PERSPECTIVE

Leaky gut, dysbiosis, and enteric glia activation: the trilogy behind the intestinal origin of Parkinson's disease

Early diagnosis of Parkinson's disease (PD): how we can improve the therapeutic approach: PD is a neurodegenerative disease characterized by motor dysfunctions (tremor, rigidity, bradykinesia and impaired posture/balance) elicited by selective depletion of dopaminergic (DA) neurons in substantia nigra pars compacta. DA neuron loss is associated with neuronal inclusions of the phosphorylated α-synuclein protein called Lewy body (Shults, 2006). Although the underlying neurodegenerative process is not affected, the management of PD patients has been revolutionized with the introduction of levodopa and DA drugs in the routine therapy, which ensures initial symptomatic relief of motor functions through the DA supply in the nigrostriatal circuit. These drugs are currently the best option for treating PD, although their chronic use is associated with progressive dopamine resistance and loss of effectiveness in the recovery of motor dysfunctions. Alternative therapeutic strategies, including agonists of DA receptors, monoamine oxide B inhibitors, and even deep brain stimulation techniques have been developed to overcome these clinical limitations. Unfortunately, these therapeutic approaches cannot restore PD-compromised functions, as irreversible DA neurodegeneration has occurred in substantia nigra pars compacta when first motor symptoms appear. The nigrostriatal system is traditionally considered as the first region affected by neuronal impairment in Parkinsonisms; however, α-synuclein aggregation appears in a pre-motor stage of the disease in the enteric nervous system (ENS), strongly highlighting an extra-central nervous system (CNS) origin for this neurodegenerative disorder and completely overturning the concept of PD as a central disease. The "ability" of the gut to show the pre-symptomatic evolution of PD is a very fascinating hypothesis to anticipate PD diagnosis and develop more efficient anti-Parkinsonian drugs. In this perspective article, we summarize recent evidence showing enteric glia involvement triggering intestinal neuroinflammation in the asymptomatic stage of PD and improvements in the therapeutic approach that we could achieve by targeting these glial cells.

ENS as origin-site of PD: In the broad spectrum of non-motor symptoms commonly associated with PD, gastrointestinal (GI) dysmotility was mistakenly considered as only a related autonomic disorder to this disease. However, GI dysfunctions occur up to 30% of PD patients in the pre-motor stage of disease evolution, preceding CNS involvement and motor symptom development, and only exacerbating in advanced Parkinsonism (Jost, 2010). Therefore, a GI-origin hypothesis for PD has attracted researchers' attention into the nervous system of the intestine, known as the ENS, as the possible onset site of Parkinsonism. ENS is considered as "the second brain" counting five times as many as the 100 million neurons in the human spinal cord. Anatomically arranged in an interconnected system of neurons and glia organized in two main plexuses throughout the intestine, the myenteric (Auerbach's) plexus and submucosal (Meissner's) plexus, ENS is responsible for maintaining gut homeostasis by regulating GI functions locally. The first Lewy body is detected at the non-nigral level in the peripheral autonomic nervous system, particularly in dopamine-negative neurons of myenteric plexuses and terminal axons of submucosal plexuses. Misfolded α-synuclein is associated with an increased expression of PD-related proteins, like leucine-rich repeat kinase 2 (LRRK-2), and GI malfunctioning onset (Braak et al., 2006). Although the following hypothesis requires to be confirmed, factors fostering gut microbiota alterations, epithelial mucosa disruption and consequent immunological priming of ENS, could converge in a spatiotemporal propagation of misfolded α-synuclein from the enteric to the CNS through a derangement of the gut-brain axis (Holmqvist et al., 2014). This would establish that ENS acts as a gateway to "putative PD pathogens", thereby triggering PD pathogenesis to the intestinal level.

Enteric glia in PD etiology: Enteric glial cells (EGCs) are a unique type of peripheral neuroglia localized in the myenteric and submucosal ganglia of the ENS and in extra-ganglionic sites, such as muscular layer and mucosal lamina propria. Traditionally, EGCs were thought to contribute primarily to the structural and nutritional support of enteric neurons. In addition to their conventional roles, they are essential for the homeostatic regulation of a wide range of GI functions, including gut motility, mucosal integrity, synaptic transmission, neuroprotection, and neurogenesis, through a complex interplay with neurons, immune, and epithelial cells. A series of studies have demonstrated that EGCs express the major histocompatibility complex II and respond to harmful stimuli mainly through the Toll-like receptors-2 and -4, critically regulating the neuro-immune axis and defending the host against pathogens in the gut (Cabarrocas et al., 2003). Although a glial heterogeneity has been demonstrated in the gut, some physiological glial markers, such as vimentin, glial fibrillary acidic protein (GFAP), Sox-10, and S100B, change dynamically based on the state of the intestinal mucosa. GFAP and S100B expression increase significantly in EGCs triggered by cellular danger cues, and their upregulation has been associated with a glia-mediated neuroinflammatory response in the ENS. In this condition, pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6 and tumor necrosis factor-α, growth factors as glial cell-derived neurotrophic factor and nerve growth factor, and other immunomodulatory signal molecules, including nitric oxide, are released by activated EGCs, amplifying the pro-inflammatory environment, intestinal barrier damage and neurochemical changes. As these glial cells are involved in regulations of motility, epithelium permeability, and immune response in the gut, EGC-driven neuroinflammation was postulated responsible for synaptic dysfunctions and intestinal motility disorders in the early stages of PD progress. Supporting to this hypothesis, Lewy pathology (Lewy bodies and neuritis) and LRRK-2 protein upregulation are detected with an increase in enteroglia-related pro-inflammatory markers, such as GFAP, S100B, Sox-10, IL-6, IL-1β, and tumor necrosis factor-α in the colonic mucosa of PD patients (Clairembault et al., 2015). Furthermore, the "pathological loop" between glial activation by the aggregated α-synuclein and the induction of α-synuclein misfolding by the activated enteric glia highlights the EGCs involvement in the etiology of PD (Devos et al., 2013). The in-depth study of pathological conditions leading to "reactive EGCs" in the pre-motor stage of PD evolution, could thus provide new insights for preventing or delaying the onset of the neurodegenerative process and its spread from ENS to CNS. Thereafter, several inflammatory conditions that alter gut homeostasis, including chronic colitis, autoimmune diseases, and metabolic diseases, pathologically increase intestinal permeability by inducing a syndrome known as "leaky gut" (Jogn et al., 2011) (**Figure 1A**). Luminal noxiae translocation through the impaired colonic mucosa leads to the immunological priming of EGCs, fostering enteric neuroinflammation. The increase in oxidative stress through the activation of enteric glial TLRs could induce the initial α-synuclein misfolding and LRRK-2 upregulation. Finally, the amplification of such pathological markers in the CNS through gut-brain axis afferents might elicit late motor dysfunctions (**Figure 1B**). Taken together, it is strongly suggested that leaky guts syndrome commonly observed in PD patients and experimental Parkinsonism, and EGCs activation are not independent but intimately interconnected conditions at the origin of PD.

Dysbiosis, leaky gut and EGCs priming: the vicious loop leading to gut-brain axis derangement in PD: In this complex scenario, microbiota in the intestinal lumen may act as a trigger for neuroinflammation in the ENS by crossing the impaired epithelial wall. Interestingly, gut microbiota alteration and leaky gut syndrome are strongly associated and highlighted as key factors in gut inflammation and PD pathogenesis. This close link is consistent with findings of the altered composition of gut microbiomes in PD patients and enhanced motor disorders in α-synuclein-overexpressing mice colonized by microbiota from PD patients (Sampson et al., 2016). *Faecalibacterium* is significantly decreased and *Ralstonia* is significantly increased in the mucosa of PD subjects, while a significant reduction in microbiota strains producing short-chain fatty acids, such a *Blautia*, *Coprococcus* and *Roseburia*, is found. This has been related to increased susceptibility to developing the leaky gut syndrome. Furthermore, small intestinal bacterial overgrowth has been closely associated with PD development, since nearly 25% of PD patients have small intestinal bacterial overgrowth, and its downregulation mediates the improvement of motor fluctuations. Although specific bacterial strains have not yet been directly involved in the onset of Parkinsonism, gut-brain axis disarrangement resulting from immunological priming of EGCs by altered gut microbiota is increasingly confirmed (Clairembault et al., 2015). To reinforce this hypothesis, TLR-4 knock-out mice are partially protected from rotenone-induced Parkinsonism, suggesting that inhibiting the EGC-mediated immune response, prevention of parkinsonian symptoms is achieved (Perez-Pardo et al., 2019). In light of this evidence that emphasizes the role of intestinal dysbiosis and leaky gut syndrome in the onset of the typical PD intestinal synaptopathy, prevention of increased permeability of the mucosa and the related glial-sustained neuroinflammation, could potentially prevent unidirectional propagation of misfolded α-synuclein from the ENS to CNS through gut-brain connections.

The future of PD therapy by targeting leaky gut and EGC-driven neuroinflammation: According to Braak's specific chronological and regional pattern for neuropathological changes in PD, the neurodegenerative process initiated in the ENS could spread progressively into the CNS as the disease progress. Microbiota alteration and leaky gut are considered key events for the onset of neurodegeneration, which is strongly linked to glial dysfunction and GI dysmotility commonly observed in PD patients. Abnormal activation of EGCs in response to altered microbiota crossing the impaired

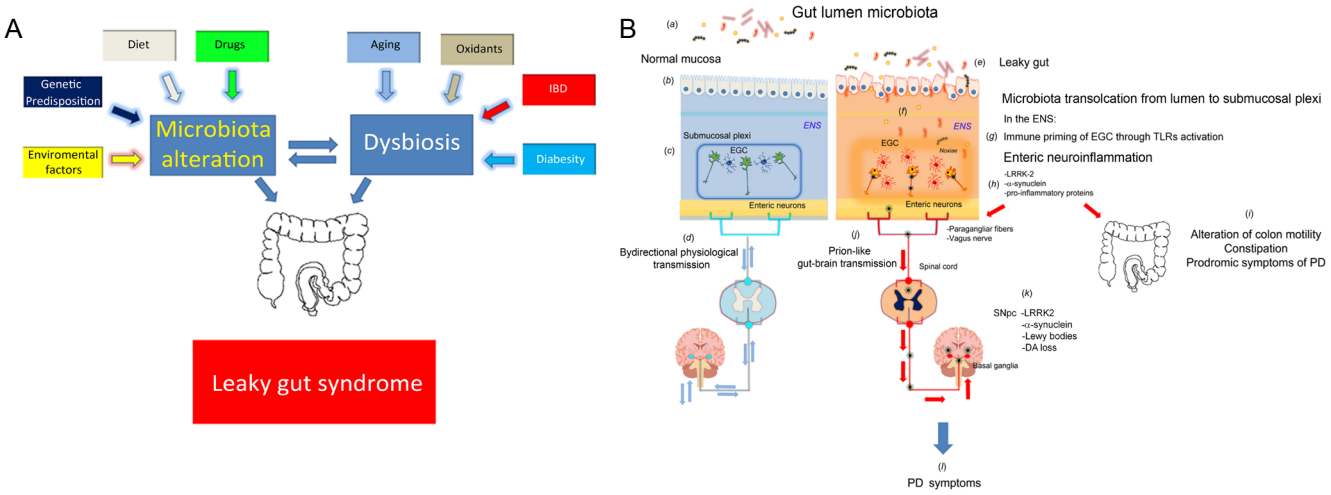

Figure 1 Leaky gut syndrome and enteric glia activation at the basis of PD.
(A) Converging factors and pathological conditions increasing the susceptibility to develop dysbiosis and related leaky gut in the initial stages of PD evolution. The disruption of the "healthy" gut microbiota that occurs by genetic predisposition, massive antibiotic therapy, environmental factors, and several intestinal inflammatory diseases, has been described as one of the most critical conditions for the leaky gut syndrome. (B) In normal mucosa (a) bacteria and other noxiae are confined in the gut lumen (b) by mucosal tight junctions and no EGCs activation occurs in submucosal and myenteric plexuses (c). In this condition, (d) physiological bidirectional communication between ENS and CNS is ensured by extrinsic nerves. In leaky gut syndrome (e), epithelial membrane integrity is lost and bacteria/noxiae translocation from the lumen into the mucosa (f) results in EGCs priming through TLRs activation (g). The EGCs-sustained neuroinflammation increases the expression of LRRK-2 and misfolded α-synuclein within the ENS (h). In these conditions, symptomatic alteration of GI motility can be detected as a prodromic symptom in PD patients (i). By a prion-like transmission through extrinsic paraganglia fibers and/or vagus nerve (j), misfolded proteins move from the ENS to the CNS by an unidirectional way (k). Accumulation of LRRK-2 and α-synuclein, in the nigrostriatal area (l), may thus predispose parkinsonian neurodegeneration and appearance of motor symptoms. DA: Dopaminergic; EGC: enteric glial cell; ENS: enteric nervous system; IBD: inflammatory bowel diseases; LRRK2: leucine-rich repeat kinase 2; PD: Parkinson's disease; SNpc: substantia nigra pars compacta; TLRs: Toll-like receptors.

mucosal barrier would be responsible for neuroinflammation and related neuropathological changes in the ENS. This cascade of events leads to an initial α-synuclein misfolding and LRRK-2 upregulation, thereby developing GI dysfunctions featuring the early stage of PD. Although the exact mechanism(s) of PD pathology transmission to the nigrostriatal area has not clearly understood, a propagation of α-synuclein pathology in "prion-like" manner through a glial-glial pathway has been postulated (**Figure 1B**). A glia-glia syncytium through the connexin-43 (Cx43) hemichannels has been reported as a pathway of cell-to-cell communication between the gastrointestinal tract and the CNS. The expression of Cx43 in the glial gap junctions is deeply regulated by inflammation and dependent on intracellular Ca^{2+} concentration. Moreover, significant spatiotemporal overexpression of Cx43 in S100B-positive cells in the submucosal plexus, spinal cord, and frontal cortex was progressively observed in HIV-1 Tat-induced diarrhea model, suggesting that the communication between EGCs and central astrocytes involves these glial gap junction proteins (Esposito et al., 2017). Although further investigations will confirm the exact EGCs role in the onset and progression of PD from the gut to CNS, it is clear how the PD therapeutic approach could be completely revisited by gut-targeted therapies. The administration of eubiotics bacteria to modulate the dysbiosis, as well as the introduction of drugs that inhibit leaky gut syndrome and EGC-driven neuroinflammation, could potentially prevent the rise of neurodegeneration through the gut-brain vagal afferents. In this way, we could overcome the concept of central neuroprotection and, in the future, develop gut-targeted approaches, maximizing the therapeutic success and limiting the side effects on the CNS of actual antiparkinsonian drugs.

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