● PERSPECTIVE

TET2, an "ambiguous" player in inflammation

Microglial cells, the "macrophages" from the central nervous system (CNS), perform a variety of roles necessary to keep the homeostasis in the healthy brain. However, microglial cells are best known for their role as "first responders" through initiation of an innate immune response against a wide variety of deleterious stimuli in the brain. This controlled inflammatory response is beneficial and disappears once the deleterious stimuli are gone. But, it is also well-acknowledged that uncontrolled activation may transform into a chronic neuroinflammatory response which is partially responsible for the progression of the disease, for instance in Parkinson's disease (PD) and Alzheimer's disease (AD) (Shen et al., 2018). For this reason, microglia have become a target in the search for new therapeutic strategies to hinder the progression of different neurodegenerative diseases, such as PD or AD.

Most cases of PD or AD are considered idiopathic, and the brain's environment (for instance, the presence of toxic aggregates of proteins) may modify the expression and/or activity of epigenetic modifying enzymes (Carrillo-Jimenez et al., 2019). These changes may affect the progression of such diseases by altering cellular signaling pathways, including the control of the neuroinflammatory response. Little is known about epigenetic modifying enzymes and their roles in microglial cells, although some studies have shown the importance of DNA methylation of cytosine guanine dinucleotides at promoters of cytokines such as interleukin (IL)-1β in the neuroinflammatory response (Carrillo-Jimenez et al., 2019). DNA methylation is a stable and widespread epigenetic modification that allows inheritance of information in cells from one generation to the next. This epigenetic process is the result of a balance in the activity between DNA methyl transferase and Ten-eleven translocation (TET) enzymes. TETs are involved in the active and the passive demethylation process via oxidation of the methyl group of 5-methylcytosine to 5-hydroxymethylcytosine (Lio and Rao, 2019). In fact, 5-methylcytosine and 5-hydroxymethylcytosine are nowadays considered by many as the 5th and 6th base of DNA, respectively. Generally, the appearance of 5-hydroxymethylcytosine is strongly associated with active sites for gene transcription (Lio and Rao, 2019) and abnormal levels of TET activity (either via altered level of protein expression or mutations in the catalytic site) may promote changes in cell cycle that may lead to cell transformation. In fact, loss-of-function of TETs has been linked with the origin of several hematopoietic cancers of myeloid and lymphoid origin. Essentially, TET activity is considered to be key in the control of differentiation and development processes in myeloid and lymphoid cells, as well as a regulator of their immune functions (Lio and Rao, 2019).

Regarding the regulation of immune functions, TETs have been described as decisive players in various aspects of the inflammatory response driven by myeloid and lymphoid cells (Ichiyama et al., 2015; Zhang et al., 2015; Carrillo-Jimenez et al., 2019; Lio and Rao, 2019). These studies described that TETs can regulate the inflammatory response depending on or independently of its dioxygenase activity based on the nature of the inflammatory stimuli and type of cell involved. For instance, in two subsets of CD4$^+$ T helper cells, Th1 and Th17, TET2 plays a critical role in the control of cytokine expression (**Figure 1**) in a process depending on its dioxygenase activity (Ichiyama et al., 2015). On the other hand, in peripheral macrophages and dendritic cells, TET2 is necessary to resolve the inflammatory response in a murine model of peripheral inflammation induced by intraperitoneal injection of lipopolysaccharide (LPS) or dextran sulfate sodium. In this case, LPS induces the relocation of TET2 to the IL-6 promoter via formation of a complex with IkappaB zeta (Iκbζ), an IL-6-specific transcription factor. Once the TET2/Iκbζ complex is formed, it recruits histone deacetylase 2 that suppresses IL-6 expression (Zhang et al., 2015) (**Figure 1**). In this study, lack of TET2 promotes an increase in the expression of IL-6 and mice lacking TET2 in myeloid cells become more susceptible to endotoxin shock and dextran sulfate sodium-induced colitis.

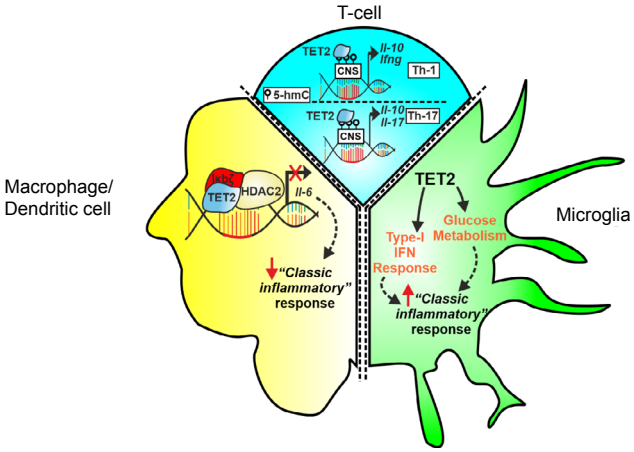

Figure 1 Schematic representing TET2 regulation of the immune response in a T-cell (blue area), peripheral macrophage/dendritic cell (yellow area) and microglia (green area).
CNS: Central nervous system; HDAC2: histone deacetylase 2; 5-hmC: 5-hydroxymethylcytosine; IL: interleukin; TET2: ten-eleven translocation 2; type I IFN: type I interferon. Figure 1 was adapted from Ichiyama et al., 2015; Zhang et al., 2015; Carrillo-Jimenez et al., 2019.

In microglia cells, we observed that TET2 regulates the proinflammatory response induced by LPS treatment both *in vitro* and *in vivo* (Carrillo-Jimenez et al., 2019). At the timepoint used in our *in vitro* study (early activation, 3 hours after LPS treatment), we did not observe any LPS-induced changes of 5-methylcytosine or 5-hydroxymethylcytosine in cytosine guanine dinucleotides. This result is in agreement with Zhang et al. (2015), where they also observed that regulation of the inflammatory response mediated by TET2 is independent of its enzymatic activity (**Figure 1**). To explain the mechanisms affected by TET2 in microglia under inflammatory conditions, we observed in our transcriptomic data that TET2 affects the expression of several signaling pathways governing different aspects of the inflammatory response, including among others, the LPS-induced type I interferon (type I IFN) response and control of the cell cycle. Although TET2's role in LPS-induced inflammatory response can be described as opposing [proinflammatory in (Carrillo-Jimenez et al., 2019) or anti-inflammatory in (Zhang et al., 2015)] there is agreement regarding a "delayed" effect of TET2 over the expression of classical inflammatory markers such as IL-6. The difference between microglia and peripheral macrophages in the regulation of the inflammatory response by TET2 can be added to previous studies where differences in the immune response between peripheral macrophages and microglia have been already described (Zarruk et al., 2018). These differences in response might be explained by their developmental origins. Despite both being cell types of myeloid origin (Shen et al., 2018), differences in the environment arise during embryonic development that affect their differentiation. Briefly, primitive yolk sac-primitive macrophages colonize the embryonic neuroepithelium where they generate microglia, which then invade the CNS and colonize it (Shen et al., 2018). Some unidentified factors during development in the CNS environment affect microglia behaviour differently from peripheral macrophages (Zarruk et al., 2018). How then does TET2 affect the "classical" inflammatory response in microglia cells? In our case, we found two signaling pathways that are affected by TET2 knockdown and could explain this "delayed" effect over the inflammatory response.

LPS provokes a metabolic shift from oxidative phosphorylation towards aerobic glycolysis, therefore changes in this metabolic shift could affect the inflammatory response. In our system, we observed that the expression of two genes related to glucose metabolism were affected, hexokinase 3 and 6-phosphofructo-2-kinase/fruc-

tose-2,6-biphosphatase 3. These two genes play key roles during the aerobic glycolysis process. We also observed that lack of TET2 diminishes aerobic LPS induced glycolysis and glucose uptake which could explain the effect observed on proinflammatory cytokines. Besides TET2's effect on the LPS-induced metabolic shift, we observed that the expression of genes related to type I IFN response such as Ifit2 or small IFN-induced GTPases like guanylate-binding proteins 2 and 3, were diminished after TET2 knockdown (Carrillo-Jimenez et al., 2019). Ifit2 is required to amplify the secretion of LPS-induced proinflammatory cytokines, including tumor necrosis factor-α or IL-6 in animal models associated with LPS-induced endotoxemia (Carrillo-Jimenez et al., 2019). Also, guanylate-binding proteins 2 and 3 have been reported to be necessary for the full activity of the non-canonical caspase-11 inflammasome upon vacuolar Gram-negative bacteria infection (Carrillo-Jimenez et al., 2019).

The results that we present in our paper, although performed in LPS-intraperitoneally injected mice, could represent and advance the study of the neuroinflammatory response in different neurodegenerative diseases. Our GO analysis of siTET2-downregulated genes not affected by LPS treatment showed an enrichment for genes involved in the immune response suggesting that TET2 could also be involved in the control of other inflammatory signaling pathways independently of TLRs. Microglia treated with α-synuclein or β-oligomers, both key components of protein aggregates in PD and AD respectively, promotes TET2 expression similar to LPS thus leaving open the possibility that TET2 could also be involved in the regulation of the inflammatory response. Furthermore, we observed that microglia cells associated with β-plaques in a murine model of AD (5×FAD) presented higher levels of TET2 expression than microglia located further away from the plaque. Furthermore, in a small cohort of AD patients (three patients), we observed high expression of TET2 in microglia surrounding the plaque in one patient. We could not link the lack of statistical significance in the other two patients with either gender, age or Braak Stage. However, other factors that we did not analyze such as the type of plaque, could explain this difference. Further studies with more patients and using a different array of markers to categorize the different plaques are required to show this.

At the transcriptomic level, we observed that lack of TET2 affects signaling pathways related to disease associated microglia, including cell cycle regulation and type I IFN response (Mathys et al., 2017). Activation of type I IFN response, that traditionally has been linked with defense against viral and bacterial infections, has been demonstrated in the CNS of animal disease models including ischemia, spinal cord injury or transgenic mouse models such for AD mouse models. Additionally there is evidence of the type I IFN response in postmortem brains of AD patients (Carrillo-Jimenez et al., 2019).

Altogether, and taking into consideration the results published by other groups and ourselves, it seems plausible that microglial TET2 plays a role in the neuroinflammatory response in different neurodegenerative conditions such as PD or AD. Although further studies are required to confirm TET2's relevance in neurodegenerative diseases.

So, is there any way to modify TET2 so that it could be used as a therapeutic tool? Recently, it has been shown that vitamin C could be used to enhance 5-hydroxymethylcytosine production (Cimmino et al., 2017). Vitamin C is a co-factor of Fe^{2+} and α-ketoglutarate-dependent dioxygenases, and mimic TET2 restoration in a model of aberrant hematopoietic stem and progenitor cell self-renewal hematopoiesis (Cimmino et al., 2017). However, this approach would not be advisable in situations where TET2 is regulating the inflammatory response independently of its enzymatic activity. In this case, we should focus on treatments that will disrupt the TET2 interaction with other proteins responsible for its regulation of the inflammatory response (such as histone deacetylase 2 and/or Iκbζ as described in (Zhang et al., 2015)). In the latter scenario, we could use aptamers, which are small single-stranded DNA or RNA oligonucleotides that modulate protein functions by interfering protein-protein interactions (Xiang et al., 2015). Recent developments in aptamer technology have allowed generation of molecules that can cross the blood-brain barrier (Dowdy, 2017).

These results presented here and elsewhere open a new and exciting field in the study of TET2 in many immune cell types and in various organs such as the brain.

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