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Joanna Zareba and Francesca Peri

Abstract

Neuronal-immune interactions are known to play crucial roles in brain development and homoeostasis. Of great relevance in this context are microglia, brain macrophages that phagocytose neurons that die during development, and many neurological disorders. Single-cell RNA sequencing methods have significantly advanced our understanding of microglial heterogeneity and transcriptional response to environmental changes. Here, we review recent work showing how microglia adopt a similar molecular signature during development and disease characterised by the expression of genes linked to phagocytosis and lipid uptake and metabolism. These studies show that in many neurodegenerative conditions, microglia accumulate cholesterols and lipid-rich debris, pointing to lipid processing and transport as promising targets for developing new therapeutical treatments against neurodegenerative disorders.

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Introduction

Microglia have long been viewed as dormant cells capable only of reacting to the presence of pathogens, but today we know that these cells are major players in the development and pathophysiology of the central nervous system (CNS) [1,2]. In fact, microglia are highly motile and survey constantly the brain parenchyma by extending and retreating dynamic cellular processes [3,4]. However, in several pathological contexts, they display a different morphology usually characterised by a more ameboid shape. To date, the regulation and function of this phenotypic switch remain unclear, along with its role in disease prognosis.

Microglia at the single-transcriptome level: disease-associated microglia (DAM), proliferative-region-associated microglia (PAM) and others

Single-cell transcriptomics approaches have shown that microglia in response to pathological conditions acquire distinct gene expression profiles providing new molecular descriptives for these cells and facilitating our understanding of their heterogeneity and phenotypic remodelling.

In 2017 Keren-Shaul et al. [5] found that microglia around beta-amyloid plaques are characterised by the upregulation of typical phagocytic genes and lipid metabolic factors such as lipoprotein lipase (LPL), and apolipoprorein E (ApoE), a cholesterol transporter. These microglia were named DAM, which stands for Disease-Associated Microglia. By using an Alzheimer's disease (AD) mouse model, the authors were able to identify two steps in the DAM phenotype acquisition; the first is characterised by the downregulation of typical microglial markers such as Cx3cr1 and P2Y12, whereas the second is defined by the upregulation of genes connected with phagocytosis and lipid metabolism [5]. Interestingly, this second step depends on the triggering receptor expressed on myeloid cells 2 (TREM2), a cell surface transmembrane receptor present only on microglia and known to enhance phagocytosis, inflammatory signalling and myeloid proliferation and survival [6,7]. TREM2 is a well-known AD risk factor in humans, and TREM2 mouse knockouts have a worse AD prognosis at later stages, suggesting a possible beneficial role for DAM in AD's [8]. Thus, these data suggest that highly phagocytic microglia capable of processing lipids might arise to fight AD progression. Interestingly DAM-like microglia are also detected in a mouse model for amyotrophic lateral sclerosis, [5], pointing to the fact that this disease-associated phenotype is not AD specific but is perhaps shared between different pathologies. Another condition where microglia have been seen to acquire a DAM-like signature is during demyelination of subcortical white matter in mice triggered by lysolecithin injection [9]. In this context, injury-responsive microglia (IRM) are similar to DAM as they downregulate P2ry12 and Cx3cr1 and upregulate Spp1, LPL and ApoE. In addition, injury-responsive microglia are also found to regulate interferon response genes, including Ifi27l2a and Cxcl10, indicating that although there are perhaps similarities in how microglia respond to disease conditions, there are

also specific adaptations to different pathologies [9]. scRNA methods have also been used in humans to profile freshly isolated microglia coming, for example, from cerebral cortex samples obtained during autopsies [10]. Here, several microglial subsets were found to have DAM-like signatures, indicating a high degree of conservation [10].

Although for many years there has been a strong interest in understanding diseased microglia, a recent study has looked at microglia that populate the developing corpus callosum and cerebellar white matter during the first postnatal week when these cells are highly phagocytic and engulf newly born oligodendrocytes. This led to the identification of PAM (proliferative-region-associated microglia), which have a transcriptome that is surprisingly similar to that of DAM's [11] characterised by the expression of typical phagocytic (macrophage scavenger receptor 1 (Msr1), leukocyte immunoglobulin like receptor B4 (Lilrb4) and so on) and lipid metabolic (NPC intracellular cholesterol transporter 2 (NPC2), ApoE, ATP binding cassette subfamily A member 1 (ABCA1), and so on) genes (Figure 1). Contrary to DAM, the PAM signature does not depend on TREM2; nevertheless, PAM and DAM are related, highly phagocytic microglial states. This suggests that although triggers can be different, there is a significant overlap in the molecular and cellular mechanisms that allow microglia to remove and process dead cells and debris in different contexts.

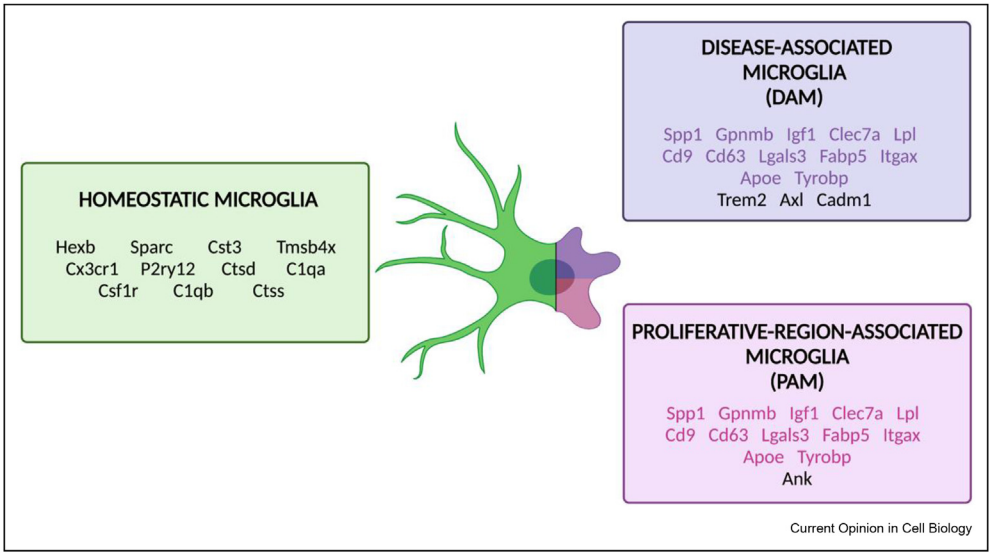
In line with this, the zebrafish presents an interesting case. *In vivo* studies have shown that these cells perform similar functions to their mammalian counterparts, such as engulfing apoptotic neurons [12] and migrating towards neuronal damage [13]. Interestingly, a study conducted in the adult fish brain has revealed that microglia can be either highly branched or more ameboid [14]. The latter group is highly phagocytic and characterised by the expression of many lipid metabolic genes previously found in DAM and PAM [14]. The presence of these 'DAM/PAM-like' microglia in the zebrafish adult brain might be a consequence of the fact that the adult fish brain is highly neurogenic.

Thus, there are striking similarities between developmental and diseased microglia. PAM and DAM might represent similar highly phagocytic states in these cells, indicating that the way microglia react to increased cell death can be comparable in development and disease. What also emerges from these and other studies is the central role of lipid metabolism in these contexts.

Fat storage in microglia

The realisation that lipids play a crucial role in microglia highlights the importance of understanding how different lipids are stored, processed and moved around these cells. It is well-known that the brain is a lipid-rich organ with a large fraction of lipids that are enriched specifically in neural tissues, indicating essential requirements for these molecules in brain architecture and functionality [15]. In this context, a quantitative cell-type resolved brain lipidomic approach has revealed that contrary, for example, to neurons that are rich in cholesterol and ceramides, microglia under physiological

Figure 1



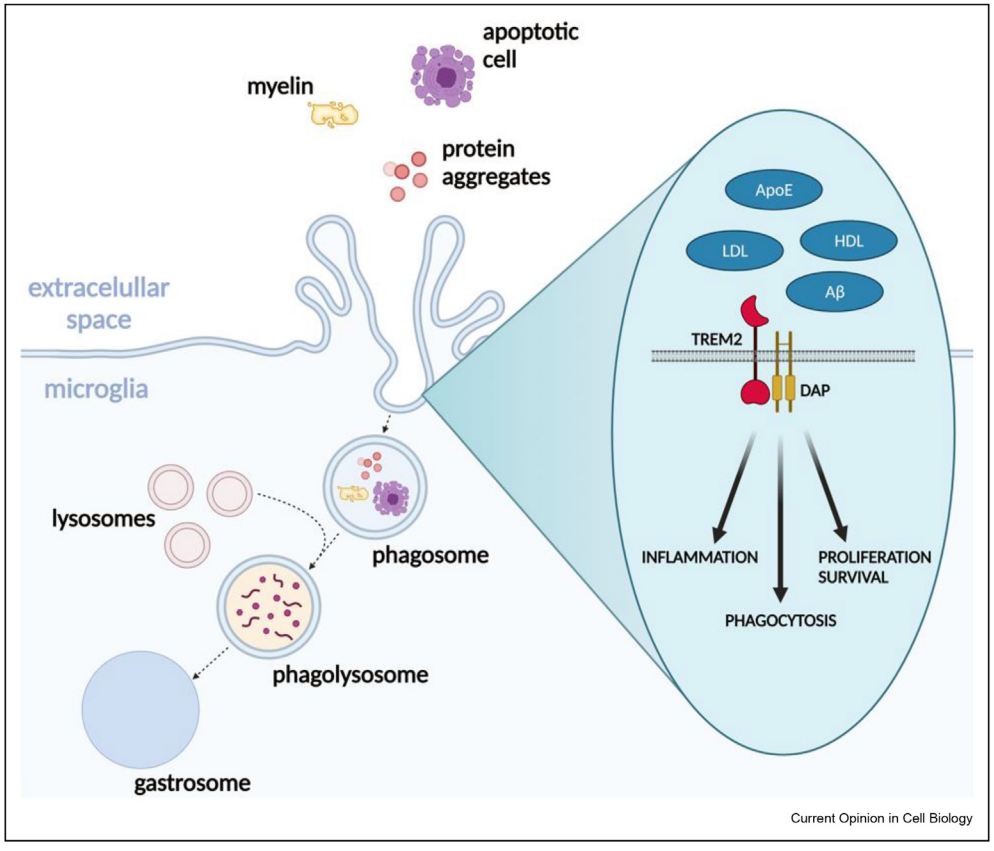
Microglia at the single-transcriptome level. Single-cell transcriptomics approaches have provided important molecular descriptives and identified several relevant microglial cellular states in different contexts. Schematic drawing of homoeostatic microglia, diseased-associated microglia (DAM) and proliferative-associated microglia (PAM) in development. List of genes that are upregulated in these different cellular states. Created with BioRender.com.

conditions exhibit high concentrations of sphingolipids such as sphingomyelin and phosphatidylglycerol [16]. Consequently, in microglia, biological processes associated with sphingolipids metabolism, including biosynthesis and transport, are also enhanced. Recently, Marschallinger et al. [17] showed that ageing microglia in the hippocampus accumulate lipid droplets. They termed these cells lipid-droplet-accumulating microglia (LDAM) and showed that they localise preferentially around amyloid plaques in the AD brain. These droplets contain glycerolipids, such as triacylglycerols and diacylglycerols, preferentially. Transcriptome analysis of lipid-droplet-high compared with lipid-droplet-low microglia revealed that LDAM could be considered proinflammatory [17]. This finding raises a typical causality dilemma of what comes first if inflammation-causing lipid droplet formation or lipid droplet accumulation induces inflammation in microglia. Finally, this study shows that microglia with an elevated lipid-droplet content are also defective in phagocytosis [17] (Figure 2). This is an interesting finding as it is in line with another study looking at how microglia digest apoptotic neurons during development [18]. Here, live microscopy and vesicular tracking have revealed the existence in fish microglia and mammalian macrophages of a previously undescribed cellular compartment that, by fusing with phagosomes, allows the efficient processing of apoptotic cells. This compartment named 'gastrosome' is a single vesicle, a unique compartment that contains membrane fragments and cellular debris [18]. Interestingly, interfering with the size and content of the gastrosome feeds back on cellular morphology, causing microglia to expand. These enlarged ameboid microglia are poor phagocytes that do not migrate towards neuronal injuries and fail to remove apoptotic neurons [18]. Thus, intracellular processing defects in microglia impact phagocytosis, indicating that efficient uptake also depends on effective digestion and processing of the engulfed material. Thus, altering intracellular processing in these cells 'at-will' could be a powerful way to modulate microglial phagocytic activity in the context of many neurodegenerative disorders.

Fat processing in microglia

The fact that genes encoding for ApoE and Lpl, for example, are upregulated in highly phagocytic microglia suggests an essential role for lipid transport and metabolism in these cells. Developmental and diseased microglia could boost the expression of these factors to support their high energy needs. In line with this, loss of

Figure 2



The phagocytic microglia. Schematic drawing of phagocytosis in microglia, highlighting the relevant cellular and molecular components. Created with BioRender.com.

lipases in microglia leads to decreased ATP production and mitochondrial defects, suggesting a role for lipid processing in energy production [19,20]. Alternatively, upregulation of PAM/DAM factors could be required to process and transport the ingested lipid-rich material. In agreement with this, in multiple sclerosis (MS) models, ApoE production increase correlates with the transport of cholesterols that accumulate upon phagocytosis of myelin debris [21]. Ageing microglia fail to upregulate ApoE, leading to the accumulation of cholesterols that forms visible crystals in these cells [21]. Many lipases play essential roles in microglia by catalysing the release of lipids from membranes and lipoproteins. Although the role of LPL in microglia remains unclear, this enzyme promotes hydrolysis of triacylglycerols and is known to colocalise with Ab plaques in Alzheimer's brains to favour uptake of Aβ [22]. Recent work from Loving et al. [23] has shown that LPL knockdown in BV-2 microglia correlates with a reduced cholesterol flux and accumulation of lipid droplets in these cells.

An interesting lipid-rich target for microglia is myelin. After internalisation, this substrate gets degraded, processed and transported within microglia (Figure 2). These steps are mediated by lipid-related pathways, initiated, for example, by the liver X receptor, a key transcriptional regulator of lipid and cholesterol metabolism [24,25]. As shown by Cantuti-Calstelvetri et al [21], this factor is crucial to prevent the accumulation of cholesterol crystals in microglia, a feature of demyelination and ageing. In their study, young mouse mutants for liver X receptor-α treated with the demyelinating agent cuprizone accumulate cholesterol crystals in microglia, have massive microglial infiltration, and fail to repair lesions. A similar phenotype is also seen in APoE mutants in response to demyelination. Here, administration of cyclic oligosaccharide 2-hydroxypropyl-β-cyclodextrin, a compound that increases cholesterol efflux, attenuates this phenotype, highlighting the importance of efficient cholesterol transport downstream of myelin engulfment for microglia to promote lesion repair [25].

A recent study by Nugent et al. [26] has combined transcriptomics and lipidomics to examine how TREM2-defective microglia react to demyelination caused by feeding mice with cuprizone for either short (5 weeks) or long (12 weeks) periods. As expected, transcriptome analysis shows that TREM2-defective microglia fail to activate typical DAM factors involved in lysosomal function, lipid hydrolysis and cholesterol transport. Interestingly, brain lipidomics approaches using liquid chromatography-mass spectrometry revealed defective intracellular lipid metabolism only in microglia from TREM2 mutant animals fed with cuprizone for 12 weeks, with these cells showing high levels of cholesterol esters (CE) and oxidised CE when compared with wild type cells [26]. *In vitro* studies using bone marrow-derived macrophages and human induced pluripotent stem cells (iPSC) derived microglia showed that these phenotypes cannot be ascribed to phagocytic defects but can be rescued by reducing CE synthesis from free cholesterol via inhibition of the acetyl-CoA acetyltransferase 1, an enzyme located on the endoplasmic reticulum. In the same way, upregulation of cholesterol transporters such as ABCA1 and ATP binding cassette subfamily G member 1 (ABCG1) can reduce CE accumulation *in vitro* [27]. The link between TREM2 and cholesterol transport defects in these microglia remains, however, unclear. A possible scenario is that TREM2 activation by either one of its ligands, ApoE, high-density lipoprotein (HDL), low-density lipoproteins (LDL) [27,28] or β-amyloid [29], could promote its association with DAP12 [30], phosphorylation of DAP12 by Src family kinases, and finally metabolic changes in microglia via the upregulation of genes involved in lipid processing and transport (Figure 2). Thus, a clear outcome of this and other studies previously discussed is that drugs that boost cholesterol export in microglia can be beneficial to flight age-related microglial defects.

Conclusion and outlook

To date, despite many studies focussing on microglia, essential questions remain unanswered. One, for sure, is if, in disease contexts, highly phagocytic PAM, DAM and IRM are our friends or worse enemies. We still do not know if these microglial states are context-dependent and how they evolve with disease progression. Addressing these questions will require mechanistic investigations to link these cellular states to specific functions and behaviours in microglia.

Another challenge is to develop methods to manipulate microglia. Data presented here indicate that cargo processing and lipid metabolism in these cells could represent powerful ways to affect higher-order microglial activities, thus modulating the impact these cells have in the context of many neurodegenerative disorders.

Conflict of interest statement

Nothing declared.

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