M. Krichevsky, H. L. Weiner, MicroRNA-124 promotes microglia quiescence and suppresses EAE by deactivating macrophages via the C/EBP-α-PU.1 pathway. *Nat Med* **17**, 64-70 (2011).

80. B. Bian *et al.*, Exosomes derived from neural progenitor cells preserve photoreceptors during retinal degeneration by inactivating microglia. *J Extracell Vesicles* **9**, 1748931-1748931 (2020).

81. C. Zhao *et al.*, MicroRNA let-7b regulates neural stem cell proliferation and differentiation by targeting nuclear receptor TLX signaling. *Proceedings of the National Academy of Sciences* **107**, 1876 (2010).

82. N. Iraci *et al.*, Extracellular vesicles are independent metabolic units with asparaginase activity. *Nature chemical biology* **13**, 951-955 (2017).

83. L. Peruzzotti-Jametti *et al.*, Macrophage-derived extracellular succinate licenses neural stem cells to suppress chronic neuroinflammation. *Cell Stem Cell* **22**, 355-368 (2018).

84. L. Peruzzotti-Jametti *et al.*, Neural stem cells traffic functional mitochondria via extracellular vesicles. *PLoS Biol* **19**, e3001166 (2021).

85. R. L. Webb *et al.*, Human Neural Stem Cell Extracellular Vesicles Improve Tissue and Functional Recovery in the Murine Thromboembolic Stroke Model. *Translational Stroke Research* **9**, 530-539 (2018).

86. S. Pluchino *et al.*, Neurosphere-derived multipotent precursors promote neuroprotection by an immunomodulatory mechanism. *Nature* **436**, 266-271 (2005).

87. N. Fainstein *et al.*, Neural precursor cells inhibit multiple inflammatory signals. *Molecular and Cellular Neuroscience* **39**, 335-341 (2008).

88. M. Cusimano *et al.*, Transplanted neural stem precursor cells instruct phagocytes and reduce secondary tissue damage in the injured spinal cord. *Brain : a journal of neurology* **135**, 447-460 (2012).

89. M. Cusimano *et al.*, Transplanted neural stem/precursor cells instruct phagocytes and reduce secondary tissue damage in the injured spinal cord. *Brain* **135**, 447-460 (2012).

90. L. Peruzzotti-Jametti, S. Pluchino, Targeting mitochondrial metabolism in neuroinflammation: towards a therapy for progressive multiple sclerosis. *Trends in molecular medicine* **24**, 838-855 (2018).

91. C. M. Willis *et al.*, Harnessing the Neural Stem Cell Secretome for Regenerative Neuroimmunology. *Frontiers in cellular neuroscience* **14**, 590960-590960 (2020).

92. A. M. Nicaise, C. M. Willis, S. J. Crocker, S. Pluchino, Stem Cells of the Aging Brain. *Front Aging Neurosci* **12**, 247 (2020).

93. C. Franceschi *et al.*, Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci* **908**, 244-254 (2000).

94. D. J. Baker, R. C. Petersen, Cellular senescence in brain aging and neurodegenerative diseases: evidence and perspectives. *J Clin Invest* **128**, 1208-1216 (2018).

95. F. H. Gage, Mammalian neural stem cells. *Science* **287**, 1433-1438 (2000).

96. A. M. Nicaise, C. M. Willis, S. J. Crocker, S. Pluchino, Stem Cells of the Aging Brain. *Front Aging Neurosci* **12**, 247-247 (2020).

97. C. M. Dong *et al.*, A stress-induced cellular aging model with postnatal neural stem cells. *Cell Death & Disease* **5**, e1116-e1116 (2014).

98. M. A. Storer *et al.*, Interleukin-6 Regulates Adult Neural Stem Cell Numbers during Normal and Abnormal Post-natal Development. *Stem Cell Reports* **10**, 1464-1480 (2018).

99. Y. Zhang *et al.*, Hypothalamic stem cells control ageing speed partly through exosomal miRNAs. *Nature* **548**, 52-57 (2017).

100. D. J. Baker *et al.*, Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. *Nature* **479**, 232-236 (2011).

101. M. J. Yousefzadeh *et al.*, Tissue specificity of senescent cell accumulation during physiologic and accelerated aging of mice. *Aging Cell* **19**, e13094 (2020).

102. M. Ogrodnik *et al.*, Whole-body senescent cell clearance alleviates age-related brain inflammation and cognitive impairment in mice. *Aging Cell* **20**, e13296 (2021).

103. J.-P. Coppé, P.-Y. Desprez, A. Krtolica, J. Campisi, The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annual review of pathology* **5**, 99-118 (2010).

104. J. P. Coppé *et al.*, Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol* **6**, 2853-2868 (2008).

105. Y. Ovadya *et al.*, Impaired immune surveillance accelerates accumulation of senescent cells and aging. *Nat Commun* **9**, 5435 (2018).

106. W. Xue *et al.*, Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature* **445**, 656-660 (2007).

107. J. C. Acosta *et al.*, A complex secretory program orchestrated by the inflammasome controls paracrine senescence. *Nat Cell Biol* **15**, 978-990 (2013).

108. K. Sofiadis *et al.*, HMGB1 coordinates SASP-related chromatin folding and RNA homeostasis on the path to senescence. *Mol Syst Biol* **17**, e9760 (2021).

109. K. M. Aird *et al.*, HMGB2 orchestrates the chromatin landscape of senescence-associated secretory phenotype gene loci. *J Cell Biol* **215**, 325-334 (2016).

110. A. R. Davalos *et al.*, p53-dependent release of Alarmin HMGB1 is a central mediator of senescent phenotypes. *J Cell Biol* **201**, 613-629 (2013).

111. J. Huang *et al.*, DAMPs, ageing, and cancer: The 'DAMP Hypothesis'. *Ageing Res Rev* **24**, 3-16 (2015).

112. R. Solano Fonseca *et al.*, Neurogenic Niche Microglia Undergo Positional Remodeling and Progressive Activation Contributing to Age-Associated Reductions in Neurogenesis. *Stem Cells Dev* **25**, 542-555 (2016).

113. Y. Shigemoto-Mogami, K. Hoshikawa, J. E. Goldman, Y. Sekino, K. Sato, Microglia enhance neurogenesis and oligodendrogenesis in the early postnatal subventricular zone. *J Neurosci* **34**, 2231-2243 (2014).

114. Y. Ziv, M. Schwartz, Immune-based regulation of adult neurogenesis: implications for learning and memory. *Brain Behav Immun* **22**, 167-176 (2008).

115. J. R. Pineda *et al.*, Vascular-derived TGF-β increases in the stem cell niche and perturbs neurogenesis during aging and following irradiation in the adult mouse brain. *EMBO Mol Med* **5**, 548-562 (2013).

116. J. Marschallinger *et al.*, Lipid-droplet-accumulating microglia represent a dysfunctional and proinflammatory state in the aging brain. *Nat Neurosci* **23**, 194-208 (2020).

117. J. V. Pluvinage *et al.*, CD22 blockade restores homeostatic microglial phagocytosis in ageing brains. *Nature* **568**, 187-192 (2019).

118. L. E. Clarke *et al.*, Normal aging induces A1-like astrocyte reactivity. *Proc Natl Acad Sci U S A* **115**, E1896-e1905 (2018).

119. E. Kokovay *et al.*, VCAM1 is essential to maintain the structure of the SVZ niche and acts as an environmental sensor to regulate SVZ lineage progression. *Cell Stem Cell* **11**, 220-230 (2012).

120. M. Segel *et al.*, Niche stiffness underlies the ageing of central nervous system progenitor cells. *Nature* **573**, 130-134 (2019).

121. M. Eren *et al.*, PAI-1-regulated extracellular proteolysis governs senescence and survival in Klotho mice. *Proc Natl Acad Sci U S A* **111**, 7090-7095 (2014).

122. S. Özcan *et al.*, Unbiased analysis of senescence associated secretory phenotype (SASP) to identify common components following different genotoxic stresses. *Aging (Albany NY)* **8**, 1316-1329 (2016).

123. F. Gattazzo, A. Urciuolo, P. Bonaldo, Extracellular matrix: a dynamic microenvironment for stem cell niche. *Biochim Biophys Acta* **1840**, 2506-2519 (2014).

124. A. J. Keung, E. M. de Juan-Pardo, D. V. Schaffer, S. Kumar, Rho GTPases mediate the mechanosensitive lineage commitment of neural stem cells. *Stem Cells* **29**, 1886-1897 (2011).

125. K. Saha *et al.*, Substrate modulus directs neural stem cell behavior. *Biophys J* **95**, 4426-4438 (2008).

126. M. Tavazoie *et al.*, A specialized vascular niche for adult neural stem cells. *Cell stem cell* **3**, 279-288 (2008).

127. L. Katsimpardi *et al.*, Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. *Science* **344**, 630-634 (2014).

128. J. V. Pluvinage, T. Wyss-Coray, Systemic factors as mediators of brain homeostasis, ageing and neurodegeneration. *Nature reviews. Neuroscience* **21**, 93-102 (2020).

129. B. Lehallier, M. N. Shokhirev, T. Wyss-Coray, A. A. Johnson, Data mining of human plasma proteins generates a multitude of highly predictive aging clocks that reflect different aspects of aging. *Aging cell* **19**, e13256 (2020).

130. L. K. Smith *et al.*, beta2-microglobulin is a systemic pro-aging factor that impairs cognitive function and neurogenesis. *Nature medicine* **21**, 932-937 (2015).

131. J. Rebo *et al.*, A single heterochronic blood exchange reveals rapid inhibition of multiple tissues by old blood. *Nature communications* **7**, 13363 (2016).

132. J. M. Castellano *et al.*, Human umbilical cord plasma proteins revitalize hippocampal function in aged mice. *Nature* **544**, 488-492 (2017).

133. S. A. Villeda *et al.*, Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. *Nature medicine* **20**, 659-663 (2014).

134. E. J. Lee, H. S. Kim, The anti-inflammatory role of tissue inhibitor of metalloproteinase-2 in lipopolysaccharide-stimulated microglia. *Journal of neuroinflammation* **11**, 116 (2014).

135. V. Silva-Vargas, A. R. Maldonado-Soto, D. Mizrak, P. Codega, F. Doetsch, Age-Dependent Niche Signals from the Choroid Plexus Regulate Adult Neural Stem Cells. *Cell stem cell* **19**, 643-652 (2016).

136. K. Baruch *et al.*, Aging. Aging-induced type I interferon response at the choroid plexus negatively affects brain function. *Science* **346**, 89-93 (2014).

137. T. Croese, G. Castellani, M. Schwartz, Immune cell compartmentalization for brain surveillance and protection. *Nature immunology* **22**, 1083-1092 (2021).

138. F. Erdo, L. Denes, E. de Lange, Age-associated physiological and pathological changes at the blood-brain barrier: A review. *J Cereb Blood Flow Metab* **37**, 4-24 (2017).

139. B. W. Dulken *et al.*, Single-cell analysis reveals T cell infiltration in old neurogenic niches. *Nature* **571**, 205-210 (2019).

140. D. Mrdjen *et al.*, High-Dimensional Single-Cell Mapping of Central Nervous System Immune Cells Reveals Distinct Myeloid Subsets in Health, Aging, and Disease. *Immunity* **48**, 380-395 e386 (2018).

141. W. N. Jin *et al.*, Neuroblast senescence in the aged brain augments natural killer cell cytotoxicity leading to impaired neurogenesis and cognition. *Nature neuroscience* **24**, 61-73 (2021).

142. P. S. Minhas *et al.*, Restoring metabolism of myeloid cells reverses cognitive decline in ageing. *Nature* **590**, 122-128 (2021).

143. M. J. Yousefzadeh *et al.*, An aged immune system drives senescence and ageing of solid organs. *Nature* **594**, 100-105 (2021).

144. C. Lopez-Otin, M. A. Blasco, L. Partridge, M. Serrano, G. Kroemer, The hallmarks of aging. *Cell* **153**, 1194-1217 (2013).

145. M. Deleidi, M. Jaggle, G. Rubino, Immune aging, dysmetabolism, and inflammation in neurological diseases. *Front Neurosci* **9**, 172 (2015).

146. J. P. Coppé, P. Y. Desprez, A. Krtolica, J. Campisi, The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu Rev Pathol* **5**, 99-118 (2010).

Declaration of competing interests:

SP is co-founder and shareholder (>5%) of CITC Ltd.; co-founder and Non-Executive Director (NED) at asitia Therapeutics and iSTEM Therapeutics; and CEO at ReNeuron.

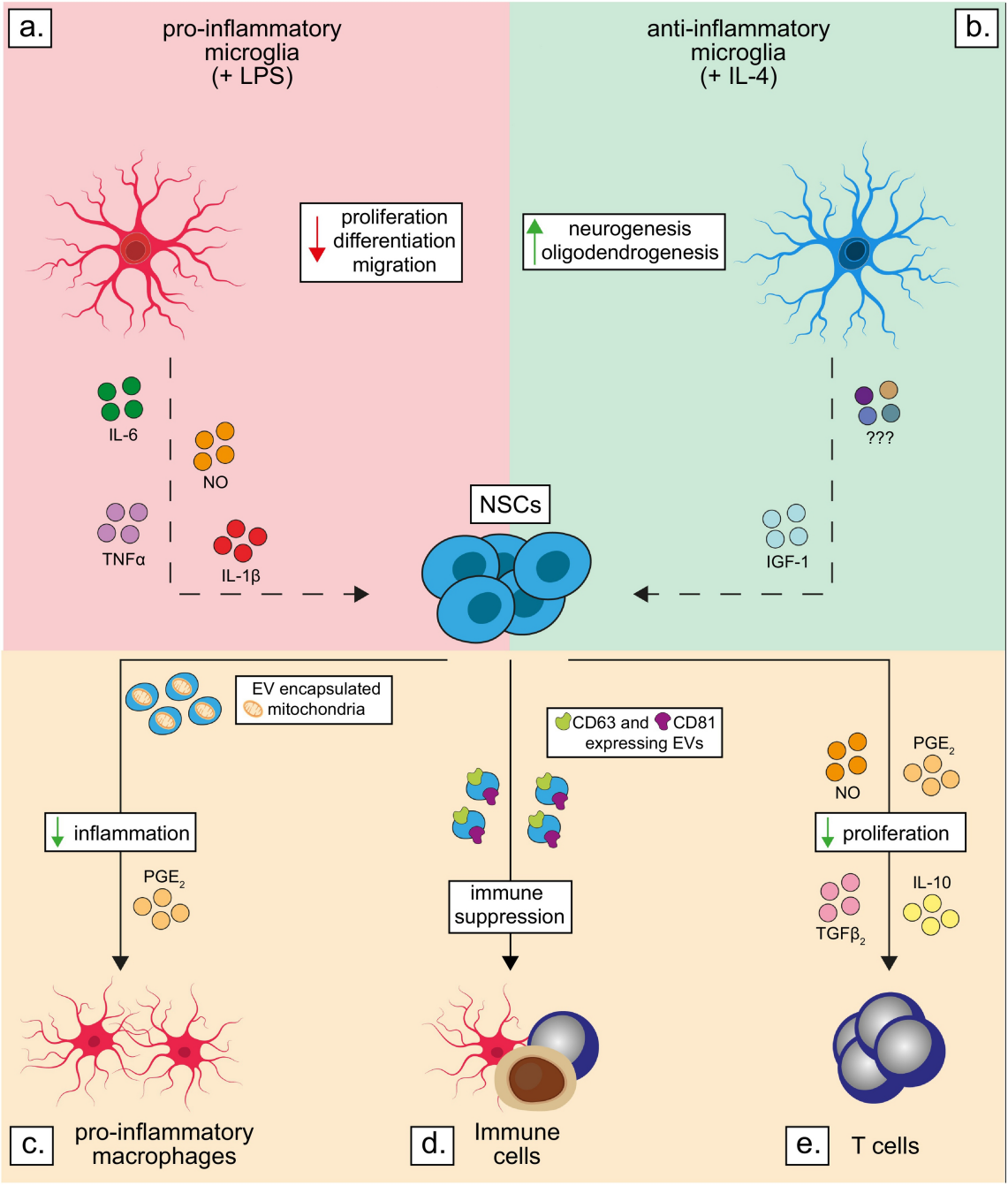
a.
pro-inflammatory
microglia
(+ LPS)
b.
anti-inflammatory
microglia
(+ IL-4)
proliferation
differentiation
migration
neurogenesis
oligodendrogenesis
IL-6
NO
TNFα
IL-1β
???
IGF-1
NSCs
EV encapsulated
mitochondria
CD63 and CD81
expressing EVs
NO
PGE2
inflammation
PGE2
immune
suppression
proliferation
IL-10
TGFβ2
c.
pro-inflammatory
macrophages
d.
Immune
cells
e.
T cells

Figure 1

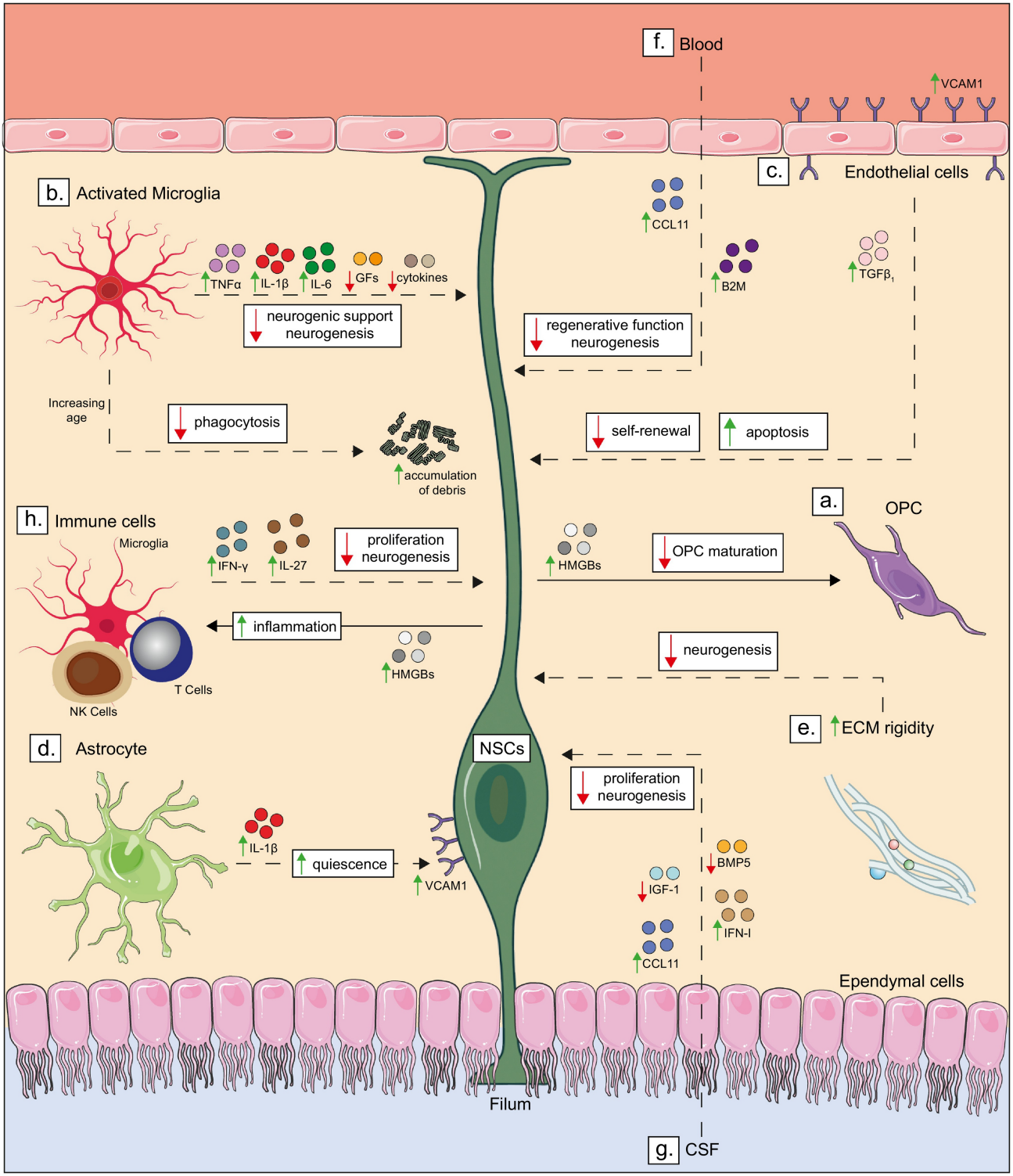
f. Blood
VCAM1
c. Endothelial cells
b. Activated Microglia
CCL11
B2M
TGFβ1
TNFα
IL-1β
IL-6
GFs
cytokines
neurogenic support neurogenesis
regenerative function neurogenesis
Increasing age
phagocytosis
accumulation of debris
self-renewal
apoptosis
h. Immune cells
Microglia
IFN-γ
IL-27
proliferation neurogenesis
HMGBs
OPC maturation
a. OPC
inflammation
HMGBs
T Cells
NK Cells
neurogenesis
e. ECM rigidity
d. Astrocyte
NSCs
proliferation neurogenesis
IL-1β
quiescence
VCAM1
BMP5
IGF-1
IFN-I
CCL11
Ependymal cells
Filum
g. CSF

Figure 2