Microbiota-gut-brain axis in health and disease: is NLRP3 inflammasome at the crossroads of microbiota-gut-brain communications?

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Abstract

Growing evidence highlights the relevance of microbiota-gut-brain axis in the maintenance of brain homeostasis as well as in the pathophysiology of major neurological and psychiatric disorders, including Parkinson's disease (PD), Alzheimer's disease (AD), multiple sclerosis (MS), autism spectrum disorder (ASD) and major depressive disorder (MDD). In particular, changes in gut microbiota can promote enteric and peripheral neurogenic/inflammatory responses, which, in turn, could contribute to neuroinflammation and neurodegeneration in the central nervous system (CNS). Of note, the nucleotide-binding oligomerization domain leucine rich repeat and pyrin domain-containing protein 3 (NLRP3) inflammasome acts as a key player in both coordinating the host physiology and shaping the peripheral and central immune/inflammatory responses in CNS diseases. In this context, there is pioneering evidence supporting the existence of a microbiota-gut-inflammasome-brain axis, in which enteric bacteria modulate, via NLRP3 signaling, inflammatory pathways that, in turn, contribute to influence brain homeostasis.

The present review provides an overview of current knowledge on the role of microbiota-gut-inflammasome-brain axis in the major CNS diseases, including PD, AD, MS, ASD and MDD. In particular, though no direct and causal correlation among altered gut microbiota, NLRP3 activation and brain pathology has been demonstrated and in-depth studies are needed in this setting, our purpose was to pave the way to a novel and pioneering perspective on the pathophysiology of CNS disorders. Our intent was also to highlight and discuss whether alterations of microbiota-gut-inflammasome-brain axis support a holistic view of the pathophysiology of CNS diseases, even though each disorder displays a different clinical picture.

***Abbreviation*:**

Aβ, amyloid beta; α-syn, alpha-synuclein; AD, Alzheimer's disease; ASC, inflammasome adaptor protein apoptosis-associated speck-like protein containing CARD; ASD, autism spectrum disorder; ATP, adenosine triphosphate; BBB, blood brain barrier; CNS, central nervous system; CRS, chronic restraint stress; CUS, chronic unpredictable stress; DAMPs, damage-associated molecular pattern molecules; FTM, fecal microbiota transplantation; GF, germ free; GPCRs, G-protein-coupled receptors; HIPK2, homeodomain-interacting protein kinase 2; HMGB1, high mobility group box 1 protein; IEB, intestine al epithelial barrier, IECs, intestinal epithelial cells; IL, interleukin; IRF, interferon regulatory factor; IFN, interferon; MLLKL, mixed lineage kinase domain-like protein; MDD, major depressive disorder; MS, multiple sclerosis; NF-kB, nuclear factor kB; NLRP3, nucleotide-binding oligomerization domain leucine rich repeat and pyrin domain-containing protein 3; OTUs, operational taxonomic units; PAMPs, pathogen-associated molecular pattern molecules; PD, Parkinson's disease; ROS, reactive oxygen species; SCFAs, short chain fat acids; TLR, toll-like receptor; TRIF, toll/IL-1 receptor homology-domain-containing adapter-inducing interferon-β

Keyword: Gut microbiota, NLRP3 inflammasome, enteric inflammation, gut-brain axis, neurological disorders, psychiatric diseases, animal models

1. Introduction

Several lines of evidence highlight the relevance of the microbiota-gut-brain axis in the maintenance of brain homeostasis as well as the pathophysiology of major neurological and psychiatric disorders, including Parkinson's disease (PD), Alzheimer's disease (AD), multiple sclerosis (MS), autism spectrum disorder (ASD) and major depressive disorder (MDD) (Foster *et al.*, 2017; Fung *et al.*, 2017). It has been hypothesized that changes in gut microbiota composition can alter the intestinal mucosal barrier and trigger enteric and peripheral neurogenic/inflammatory responses, which, in turn, could contribute to neuroinflammation and neurodegeneration in the central nervous system (CNS) (Pellegrini *et al.*, 2018). Indeed, PD, AD, MS, ASD and MDD patients display different colonic bacterial composition from healthy controls along with patterns of peripheral and central immune/inflammatory cell activation (Pellegrini *et al.*, 2018). Of note, recent evidence supports the contention that the nucleotide-binding oligomerization domain leucine rich repeat and pyrin domain-containing protein 3 (NLRP3) inflammasome multiprotein complex, through the processing and release of interleukin (IL)-1β and IL-18, can act as a key player both in coordinating the host physiology and shaping the peripheral and central immune/inflammatory responses in CNS diseases (Gordon *et al.*, 2018; Heneka *et al.*, 2018; Kaufmann *et al.*, 2017). In this context, there is pioneering evidence supporting the occurrence of dynamic interplays between the gut microbiota and NLRP3 inflammasome, currently referred as microbiota-gut-inflammasome-brain axis, where enteric bacteria modulate, via inflammasome signaling, peripheral inflammatory pathways that, in turn, contribute to influence brain homeostasis (Rogers *et al.*, 2016). There is also evidence that various stimuli associated with inflammatory and/or neurodegenerative processes, including an increase in extracellular ATP, β-amyloid (Aβ) fibers, alpha-synuclein (α-syn) aggregates, reactive oxygen species (ROS) and deubiquitination, promote NLRP3 inflammasome activation that, in turn, can alter the gut microbiota composition, with a shift towards a pro-inflammatory phenotype (Hirota *et al.*, 2011; Pellegrini *et al.*, 2017). However, despite the above knowledge, most of the published reviews have been focused on either the role of microbiota-gut-brain axis or NLRP3 inflammasome activation

in the CNS, regarded as distinct determinants involved in the pathophysiology of neurological and psychiatric disorders.

Based on the above background, the present review provides an overview of current knowledge on the role of microbiota-gut-inflammasome-brain axis in the major neurological and psychiatric diseases, including PD, AD, MS, ASD and MDD. In particular, despite no direct and causal relationship among altered gut microbiota, NLRP3 inflammasome activation and brain pathology has been established, and further studies are needed to better clarify the mechanisms underlying such immune-bacteria interplay, this review article was intended to provide new insights into the role of microbiota-gut-inflammasome-brain axis in CNS neurobiology. In addition, based on pre-clinical and human evidence, our intent was to discuss whether alterations of bidirectional signaling between gut microbiota and NLRP3 inflammasome support a holistic view of the pathophysiology of CNS disorders.

2. Mechanisms underlying NLRP3 inflammasome activation

NLRP3 inflammasome, a tripartite protein of the nucleotide-binding domain and leucine-rich repeat (NLR) family, is the best characterized inflammasome complex. It contains an amino-terminal pyrin domain (PYRIN) domain, a nucleotide-binding NACHT domain with ATPase activity and a carboxy-terminal leucine-rich repeat (LRR) domain (Duncan *et al.*, 2007). NLRP3 is an immune sentinel, which senses changes in cellular homeostasis. At present, mechanisms of both canonical and non-canonical NLRP3 inflammasome activations have been characterized (Latz *et al.*, 2013).

The canonical NLRP3 activation involves two parallel and independent steps: transcription and oligomerization (Figure 1) (Latz *et al.*, 2013). The first step is regulated by innate immune signaling via toll-like receptor (TLR)-adaptor molecule myeloid differentiation primary response 88 (MyD88) and/or cytokine receptors (i.e. tumor necrosis factor receptor and interferon-α/β receptor), that promote pro-IL-1β and NLRP3 transcription through nuclear factor-κB (NF-κB) activation. The second step, characterized by the oligomerization and activation of the NLRP3 multiprotein complex,

leads to caspase-1 activation and, in turn, IL-1β and IL-18 processing and release (Bauernfeind *et al.*, 2009; Latz *et al.*, 2013). Viral RNA, extracellular osmolarity, aggregates of α-syn or Aβ proteins, post-translational NLRP3 modification, including phosphorylation and ubiquitination, can contribute to NLRP3 inflammasome oligomerization and activation (Pellegrini *et al.*, 2019). Moreover, exposure to pore-forming gasdermin D by lysosomal damage and cathepsin release, activation of mixed lineage kinase domain-like protein (MLKL), increase in extracellular ATP and consequent P2X7 purinergic receptor activation, increase in the permeabilization of cell membranes to potassium efflux, increase in mitochondrial reactive oxygen species (ROS) and cardiolipin externalization can all activate NLRP3 inflammasome assembly (Mangan *et al.*, 2018; Pellegrini *et al.*, 2017). Caspase-1 activation promotes also pyroptosis, a key defense mechanism against pathogens, which induces phagocytosis and promotes the release of cytosolic proteins, including high mobility group box 1 (HMGB1), an alarmin thought to be involved in inflammatory chronic diseases (Aachoui *et al.*, 2013; Lu *et al.*, 2013).

Besides caspase-1-dependent NLRP3 activation, a non-canonical activation, depending on caspase-11 in mice (caspase 4 and caspase 5 in humans), has been described (Figure 1) (Pellegrini *et al.*, 2017). The first transcription step of non-canonical NLRP3 activation is regulated by Gram-negative bacteria (i.e., *Citrobacter rodentium*, *Escherichia coli*, *Legionella pneumophila*, *Salmonella typhimurium*, and *Vibrio cholerae*), which activate the TLR4–MyD88 and toll/IL-1 receptor homology-domain-containing adapter-inducing interferon-β (TRIF) pathways, with consequent transcription of IL-1β, IL-18, NLRP3, interferon regulatory factor (IRF)-3 and IRF7 genes through NF-κB activation. The IRF3–IRF7 complex induces interferon (IFN)-α/β release, which, activating the IFN-α/β receptor 1 (IFNAR)/IFNAR2-JAK/STAT pathway, leads to the transcription of caspase-11 gene. In the second step, Gram-negative bacteria, through the induction of unidentified proteins or receptors, activate caspase-11, which promotes the release of IL-1β through activation of the canonical NLRP3-ASC-caspase-1 signaling as well as pyroptosis, HMGB1 and IL-1α release (Figure 1) (Pellegrini *et al.*, 2017).

Of note, a non-canonical caspase-8 dependent NLRP3 activation has been also characterized (Figure 1) (Antonopoulos *et al.*, 2015; Chen *et al.*, 2015; Chung *et al.*, 2016; Gringhuis *et al.*, 2012; Gurung *et al.*, 2014). In particular, pathogen-associated molecular pattern molecules (PAMPs) and/or damage-associated molecular pattern molecules (DAMPs) activate TLR4, that, in turn, promote caspase-8 and its receptor-interacting protein 1 (RIP1)–FAS-associated death domain protein (FADD), with consequent canonical NLRP3 activation (Gurung *et al.*, 2014). In addition, mycobacteria, fungi or β-glucans, through dectin-1 activation, promote both IL-1β transcription and the formation and activation of a mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1)–caspase-8–ASC complex that promote the processing and release of IL-1β (Gringhuis *et al.*, 2012). Of note, caspase-8 acts as a direct IL-1β-converting enzyme (Antonopoulos *et al.*, 2015; Chung *et al.*, 2016; Gringhuis *et al.*, 2012).

Taken together, this body of evidence points out that different stimuli can promote canonical and/or non-canonical NLRP3 inflammasome activations. In particular, bacteria, mycobacteria, fungi and their products as well as protein aggregates and mitochondrial damage can promote activation and oligomerization of NLRP3 inflammasome complex. However, a better *in vitro* characterization of which bacterial, fungal or mycobacterial species promote activation of canonical caspase-1 dependent and/or non-canonical caspase-11 and caspase-8-dependent NLRP3 activation should be performed.

3. Gut microbiota

The gut microbiota comprises tens of trillions of microorganisms, including bacteria, viruses, fungi and protozoans. Even though the gut microbiota composition in humans can vary widely, depending on several factors, such as diet, environment or genetics, the main bacterial species in the gastrointestinal tract include four main phyla: Bacteroidetes, Firmicutes, Actinobacteria and Proteobacteria (Rodriguez *et al.*, 2015). The main Bacteroidetes genera include *Bacteroides* and *Prevotella,* while *Clostridium, Blautia, Faecalibacterium, Eubacterium, Roseburium* and *Ruminococcus* represent the main genera of Firmicutes. Actinobacteria are represented by the genera

Bifidobacteria, Atopobium and *Collinsella*, while Proteobacteria consist mainly of *Enterobacteriaceae* (Rodriguez et al., 2015).

The gut microbiota, through interactions with the intestinal epithelial barrier (IEB) and immune system contributes to both the maintenance and breakdown of gut homeostasis. Indeed, enteric bacteria interact directly with intestinal epithelial cells (IECs), including M cells, goblet cells, Paneth cells, enterochromaffin cells and columnar epithelial cells, thus contributing directly to preserve the integrity of the IEB, through the regulation of epithelial cell growth and differentiation, tight junction protein expression and mucosal permeability (Sharma *et al.*, 2010). Changes in gut microbiota composition can lead to impairments of IEB and alterations of mucosal permeability, with consequent translocation of enteric bacteria and their products into the lamina propria that, in turn, could trigger immune/inflammatory responses (Bischoff *et al.*, 2014). For instance, a reduced abundance of *Faecalibacterium prausnitzii* in inflammatory bowel disease (IBD) patients has been found to correlate with an increase in mucosal permeability. In addition, a decrease in short chain fat acids (SCFAs) levels in IBD patients is associated with tight junction alterations and impaired mucosal permeability. These findings highlight the significance of gut microbiota in the maintenance of the integrity of IEB. However, further extensive investigations are required to clarify in detail the molecular and cellular mechanisms underlying the gut microbiota-intestinal epithelium interplay under both physiological and pathological conditions.

Of note, gut microbiota can modulate directly also the immune system, contributing to maintenance/breakdown of immune tolerance (Shi *et al.*, 2017). The main components of the intestinal innate immune system include Paneth cells, dendritic cells, macrophages, neutrophils, natural killer cells and mast cells. Most of innate immune responses are mediated by pattern recognition receptors such as transmembrane surface or endosome toll-like receptors (TLRs) or cytosolic nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) that recognize the microbe-associated molecular patterns (MAMPs) expressed by gut microbiota. Indeed, enteric

bacteria and/or MAMPs can modulate innate immune/inflammatory responses via TLRs and/or NLRs thus regulating microbe–host interactions and immune tolerance.

With regard for the adaptive immune system, it includes mainly T lymphocytes, in particular CD4+ and CD8+ T cells, located mostly in the intestinal lamina propria and intraepithelial compartment, respectively (Wu and Wu, 2012). Naive CD4+ T cells can differentiate into seven subtypes: T helper 1 (Th1), Th2, Th9, Th17, Th22, regulatory T cells (Treg) or T follicular helper (Tfh) cells (Wu and Wu, 2012).

The gut microbiota is able to modulate also the adaptive immune response. Indeed, treatment with SCFAs has been found to exert beneficial effects on bowel inflammation, ameliorating the outcomes of T cell-transfer-induced colitis. Such an effect has been ascribed to the ability of SCFAs of promoting the differentiation of T cells into anti-inflammatory FoxP3+IL-10-producing Treg cells. Besides T cells, adaptive immune responses are supported by gut-associated B cells. These cells, located in Peyer's patches, include mainly of immunoglobulin (Ig) A-secreting plasma cells. In this respect, the gut microbiota has been found to influence both B cell activity and IgA production. Accordingly, a decreased number of plasma cells and IgA levels has been detected in the intestine of germ free (GF) animals. Taken together, these results indicate that gut microbiota, IEB and immune system interact continuously to preserve the digestive homeostasis.

Of interest, the bacteria-immune interplay is emerging as a pivotal pathway involved in the maintenance of host physiology (Fung *et al.*, 2017; Grosicki *et al.*, 2017; Winer *et al.*, 2016). Indeed, alterations of enteric bacteria compositions towards pathogenic phenotypes and the activation of immune/inflammatory cells are the main factors implicated in the pathogenesis of digestive and extra-digestive diseases (e.g. immune-mediated diseases, neurological and psychiatric disorders, and dysmetabolic syndromes) (Fung *et al.*, 2017; Obata and Pachnis, 2016; Sommer and Backhed, 2013; Winer *et al.*, 2016). Thus, the interplay among gut microbiota, IEB and immune system contributes to the maintenance of intestinal homeostasis and coordination of host physiology (Fung *et al.*, 2017; Grosicki *et al.*, 2017; Winer *et al.*, 2016).

4. Interplay between gut microbiota and NLRP3 inflammasome in the maintenance of intestinal homeostasis

There is consistent evidence that a bidirectional interplay between inflammasome multiprotein complexes and gut microbiota contributes to the maintenance of intestinal homeostasis (Figure 1). Indeed, the overactivation of inflammasomes in both hematopoietic- and non-hematopoietic cells, including macrophages and IECs, and changes in gut microbiota composition represent one of the main factors involved in the pathogenesis of several bowel diseases (e.g., IBDs, colorectal cancer and intestinal infections) (Pellegrini *et al.*, 2017; Yao *et al.*, 2017). In this context, though several inflammasomes have been characterized, the NLRP3 subtype is now regarded as the central node in the preservation of immune-bacterial quorum sensing as well as in the maintenance of IEB integrity (Gagliani *et al.*, 2014; Pellegrini *et al.*, 2017; Yao *et al.*, 2017).

NLRP3 inflammasome activation influence the composition of gut microbiota (Chung and Kasper, 2010). Indeed, fecal microbiota in NLRP3 knockout ($^{-/-}$) mice differs, in terms of both load and species, from wild type (WT) animals (Hirota *et al.*, 2011). In particular, gut microbiota from NLRP3$^{-/-}$ mice is characterized by an increase in Firmicutes along with a decrease in Bacteroidetes. In addition, NLRP3$^{-/-}$ animals display an increase in Lachnospiraceae, Ruminococcaceae and Prevotellaceae (Hirota *et al.*, 2011). Likewise, caspase-1$^{-/-}$ animals display relevant alterations of enteric bacteria, mainly characterized by a decrease in Firmicutes/Bacteroidetes ratio along with an increase in Prevotellaceae (Brinkman et al., 2011). These findings indicate a role of canonical NLRP3 inflammasome signaling in the maintenance of bacterial quorum sensing mainly through the regulation of Firmicutes/Bacteroidetes ratio. Recent papers reported also that fecal microbiome of NLRP3$^{-/-}$ and ASC$^{-/-}$ animals differ from both WT and IL-18$^{-/-}$ mice, thus suggesting that downstream pathways of inflammasome activation modulates differently the gut microbiota composition (Chen, 2017; Hirota *et al.*, 2011).

Of interest, a pioneering study by Yao et al., (2017) showed that the hyperactivation of a genetically mutated form of NLRP3 shifted the gut microbiota composition towards an anti-inflammatory

phenotype, that, in turn, induced T cell differentiation in Tregs, preserving the intestinal microenviroment integrity and conferring resistance to the development of bowel inflammation (Yao *et al.*, 2017). In particular, mice carrying a gain-of-function mutation in the NLRP3 gene (NLRP3R258W) displayed an inflammasome activation exclusively of mononuclear phagocytes in intestinal *lamina propria*. This specific NLRP3 activation in the gut enhanced the release IL-1β, but not IL-18, that, in turn, increased the local levels of antimicrobial peptides with consequent shift of gut microbiota toward an anti-inflammatory phenotype (Yao et al., 2017). The 'anti-inflammatory' reshaped gut microbiota, stimulated the differentiation of T cells in Treg. In particular, the microbiota of NLRP3R258W mice was characterized by an increase in some operational taxonomic units (OTUs), including *Clostridium XIVa* and *Lactobacillus murinus*, closely related to species or genera reported to enhance Tregs population (Atarashi *et al.*, 2011; Geuking *et al.*, 2011; Tang *et al.*, 2015). In addition, NLRP3R258W mice displayed a decrease in *Akkermensia muciniphila* and Prevotella, regarded as colitogenic bacteria (Seregin *et al.*, 2017). These findings suggest that NLRP3 modulates the gut microbiota increasing Tregs-promoting bacteria and the decreasing pathogenic species, thus contributing to the maintenance of immune-bacterial tolerance (Yao *et al.*, 2017).

Of interest, the bidirectional interplay between NLRP3 inflammasome and gut microbiota play also a crucial role in the maintenance of IEB integrity (Rooks and Garrett, 2016; Sharma *et al.*, 2010). In particular, IL-18 release, following canonical NLRP3 activation, has been found to regulate crypt bactericidal capacity, β-defensin release, tight junction protein expression, mucosal permeability and mucus production, thus contributing to preserve the protective, secretory and absorptive functions of the enteric epithelium (Pellegrini *et al.*, 2017; Rooks and Garrett, 2016). Mice with gene deletion of inflammasome components, including ASC and caspase-1 subunits, displayed a decrease in antimicrobial peptide levels and impairments of IEB, as compared with WT animals. In these animals, the administration of IL-18 was associated with a restoration of antimicrobial peptide levels and an improvement of mucosal permeability, thus suggesting a pivotal role of IL-18 in the preservation of IEB integrity (Chen, 2017). In this context, metabolic products of enteric bacteria, mainly SCFAs,

have been shown to promote NLRP3 activation and the consequent IL-18 release (Rooks and Garrett, 2016). Indeed, animals fed with high fiber diet displayed a remodeling of gut microbiota, characterized by an increase in SCFAs, along with an activation of inflammasome pathways that, in turn, contributed to epithelial repair and protection, as compared with mice fed with standard diet (Macia *et al.*, 2015). The molecular mechanisms underlying the relationship between SCFAs and NLRP3 activation rely mainly on G-protein-coupled receptors (GPCRs), including GPR43 and GPR109A expressed on IECs. In particular, SCFAs activate GPRCs that, in turn, promote the assembly and activation of NLRP3 inflammasome through the increase in Ca^{2+} mobilization and K^{+} efflux (Macia *et al.*, 2015). These findings suggest that the actions of SCFA-producing bacteria on NLRP3 inflammasome preserves the intestinal epithelium, contributing to the maintenance of gut homeostasis. In support of this view, mice treated with an antibiotic cocktail displayed alterations of gut microbiota, characterized by an increase in *Proteobateria* and *Tennericutes*, along with a decrease in Bacteroidetes and Firmicutes, decrease in SCFA levels, and impaired IEB (Feng *et al.*, 2019; Rakoff-Nahoum *et al.*, 2004). Thus, based on current knowledge, it appears that bidirectional interactions between gut microbiota and NLRP3 inflammasome contribute to preserve the homeostasis of intestinal microenvironment, through the maintenance of immune-bacterial tolerance and IEB integrity.

Of interest, the NLRP3 inflammasome–gut microbiota interplay is emerging also as a key network involved in the coordination of host physiologies, including the modulation of adipose tissue, liver, kidney and brain functions (Ayres, 2013). Indeed, a concomitance of altered gut microbiota towards pathogenic phenotypes and overactivation of NLRP3 inflammasome pathways, besides contributing to the pathogenesis of intestinal diseases (i.e. IBDs and colorectal cancer), represents the main determinant involved in the onset of several extra-digestive diseases, including metabolic disorders and related co-morbidities, liver diseases and neurological as well as psychiatric disorders (Belizario *et al.*, 2018; Inserra *et al.*, 2018; Wang *et al.*, 2019). Therefore, the NLRP3 inflammasome–gut

microbiota network acts as a pivotal player both in regulating the maintenance of intestinal homeostasis and coordinating the host physiology.

5. Interplay between gut microbiota and NLRP3 inflammasome in the maintenance of brain homeostasis

Several lines of evidence point out the gut microbiota as a pivotal player in the maintenance of brain homeostasis (Foster *et al.*, 2017; Fung *et al.*, 2017). Indeed, alterations of enteric bacteria, besides compromising the intestinal microenvironment integrity, have been found to alter the brain physiology (Luczynski *et al.*, 2016a; Luczynski *et al.*, 2016b). In addition, studies in GF mice have shown that enteric bacteria can influence several biological processes in the CNS (i.e. development, neurogenesis, neurotransmission and immune cell activity) and preserve the integrity of blood brain barrier (BBB) (Erny *et al.*, 2015; Luczynski *et al.*, 2016a). The mechanisms underlying the microbiota gut-brain axis rely mainly on interactions of enteric bacteria with IEB, immune system and nerve pathways (Pellegrini *et al.*, 2018). In particular, the immune-bacterial interplay represents one of the main networks involved in gut-brain communication. Specific bacterial products (e.g., SCFAs, vitamins or neurotransmitters) can indeed translocate into the blood stream and spread upwards to the brain, where they can influence the central circuits through the regulation of CNS immune/inflammatory cell activities, including the microglia. In addition, bacterial products can directly activate circulating immune cells, which, in turn, migrate to the CNS and modulate the brain physiology (Fung *et al.*, 2017; Rooks and Garrett, 2016; Rothhammer *et al.*, 2016; Wikoff *et al.*, 2009). This body of evidence suggests that the bacteria-immune interplay contributes to communications between the gut-brain axis. In this context, the NLRP3 inflammasome, regarded as an immune sentinel sensing enteric bacteria, has been shown to act at the crossroad between gut microbiota and immune system in the regulation of brain physiology, thus representing a novel mechanism underlying the gut-brain communications referred as ‘microbiota-gut-inflammasome-brain axis’ (Wong *et al.*, 2016). Most of current evidence about the role of microbiota-inflammasome

interplay in the coordination of brain physiology comes from preclinical studies in mice subjected to manipulation of the gut microbiota as well as in animals with gene deletion of inflammasome components, including caspase-1, ASC, NLRP3 and IL-1β. Lowe et al., (2018) showed that the depletion of gut microbiota with an antibiotic cocktail influenced the inflammasome signaling in the intestine, blood and brain (Lowe *et al.*, 2018). In particular, depleted mice displayed an increase in mRNA expression of caspase-1, ASC, IL-18 and IL-1β in both intestinal and brain tissues as well as an increment of circulating IL-1β levels, thus suggesting that the gut microbiota influences the expression of inflammasome components (Lowe *et al.*, 2018). Wong et al., (2016) observed that caspase-$1^{-/-}$ mice displayed altered enteric microbiota with altered behavior and locomotion, as compared with WT animals (Wong *et al.*, 2016). However, these authors did not demonstrate a relationship between the lack of inflammasome signaling, changes in enteric bacteria and behavioral and motor alterations. Of note, a recent pioneering study by Zhang et al., (2019) showed that NLRP3 gene deficiency in mice altered the gut microbiota composition and affected both mood-related behaviors and locomotor activities. Moreover, cohousing WT and $NLRP3^{-/-}$ animals reshaped the enteric bacteria and prevented the effects of NLRP3 inflammasome gene depletion on both locomotion and behavior (Zhang *et al.*, 2019). Taken together, these findings suggest that the gut microbiota, through a modulation of NLRP3 inflammasome pathways, contributes to the regulation of brain functions. One of the hypothesized molecular mechanisms through which the microbiota-gut-inflammasome-brain axis could influence CNS functions has been ascribed to the ability of regulating astrocyte functions, through the modulation the plasma levels of circular RNA homeodomain-interacting protein kinase 2 (HIPK2) (Huang *et al.*, 2017; Zhang *et al.*, 2019). However, future investigations are clearly needed to elucidate the molecular and cellular mechanisms underlying the interactions among gut microbiota and NLRP3 inflammasome as well as their role in the regulation of gut-brain communications both in physiological and pathological conditions.

6. Role of gut microbiota-NLRP3 inflammasome interplay in the pathophysiology of neurological and psychiatric diseases

Recent investigations have been focused on the role of gut microbiota or NLRP3 inflammasome complex in the pathophysiology of neurological and psychiatric diseases, including PD, AD, MS, ASD and MDD. In this regard, while a number of exhaustive review articles have provided well integrated overviews about the involvement of the gut microbiota or NLRP3 inflammasome in these disorders, the relevance of their mutual interplays has been fairly disregarded (Heneka *et al.*, 2018; Liu *et al.*, 2019; Ma *et al.*, 2019; Peirce and Alvina, 2019). Thus, in an attempt to filling this gap, the most prominent clinical and experimental data on the role played by gut-microbiota-NLRP3 inflammasome interactions in the pathophysiology of CNS disease have been addressed in the following sections and summarized in Tables 1, 2 and 3.

6.1 Evidence in humans

Changes in gut microbiota composition and the occurrence of enteric inflammation have been documented in CNS patients at different stages of disease (Pellegrini *et al.*, 2018). Besides bowel motor dysfunctions, these changes could promote conditions of peripheral inflammation and contribute to neuroinflammation and neurodegeneration in the CNS, via bacteria-immune-gut-brain axis (Pellegrini *et al.*, 2018). In this context, NLRP3 inflammasome signaling is emerging as an immune sentinel pivotally involved in shaping intestinal, peripheral and central immune/inflammatory responses in CNS diseases. In support of this view, clinical studies have shown that patients with PD, AD, MS, ASD and MDD are characterized by changes in gut microbiota composition as well as enteric, peripheral and brain activation of NLRP3 inflammasome (Bedarf *et al.*, 2017; Cao *et al.*, 2015; Cheung *et al.*, 2019; Chiang and Lin, 2019; Fang *et al.*, 2016; Gordon *et al.*, 2018; Keane *et al.*, 2018; Keshavarzian *et al.*, 2015; Liu *et al.*, 2019; Ming *et al.*, 2002; Qin *et al.*, 2016; Saresella *et al.*, 2016; Tan *et al.*, 2014; Vogt *et al.*, 2017; Zhou *et al.*, 2016) (Table 1).

Tough current evidence about the implication of changes in gut microbiota in CNS diseases is heterogeneous and often conflicting, likely owing to for different methodological approaches, diet and geographical and/or clinical background of the study populations, patients with PD, AD, MS, ASD and MDD display patterns of "pro-inflammatory" dysbiosis, characterized mainly by a decrease in families (i.e. Prevotellaceae and Lachnospiraceae) and genera (i.e. *Akkermansia, Roseburia,, Faecalibacterium, Blautia, Coprococcus* and *Roseburia*) involved in the production of SCFAs (Cheung *et al.*, 2019; Mowry and Glenn, 2018; Pistollato *et al.*, 2016; Scheperjans *et al.*, 2015; Unger *et al.*, 2016; Vuong and Hsiao, 2017). Indeed, these patients are characterized by significant decrease in fecal and circulating SCFA levels, that might compromise both the intestinal and brain barrier, and facilitate the activation of immune/inflammatory pathways (Adams *et al.*, 2011; Keshavarzian *et al.*, 2015; Skonieczna-Zydecka *et al.*, 2018). Such alterations could depend on a NLRP3 hypo-functionality at level of epithelial and endothelial cells, known to be pivotal to the preservation of barrier integrity, along with an overactivation of NLRP3 signalling in immune/inflammatory cells, including macrophages, monocytes and microglial cells (Figure 1). In support of this view, recent studies have shown a decrease in SCFA-producing bacteria, and an increase in colonic IL-1β levels along with impaired IEB in PD patients (Devos *et al.*, 2013; Perez-Pardo *et al.*, 2019). Therefore, it is conceivable that a 'pro-inflammatory' dysbiosis in CNS patients, mainly characterized by a decrease in SCFA-producing bacteria and an increment of pathogenic bacteria strains, could promote the overactivation of NLRP3 inflammasome signaling in immune/inflammatory cells, that, in turn, could impair both the IEB and BBB as well as shape both peripheral and central neurogenic/immune-inflammatory responses, thus contributing to CNS pathology. Indeed, an overactivation of inflammasome signaling, characterized by an increase in NLRP3, ASC, caspase-1 and IL-1β, in the serum, peripheral monocytes and cerebrospinal fluid has been documented in PD, AD, MS and ASD patients (Cao *et al.*, 2015; Gordon *et al.*, 2018; Keane *et al.*, 2018; Ming *et al.*, 2002; Qin *et al.*, 2016; Saresella *et al.*, 2016; Tan *et al.*, 2014; Zhou *et al.*, 2016). In addition, an increase in both mRNA and protein expression of NLRP3 and caspase-1, along with an increment of IL-1β levels, in peripheral

blood mononuclear cells from MDD patients have been found to correlate with the severity of disease (Alcocer-Gomez *et al.*, 2014; Kim *et al.*, 2016). Heneka et al. and Venegas et al. reported an increased ASC-bound amyloid β protein, caspase-1 activity and IL-1β levels in microglial cells from the frontal cortex and hippocampus of AD patients since the earliest stage of disease (Heneka *et al.*, 2013; Venegas *et al.*, 2017). Likewise, others showed an overactivation of inflammasome pathways, characterized by an increase in NLRP3, cleaved caspase-1 and ASC expression, along with an increase in IL-1β, in brain tissues from PD and MS patients (Gordon *et al.*, 2018; Keane *et al.*, 2018). Taken together, current evidence suggests that changes in gut microbiota, characterized by an increase in pathogenic bacterial strains, and NLRP3 inflammasome activation may represent common paths to different CNS disorders. Indeed, patients with PD, AD, MS, ASD and MDD display similar changes in gut microbiota composition as well as enteric, circulating and central activation of inflammasome pathways. However, this knowledge does not allow to establish a clear relationship between altered gut microbiota, activation of NLRP3 inflammasome signaling and brain pathology. In this regard, few studies have evaluated, in the same cohort of patients with different CNS diseases, putative correlations between alterations of enteric bacteria and activation of NLRP3 inflammasome signalling (Carissimi *et al.*, 2019; Cattaneo *et al.*, 2017; Lin *et al.*, 2019; Perez-Pardo *et al.*, 2019). Lin et al. (2019) showed that, in patients with PD, alterations of gut microbiota, characterized by a decrease in *Verrucomicrobia, Mucispirillum, Porphyromonas, Lactobacillus,* and *Parabacteroides,* along with an increased abundance in *Prevotella*, correlated with an increase in circulating pro-inflammatory cytokine levels, including IL-1β, thus suggesting a possible involvement of inflammasome activation (Lin *et al.*, 2019). Likewise, Perez-Pardo et al., (2019) reported alterations of intestinal bacteria, characterized by a decrease in SCFAs, along with an increase in colonic IL-1β levels, in PD patients (Perez-Pardo *et al.*, 2019). Carissimi et al., (2019) showed that ASD patients displayed decreased gut microbiota biodiversity, impaired SCFA catabolism and an increase in plasma IL-1β levels. In addition, these authors observed an increase in fecal HMGB1 levels and this pattern appeared to correlate with the severity of GI symptoms (Carissimi *et al.*, 2019). The release

of the alarmin HMGB1 is regulated by both caspase-1 and caspase-11, whose activation depend mainly on NLRP3 oligomerization. However, the authors did not evaluate a concomitance of intestinal and circulating activation of other components of the inflammasome pathway, including NLRP3, ASC, caspase-1 and -11, and, most importantly, they did not clarify the role of the bacteria-inflammasome interplay in ASD progression. Of note, Cattaneo et al., (2017) showed that an increase in the abundance of pro-inflammatory bacteria *Escherichia/Shigella,* along with a decreased abundance of anti-inflammatory *E. rectale* taxon was associated with peripheral NLRP3 signaling activation in patients with cognitive impairment and brain amyloidosis, thus suggesting that changes in enteric bacteria could promote peripheral inflammasome activation (Cattaneo *et al.*, 2017).

Overall, current evidence suggests that changes in gut microbiota, characterized by an increase in 'pro-inflammatory' bacteria strains along with decreased SCFA-producing bacteria and NLRP3 inflammasome, could contribute to neurogenic/immune-inflammatory responses in CNS diseases via gut-microbiota-inflammasome-brain axis. However, it remains to be clarified whether changes in enteric bacteria promote NLRP3 activation or whether dysregulations of inflammasome signalling could induce alterations of enteric bacteria, thereby contributing to brain disorders. In this regard, since dysregulation of enteric NLRP3 activation has been found to alter the intestinal microbiota, and that the accumulation CNS disease-related proteins, including Aβ, tau or α-syn and their heterocomplexes in the intestine, red blood cells and brain can promote NLRP3 overactivation (Gordon *et al.*, 2018; Piccarducci *et al.*, 2019), it is conceivable that an overactivation of NLRP3 pathways can determine the shift of gut microbiota towards 'pro-inflammatory' phenotypes, thus promoting both peripheral and central neuroinflammatory and neurodegenerative processes. However, the molecular mechanisms underlying the bacteria-NLRP3 inflammasome interplay in the pathophysiology of CNS diseases remain to be elucidated. Thus, future research efforts should be dedicated to better clarify the pathophysiological role of microbiota-gut-inflammasome-brain axis in patients with CNS disorders.

6.2 Evidence in pre-clinical models

In an attempt of understanding the putative role of microbiota-gut-inflammasome-brain axis in the pathophysiology of neurological and psychiatric disorders, research efforts have been made in animal models of CNS diseases. In this context, the majority of studies have shown that animal models of CNS disorders are characterized by altered gut microbiota or inflammasome signaling activation, investigated as distinct determinants of the pathophysiology of CNS diseases (Gordon *et al.*, 2018; Sampson *et al.*, 2016). For instance, a pioneering study by Sampson et al. (2016) showed that genetic Thy1-α-syn PD mice, receiving gut microbiota from PD patients, displayed a worsening of GI dysfunctions, along with enhanced motor impairment, microglia activation and α-syn deposition in the brain, as compared with PD mice subjected to FTM from healthy controls. In addition, the authors showed that, under GF conditions, or following the depletion of gut microbiota by an antibiotic cocktail, PD animals were characterized by a decrease in microglia activation and α-syn aggregation, along with an improvement of motor deficiencies. Moreover, the administration of SCFA mixtures to GF transgenic PD mice was associated with worsening of GI dysfunctions, motor impairment and α-syn inclusions, as compared with GF PD mice. These findings suggest that the gut microbiota may contribute to PD, and that treatment with antibiotics or gut microbiota depletion can counteract the progression of CNS pathology, while treatment with microbially-produced SCFAs restores all the major features of disease observed in GF transgenic PD mice (Sampson *et al.*, 2016). Conversely, several papers have reported that SCFA treatment exerted beneficial effects in animal models of PD, AD, depression, and traumatic brain injury (Ho et al., 2018; Li et al., 2016; Liu et al., 2017; Macfabe, 2012; Sun et al., 2016; Sun et al., 2019; Sun et al., 2018). These conflicting results, about the effects of SCFAs on CNS pathologies, could depend on different methodological approaches or disease phenotypes. Nevertheless, such findings highlight the relevance of gut microbiota in the pathophysiology of CNS disorders.

Besides the gut microbiota, a pivotal role for NLRP3 inflammasome in the onset of brain pathologies has been described (Bellezza *et al.*, 2018; Gordon *et al.*, 2018; Heneka *et al.*, 2013; Tha *et al.*, 2000;

Venegas *et al.*, 2017). For instance, Gordon et al. (2018) observed an increase in the expression of NLRP3 inflammasome components in the *substantia nigra* of mice with PD induced by intranigral injection of 6-hydroxydopamine or α-syn preformed fibril, and that the pharmacological inhibition of inflammasome with MCC950, a known selective NLRP3 inhibitor, counteracted microglia activation, nigrostriatal degeneration, α-syn accumulation and motor deficiencies in PD animals (Gordon *et al.*, 2018). Likewise, Venegas et al. reported that central NLRP3 activation contributed to Aβ deposition and disease progression since the earliest stage of AD (Venegas et al., 2017).

Of note, the above studies, despite of high interest, investigated the role of gut microbiota and NLRP3 inflammasome as distinct determinants of CNS diseases, leaving out the possible relevance of their interplay. In this context, most of current knowledge comes from studies on CNS animals with manipulation or depletion of gut microbiota, as well as pharmacological modulation or gene deletion of the inflammasome components (Table 3). A recent report by Guo et al., (2018) showed that mice with chronic restraint stress (CRS)-induced anxiety-like and depressive-like behaviors displayed gut dysbiosis, characterized by an increase in Firmicutes and decrease in Bacteriodetes and Proteobacteria, along with central and peripheral inflammatory responses, characterized by an increase in IL-1β levels in the hippocampus and serum, thus suggesting the concomitance of alterations of enteric bacteria and inflammasome signaling activation (Guo *et al.*, 2018). In addition, a treatment with rosemary extracts exerted beneficial effects in CRS animals, through the modulation of both gut microbiota and immune/inflammatory responses, including a decrease in circulating and central IL-1β levels (Guo *et al.*, 2018). However, the authors did not demonstrate a relationship between the antidepressant effects of rosemary extracts, the modulation of gut microbiota and the inhibition of central and peripheral NLRP3-mediated inflammatory responses (Guo et al., 2018). In a subsequent paper, the same authors showed that treatment of CRS animals with the probiotic *Bifidobacterium adolescentis* counteracted the IL-1β-induced CNS inflammatory responses and ameliorated the behavioral alterations, suggesting that the manipulation of gut microbiota can exert anti antidepressant-like effects through the inhibition of inflammasome-induced inflammatory

responses in the brain (Guo et al., 2019). Of note, in a recent paper, Zhou et al., (2019) documented that the intake of fasting mimicking diet by 1-methyl-4-phenyl-1,2,3,6-tetrathydropyridine (MPTP)-induced PD mice ameliorated their motor dysfunctions, attenuated the loss of dopaminergic neurons in the *substantia nigra* and decreased brain IL-1β, likely through an increase in SCFA-producing bacteria. In addition, FMT from control mice fed with fasting mimicking diet to PD mice, following depletion of their gut microbiota with an antibiotic cocktail, increased dopamine levels and improved brain inflammation, suggesting that the modulation of gut microbiota can exert beneficial effects in PD animals, by counteracting IL-1β-induced central inflammatory responses (Zhou et al., 2019). Taken together, these results point out an involvement of gut microbiota–inflammasome signalling interplay in CNS inflammation and neurodegeneration, and that the modulation of gut microbiota could represent a useful therapeutical option for the management of psychiatric and neurological disorders, through the modulation of central and peripheral inflammasome-mediated inflammatory responses. However, the authors did not evaluate a direct and casual relationship of microbiota-gut-inflammasome-brain axis with CNS alterations.

Of interest, a recent paper by Lowe et al., (2018) showed that alcohol-induced neuroinflammation in mice was associated with central, circulating and intestinal inflammasome signaling activation, and that treatment with an antibiotic cocktail counteracted significantly both peripheral and central neuroinflammation by inhibition of NLRP3 inflammasome activation (Lowe et al., 2018). These findings suggest a pivotal role of microbiota-gut-inflammasome-brain axis in the onset of central neurogenic/inflammatory responses. In support of this view, a pioneering study by Wong et al., (2016) showed that caspase-$1^{-/-}$ gene deletion decreased anxiety-like and depressive-like behaviors in CRS mice. In addition, treatment with minocycline, a tetracycline antibiotic able to modulate the gut microbiota and inhibit caspase-1, ameliorated the stress-induced depressive-like behavior in CRS mice (Wong *et al.*, 2016). In particular, minocycline increased the relative abundance of *Akkermansia* and *Blautia*, both regarded as beneficial bacteria involved in the maintenance of IEB and the modulation of immune/inflammatory responses (Wong *et al.*, 2016). Likewise, Zhang et al., (2019)

showed that NLRP3$^{-/-}$ mice with chronic unpredictable stress (CUS)-induced depression displayed an improvement of behavioral alterations, as compared with CUS animals. In addition, cohousing WT and NLRP3$^{-/-}$ mice, as well as FMT from NLRP3-/- animals, ameliorated significantly the depressive-like behaviors in CUS mice (Zhang et al., 2019).

In a recent paper, Shen et al. (2020) showed that FMT from AD patients to APP/PS1 double transgenic AD mice was associated with an increased expression of intestinal and circulating NLRP3 inflammasome components, including NLRP3, caspase-1 and IL-1β, as compared with APP/PS1 subjected to FTM from healthy subjects (Shen *et al.*, 2020). AD mice receiving gut microbiota from AD patients displayed also an impairment of cognitive functions and central neurogenic/inflammatory responses, that was more severe than AD mice receiving enteric bacteria from healthy subjects (Shen *et al.*, 2020). In addition, the authors observed that AD mice, treated with minocycline or receiving gut microbiota from healthy subjects, were characterized by a decrease in intestinal, circulating and central NLRP3 inflammasome activation, improvements of cognitive functions and suppression of neurogenic/inflammatory responses. Of note, in the same paper, Shen et al. (2020) showed that FTM from AD patients to WT mice was associated with activation of inflammasome signalling in the gut, blood and brain along with microglia activation, while no changes in the cognitive impairment were detected. To explain these findings, it is conceivable that WT mice need more time to develop cognitive impairments than APP/PS1 double transgenic AD mice (Shen *et al.*, 2020). Therefore, additional experiments, aimed at investigating whether WT mice receiving gut microbiota from AD patients, besides the occurrence of neuroinflammatory processes in the intestine, blood and brain, develop also cognitive impairment and/or the deposition AD-related proteins, should be performed. In addition, a characterization of enteric bacteria composition in AD patients should be implemented, in order to identify the bacterial strains responsible for the activation of NLRP3 inflammasome signaling and the progression of neurodegeneration.

Taken together, these findings suggest the relevance of gut microbiota-NLRP3 inflammasome network in the onset of central neuroinflammation and neurodegeneration. However, further studies,

aimed at evaluating how specific alterations of the gut microbiota and activation of inflammasome pathways contribute to central neuroinflammation and neurodegeneration in different animal models of CNS disorders, should be implemented.

7. Conclusions and future perspectives

Current evidence from human studies shows that patients with major neurological and psychiatric disorders are characterized by a gut dysbiosis, which includes a decrease in SCFA-producing bacteria and an increase in pro-inflammatory' bacterial strains, and a condition of NLRP3 inflammasome signaling activation that could contribute to neuroinflammation and neurodegeneration in the CNS.

Of note, studies in animal models of CNS diseases, with particular regard for PD, depression and anxiety, are allowing the characterization of the pathophysiological role of the gut-microbiota-inflammasome-brain axis in the occurrence of brain pathologies. Accordingly, the manipulation or targeted-depletion of the gut microbiota, including FMT, as well as gene deletion or the pharmacological modulation of inflammasome signaling have been found to exert beneficial effects in these models.

Based on current knowledge, it is conceivable that changes in the gut microbiota, leading to the activation of inflammasome pathways, could trigger peripheral and central inflammatory responses, thus contributing to the onset of CNS disorders. In particular, the NLRP3 inflammasome complex, sensing changes in enteric bacteria and its metabolites, behaves as a bacteria-immune sentinel, deputed to regulate the gut-microbiota-brain communications. In parallel, the overactivation of NLRP3 could alter further the enteric bacteria, thus generating a sort of vicious circle that might lead to the chronicization of peripheral and central neuroinflammatory and neurodegenerative processes. In support of this view, it is noteworthy that the deposition of CNS disease-related proteins, including Aβ, tau or α-syn and their heterocomplexes in the intestine, red blood cells and brain from CNS patients can activate NLRP3 inflammasome assembly (Gordon *et al.*, 2018; Heneka *et al.*, 2013; Piccarducci *et al.*, 2019). However, the molecular mechanisms underlying the alterations of gut-

microbiota-inflammasome-interplay in CNS disorders as well as its role in the pathophysiology of brain pathology remain to be elucidated. In addition, given the role of other inflammasomes, including NLRP6, NLRP1, AIM2, and NLRC4, in the onset of immune/inflammatory responses both in intestinal and brain disorders, their involvement in the regulation of microbiota-gut-brain axis should be deeply investigated as well.

A substantial gap in our knowledge pertains also to the possible influence of diet on the gut microbiota-inflammasome interplay, both under physiological conditions and in the presence of CNS disorders. In this regard, a number of exhaustive review articles have provided well-integrated overviews about the effect of diet on behavioral, cognitive and neurochemical alterations associated with neurological and psychiatric disorders, including AD, PD, MS, schizophrenia, autism and anxiety, through the modulation of gut microbiota (Pistollato *et al.*, 2016; Sandhu *et al.*, 2017; Tengeler *et al.*, 2018). For instance, western diet, including saturated fatty acids, sugar and proteins, through a decrease in the abundance of *Lactobacillus*, Ruminococcaceae, Lachnospiraceaeae and SCFA-producing bacteria strains (e.g. *Ruminococcus bromii, Faecalibacterium prausnitzii, Eubacterium rectale, Eubacterium hallii, and Anaerostipes coli SS2/1),* along with an increase in bile-tolerant bacteria, like *Bacteroides, Alistipes*, and *Bilophila*), has been found to be associated with an increased risk of developing dementia (Beilharz *et al.*, 2016; Daniel *et al.*, 2014; David *et al.*, 2014; Singh *et al.*, 2017; Whitmer *et al.*, 2005). Indeed, a probiotic mixture containing multiple strains of Bifidobacterium and Lactobacillus was able to prevent diet-induced hippocampal-dependent spatial memory deficits (Beilharz *et al.*, 2018). Conversely, the mediterranean diet, including polyunsaturated fatty acids (e.g. docosahexaenoic acid, eicosapentaenoic acid, omega-3 and -6 fatty acids), plant proteins, vitamins, polyphenols and fibers, acting mainly through an increase in the abundance of *Bifidobacterium* and *Lactobacillus* and a decrease in *Bacteroides* and *Clostridium*, has been found to improve cognitive functions and memory in healthy postmenopausal women, to counteract behavioral symptoms in patients with autism spectrum disorder as well as to reduce amyloid aggregation and the incidence of amyloid-related diseases in elderly patients (Bastianetto *et*

al., 2008; Henderson *et al.*, 2012; Meguid *et al.*, 2008; Richardson, 2006; Rigacci and Stefani, 2015; Singh *et al.*, 2017; Stefani and Rigacci, 2014). In addition, SCFA-derived indigestible fibers, obtained by fermentation of enteric bacteria, mainly belonging to Bacteroidetes and Firmicutes phyla including *Ruminococcus bromii, Faecalibacterium prausnitzii, Eubacterium rectale, Eubacterium hallii, and Anaerostipes coli SS2/1*, have been shown to exert beneficial effects in animal models and patients with CNS diseases (Joseph *et al.*, 2017; Sharma *et al.*, 2015; van de Wouw *et al.*, 2018). However, there is also evidence showing high levels of fecal SCFAs in CNS diseases and detrimental effects arising from SCFA tretment in animals models of brain disorders (Kelly et al., 2016; MacFabe, 2015; Sampson et al., 2016). Therefore, the role of SCFAs in gut-brain communication remains to be clarified and deserves further investigations. Of note, other dietary fibers, regarded as prebiotics, such as galactooligosaccharide, fructooligosaccharide, and polydextrose were reported to decrease anxiety and improve cognition in mice and humans, by incresing the abundance of probiotic bacteria (e.g Bifidobacteria and Lactobacilli) (Allen *et al.*, 2016; Canfora *et al.*, 2017; Savignac *et al.*, 2015; Thongaram *et al.*, 2017).

Of interest, several studies have reported that diet can influence also NLRP3 inflammasome activation (Camell *et al.*, 2015; Pinto *et al.*, 2018). For instance, western diet, rich in cholesterol and high fats, can promote the assembly of NLRP3 inflammasome complex with the consequent release of IL-1β levels, that, in turn, promotes the activation of immune/inflammatory cells (Camell *et al.*, 2015). Conversely, ketogenic diet, containing medium chain triglyceride oil, magnesium, electrolytes, fiber, polyunsatured fatty acids, digestive enzymes and L-theanine, has been found to improve memory encoding, through the inhibition of both steps of NLRP3 activation (Pinto *et al.*, 2018).

Based on the above considerations, it is conceivable that, a diet poor in fibers, regarded as the main substrates for SCFA production (Cryan and Dinan, 2012; Koh *et al.*, 2016), could promote changes in the gut microbiota, with consequent central and peripheral NLRP3-induced immune/inflammatory responses. In support of this view, Savignac et al., (2016) reported that treatment with indigestible

galactooligosaccharide counteracted sickness behaviour and anxiety induced by LPS in mice, through a modulation of gut microbiota composition and a decrease in brain IL-1β levels (Savignac *et al.*, 2016). However, the effects of diet and SCFAs on gut-microbiota-inflammasome-brain axis, both under physiological conditions and in the presence of CNS disorders, remain to be clarified and deserve further investigations. In addition, given the relevance of the interaction between SCFA receptors and NLRP3 in maintaining IEB integrity, the role of this bacteria-immune interplay in the preservation of BBB should be elucidated.

Another considerable gap concerns whether individual factors, including genetic and epigenetic background and environmental exposure, specific to each brain disease (e.g. neuropsychiatric disorders tend to occur over the development, while neurodegenerative diseases during aging) (De Felice *et al.*, 2015; Dzamko *et al.*, 2015; Iqbal and Grundke-Iqbal, 2010; Vesikansa, 2018), could influence differently the gut-microbiota-inflammasome-brain-axis. Indeed, though several studies have shown that patients with CNS disorders display several changes in gut microbiota composition that could contribute to brain pathology, the direct influence of genetic, epigenetic and environmental factors on the alterations of enteric bacteria remains fairly unclear. Nevertheless, several lines of evidence have shown that patients with CNS disorders are all characterized by central and peripheral activation of NLRP3 pathways, thus suggesting that, despite each disease displays distinct temporal, clinical, neuropathological, genetic features and environmental context, NLRP3 activation represents a common immune sensor underlying the neurogenic/inflammatory responses in such diseases. Therefore, it is conceivable that, despite distinct genetic, epigenetic and environmental factors as well as different changes in enteric bacteria composition, CNS patients display a 'pro-inflammatory' dysbiosis characterized by the release of specific pathogenic bacterial products that trigger intestinal, circulating and central neurogenic/inflammatory responses through the activation of NLRP3 inflammasome pathways. However, further investigations, aimed at elucidating the influence of the different temporal factors of each disease on the gut-microbiota-inflammasome-brain-axis, should be implemented.

In conclusion, based on current knowledge, some important issues remain to be addressed: (1) What is the role of microbiota-gut-inflammasome-brain axis in the pathophysiology of CNS disease? (2) Are other inflammasomes involved in this bacteria-immune interplay? (3) Can the alterations of enteric bacteria-inflammasome network represent an early biomarker of CNS diseases? (4) Can the pharmacological modulation of enteric microbiota and/or inflammasome complex be a suitable therapeutical option for the management of brain disorders? (5) What is the role of diet? (6) What is the impact of temporal factors, including individual genetic, epigenetic and environmental features, which can increase the susceptibility to develop brain diseases, on gut-microbiota-inflammasome-brain axis? (7) As CNS disorders result from multifactorial etiologies, characterized by complex interactions among genetic and epigenetic background, environmental factors, gut microbiota and host immune system (Vesikansa, 2018), what are the pathophysiological mechanisms underlying CNS diseases un-related to gut microbiota or inflammasome signalling that could contribute to brain pathology?

To elucidate these issues, studies aimed at investigating the alterations of the bacteria-inflammasome interplay both in animal models and patients with CNS disorders classified on the basis of genetic and epigenetic background, gender, diet and different environmental factors, should be implemented. Clarifying these aspects could pave the way to a holistic view of the major neurological and psychiatric disorders, and promote the identification of common therapeutic strategies for their management.

Declarations of Competing Interest

The Authors declare no competing interest

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Table 1. Summary of the main changes in gut microbiota and activation of NLRP3 signaling observed at CNS, circulation and intestinal in patients with neurological disorders

Neurological disorder	Main changes in gut microbiota composition	CNS NLRP3 activation	Circulating NLRP3 activation	Intestinal NLRP3 activation	References
PD	↑↓ Bacteroidetes (conflicting evidence) ↓= Firmicutes (conflicting evidence) *Blautia, Coprococcus, Roseburia, Escherichia coli, Akkermansia, Bifidobacterium, Flavonifractor and Lactobacillus, Christensenella, Catabacter, Oscillospira,, Christensenella minuta, Catabacter hongkongensis, Lactobacillus mucosae, Ruminococcus bromii, and Papillibacter cinnamivorans, Prevotella* ↓ *Ralstonia, Faecalibacterium prausnitzii, Clostridium coccoides and Bacteroides fragilis, Dorea, Bacteroides, Bacteroides massiliensis, Stoquefichus massiliensis, Bacteroides coprocola, Blautia, glucerasea, Dorea longicatena, Bacteroides dorei, Bacteroides pebeus, Coprococcus eutactus, Rominococcus callidus, Verrucomicrobia, Mucispirillum, Porphyromonas, Lactobacillus,* and *Parabacteroides* ↑ Enterobacteriaceae, Lachnospiraceae Lactobacillaceae, Verrucomicrobiaceae ↓ Prevotellaceae *(Prevotella copri),* Erysipelotrichaceae *(Eubacterium biforme),* Barnesiellaceae and Enterococcacea ↓ Fecal SCFAs levels (butyrate, acetate, propionate)	↑ NLRP3, caspase-1 and ASC ↑ IL-1β	↑ NLRP3, caspase-1 and ASC mRNA ↑ IL-1β	↑ IL-1β	Devos *et al.*, 2013; Perez-Pardo *et al.*, 2019 Gordon *et al.*, 2018; Wang *et al.*, 2016 Li et al., 2019 Qin *et al.*, 2016 Zou e al., 2016 Keshavarzian *et al.*, 2015
AD and Cognitively impaired elderly	↓ *Bacteroides, Blautia and Escherichia/Shigella* ↑ *SMB53 and Dialister and E. rectale* taxon ↓ Gut microbiota biodiversity, Impaired SCFA catabolism	↑ NLRP3, ASC and Caspase-1 ↑ IL-1β	↑ IL-1β ↑ NLRP3	↑ HMGB1	Heneka *et al.*, 2013; Venegas *et al.*, 2017 Catteneo et al., 2017 Saresella *et al.*, 2016;
MS	↓ Bacteroides (*Bacteroides stercoris, Bacteroides coprocola, and Bacteroides coprophilus*) ↑ *Psuedomonas, Mycoplana, Haemophilus, Blautia, and Dorea* genera	↑ NLRP3, ASC and cleaved caspase-1 protein ↑ IL-1β	↑ ASC and caspase-1 mRNA ↑ IL-1β	n.a.	Keane *et al.*, 2018; Cao 2015 Ming *et al.*, 2002

Abbreviations: AD: Alzheimer's disease; ASC: inflammasome adaptor protein apoptosis-associated speck-like protein containing CARD; CNS: central nervous system; HMGB1; high mobility group box 1 protein; IL-1β interleukin-1 beta;

MS: multiple sclerosis; NLRP3: nucleotide-binding oligomerization domain leucine rich repeat and pyrin domain-containing protein 3; SCFAs: short chain fat acids; PD: Parkinson's disease; SCFAs: short chain fat acids; n.a: not available

Table 2. Summary of the main changes in gut microbiota and activation of NLRP3 signaling observed at CNS, circulation and intestinal level in patients with psychiatric disorders

Psychiatric disorder	Main changes in gut microbiota composition	Central NLRP3 signaling activation	Circulating NLRP3 signaling activation	Intestinal NLRP3 signaling activation	References
ASD	↓ Gut microbiota biodiversity ↓ SCFA	n.a	↑ NLRP3 and caspase-1 mRNA ↑ IL-1β ↑ IL-18	n.a	Carissimi et al., 2019 Adams et al., 2011 Saresella et al., 2016
MDD	Bacteriodetes, Proteobacteria, Firmicutes *Lachnobacteriaceae* (conflicting findings) ↑ *Enterobacteriacea* ↑ *Clostridium, Streptococco, Prevotella* ↓ *Lactobacillus and Bifidobacterium* ↓ SCFA	n.a	↑ NLRP3 and caspase-1 mRNA and protein ↑ IL-1β ↑ IL-18	n.a	Alcocer-Gomez *et al.*, 2014 Skonieczna-Zydecka *et al.*, 2018 Huang et al., 2019
Bipolar disorder	Actinobacteria, Proteobacteria ↑ *Coriobacteriaceae, Ruminococcus* ↓ *Ruminococcaceae, Bacteroides* ↓ SCFA	↑ NLRP3, ASC, active caspase-1 protein ↑ IL-1β	↑ IL-1β	n.a	Kim *et al.*, 2016 Huang et al., 2019 Tsai et al., 2012 Lotrich et al., 2014

Abbreviations: ASD: autism spectrum disorder; ASC: inflammasome adaptor protein apoptosis-associated speck-like protein containing CARD; CNS: central nervous system; IL-1β interleukin-1 beta; MDD: major depressive disorder; NLRP3: nucleotide-binding oligomerization domain leucine rich repeat and pyrin domain-containing protein 3; SCFAs: short chain fat acids; n.a.: not available

Table 3. Summary of the current evidence showing the concomitance of gut dysbiosis and NLRP3 activation in animal models of CNS disorders

Experimental models		Changes in gut microbiota-NLRP3 inflammasome complex	*References*
PD	**MPTP-induced central dopaminergic neurodegeneration**	✓↓**SCFA** ✓↑**Central IL-1β**	***Zhou et al., 2019***
MDD	**CRS-induced anxiety-like and depressive-like behavious**	✓ **Gut dysbiosis (↑Firmicutes, ↓Bacteriodetes and ↓Proteobacteria)** ✓↑**Central and circulating IL-1β**	***Zhang et al., 2019*** ***Guo et al., 2018*** ***Guo et al., 2019*** ***Wong et al., 2016***
	CUS-induced anxiety-like and depressive-like behaviors	✓↑**Bacteroides** ✓↓**Ruminococcus, Desolfuvibrio, Mucispirillum, Oscillospira, Prevotella** ✓ **NLRP3 gene deletion altered enteric bacteria (↓Bacteroides, ↑Ruminococcus, Desolfuvibrio, Mucispirillum, Oscillospira, Prevotella)** ✓ **NLRP3 gene deletion improved depressive-like behavior** ✓ **Cohousing reduced the differences in behavior and locomotion between WT and NLRP3 knock out animals**	
Neuroinflammation	**Alcohol-induced neuroinflammation**	✓↑**Central, circulating and intestinal NLRP3 inflammasome signaling activation, including NLRP3, ASC, caspase-IL-1 and IL-18 mRNA expression and IL-1β and IL-18 cytokine levels)**	***Lowe et al., 2019***

Abbreviations: ASC: inflammasome adaptor protein apoptosis-associated speck-like protein containing CARD; CNS: central nervous system; CRS: chronic restraint stress; CUS: chronic unpredictable stress; IL-1β interleukin-1 beta; MDD: major depressive disorder; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrathydropyridine; NLRP3: nucleotide-binding oligomerization domain leucine rich repeat and pyrin domain-containing protein 3; SCFAs: short chain fat acids; PD: Parkinson's disease; WT, wild type

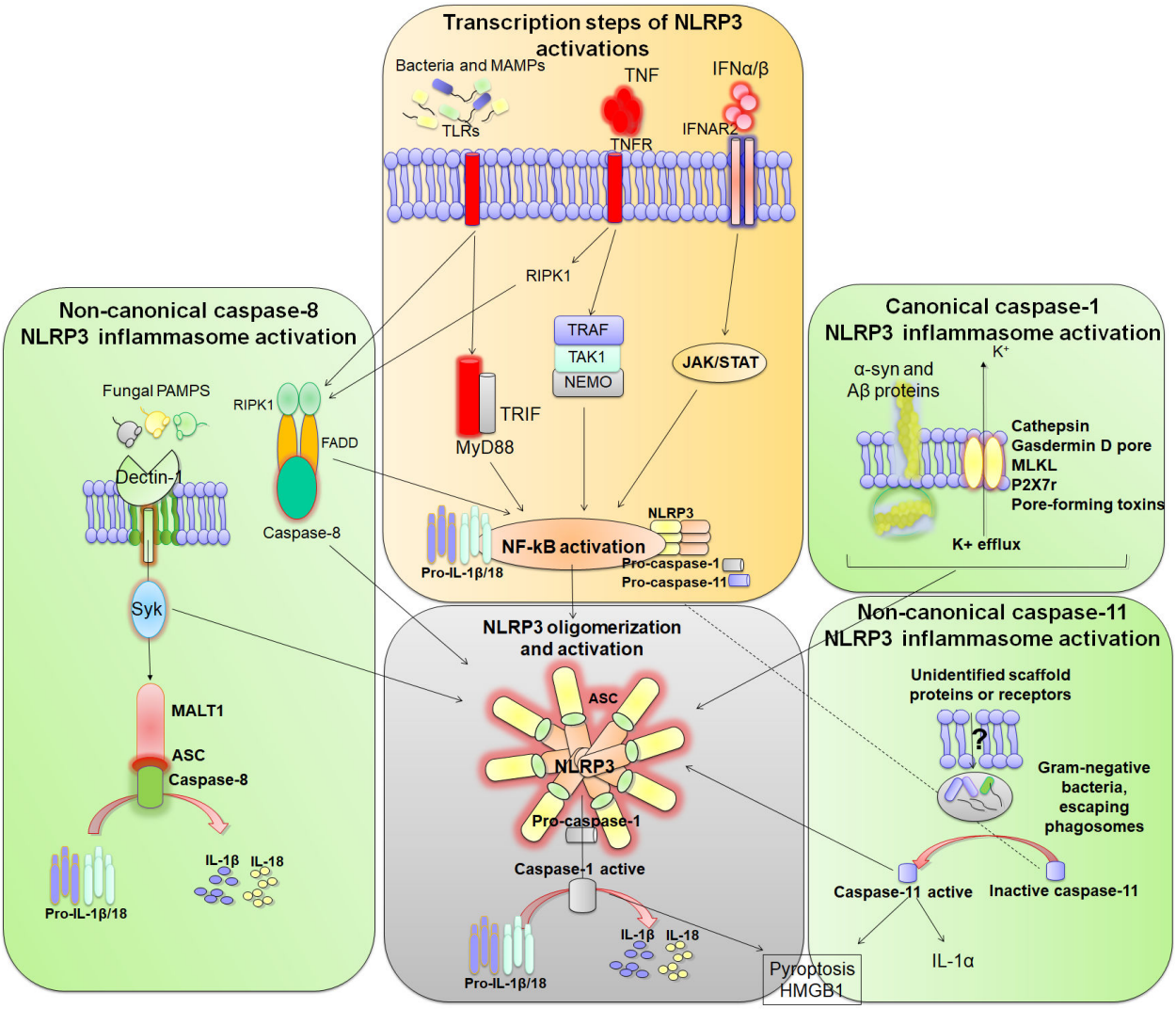

Figure 1. Mechanisms underlying NLRP3 inflammasome activation

Diagram showing the different molecular mechanisms of NLRP3 inflammasome activation. **Middle upper and lower panel**: Transcription steps of NLRP3 activation. The transcription step is regulated by TLRs–MyD88, TNF/TNFR-NEMO/TAK1/TRAF/RIPK1 and/or IFNα,β-JAK/STAT pathways, which activate pro-IL-1β, NLRP3, pro-caspase-1 and pro-caspase-11 transcription *via* NF-κB activation. TLRs stimulation by bacteria and MAMPs as well as TNFR activation can also activate also the RIPK1–FADD-caspase-8 protein complex, which, in turn, promotes NF-kB transcription. The second step results in NLRP3 inflammasome oligomerization, leading to caspase-1 activation as well as IL-1β and IL-18 release. **Left panel**: non-canonical caspase-8-dependent NLRP3 activation. TLR4 stimulation by PAMPs and/or DAMPs activates RIPK1–FADD-caspase-8 intracellular signaling, which, besides promoting the NF-kB transcription step, can activate directly canonical

NLRP3 oligomerization and assembly. In addition, fungal PAMPS (i.e. *Candida albicans*, fungal cell wall component β-glucans and mycobacteria), via dectin-1 stimulation, can promote IL-1β transcription as well as the formation and activation of a MALT1–caspase-8–ASC complex, which contributes to the processing and release of IL-1β. **Right upper panel**: Permeabilization of cell membranes to potassium efflux (i.e. MLKL activation, exposure to pore-forming Gasdermin D, P2X7 receptor activation by extracellular ATP, lysosomal damage and cathepsin release) leads to a massive release of oxidized mitochondrial DNA, increase in mitochondrial ROS and cardiolipin externalization, which, in turn, promote NLRP3 inflammasome oligomerization and activation. α-synuclein and β-amyloid protein accumulation as well as post-translational NLRP3 modifications (i.e. phosphorylation and ubiquitination) can promote also the second step of NLRP3 inflammasome activation. Caspase-1 activation promotes also pyroptosis and HMGB1 release. **Right lower panel**: non-canonical caspase-11-dependent NLRP3 activation. Unidentified scaffold proteins or receptors induced by Gram-negative bacteria cleave and activate caspase-11, which induces pyroptosis as well as HMGB1 and IL-1α release, and promotes the activation of NLRP3-ASC-caspase-1 pathway.

Abbreviations: ASC, inflammasome adaptor protein apoptosis-associated speck-like protein containing CARD; FADD, FAS associated death domain protein; HMGB1, high mobility group box 1; JAK/STAT, janus kinase/signal transducers and activators of transcription; IFN, interferon; IFNAR, interferon-α/β receptor; IL, interleukin; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; MAMPs, microbe-associated molecular pattern molecules; MLKL, mixed lineage kinase domain-like protein; MyD88, adaptor molecules myeloid differentiation primary response 88; NEMO, NF-κB essential modulator NF-κB, nuclear factor-κB; NLRP3, nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain-containing protein 3; RIPK1, receptor-interacting protein kinase 1; TAK1; transforming growth factor β activated kinase-1; TLRs, toll-like receptors; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor; TRAF, TNF receptor-associated factor 2; TRIF, toll/IL-1 receptor homology

(TIR)-domain-containing adapter-inducing interferon-β; P2X, purinergic receptor 7; PAMPs, pathogens-associated molecular patterns; ROS, reactive oxygen species.

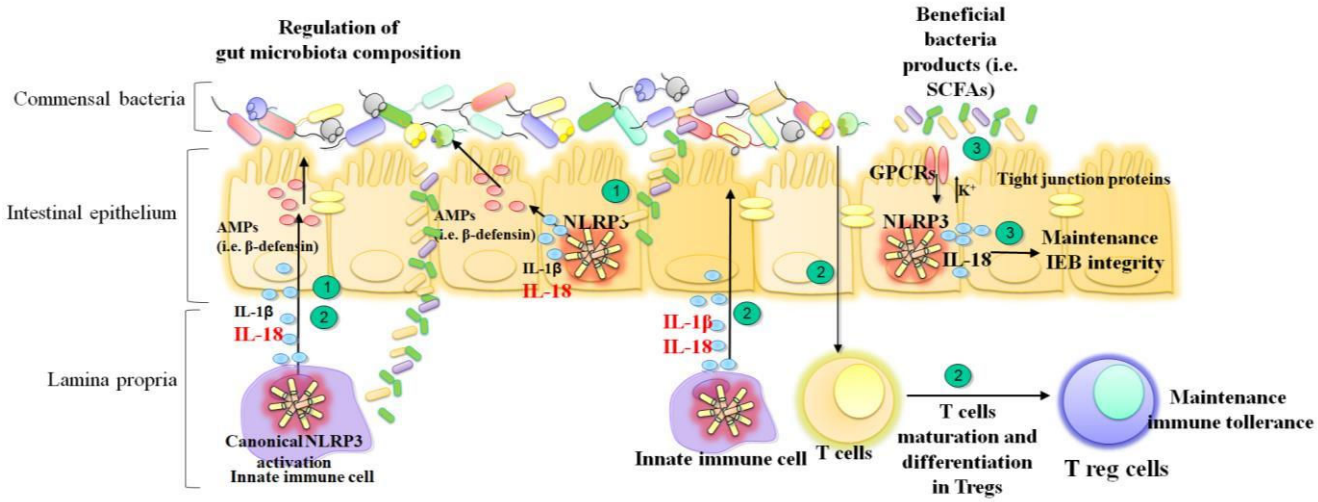

Figure 2. Role of interactions between gut microbiota and NLRP3 inflammasome in the maintenance of intestinal homeostasis

Diagram showing the physiological role of interactions between gut microbiota and NLRP3 inflammasome. 1) NLRP3 inflammasome activation in immune cells and intestinal epithelial cells by enteric bacteria induces IL-18 release. This promotes the release of antimicrobial peptides (i.e. β-defensin) that, in turn, contribute to the maintenance gut eubiosis; 2) NLRP3 reshapes gut microbiota that, in turn, promotes T cell maturation and differentiation in T regs, thus contributing to the maintenance of immune-bacterial tolerance 3) Metabolic products of enteric bacteria, such as SCFAs through GPCRs, including GPR43 and GPR109A on IECs promote NLRP3 activation, that, in turn, via release of IL-18, regulates tight junction protein expression, mucosal permeability and mucus production, thus preserving IEB integrity.

Abbreviation: NLRP3: nucleotide-binding oligomerization domain leucine rich repeat and pyrin domain-containing protein 3; GPCRs: G-protein-coupled receptors; IECs, intestinal epithelial cells; IEB, intestinal epithelial barrier; SCFAs: short chain fatty acids; IL-18: interleukin-18

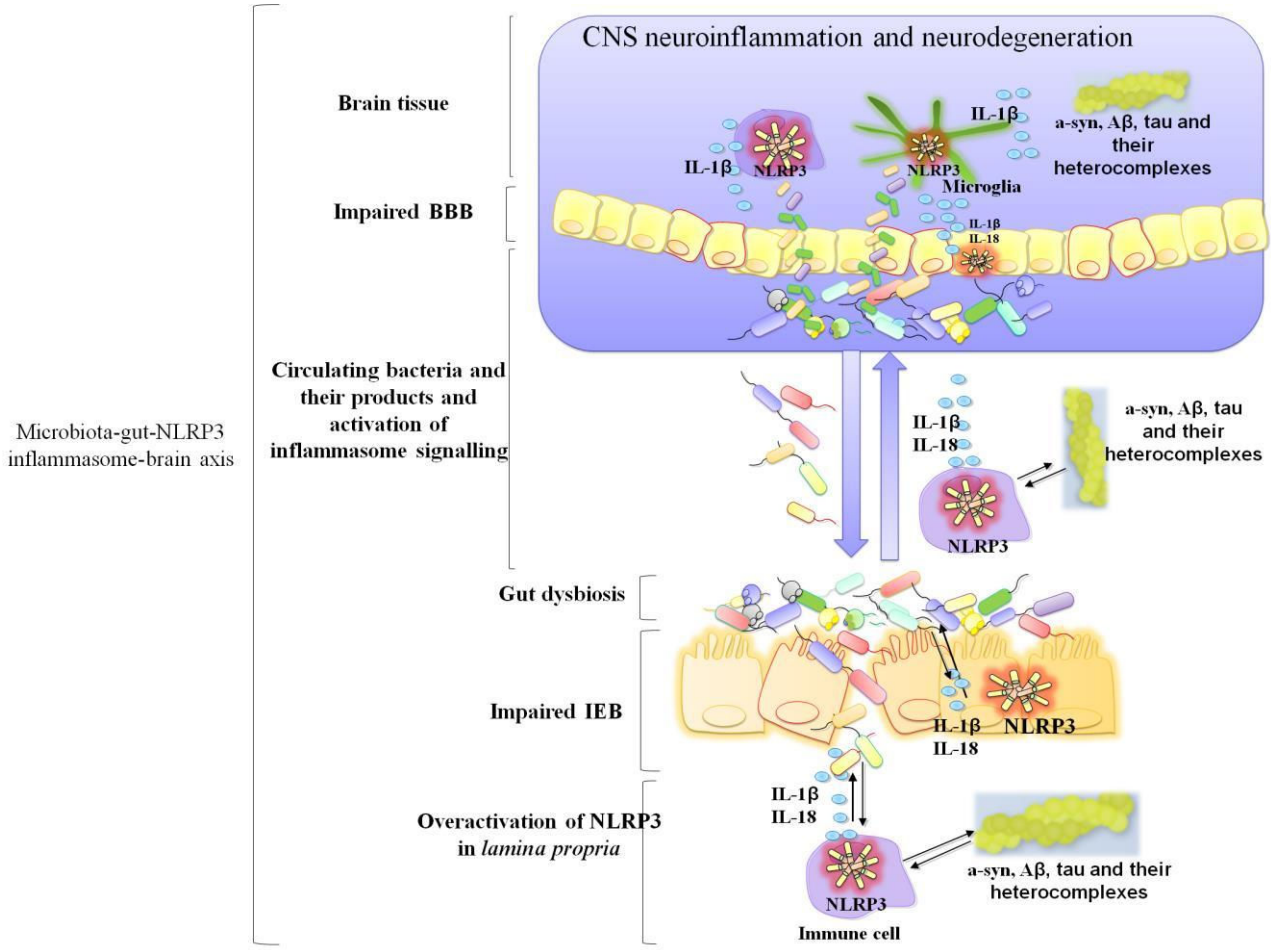

Figure 3. Microbiota-gut-NLRP3 inflammasome-brain axis in CNS disorders

Diagram showing the role of microbiota-gut-NLRP3 inflammasome-brain axis in the main neurological and psychiatric disorders, including PD, AD, MS, MDD and ASD. Enteric bacteria can promote NLRP3 activation that, in turn, shapes peripheral and central neurogenic/inflammatory responses, thus contributing to the onset of CNS neuroinflammation and neurodegeneration. In this regard, a pivotal issue should to be addressed. In particular, since it has been observed that patients with CNS disorders, such as PD, are characterized by an increase in IL-1β and CNS-related protein inclusions, including α-syn, Aβ and tau proteins, in intestinal tissues, might the overactivation of NLRP3 in the gut, following CNS protein deposition, alter the enteric bacteria composition? This immune-bacteria loop could generate a sort of vicious circle that could drive the chronicization of ongoing peripheral and central neuroinflammatory and neurodegenerative processes.

Abbreviation: Aβ, amyloid beta; α-syn, alpha-synuclein; ASD: autism spectrum disorders, AD: Alzheimer's disease; α-syn: a-synuclein; Aβ: amyloid beta; CNS: central nervous system; IEB, intestinal epithelial barrier; MDD: major depressive disorder; MS: multiple sclerosis, PD: Parkinson's disease; NLRP3, nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain-containing protein 3.