Perspective

Microglia-oligodendrocyte intercellular communication: role of extracellular vesicle lipids in functional signalling

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The term microglia refers to the group of resident brain immune-cells that are responsible, mainly, for the immune response and the homeostasis of the brain. Unlike monocyte-derived macrophages that infiltrate the brain, microglia are long-lived cells which arise exclusively from the embryonic yolk sac (Stratoulias et al., 2019). Impairment in microglia functions is at the basis for the development of multiple brain diseases, including multiple sclerosis and neurodegenerative diseases. In the last decade, the number of research articles and reviews dealing with the role of microglia in the pathogenesis of brain disorders has exponentially increased. Indeed, microglia cells play a major role in both the brain homeostasis and the onset and maintenance of the inflammatory processes within the central nervous system (CNS) that often accompany the diseases. The complex mechanisms by which microglia exert their action in pathological conditions have not been completely clarified, yet, but their ability to mediate the intercellular communication with all of the other cell populations in the CNS tissue has clearly emerged in both physiological and pathological conditions (Paolicelli et al., 2018).

In this context, microglia were demonstrated to communicate not only by cell-to-cell contact and by releasing or receiving soluble factors in a paracrine manner, but also through membrane bound particles, now known as extracellular vesicles (EVs) (Verderio et al., 2012; Prada et al., 2013). According to their size and subcellular origin, EVs have been divided into exosomes (50–100 nm) and microvesicles (up to 1 micrometer), but currently they are preferentially distinguished in small EVs (< 100–200 nm) and medium-large EVs (> 200 nm) based on their physical characteristics (Thery et al., 2018). Like those released by other body cells, microglia-derived EVs (MEVs) were reported to be loaded with pro-peptides, cytosolic proteins, microRNAs and mRNAs, to travel in the cerebrospinal fluid and in blood and to modulate the activity of neighbouring microglial population as well as of different cell types in the surrounding tissue. It is worth noting that EVs are important vehicles of bioactive molecules and essential stimuli released by microglia and directed towards other CNS resident cells, but EVs can vehicle also crucial molecular mediators, able to influence microglia activation state and phenotype.

Since 2012, researchers have started hypothesizing a role of microglia-derived EVs during neuroinflammatory/neurodegenerative diseases, suggesting a direct involvement of MEVs in the transfer of proteins, lipids and nucleic acids (especially miRNAs) in-between microglia cells and to other CNS cells, including neurons, astrocytes, and oligodendrocytes (Verderio et al., 2012; Paolicelli et al., 2018). The complex cargo and structure of EVs together with their size (that can vary from 1 μm to few tens of nm) have hampered the investigation of their content with standard procedures and technologies, making the identification of the main bioactive molecules that exert the MEV function challenging. Besides, microglia themselves have demonstrated a kaleidoscopic nature, with emerging evidence of their dual responses to environmental changes, which make them both friends and foes of the human brain. Actually, microglia are essential to guarantee the plasticity and regeneration of the CNS and to sustain the positive immune response to pathogens; nonetheless, their response might become detrimental and hazardous for the brain when a chronic inflammatory state is established (Stratoulias et al., 2019).

In 2019, we investigated the role of MEVs in a murine model of multiple sclerosis, demonstrating that the beneficial role of microglia on the differentiation of oligodendrocytes progenitor cells (OPC) into myelin-forming cells can be blocked in presence of an inflammatory stimulus that leads MEV to activate a harmful astrocyte activation (**Figure 1**) (Lombardi et al., 2019). At the same time, our data demonstrated that MEVs released from inflammatory cells co-cultured with immunomodulatory mesenchymal stem cells promoted remyelination, thus reverting the detrimental action of inflammatory MEVs on OPCs. The reasons underlying these phenomena were investigated starting from the physico-chemical characterization of MEVs released by cells with inflammatory and pro-regenerative phenotypes. After verifying that no major changes could be addressed to the total MEV production in terms of size and concentration, we detected clear differences in the molecular composition of MEVs derived from differently activated microglia by Raman spectroscopy, a sensitive optical technique that can provide information on the chemical content of EVs (Gualerzi et al., 2017). Main differences were identified in the spectral intervals that can be attributed to lipid components. The hypothesis that

the surface lipid compartment of MEVs may induce OPC maturation was demonstrated by replicating the MEV differentiating activity using lipids extracted from MEVs to treat OPCs *in vitro*. Still, the lipids driving OPC differentiation remained to be fully defined. The sphingolipid sphingosine 1 phosphate (S1P) was unveiled as key molecule favouring OPC migration, the first fundamental step in the remyelination process. Starting from previous studies showing that S1P can be implicated in the pro differentiating activity of MEVs (Cui et al., 2014), our observations revealed for the first time that MEV-associated S1P is a crucial mediator attracting OPCs. Conversely, surface lipids responsible for the pro-myelinating action of EVs remained unknown.

It is now well-known that specific classes of lipids, the so-called bioactive lipids (i.e., eicosanoids, specialized pro-resolving mediators, lysophospholipids/sphingolipids and endocannabinoids), represent key players in both onset and resolution of the inflammatory processes that become dysregulated in a plethora of inflammation based diseases, including brain disorders (Chiurchiù et al., 2018). Indeed, lipid mediators have emerged as internal regulatory signals that activate many aspects of the inflammation and resolution cascades and it was demonstrated that the lipid and lipoprotein metabolism is tightly regulated in microglia, with profound changes occurring during damage and disease (Loving et al., 2020). However, the lipid-related aspects of MEVs have not obtained sufficient attention. Herein, we would like to stress the importance of investigating the role of lipids and of specific lipid subfamilies in the intercellular communication within the CNS and, in particular, their involvement in MEV-mediated responses to inflammation and injury.

Endocannabinoids are a lipid class worthy of deep investigation in microglia-OPC crosstalk. They were already demonstrated to be expressed on MEV surface (Gabrielli et al., 2015), owing to microglia the ability to influence synaptic activity in the CNS. In addition, endocannabinoids were previously shown to promote OPC maturation towards myelin-forming cells (Ilyasov et al., 2018), thus representing possible candidates mediating OPC differentiation to myelin forming cells.

Reported results about the lipid composition of EVs are currently highly variable, possibly because of the variations in cell types and growth conditions, as well as the methods used for lipid isolation and analyses (mainly thin layer chromatography, gas liquid chromatography and mass spectrometry) (Skotland et al., 2020). Pre-analytical conditions, including storage temperature, can influence lipid degradation and in particularly lipid oxidation, but so far, the impact of these variables in the EV lipidome has not been thoroughly investigated. To test the role of lipids in EV activity, dyes (including fluorescent dyes) were proposed (Thery et al., 2018), but no quantitative
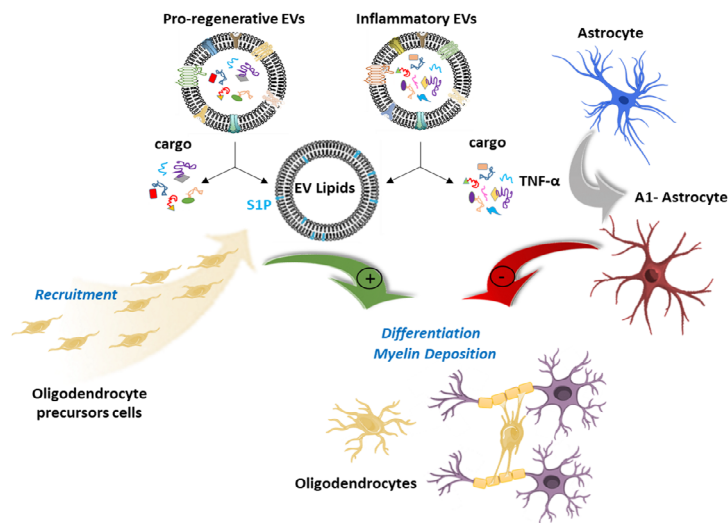
Figure 1 | Schematic representation of the main results reported in a study of Lombardi et al. (2019).
Microglia activated towards a pro-regenerative or inflammatory phenotype release MEVs loaded with specific set of molecules (cargo and membrane associatedproteins). The inflammatory MEV cargo promotes astrocyte activation towards the A1 harmful phenotype that can inhibit oligodendrocyte differentiation and myelin deposition. On the contrary, the EV lipids, including S1P, of both pro-regenerative and inflammatory MEVs promote oligodendrocyte recruitment and differentiation, the important steps for the remyelination process. EV: Extracellular vesicle; MEV: microglia-derived extracellular vesicle; S1P: sphingosine 1 phosphate; TNF: tumour necrosis factor.

information can be derived from these assays. Biophotonic techniques like Fourier transform infrared spectroscopy and Raman spectroscopy have demonstrated remarkable potentiality, providing information about total lipids, protein-to-lipid ratio and qualitative differences in the chemical composition of EVs. In our work, using tiny volumes of samples, the Raman analysis allowed us to perform a fast, label-free profiling of EVs from activated microglia and to obtain a molecular overview of MEVs that gave hints to focus on the lipid compartment (Lombardi et al., 2019). Still, considering the very low amount of sample and the complexity of the results, EV lipid profiling is a great challenge and mass spectrometry, combined to well-controlled extraction methods, is currently the most reliable analysis to obtain quantitative data about lipids in EVs. Although new platforms (e.g., LIPID MAPS, http://www.lipidmaps.org) and software (e.g., LipidXplorer, https://lifs.isas.de/wiki/index.php/Main_Page) have been established to support the spectrometry analysis of lipids, a comprehensive analysis of large lipidomics data-sets is a challenging and time consuming task. The application of innovative techniques, like the spectroscopic ones, to select samples with negligible contaminants, to verify the reproducible isolation of EVs and to direct researchers' attention on specific lipid subfamilies might be of great help to target the lipidomic analysis and limit misleading results.

In conclusion, despite being aware of the intrinsic difficulties of the molecular analysis of EVs, which can provide ambiguous results especially in lipid analysis, we believe that new innovative and multidisciplinary approaches are needed to unravel the role of these macromolecules in the EV function. Indeed, further quantitative data about the lipid species present in MEVs are needed to understand the function of MEV associated lipids in intercellular communication and CNS homeostasis and to uncover the disease-related variability of their lipid compositions.

In a therapeutic perspective, the ambivalent role of MEVs can inspire new directions in drug discovery and the identification of those components leading to their beneficial, reparative actions is of crucial importance for the development of new drugs. Deepening the lipid hypothesis and identifying those lipids responsible for the pro-differentiating action of MEVs may help to design new therapeutics to foster myelin repair. Moreover, in a diagnostic perspective, understanding the key players in the onset and progression of inflammatory brain disorders can help the discovery of new biomarkers for the stratification of patients, in view of the personalized tailoring of therapeutic and rehabilitation plans.

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References

Chiurchiù V, Leuti A, Maccarrone M (2018) Bioactive lipids and chronic inflammation: managing the fire within. Front Immunol 9:38.

Cui QL, Fang J, Kennedy TE, Almazan G, Antel JP (2014) Role of p38MAPK in S1P receptor-mediated differentiation of human oligodendrocyte progenitors. Glia 62:1361-1375.

Gabrielli M, Battista N, Riganti L, Prada I, Antonucci F, Cantone L, Matteoli M, Maccarrone M, Verderio C (2015) Active endocannabinoids are secreted on extracellular membrane vesicles. Embo Rep 16:213-220.

Gualerzi A, Niada S, Giannasi C, Picciolini S, Morasso C, Vanna R, Rossella V, Masserini M, Bedoni M, Ciceri F, Bernardo ME, Brini AT, Gramatica F (2017) Raman spectroscopy uncovers biochemical tissue-related features of extracellular vesicles from mesenchymal stromal cells. Sci Rep 7:9820.

Ilyasov AA, Milligan CE, Pharr EP, Howlett AC (2018) The endocannabinoid system and oligodendrocytes in health and disease. Front Neurosci 12:733.

Lombardi M, Parolisi R, Scaroni F, Bonfanti E, Gualerzi A, Gabrielli M, de Rosbo NK, Uccelli A, Giussani P, Viani P, Garlanda C, Abbracchio MP, Chaabane L, Buffo A, Fumagalli M, Verderio C (2019) Detrimental and protective action of microglial extracellular vesicles on myelin lesions: astrocyte involvement in remyelination failure. Acta Neuropathol 138:987-1012.

Loving BA, Bruce KD (2020) Lipid and lipoprotein metabolism in microglia. Front Physiol 11.

Paolicelli RC, Bergamini G, Rajendran L (2019) Cell-to-cell communication by extracellular vesicles: focus on microglia. Neuroscience 405:148-157.

Prada I, Furlan R, Matteoli M, Verderio C (2013) Classical and unconventional pathways of vesicular release in microglia. Glia 61:1003-1017.

Ramirez MI, Amorim MG, Gadelha C, Milic I, Welsh JA, Freitas VM, Nawaz M, Akbar N, Couch Y, Makin L, Cooke F, Vettore AL, Batista PX, Freezor R, Pezuk JA, Rosa-Fernandes L, Carreira ACO, Devitt A, Jacobs L, Silva IT, et al. (2018) Technical challenges of working with extracellular vesicles. Nanoscale 10:881-906.

Stratoulias V, Venero JL, Tremblay ME, Joseph B (2019) Microglial subtypes: diversity within the microglial community. EMBO J 38:e101997.

Skotland T, Sagini K, Sandvig K, Llorente A (2020) An emerging focus on lipids in extracellular vesicles. Adv Drug Deliv Rev doi:10.1016/j.addr.2020.03.002.

Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, Antoniou A, Arab T, Archer F, Atkin-Smith GK, Ayre DC, Bach JM, Bachurski D, Baharvand H, Balaj L, Baldacchino S, Bauer NN, Baxter AA, Bebawy M, Beckham C, et al. (2018) Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles 7:1535750.

Verderio C, Muzio L, Turola E, Bergami A, Novellino L, Ruffini F, Riganti L, Corradini I, Francolini M, Garzetti L, Maiorino C, Servida F, Vercelli A, Rocca M, Dalla Libera D, Martinelli V, Comi G, Martino G, Matteoli M, Furlan R (2012) Myeloid microvesicles are a marker and therapeutic target for neuroinflammation. Ann Neurol 72:610-624.

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