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Title:

Microglia and Mast Cells Generate Proinflammatory Cytokines in the Brain and Worsen Inflammatory State: Suppressor Effect of IL-37

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Abstract:

Brain microglia cells are responsible for recognizing foreign bodies and act by activating other immune cells. Microglia react against infectious agents that cross the blood-brain barrier and release pro-inflammatory cytokines including interleukin (IL)-1β, IL-33 and tumor necrosis factor (TNF). Mast cells (MCs) are immune cells also found in the brain meninges, in the perivascular spaces where they create a protective barrier and release pro-inflammatory compounds, such as IL-1β, IL-33 and TNF. IL-1β binds to the IL-1R1 receptor and activates a cascade of events that leads to the production of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and activation of the immune system. IL-33 is a member of the IL-1 family expressed by several immune cells including microglia and MCs and is involved in innate and adaptive immunity. IL-33 is a pleiotropic cytokine which binds the receptor ST2 derived from TLR/IL-1R super family and is released after cellular damage (also called "alarmin"). These cytokines are responsible for a number of brain inflammatory disorders. Activated IL-1β in the brain stimulates microglia, MCs, and perivascular endothelial cells, mediating various inflammatory brain diseases. IL-37 also belongs to the IL-1 family and has the capacity to suppress IL-1β with an anti-inflammatory property. IL-37 deficiency could activate and enhance myeloid differentiation (MyD88) and p38-dependent protein-activated mitogenic kinase (MAPK) with an increase in IL-1β and IL-33 exacerbating neurological pathologies. In this article we report for the first time that microglia communicate and collaborate with MCs to produce pro-inflammatory cytokines that can be suppressed by IL-37 having a therapeutic potentiality.

Key words: microglia; mast cells; macrophages; cytokines; IL-37

1. Introduction

The brain network mediates and controls complex behaviors, but despite a huge number of publications on this subject, much about how brains work remains a mystery. Brain cells include the epithelial cells of blood vessels, neurons and glia cells. The latter cells are responsible for the

protection and nutrition of neurons. Therefore, the brain includes resident immune cells such as microglia cells, which are important for physiological functions, but also for pathological conditions (Conti et al., 2019; Tsilioni et al. 2019). Microglia represent the immune cells of the brain that play an important role both in physiological and various neurodegenerative diseases (Tsilioni et al., 2019). Microglia cells make up 20% of the total glial cell population of the brain and are the predominant bodies in the posterior gray and white matter (Ruiz-Sauri et al., 2019). They are also distributed in the spinal cord and are responsible for performing innate cellular immunity in the central nervous system (CNS) against damaged neurons, plaques and infectious agents. Microglia cells recognize foreign bodies and they phagocyte them acting as antigen presenting cells (APCs) (Almolda et al., 2011). The infectious agents that cross the blood-brain barrier, which protects the brain, react with microglia cells which, in turn, produce inflammatory cytokines of the Th1 type (Winklewski et al., 2016). The key component of innate immunity response is the generation of pro-inflammatory cytokines produced by microglia residing in the brain. These cytokines contribute to acute and chronic diseases of the CNS.

Cytokines generated by mast cells (MCs) and microglia cells cause inflammation in the hypothalamus and amygdala, thus explaining most of the symptoms in different brain pathologies (Conti et al., 2018a). Therefore, the production of pro-inflammatory molecules aggravates the subject's pathological state. It has been reported that the neurotransmitter neurotensin (NT) activates the immune cells of the brain, including human microglia and the human cell line-SV40 (Theoharides et al., 2015a). The role of cytokine interactions with neuropeptides in the pathogenesis of brain inflammation is very powerful (Petra et al., 2015). In addition, the same authors reported that in microglia, neurotensin increases the gene expression and release of IL-1β and chemokine CXCL8, CCL2, and CCL5 (Conti et al., 2018a). Moreover, in several neurological diseases, the activation of microglia with increase in MiR-155p5 (miroRNA) leads to brain inflammation, with a pathogenesis still obscure (Almehmadi et al. 2019). Therefore, the neurological diseases involve neuropeptides and cytokines that can be important elements as the target of effective therapeutic

strategy (Caraffa et al. 2019). However, in neurological diseases therapies are difficult to apply, since they are not specific and likewise often ineffective. Accordingly, an elaborate knowledge of pathological mechanisms and immune response are necessary for improving the pharmacological procedure (Glezer et al., 2007). This article presents new concepts concerning the inhibitory role of IL-37 in the innate response mediated by pro-inflammatory cytokines produced by microglia and MCs.

2. Mast cells

MCs are immune cells resident in various areas of the brain, located mainly perivascularly near neurons and hypothalamus, and are mostly tryptase-chymase positive phenotype (Galli et al. 2005). MCs in the meninges play an important role in contributing to neuroinflammatory diseases and are a potential therapeutic target (Theoharides, 2017). In addition, MCs are numerous in the meninges and abluminal side of the blood vessels, where they communicate with microglia and endothelial cells, and orchestrate the interaction between meninges and the immune system (Reuter et al. 2001). In neurological diseases, there is a cascade of events which occurs, with the production of inflammatory compounds (Conti et al., 2016), including the immune response, inflammation, cytotoxicity, and neuronal and glial cell death (Streit et al., 2005). MCs play an innate and adaptive immunoregulatory role, and in pathological cases they release proinflammatory compounds including cytokines (Antonopoulos et al., 2019). In addition, MCs can be activated by lipopolysaccharides and IL-1 without degranulation (Blume-Jensen et al., 2001), and produce proteases and chemokines, which mediate immunity and inflammation (Theoharides et al., 2019a) (Fig. 1). They are expressed on c-kit receptor surface, and the FcεRI high affinity receptor (Kd = 10^{-10} M) for immunoglobulin E (IgE) (Galli et al., 2005). Furthermore, MCs express Toll-like receptors (TLR) such as TLR-2 and TLR-4, which bind micorganisms and their products, and wave to the activation of a mitogen-activated protein kinase (MAPK) cascade and a phophatidylinositol-specific phospholipase Cγ (PI-PLCγ) (Galli et al., 2005). These reactions lead to the enhancement

of the transcription nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), with consequent generation of pro-inflammatory cytokines. It is well known that there is an interplay between the immune system and the brain, but a detailed understanding is still not clear (Arac et al., 2019). Immune cells, including MCs located in the brain, modulate cerebral pathology and create a protective barrier (Theoharides et al., 2016). Brain blood vessels contain MCs, that pass through the meninges and enter the brain, acting as information filters, modulating immune cells. In human, the meninges, essentially composed of connective tissue, protect the encephalon, and are composed of 3 overlapped membranes that cover the elements of the CNS, namely the brain (Ramachandran, 2018). The function of immune cells residing in the meninges is very important for the protection of the tissue complex. MCs that are not circulating and reside in the meninges, in the perivascular spaces, play a pro-inflammatory role by generating TH1 cytokines that can be different both in quality and quantity in distinct brain pathologies (Forsythe, 2016). In addition, activated MCs producing inflammatory molecules also generate chemokines that can recruit other immune circulatory cells in the brain tissue (Tettamanti et al., 2018). Brain MCs certainly participate in and exacerbate the development of pathologies affecting the CNS by producing inflammatory cytokines acting by the cross-talk with other immune cells (Arac et al., 2019).

Neurotransmitters, including nerve growth factor (NGF), substance P (SP) and neurotensin, trigger MCs to release pro-inflammatory cytokines (Taracanova et al., 2018). In fact, the hypothalamus and the amygdale interact with MCs, which release pro-inflammatory cytokines (Conti et al., 2018b). In the pathological states, such as stress and other psychiatric diseases, brain inflammatory genes can be activated in MCs and microglia with cytokine release such as IL-1β and IL-33 (Conti et al., 2018c). Therefore, some neurotransmitters can take part in triggering inflammation, which increases considerably in synergy with pro-inflammatory cytokines also including IL-1β and IL-33 (Taracanova et al., 2018). Moreover, the stimulation of MCs *in vitro* with neuropeptides causes the release of preformed substances stored in the granules of the cell and pro-inflammatory mediators such as cytokines and chemokines that are produced in the later stage (Kempuraj et al., 2010).

MCs and microglia also interact with and activate proteinase-activated receptor 2 (PAR2), cytokines and chemokines, such as CCL5 on microglia cells (Hendriksen et al., 2017). Immune and inflammatory dysfunction in brain pathologies indicate the presence of markers of cytokines and chemokines such as tumor necrosis factor (TNF), IL-6 and MCP-1 (chemotactic protein 1 of monocytes), the latter being also chemotactic for MCs (Kritas et al., 2018). In MCs, TNF is the only cytokine stored in the granules, released immediately subsequent to stimulation, and it is also generated after TNF mRNA synthesis (Piliponsky et al., 2010). The activation of MCs with production of pro-inflammatory cytokines can cause cellular neuronal and glial death, with brain dysfunction (Skaper et al., 2013). MCs contribute to the inflammatory state in many CNS disorders and represent a challenge for the development of new therapies. Therefore, the pathogenesis of neuropsychiatric disorders is mediated by brain inflammation (Theoharides et al., 2015b).

We believe that the pathological action in brain diseases is due to activated microglia, in collaboration with the immune cells when they can cross the disrupted blood-brain barrier.

The activation of microglia cells and astrocytes by MC mediator release are therefore crucial in the development of neuroinflammation (Caraffa et al., 2018). Thus, the stimulation of MCs with neurotransmitters such as neurotensin or substance P increases the gene expression of cytokines including IL-1β, TNF and chemokines (Theoharides et al., 2010). This starts partially through the activation pathway of myeloid differentiation (MyD88) after receptor stimulation, which can be inhibited with IL-37 and have a therapeutic action for the treatment of inflammatory diseases (Mukai et al., 2018). Thus, the stimulation of MCs with neurotransmitters, such as neurotensin or substance P, increases the gene expression of cytokines including IL-1β, TNF and chemokines (Oehlke et al., 2005). This starts partially through the activation pathway of MyD88 after receptor stimulation, which can be inhibited with IL-37 and have a therapeutic action for the treatment of inflammatory diseases (Conti et al., 2019).

Since IL-1R1, the receptor of IL-1β, is expressed in MCs and microglia and mediates brain inflammation, blocking IL-1β with the new cytokine IL-37 could represent a novel therapeutic

strategy, and could be useful in diseases mediated by MCs such as autism, migraine, depression and Alzheimer's disease (Theoharides et al., 2016). These concepts expressed here are of increasing interest to the scientific community and in recent years many works have addressed these issues.

3. IL-1

IL-1 family is composed of 11 members - which mainly participate in innate immunity responses, but can also contribute to acquired immunity (Dinarello, 2018). Among these cytokines are the pro-inflammatory ones including IL-1β and IL-33 and the anti-inflammatory IL-37 (Dinarello, 2019) (Fig. 2). These cytokines may promote inflammation or limit inflammation (Gugliandolo et al., 2019). The cytokine IL-1β plays an immune function, and participates in and promotes inflammatory diseases, and its receptor IL-1R1 is distributed throughout the brain (Theoharides et al. 2019b). IL-1β is produced by competent immune cells, but also by competent non-immune cells (Dinarello, 2018b). Human brain produces IL-1β which mediates various inflammatory pathologies in loco, with the activation of microglia and perivascular endothelial cells (Shaik-Dasthagirisaheb et al., 2016) (Fig. 3). IL-1β binds its receptor and activates a cascade of events that leads to the production of NF-κB, gene transcription and activation of immune system (Dinarello, 2019). IL-1 and its receptor play a key role in the inflammation that occurs in many diseases and have the ability to induce other cytokines and chemokines (Dinarello, 2018). The production of IL-1β is induced in macrophages by bacterial infections and their products can pass through the endothelium of blood vessels and affect cytokines released by cerebral perivascular blood vessel macrophages and brain microglia cells (Kaplanski et al., 1993). As we reported earlier, IL-1β releases neurotransmitters that mediate some neurological pathologies influencing the behavior through dopamine and serotonin neurotransmitter production (Taracanova et al., 2018). We also mentioned previously that IL-1β and IL-33, together with IL-1β, share the IL-1R3 receptor that promotes inflammation, as well as cytokine and chemokine generation (Højen et al., 2019). IL-1 receptor

accessory protein (IL-1RAcP) recruits the adaptor molecules MyD88, IRAK1, IRAK4 and TRAF6, which activate the downstream NF-κB, p38 and ERK (Lockett, et al., 2008). Therefore, IL-1β can be bound first to the IL-1R1 receptor that changes its conformation and then to IL-1R3. This complex leads to the formation of the Toll/interleukin-1 receptor/resistance protein that recruits MyD88 with the start of the inflammatory process (Dinarello, 2019). MyD88 stimulates IL-1R-associated kinases (IRAKs), followed by IKKÎ² kinases, IKKÎ² and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) producing IL-1β mRNA and pro-IL-1β which accumulates in the cytoplasm, being cleaved by caspase-1 and subsequently transforms into mature IL-1β (Dinarello, 2019) (Fig. 2). In pain involving the thalamus, activated MCs produce histamine, IL-6, TNF, tryptase, corticotrophin releasing hormone (CRH) and neurotransmitters, which induce the microglia to produce IL-1β and CXCL8 (Theoharides et al., 2019).

In neuroinflammation there are alterations of the cross-talk between glia cells and neurons, due to the activation of astrocytes and microglia (Batlle et al., 2015). This activation leads to the stimulation of NF-κB and pro-inflammatory cytokine generation of innate immunity, including IL-1β that affects neuronal receptors producing protein kinases and neurological disorders (Mattson, 2005). These facts certainly offer a potential therapeutic approach for neurological diseases.

4. IL-33

IL-33 (or IL-1F11/IL-1R4) is a member of the IL-1 family expressed by several immune cells including innate lymphoid cells 2, T helper 2 (Th2) cells, and MCs which are involved in neurological disorders, and innate and adaptive immunity (Rider et al., 2017). IL-33 is a 30 kDa cytokine which binds and signals through specific receptor ST2 derived from TLR/IL-1R super family (Garlanda et al., 2013). ST2 receptor forms heterodimer with IL-1 receptor accessory protein (IL-1RAcP) that also binds IL-33, a ligand for the former orphan receptor ST2 and associated with Th2 immune response (Garlanda et al., 2013). IL-33 activates immune cells including MCs, microglia and astrocytes, important cells in the mediation of neuroinflammatory states (Fattori et

al., 2017). After cellular damage, the mature IL-33 (also called "alarmin") is released from the immune cells, including the MCs (Varvara et al., 2018). IL-33 is a pleiotropic cytokine that derives from the immature IL-33 cleaved from caspase-1 and activates the NF-κB, p38 and JNK immune-cascade (Dinarello, 2005). IL-33 is primarily involved in Th2 cell-mediated diseases, while IL-18 mainly regulates the Th1 cellular response (Romanelli, 2018a). This cytokine is also involved in the sensitization of nociceptor neurons and in neuropathic pain (Romanelli, 2018b). It has been observed that pro-inflammatory cytokines, such as IL-33 and IL-1β released by microglia, astrocytes, neurons, T cells and activated MCs, mediate neurodegeneration and neuroinflammation (Kempuraj et al., 2016b). Glia cells, neurons and astrocytes cultured with MCs *in vitro* interact and release IL-33, causing neuroinflammation, generating a new therapeutic target for the treatment of neuroinflammatory diseases (Corrigan et al., 2016). Inhibition of microglia leads to an improvement in the inflammatory state. Therefore, the inhibition of these cytokines can certainly represent a promise of therapeutic approach.

5. IL-37

As we have described above, cytokines actively participate in the initiation and suppression of inflammation (Dinarello et al., 2016) (Fig. 3). The innate immune response that occurs in inflammatory processes is regulated principally by the cytokine family of IL-1, among 11 members of which IL-1β is the most important and studied (Dinarello et al., 2016). IL-37 belongs to the IL-1 family and the first biological activity described for this cytokine was to suppresses the activation of IL-1β and stimulate anti-inflammatory cytokines including IL-10 (Table 1). IL-37b, formerly called IL-1F7, is a member of the IL-1 family, which binds IL-18Ra receptor and performs anti-inflammatory action (Dinarello et al., 2016). To date, five IL-37 isoforms have been discovered, ranging from "a" to "e", of which the most studied form is "b", and are generated mainly by macrophages. In this article we simply call the form “b” IL-37. IL-37 is activated after stimulation of caspase-1 that cleaves the protein (Dinarello et al., 2016). One part of the cytokine enters the

nucleus, while the other part is released together with the pro-IL-37 outside the cell. IL-37, that is lacking in mice, exerts marked anti-inflammatory properties in neurological diseases by acting on the inhibition of IL-1β generated by microglia cells, conferring functional protection to the tissue (Dinarello et al., 2016). IL-37 binds to an IL-18 binding protein (IL-18BP) and to the decoy receptor 8 (ILR8) carrying out its anti-inflammatory activity (Jiang, 2019). It has been reported that the transgenic mice expressing human IL-37 are protected from inflammatory diseases and the administration of recombinant human IL-37 in mice candidates this cytokine for clinical use as a therapeutic agent (Dinarello, 2019).

Recently, it has been found that IL-37 inhibits the IL-1β released by microglia stimulated by neurotensin and/or lipopolysaccharides, as well as MCs stimulated by substance P and/or IL-33 (Tsilioni et al., 2019). It was reported that the treatment of human microglia cells with IL-37 inhibits the gene expression of IL-1β and chemokine ligand 8 stimulated with the neuropeptide neurotensin (Tsilioni et al., 2019). In fact, some neurotransmitters and/or cytokines, including IL-1β and TNF, increase the gene expression of IL-37 *in vitro*. Moreover, pro-inflammatory cytokines such as IL-1β, TNF, and chemokine ligand 8 increase in the serum, cerebrospinal fluid, and brain of many patients with autism spectrum disorder (Tsilioni et al., 2019). Therefore, the stimulation of gene expression and secretion of IL-1β, and the chemokine ligand 8 from cultured human microglia, are increased in the brain of children with autism spectrum disorder compared to the control. The increase in IL-1β causes the stimulation of IL-37 in microglia, as a protective response of brain tissue.

IL-37 deficiency could lead to an increase in the inflammatory response with activation of MyD88, p38-dependent MAPK, and an increase in pro-inflammatory cytokine secretion, exacerbating neurological pathologies. In fact, MyD88/IRAK/TRAF6 complex, can activate MAPK including the Jun kinase pathway that leads to the formation of activator protein-1 (AP-1) complex and NF-κB and, therefore, the activation of the transcription of genes encoding most innate immune proteins such as IL-1β, TNF, IL-33 and chemokines.

The pieces of experimental evidence reported here provide a link between microglial cell activation, MCs and brain inflammation, a pathway that could be inhibited by the cytokine IL-37, providing an effective treatment against neuroinflammation.

6. Conclusion

After caspase-1 activation, IL-1β is secreted in activated microglia, and aggravates inflammatory brain disorders (Scheiblich et al. 2017; Scheiblich et al. 2014) (Natoli et al., 2017). This is in accordance with our previous study where we reported that proinflammatory cytokines IL-1β and TNF increase IL-37 gene expression in cultured human microglia (Tsilioni et al., 2019), however, the level of inflammatory cytokines in different neuroinflammatory disorders remains to be determined.

In this article, we provide a plausible pathogenetic process that links the pro-inflammatory cytokines secreted by MCs to microglia cells with IL-37, which could inhibit the secretion of not only IL-1β, but other proinflammatory cytokines and some chemokines. We also underline that IL-37 is an inhibitor of IL-1 and would certainly be useful in brain inflammatory conditions. Therefore, IL-37 presents a potential therapeutic use in all inflammatory pathologies, particularly those mediated by IL-1β. The obstacles presented by IL-37 treatment, such as the route of administration and the optimal concentration, could be overcome with further careful studies in *vitro* and in *vivo*. Since, IL-37 is an inhibitor not only of inflammation, but also of the innate immune response, its application in humans should be better studied to avoid undesirable side effects.

Therefore, IL-37, by inhibiting the production of inflammatory cytokines produced by microglia cells, can exert a new therapeutic action. However, the inhibition of IL-37 on innate immunity could, for example, cause a defect of phagocytosis, but this is only a hypothesis as there is no evidence for this to date.

Here, we highlight the connection between inflammation and neuropathologic conditions, supporting the development of IL-37 as a potential therapeutic agent of neuroinflammation. Therefore, we provide for the first time on this specific subject an update on the biological

properties of pro-inflammatory IL-1β and IL-33 produced by microglia and MCs, inhibited by IL-37, a member of the IL-1 family, with a potential therapeutic approach to inflammatory brain diseases.

References

Almehmadi, K.A., Tsilioni, I., Theoharides, T.C. 2019. Increased expression of miR-155p5 in Amygdala of children with autism spectrum disorder. Autism Res. doi: 10.1002/aur.2205.

Almolda, B., Gonzalez, B., Castellano, B., 2011. Antigen presentation in EAE: role of microglia, macrophages and dendritic cells. Front. Biosci. (Landmark Ed). 16, 1157-71.

Antonopoulos, D., Tsilioni, I., Balatsos, N.A.A., et al., 2019. The mast cell - neurofibromatosis connection. J Biol. Regul. Homeost. Agents. 33, 657-659.

Arac, A., Grimbaldeston, M.A., Galli, S.J., et al., 2019. Meningeal mast cells as key effectors of stroke pathology. Front. Cell. Neurosci. 13, 126.

Batlle, M., Ferri, L., Andrade, C., Ortega, F.J., et al., 2015. Astroglia-microglia cross talk during neurodegeneration in the rat hippocampus. Biomed. Res. Int. 2015, 102419.

Blume-Jensen P., Hunter T., 2001. Oncogenic kinase signalling. Nature. 411(6835), 355-65. Review.

Caraffa, Al., Conti, C., D Ovidio, C., et al., 2018. New concepts in neuroinflammation: mast cells pro-inflammatory and anti-inflammatory cytokine mediators. J. Biol. Regul. Homeost. Agents. 32, 449-454.

Caraffa, Al., Gallenga, C.E., Kritas, S.K., Ronconi, G., Conti, P., 2019. Impact of mast cells in systemic lupus erythematosus: can inflammation be inhibited? J. Biol. Regul. Homeost. Agents. 33, 669-673.

Conti, P., Caraffa, A., Ronconi, G., et al., 2018a. Impact of mast cells in depression disorder: inhibitory effect of IL-37 (new frontiers). Immunol. Res. 66, 323-331.

Conti, P., Caraffa, A., Ronconi, G., et al., 2018b. Impact of mast cells in mucosal immunity of intestinal inflammation: Inhibitory effect of IL-37. Eur. J. Pharmacol. 818, 294-299.

Conti, P., Caraffa, A., Ronconi, G., Conti, C.M., Kritas, S.K., Mastrangelo, F., Tettamanti, L., Theoharides, T.C., 2018c. Impact of mast cells in depression disorder: inhibitory effect of IL-37 (new frontiers). Immunol. Res. 66, 323-331.

Conti, P., D'Ovidio, C., Conti, C., Gallenga, C.E., Lauritano, D., Caraffa, A., Kritas, S.K., Ronconi, G., 2019. Progression in migraine: Role of mast cells and pro-inflammatory and anti-inflammatory cytokines. Eur. J. Pharmacol. 844, 87-94.

Conti, P., Kempuraj, D., 2016. Important role of mast cells in multiple sclerosis. Mult. Scler. Relat. Disord. 5, 77-80. doi: 10.1016/j.msard.2015.11.005.

Corrigan, F., Mander, K.A., Leonard, A.V., et al., 2016. Neurogenic inflammation after traumatic brain injury and its potentiation of classical inflammation. J. Neuroinflammation. 11, 264.

Dinarello, C.A., 2018. Introduction to the interleukin-1 family of cytokines and receptors: Drivers of innate inflammation and acquired immunity. Immunol .. 281, 5-7.

Dinarello, C.A., 2019. The IL-1 family of cytokines and receptors in rheumatic diseases. Nat. Rev. Rheumatol. 15, 612-632.

Dinarello, C.A., 2005. An IL-1 family member requires caspase-1 processing and signals through the ST2 receptor. Immunity. 23, 461-2.

Fattori, V., Hohmann M.S.N., Rossaneis, A.C., et al., 2017. Targeting IL-33/ST2 signaling: Regulation of immune function and analgesia. Expert. Opin. Ther. Targets. 21, 1141-1152.

Forsythe, P., 2016. Microbes taming mast cells: Implications for allergic inflammation and beyond. Eur. J. Pharmacol. 778, 169-75.

Galli, S.J., Kalesnikoff, J., Grimbaldeston, M.A., et al., 2005. Mast cells as "tunable" effector and immunoregulatory cells: recent advances. Annu. Rev. Immunol. 23, 749-86.

Garlanda C., Dinarello C.A., Mantovani A., 2013. The interleukin-1 family: back to the future. Immunity. 39, 1003-18.

Glezer I., Simard A.R., Rivest S., 2007. Neuroprotective role of the innate immune system by microglia. Neuroscience. 147, 867-83.

Hendriksen, E., van Bergeijk, D., Oosting, R.S., Redegeld, F.A., 2017. Mast cells in neuroinflammation and brain disorders. Neurosci. Biobehav. Rev. 79, 119-133.

Gugliandolo, A., Caraffa, AL., Gallenga, C.E., et al., 2019. Mesenchymal stem cells and IL-37: a powerful combination. J. .Biol. Regul. Homeost. Agents. 33, 1019-1022.

Højen, J.F., Kristensen, M.L.V., McKee, A.S., et al., 2019. IL-1R3 blockade broadly attenuates the functions of six members of the IL-1 family, revealing their contribution to models of disease. Nat. Immunol. 20, 1138-1149.

Jiang Y.J., 2019. Correlation between the pathogenesis of bronchial asthma and serum expressions of IL-4, IL-12, IL-37 and 25-(OH)D. J. Biol. Regul. Homeost. Agents. 33, 889-894.

Kaplanski, G., Porat, R., Aiura, K., et al. 1993. Activated platelets induce endothelial secretion of interleukin-8 in vitro via an interleukin-1-mediated event. Blood. 81, 2492-5.

Kempuraj, D., Conti, P., Vasiadi, M., et al. 2010. IL-32 is increased along with tryptase in lesional psoriatic skin and is up-regulated by substance P in human mast cells. Eur. J. Dermatol. 20(6):865-7.

Kempuraj, D., Thangavel, R., Natteru, P.A., et al., 2016. Neuroinflammation induces neurodegeneration. J. Neurol. Neurosurg. Spine.1(1). pii: 1003. Epub.

Kritas, S.K., Gallenga, C.E., D Ovidio, C., et al.. 2018. Impact of mold on mast cell-cytokine immune response. J. Biol. Regul. Homeost. Agents. 32, 763-768.

Lockett, A., Goebl, M.G., Harrington, M.A., 2008. Transient membrane recruitment of IRAK-1 in response to LPS and IL-1beta requires TNF R1. Am. J. Physiol. Cell. Physiol. 295, C313-23.

Mattson, M.P., 2005. NF-kappaB in the survival and plasticity of neurons. Neurochem. Res. 30, 883-93.

Mukai, K., Tsai, M., Saito, H., Galli, S.J., 2018. Mast cells as sources of cytokines, chemokines, and growth factors. Immunol. Rev. 282, 121-150.

Natoli R., Fernando N., Madigan M., Chu-Tan J.A., Valter K., Provis J., Rutar M., 2017. Microglia-derived IL-1β promotes chemokine expression by Müller cells and RPE in focal retinal degeneration. Mol. Neurodegener. 12, 31.

. Oehlke, J., Lorenz, D., Wiesner, B., Bienert, M., 2005. Studies on the cellular uptake of substance P and lysine-rich, KLA-derived model peptides. J. Mol. Recognit. 18, 50-9.

Petra, A.I., Panagiotidou, S., Hatziagelaki, E., Stewart, J.M., Conti, P., Theoharides, T.C., 2015. Gut-Microbiota-Brain Axis and Its Effect on Neuropsychiatric Disorders With Suspected Immune Dysregulation. Clin. Ther. 37, 984-95.

Piliponsky, A.M., Chen, C.C., Grimbaldeston, M.A., et al., 2010. Mast cell-derived TNF can exacerbate mortality during severe bacterial infections in C57BL/6-KitW-sh/W-sh mice. Am. J. Pathol. 176, 2638.

Ramachandran, R., 2018. Neurogenic inflammation and its role in migraine. Semin. Immunopathol. 40, 301-314.

Reuter, U., Bolay, H., Jansen-Olesen, I., et al. 2001. Delayed inflammation in rat meninges: implications for migraine pathophysiology. Brain. 124(Pt 12):2490-502.

Rider, P, Voronov, E, Dinarello, CA, et al. 2017. Alarmins: Feel the stress. J Immunol. 198(4):1395-1402.

Romanelli, M.M., 2018a. ST2 fibro-cytokine and IL-33 alarmin protein are expressed in obese fa/fa- Zucker rat model and correlated with pro-fibrotic gene pathways. J. Biol. Regul. Homeost. Agents. 32(4 Suppl. 1), 35.

Romanelli, M.M., 2018b. Dysfunctional epicardial adipose tissue (EAT) and maladaptive heart remodeling in patients with increased visceral adiposity: the ST2/IL-33 cardio-fat signaling. J. Biol. Regul. Homeost. Agents. 32(4 Suppl. 1), 21-25.

Ruiz-Sauri, A., Orduña-Valls, J.M., Blasco-Serra. A., et al., 2019. Glia to neuron ratio in the posterior aspect of the human spinal cord at thoracic segments relevant to spinal cord stimulation. J. Anat. doi: 10.1111/joa.13061.

Scheiblich H., Schlütter A., Golenbock D.T, et al., 2017. Activation of the NLRP3 inflammasome in microglia: the role of ceramide. J. Neurochem. 143, 534-550. Scheiblich H., Roloff F., Sing V., et al., 2014. Nitric oxide/cyclic GMP signaling regulates motility of a microglial cell line and primary microglia in vitro. Brain. Res. 1564, 9-21.

Shaik-Dasthagirisaheb, Y.B., Conti P., 2016. The role of mast cells in Alzheimer's disease. Adv. Clin. Exp. Med. 25, 781-7.

Skaper, S.D., Facci, L., Giusti, P., 2013. Glia and mast cells as targets for palmitoylethanolamide, an anti-inflammatory and neuroprotective lipid mediator. Mol. Neurobiol. 48, 340-52.

Streit, W.J., Conde, J.R., Fendrick, S.E., et al., 2005. Role of microglia in the central nervous system's immune response. Neurol. Res. 27, 685-91.

Taracanova, A., Tsilioni, I., Conti, P., et al., 2018. Substance P and IL-33 administered together stimulate a marked secretion of IL-1β from human mast cells, inhibited by methoxyluteolin. Proc. Natl. Acad. Sci. U S A. 115, E9381-E9390.

Tettamanti, L., Kritas, S.K., Gallenga, C.E., et al. 2018. IL-33 mediates allergy through mast cell activation: Potential inhibitory effect of certain cytokines. J. Biol. Regul. Homeost. Agents. 32, 1061-1065.

Theoharides, T.C., Kavalioti, M., 2018. Stress, inflammation and natural treatments. J. Biol. Regul. Homeost. Agents. 32, 1345-1347.

Theoharides, T.C., Stewart, J.M., Hatziagelaki, E., Kolaitis, G., 2015a. Brain "fog," inflammation and obesity: key aspects of neuropsychiatric disorders improved by luteolin. Front. Neurosci. 3;9, 225.

Theoharides, T.C., Stewart, J.M., Panagiotidou, S., Melamed, I., 2016. Mast cells, brain inflammation and autism. Eur. J. Pharmacol. 778, 96-102.

Theoharides, T.C., Tsilioni, I., Bawazeer, M., 2019a. Mast cells, neuroinflammation and pain in fibromyalgia syndrome. Front. Cell. Neurosci. 13, 353.

Theoharides, T.C., Zhang, B., Kempuraj, D., et al., 2010. IL-33 augments substance P-induced VEGF secretion from human mast cells and is increased in psoriatic skin. Proc. Natl. Acad. Sci. U S A. 107, :4448-53.

Theoharides, T.C., 2017. Neuroendocrinology of mast cells: Challenges and controversies. Exp. Dermatol. 26, 751-759.

Theoharides, T.C., Athanassiou, M., Panagiotidou, S., Doyle, R., 2015b. Dysregulated brain immunity and neurotrophin signaling in Rett syndrome and autism spectrum disorders. J. Neuroimmunol. 279, 33-8.

Theoharides, T.C., Tsilioni, I., Conti, P., 2019b. Mast cells may regulate the anti-inflammatory activity of IL-37. Int. J. Mol. Sci. 29, 20(15).

Tsilioni, I., Patel, A.B., Pantazopoulos, H., Berretta, S., Conti, P., Leeman, S.E., Theoharides, T.C., 2019. IL-37 is increased in brains of children with autism spectrum disorder and inhibits human microglia stimulated by neurotensin. Proc. Natl. Acad. Sci. U S A. pii: 201906817. doi: 10.1073/pnas.1906817116.

Varvara, G., Tettamanti, L., Gallenga, C.E., et al., 2018. Stimulated mast cells release inflammatory cytokines: potential suppression and therapeutical aspects. J. Biol. Regul. Homeost. Agents. 32, 1355-1360.

Winklewski, P.J., Radkowski, M., Demkow, U., 2016. Neuroinflammatory mechanisms of hypertension: potential therapeutic implications. Curr. Opin. Nephrol. Hypertens. 25, 410-6.

FIG. LEGENDS

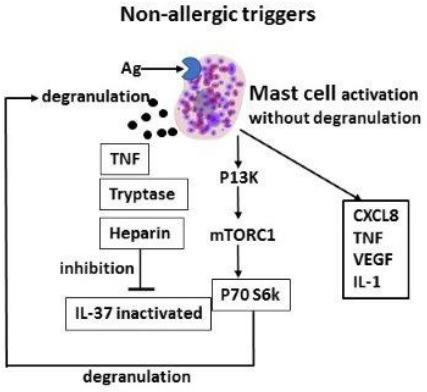
Fig. 1. MC activation by non-allergic triggers (e.g., neurotransmitters) can provoke the degranulation and, therefore, the release of TNF, tryptase and heparin, which may inactivate IL-37. In addition, the stimulation of MC may induce the generation of inflammatory cytokine/chemokine and VEGF without degranulation. CXC8= chemokine (C-X-C motif) ligand 8; P13K= phosphatidylinositol 3-kinase; mTORC1= mammalian target of rapamycin complex 1; p7056k= protein 7056K; Ag= antigen.

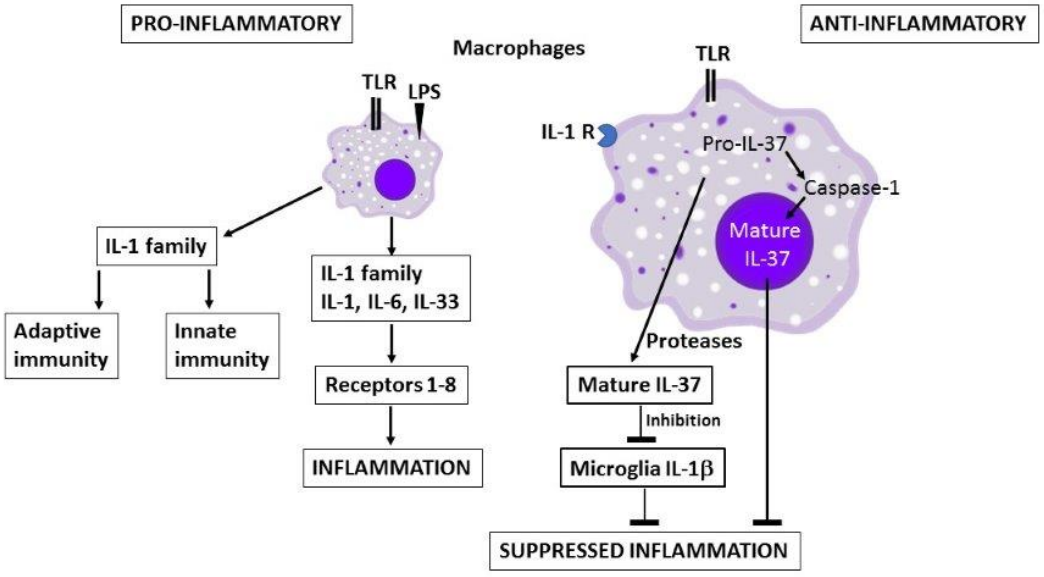
Fig. 2. Macrophages activated by lipolysaccharide (LPS) and/or Toll-like receptor (TLR) generate pro-inflammatory and anti-inflammatory cytokines, including IL-37.

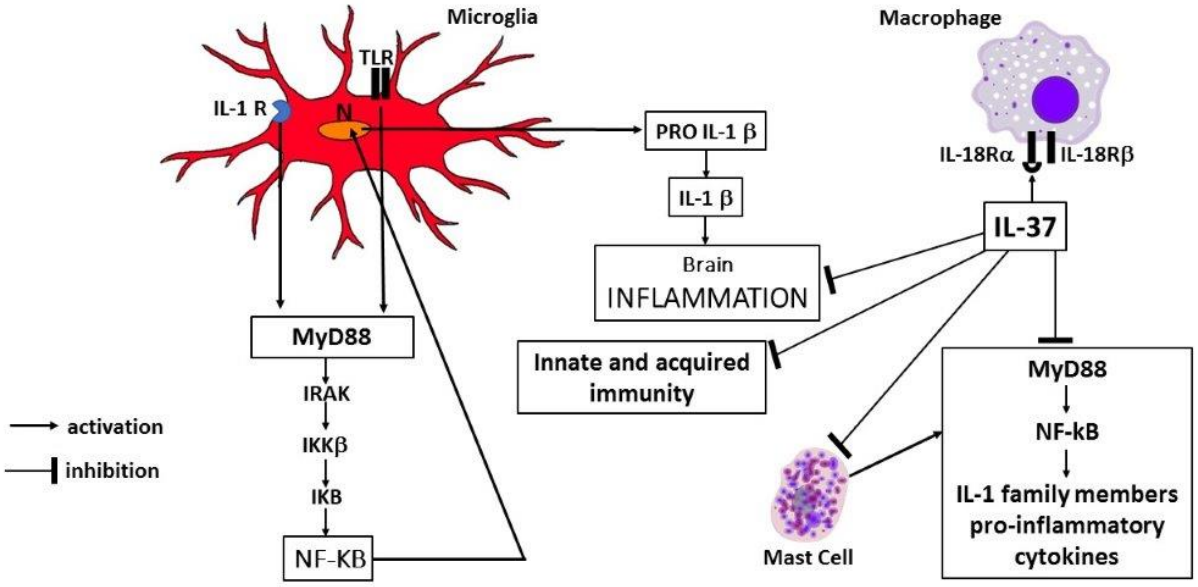
Fig. 3. Microglia cell induces MyD88 and NF-κB to produce inflammatory cytokine IL-1β by activated Toll-like receptor (TLR) and IL-1R, inducing brain inflammation. IL-37 binds to IL-18Rα and inhibits in mast cell, pro-inflammatory IL-1 family members derived from MyD88 and NF-κB, and suppresses microglia brain inflammation. N= nucleus.

Table 1. Positive and negative effects of IL-37.

IL-37	
Increases	**Decreases**
IL-10	**MHC**
Treg CD4	**CD40**
STAT 6	**IL-1b, IL-6, IL-5, IL-13, IL-17**
AMPK	**p38 MAPK**
IL-27	**TNF**
	Cell migration


Non-allergic triggers
Ag
degranulation
Mast cell activation
without degranulation
TNF
Tryptase
Heparin
inhibition
IL-37 inactivated
P13K
mTORC1
P70 S6k
CXCL8
TNF
VEGF
IL-1
degranulation


PRO-INFLAMMATORY
ANTI-INFLAMMATORY
Macrophages
TLR
LPS
TLR
IL-1 R
Pro-IL-37
Caspase-1
Mature IL-37
IL-1 family
Adaptive immunity
Innate immunity
IL-1 family IL-1, IL-6, IL-33
Receptors 1-8
INFLAMMATION
Proteases
Mature IL-37
Inhibition
Microglia IL-1β
SUPPRESSED INFLAMMATION


Microglia
TLR
IL-1 R
N
PRO IL-1 β
IL-1 β
Brain
INFLAMMATION
Innate and acquired immunity
MyD88
IRAK
IKKβ
IKB
NF-KB
activation
inhibition
Macrophage
IL-18Rα
IL-18Rβ
IL-37
MyD88
NF-kB
IL-1 family members pro-inflammatory cytokines
Mast Cell