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Microglial Cells Depletion Increases Inflammation and Modifies Microglial Phenotypes in an Animal Model of Severe Sepsis

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Abstract

Sepsis-associated encephalopathy is highly prevalent and has impact both in early and late morbidity and mortality. The mechanisms by which sepsis induces brain dysfunction include neuroinflammation, disrupted blood–brain barrier, oxidative stress, and microglial activation, but the cellular and molecular mechanisms involved in these events are not completely understood. Our objective was to determine the effects of microglial depletion in the early systemic and brain inflammatory response and its impact in phenotypes expression in an animal model of sepsis. Animals were subjected to CLP, and depletion of microglial cells was accomplished by administration of (Lipo)-encapsulated clodronate and microglial repopulation by doxycycline. Clodlip treatment was effective in decreasing microglia density in the hippocampus of animals. Pro-inflammatory cytokines were increased in the CLP+PBS, and liposomes administration increased even further these cytokines mainly 7 days, suggesting that microglial depletion exacerbates both local and systemic inflammation. In contrast, repopulation with doxycycline was able to revert the cytokine levels in both serum and cerebral structures on day 7 and 14 after repopulation. There were no differences in the correlation between M1 and M2 markers by *real-time* PCR, but immunohistochemistry showed significant increase in CD11b expression in CLP+PBS with greater expression in CLP + liposomes in the hippocampus. These results suggest that the depletion of microglia during severe sepsis development could be associated with early exacerbation of brain and systemic inflammation and repopulation is able to revert this condition, once a rapid neurological recovery is noticed until 7 days after sepsis.

Keywords Sepsis · Microglia · Clodronate-liposomes · Doxycycline

Introduction

Sepsis-associated encephalopathy is highly prevalent and has impact both in early and late morbidity and mortality [1–3]. The mechanisms by which sepsis induces brain dysfunction probably include vascular injuries, neuroinflammation, disrupted blood–brain barrier, oxidative stress, and microglial activation, but the cellular and molecular mechanisms involved in these events are not completely understood [4]. In this context, it is postulated that the modulation of microglia function could be an opportunity to decrease sepsis-associated morbidity and mortality [5–7].

Studies have demonstrated that microglia have a number of physiological, noninflammatory functions that are crucial for central nervous system (CNS) functioning in the adult brain [8]. When activated, they have been traditionally considered injurious to the brain due to production of inflammatory mediators, reactive oxygen molecules (ROS), and other toxic molecules [9, 10]. As intrinsic immune effector cells of the brain, microglia are potent mediators of cerebral inflammation in a variety of disease states [11–13].

However, microglia activation seems not to be a monotonous state; rather, recent studies characterized microglia

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in specific contexts which reveal discrete patterns in different conditions [14]. A simplistic view of these phenotypes fits microglia activation into a bimodal scheme, borrowed from earlier studies of macrophage phenotypes, the classic M1-like (proinflammatory) or alternative M2-like (anti-inflammatory) states [14]. Despite being oversimplified, this classification remains useful for understanding the function of microglia in various brain diseases [15].

In this context, ablation of microglia in transgenic mice in stroke models showed detrimental effects [16], and administration of exogenous microglia had neuroprotective effects after ischemia [17]. These effects are possibly linked to the production of neurotrophic factors by activated microglia [18]. Actually, microglia may have a dual role after CNS disease, either injurious [19] or beneficial [16], and these differences are at least in part secondary to the fact that microglia phenotypes are heterogeneous, producing distinct effects in different conditions or time points [20–24]. Thus, the type of stimulus and the local microenvironment (infectious, inflammatory, or anti-inflammatory) critically affect microglial phenotypes during disease development [16, 25, 26].

Furthermore, during systemic inflammation, the brain is affected in so many different ways [27, 28], including microglia activation [29, 30]. Inhibition of microglia activation prevented brain inflammation in an animal model of sepsis [27, 31], but whether microglia activation would impact on the control of systemic inflammation is not well known. There are evidences of a cross-talk between the brain and different systemic compartments [32–35], and some glial-derived responses seem to interfere in the systemic response after sepsis [36].

Therefore, since microglia has different roles controlling the brain, and probably, systemic inflammation, our objective was to determine the effects of microglial depletion and subsequent repopulation early during systemic and brain inflammatory response and its impact on late cognitive dysfunction in an animal model of severe sepsis.

Methods

Ethics

All experimental procedures were performed in accordance with the National Institute of Health, Guide for the Care and Use of Laboratory Animals (NRC80) and the Brazilian Society for Neuroscience and Behavior recommendations for animal care. The experimental animals were approved by the Institutional Animal Care and Use Committee of the Universidade do Extremo Sul Catarinense. Protocol numbers: 070/2016-2; 034/2018-1.

Sepsis Induction—Cecal Ligation and Perforation Model

Rats were subjected to cecal ligation and perforation (CLP) as previously described [37]. Briefly, animals were anesthetized using a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg), given intraperitoneally. Under aseptic conditions, a 3-cm midline laparotomy was performed to expose the cecum and adjoining intestine. The cecum was ligated with a 3.0 silk suture at its base, below the ileocecal valve, and was perforated once with a 14-gauge needle. The cecum was then squeezed gently to extrude a small amount of feces through the perforation site. The cecum was then returned to the peritoneal cavity, and the laparotomy was closed with 4.0 silk sutures. Animals were resuscitated with regular saline (50 mL/kg) subcutaneously (s.c.) immediately after and 12 h after CLP. All animals received antibiotics (ceftriaxone at 30 mg/kg) every 12 h s.c. for 3 days. To minimize variability between different experiments, the CLP procedure was always performed by the same investigator.

Microglia Depletion and Repopulation

Depletion of microglial cells was accomplished by i.c.v. administration of clodronate-liposomes (Clod-lip) (25 μg/rat) [38] immediately after sepsis induction. Liposomes-free of clodronate (PBS-lip) served as control. In general, Clod-lip treatment induced at least 85% reduction in microglial density [38]. To induce repopulation, doxycycline (D3447 - Sigma-Aldrich) was provided in deionized drinking water at 2 mg/ml with 5% sucrose by 14 consecutive days.

Animals were divided into four groups: (1) sham, (2) CLP, (3) CLP + liposomes, and (4) CLP + liposomes + doxycycline and were killed at 24 h, 7 days, and 14 days after sepsis. Serum and hippocampus were collected for subsequent analysis. Since repopulation using doxycycline is not effective at short periods after treatment [39], this group lacks the 24-h time point.

Perfusion Fixation of Tissue for Histology

Animals (five per group) were anesthetized with ketamine and xylazine (30 and 10 mg/kg i.p). Once the animal was unresponsive to toe pinch response, it was placed on the operating table with its back down. A scalpel was used to make an incision through the abdomen at the length of the diaphragm, followed by a cut of the rib cage up to the collarbone in both side of the ribs providing a clear view of the heart. A small incision was made in the posterior end of the left ventricle, and an olive-tipped perfusion needle was inserted through the ventricle to extend straight up about 5 mm. An incision to the rat right atrium was made to create an outlet for free flow of the solution. A hemostat was used to stabilize the needle and to clamp

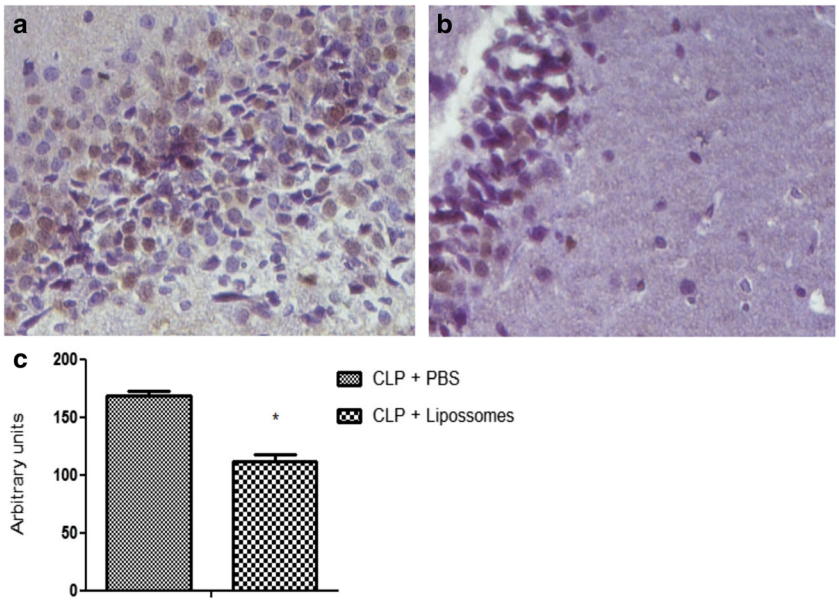


Fig. 1 Microglia density in hippocampus of septic animals treated with Clod-lip PBS. Sepsis was induced by cecal ligation and perforation (CLP) and animals were immediately after injected with clodronate-liposomes (CLP + liposomes) or liposomes free of clodronate (CLP + PBS). Twenty-four hours after treatment, animals were killed and the hippocampus were collected to the determination of IBA-1-positive cells by immunohistochemistry. Immunopositive cells appeared in brown. **a** Representative image of CLP + PBS animals. **b** Representative image of CLP + liposomes animals. **c** Iba-1-positive cells were expressed as arbitrary units by ImageJ software. $n = 5$ each group. Original magnifications × 40

the descendent aorta to optimize perfusion in the CNS. Five animals per group were perfused with 0.9% sterile saline during 10 min (flow rate 20 mL/min) followed by 10 min with paraformaldehyde (PFA) solution 4% in PBS (pH 7.4) (flow rate 20 mL/min). The brains were then carefully extracted and maintained in PFA 4% for 24 h at 4 °C, then placed in sucrose 15% for 24 h at 4 °C, and placed in sucrose 30% for 24 h at 4 °C.

Immunohistochemistry

Immunohistochemistry was performed to determine microglial density, as a marker of the effectiveness of Clod-lip treatment, and to explore markers of M1 and M2 microglia.

Briefly, 40-μm sections from the hippocampus were incubated in 0.5% hydrogen peroxide in 0.1 M PBS (pH 7.4) containing 0.3% Triton X-100 (PBST) for 30 min at room temperature to block endogenous peroxidase activity. After washing with PBST, sections were incubated for 30 min with PBST containing 2% bovine serum albumin to block non-specific protein binding. Sections were then incubated overnight at 4 °C with a rabbit monoclonal IgG antibody against IBA-1 (1:400 dilution) or a rabbit monoclonal IgG antibody against CD11-b (1:4000 dilution) or rabbit polyclonal antibody against IL-10 (1:200 dilution, Abcam, Cambridge, MA). After washing with PBST, sections were incubated at room temperature for 1 h with biotinylated anti-rabbit IgG (1:100 dilution; Abcam). Sections were incubated with 3,3′-diaminobenzidine (DAB) (Spring Bioscience). Sixteen random images per brain section were acquired at × 4 and × 20 magnification, and immunopositive area was expressed as IBA-1+/total number of cells. Only immunopositive cells with morphologic characteristics of microglia were counted. Positive control was used in all groups, according to the data sheet of antibody. The × 4 magnification images show the hippocampus as a whole. The increase × 20 provides a more detailed expansion of the CA3 region and/or dentate gyrus. These are the regions of the hippocampus with more microglial density [40, 41].

Inflammatory Markers

Concentrations of TNF-α, IL-1β, IL-6, IL-10, and CCL-2 in the hippocampus and serum were determined by ELISA on microplate reader using commercial kits (R&D Systems).

Statistics

Data collected were compared by the two-way ANOVA and expressed as mean ± standard deviation. Correlations were performed by the Pearson test. All analyses were performed with SPSS version 20 and/or GraphPad Prism 4.0. In all comparisons, $p < 0.05$ indicated statistical significance. Immunopositive cells were quantified by cells IBA-1+/total number of cells and expressed as percentage of sham.

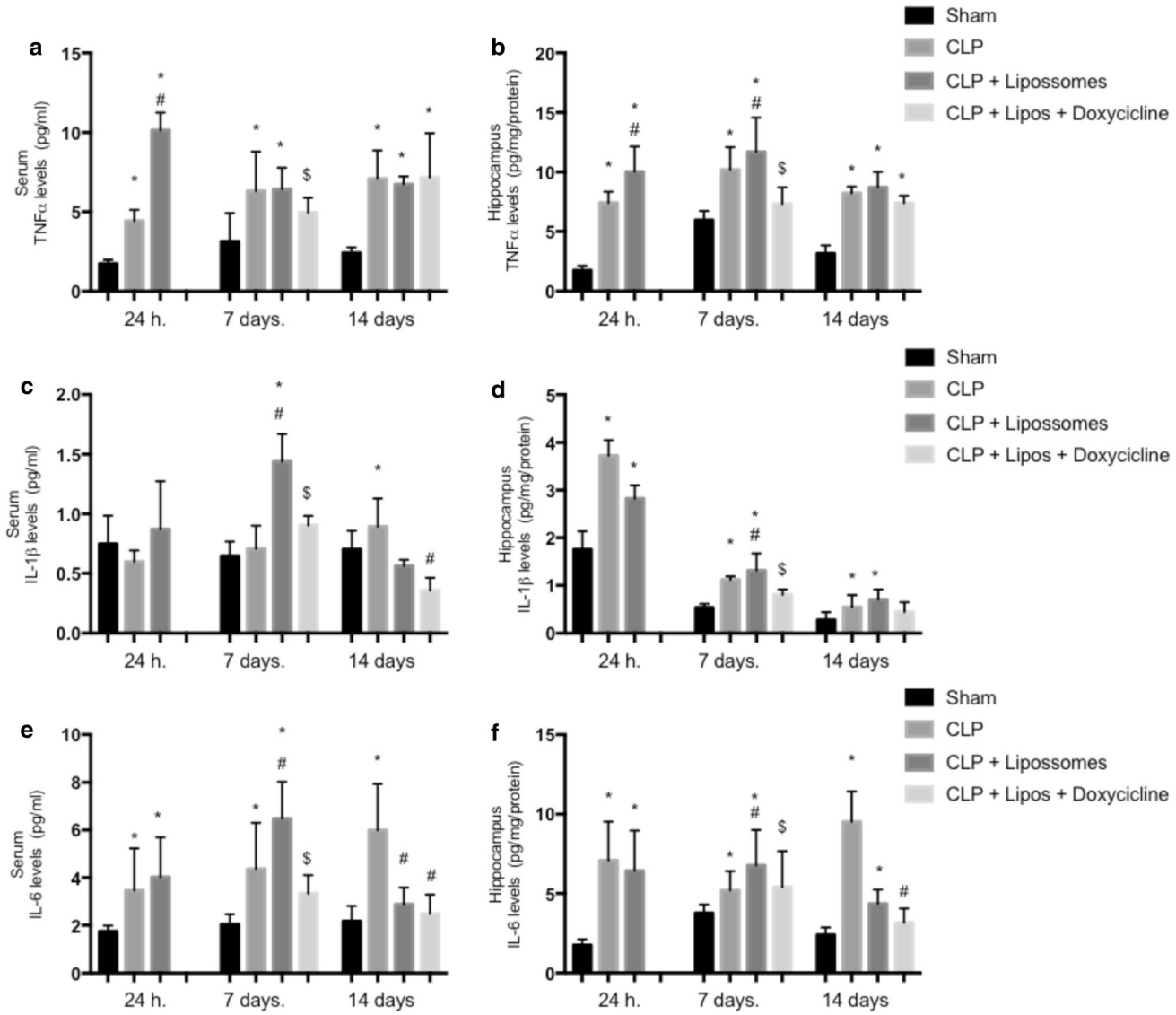


Fig. 2 Effects of microglia depletion and subsequent repopulation on local and systemic inflammation after sepsis induction. Sepsis was induced by cecal ligation and perforation and animals were immediately after treated with clodronate-liposomes (CLP + liposomes) or liposomes free (CLP + PBS). Other group received doxycycline (CLP + liposomes + doxycycline). Markers of inflammation (TNF-α (**a**, **b**), IL1β (**c**, **d**), IL-6 (**e**, **f**)) were measured in serum and hippocampus 24 h, 7 days and 14 days. $n = 5$ each group. Asterisk indicates statistically different of sham. Number sign indicates different of CLP + PBS. Dollar sign indicates different of CLP + liposomes

Results

Despite previously demonstrated in the literature that Clod-lip was effective to deplete microglia cells (Faustino et al. [38]), in Fig. 1, it was shown a representative image of Clod-lip treated septic rat. Both the qualitative (Fig. 1a, b) and the quantitative analyses (Fig. 1c) demonstrated that Clod-lip treatment was effective in decreasing microglia density in the hippocampus of septic animals in 24 h after i.c.v. treatment.

Figure 2 shows markers of inflammation (TNF-α (a, b), IL1β (c, d), IL-6 (e, f)) in the serum and hippocampus after sepsis induction, microglial depletion, and repopulation with doxycycline. Pro-inflammatory cytokines (TNF-α (a, b), IL-6 (e, f)), were increased in the CLP group, and liposome administration increased even further these cytokines mainly 7 days, suggesting that microglial depletion exacerbates both local and systemic inflammation. IL-1β levels (c, d) increased 7 days after sepsis in the CLP + liposomes group. In contrast, repopulation with doxycycline was able to revert the cytokine levels in both the serum and cerebral structures on days 7 and 14 after repopulation.

Anti-inflammatory cytokines (IL-10 (a, b), CCL-2 (c, d)) were analyzed (Fig. 3). There were no differences in IL-10 levels in the hippocampus. It was only observed a decrease in serum IL-10 levels at day 14. This was reverted by doxycycline treatment. CCL-2 was increased after sepsis, mainly at 24 h, but in doxycycline-treated

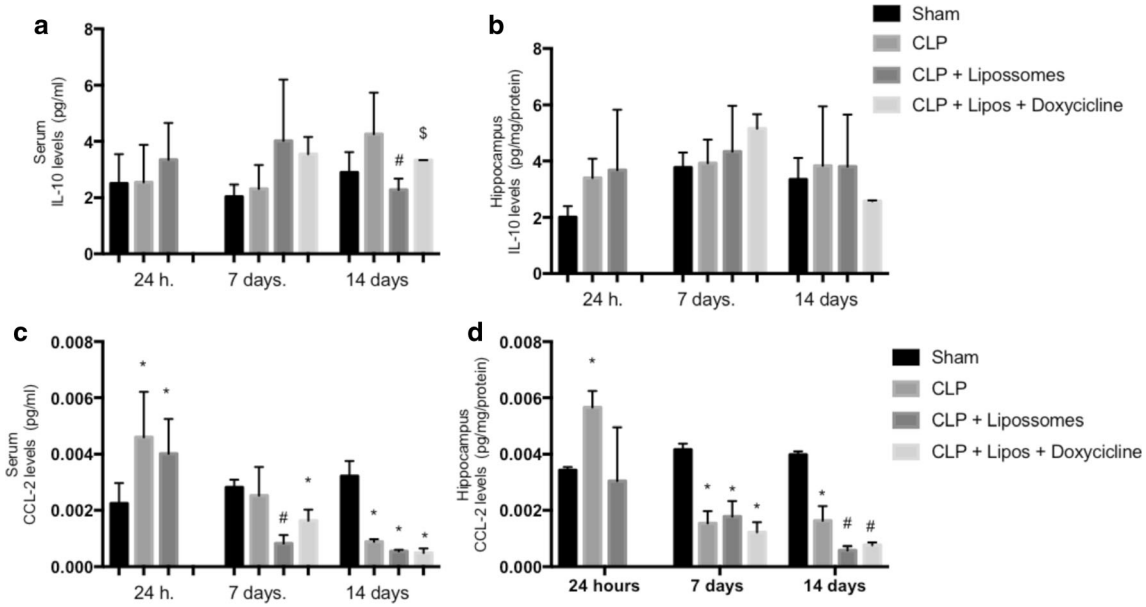


Fig. 3 Anti-inflammatories cytokines after microglial depletion and repopulation in septic animals. Sepsis was induced by cecal ligation and perforation, and animals were immediately after treated with clodronate-liposomes (CLP + liposomes) or liposomes free (CLP + PBS). One group received doxycycline (CLP + liposomes + doxycycline). Anti-inflammatory cytokines were measured (IL-10 (**a**, **b**) and CCL-2 (**c**, **d**)) in the serum and hippocampus in 24 h, 7 days, and 14 days. $n = 5$ each group. Asterisk indicates statistically different of sham. Number sign indicates different of CLP+PBS. Dollar sign indicates different of CLP + liposomes

animals, it was not observed any significant modification in its levels.

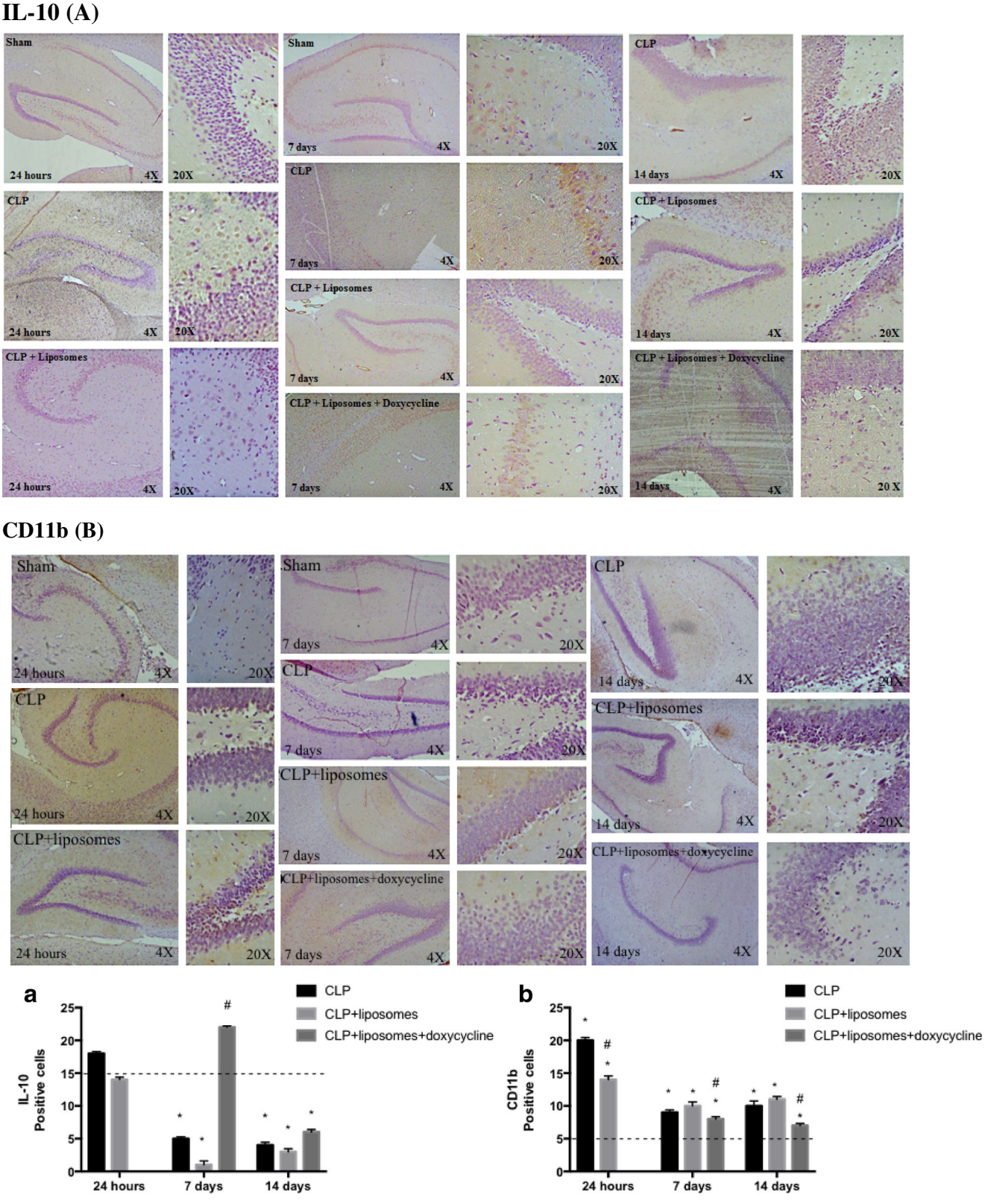
Since different microglia phenotypes could account to different brain responses, markers of M1 and M2 phenotypes were determined by immunohistochemistry. When analyzing the expression of IL-10 (Fig. 4a), there was no significant differences at 24 h between groups. At 7 days, there was a significant decrease in IL-10 hippocampus expression that was maintained up to 14 days. Repopulation by doxycycline increased IL-10-positive cells at 7 days, returning to basal levels 14 days after sepsis induction. The M1 marker CD11b (Fig. 4b) was significantly increased as early as 24 h both in the CLP and CLP + liposomes group. In 7 days, CD11b expression remains in the CLP + PBS and CLP + liposomes group and the repopulation with doxycycline is able to decrease the expression of this marker until 14 days after.

Discussion

Here it was demonstrated that microglia depletion exacerbated brain and systemic inflammation and that microglia repopulation with doxycycline prevents inflammation in a rodent sepsis model.

Microglia function is essential to brain homeostasis [8]; thus, it is plausible to suppose that microglia depletion impacts both locally (the brain) and systemically during the development of sepsis. The control of systemic responses by the brain and mainly during brain damage is a complex phenomenon. For example, it is well known that the impairment of immune responses after brain ischemia increases the susceptibility to infections [42, 43]. It has been proposed that during brain injury, the peripheral immune system became hypofunctional [44] mainly due to the influence of the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic and the parasympathetic nervous system [45]. It was recently shown that these pathways induced atrophy of the spleen and down-regulated natural killer cells [35]. On the other hand, the systemic inflammatory response is induced after both stroke and subarachnoid hemorrhage, and the intensity of this response is positively associated with the extent of tissue damage and disease severity [46–48]. Whether these processes are relevant to the development of sepsis is not known. Here, it was demonstrated that the depletion of microglia increased the systemic and local inflammatory response that accompanies sepsis.

Recently, it was demonstrated that microglia inhibition by minocycline during the early development of sepsis decreased brain inflammation [27]. However, it was here demonstrated the microglia depletion worsen brain inflammation and probably brain dysfunction, but this was not a completely unexpected finding. Evidences suggest that depletion of microglia augment the production of inflammatory mediators, mainly by an increased brain leukocyte infiltration [49–51]. Additionally, microglial depletion induced worsening of brain damage also by increased production of inflammatory mediators by astrocytes [52]. On the other hand, Jantzie et al. [53]



found that doxycycline significantly inhibits neuroinflammation in the frontal cortex, striatum, and hippocampus and decreases IL-1β and TNF-α. There are some reports in the literature suggesting that a decrease brain inflammation is associated with better functional outcomes in sepsis survivor animals [54, 55].

◀ **Fig. 4** Immunohistochemistry for IL-10 and CD11b in the hippocampus 24 h, 7 days, and 14 days after sepsis. Animals were submitted to sham or CLP and treated with PBS or clodronate-liposomes. Immunohistochemistry was performed to determine the expression of IL-10 as a marker of M2 microglial phenotype or CD11b as a marker of M1 microglial phenotype in the hippocampus. Representative images of each group in magnification of × 4 and × 20 are demonstrated. $n = 5$ each group. Sham × 4 and × 20; CLP × 4 and × 20; CLP + liposomes × 4 and × 20; CLP + liposomes + doxycycline × 4 and × 20. Representative graphic to immunohistochemistry for IL-10 (**a**) and CD11-b (**b**) in 24 h, 7 days and 14 days after sepsis and treatments. IBA-1+/total cells. Asterisk indicates different for sham. Number sign indicates different for CLP + liposomes. $p < 0.05$

Lazzarini et al. [56] and Santa-Cecilia et al. [57] showed the neuroprotective effect of doxycycline in Parkinson's disease. These authors suggest that doxycycline decreased the neurotoxicity and protects substantia nigra dopaminergic neurons in an animal model of Parkinson's disease. This effect was associated with a reduction of microglial cell activation, which suggests that doxycycline may operate primarily as an anti-inflammatory drug [56, 57]. In these studies, doxycycline attenuated the expression of key activation markers in LPS-treated microglial cultures. More specifically, doxycycline treatment decreased the expression of IBA-1 as well as the production of ROS, NO, and proinflammatory cytokines [56, 57]. Santa-Cecilia and colleagues indicate that the effect of doxycycline on LPS-induced microglial activation probably occurs via the modulation of p38 MAP kinase and NF-kB signaling pathways [57]. Repopulation resolves many aspects of chronic neuroinflammation, including microglial morphology and expression of reactive markers, as well as inflammatory transcripts. Elimination and subsequent repopulation appear to increase synaptic surrogates, such as dendritic spines and synaptophysin and PSD95 puncta numbers. Finally, and most importantly, microglial repopulation results in an almost complete reversal of behavioral impairment, suggesting that repopulation promotes an environment for recovery [58].

Faustino et al. [38] have observed low rate of phagocytosis in the absence of microglia, which further contributes to neuronal damage, thus demonstrating the protective role of microglia against neuroinflammation. Taking together these results suggests that microglia take a complex role in the control of acute brain inflammation both during primary and secondary brain injury. Generally, microglia depletion enhances local inflammation and increases injury severity, suggesting that microglia contribute to endogenous protection early after injury. On the other hand, inhibition of microglia activation, mainly by minocycline, generally attenuated brain inflammation and brain dysfunction [27, 59–61].

The depletion of microglial cells could have a major impact on the expression of microglia phenotypes. When analyzed IL-10 (M2 marker) and CD11b (M1 marker) expression by immunohistochemistry, there was a notable increase in the expression of CD11b soon after sepsis and rapid return to basal levels in the doxycycline group. Even in this context, microglia could have both positive and negative effects on brain function. Microglial activation has been linked to delayed neuronal death, presumably via releasing neurotoxic substances [62]. Wang et al. [36] recently showed that liposomes-clodronate depleted microglia but does not alter the number of neurons and astrocytes. Additionally, this effect was not selective between resting and activated microglia. Microglia activation could also contribute to microglial-mediated synaptic injury which could mediate cognitive deficits even in the absence of overt neuronal death [63].

On the other hand, microglia could also have beneficial effects, contributing to delayed repair after injury via elaboration of growth factors [64, 65]. Thus, manipulating microglia to improve brain inflammation and outcomes both in primary and secondary brain injuries requires a complex approach. The effect of microglia manipulation could depend on the severity of the insult, the timing of microglia modulation, the approach used to modulate microglia function, and probably several others unknown factors that could affect the final effect of microglia manipulation. Despite this, some clinical trials are ongoing using minocycline for different disease states [66–68].

In conclusion, these results suggest that the depletion of microglia during severe sepsis development could be associated with early exacerbation of brain and systemic inflammation. Microglial repopulation is able to revert this condition decreasing proinflammatory cytokines and CD11b expression in the brain.

Our results show some limitations. First, we did not show in this study the effect of doxycycline in microglial repopulation of the brain; however, both Rice et al. [39, 58] and Elmore et al. [69] demonstrated an increase in microglial density in animals receiving doxycycline treatment as soon as 3 days after doxycycline, and at day 7, there was a major proportion of repopulation that peaked at day 14. Another important point is that it is not usual to use IL-10 as a M2 marker in immunohistochemistry due to the secretive nature of IL-10. However, IL-10 was reported as a surface protein in macrophages [70, 71] as well as in glial cells mainly after brain insults [72].

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References

1. Mazeraud A, Pascal Q, Verdonk F, Heming N, Chrétien F, Sharshar T (2016) Neuroanatomy and physiology of brain dysfunction in sepsis. Clin Chest Med 37(2):333–345
2. Annane D, Sharshar T (2015) Cognitive decline after sepsis. Lancet Respir Med 3(1):61–69
3. Widmann CN, Heneka MT (2014) Long-term cerebral consequences of sepsis. Lancet Neurol 13(6):630–636

4. Bozza FA, D'Avila JC, Ritter C, Sonneville R, Sharshar T, Dal-Pizzol F (2013) Bioenergetics, mitochondrial dysfunction, and oxidative stress in the pathophysiology of septic encephalopathy. Shock 39(Suppl 1):10–16
5. Hoogland IC, Houbolt C, van Westerloo DJ, van Gool WA, van de Beek D (2015) Systemic inflammation and microglial activation: systematic review of animal experiments. J Neuroinflammation 12: 114
6. Hirbec H, Noristani HN, Perrin FE (2017) Microglia responses in acute and chronic neurological diseases: what microglia-specific transcriptomic studies taught (and did not teach) us. Front Aging Neurosci 9:227
7. Salter MW, Stevens B (2017) Microglia emerge as central players in brain disease. Nat Med 23(9):1018–1027
8. Li Q, Barres BA (2017) Microglia and macrophages in brain homeostasis and disease. Nat Rev Immunol 18:225–242. https://doi.org/10.1038/nri.2017.125
9. Wang X, Min S, Xie F, Yang J, Li L, Chen J (2018) Glial cell-derived neurotrophic factor alleviates sepsis-induced neuromuscular dysfunction by decreasing the expression of γ- and α7-nicotinic acetylcholine receptors in an experimental rat model of neuromyopathy. J Biochem Biophys Res Commun 496(2):260–266
10. Lai AY, Todd KG (2006) Microglia in cerebral ischemia: molecular actions and interactions. Can J Physiol Pharmacol 84:49–59
11. Lan X, Han X, Li Q, Yang QW, Wang J (2017) Modulators of microglial activation and polarization after intracerebral haemorrhage. Nat Rev Neurol 13(7):420–433
12. Ising C, Heneka MT (2018) Functional and structural damage of neurons by innate immune mechanisms during neurodegeneration. Cell Death Dis 9(2):120
13. Calcia MA, Bonsall DR, Bloomfield PS, Selvaraj S, Barichello T, Howes OD (2016) Stress and neuroinflammation: a systematic review of the effects of stress on microglia and the implications for mental illness. Psychopharmacology 233(9):1637–1650
14. Ransohoff RM (2016) A polarizing question: do M1 and M2 microglia exist? Nat Neurosci 19:987–991
15. Liu Q, Jin WN, Liu Y, Shi K, Sun H, Zhang F, Zhang C, Gonzales RJ et al (2017) Brain ischemia suppresses immunity in the periphery and brain via different neurogenic innervations. Immunity 46(3):474–487
16. Lalancette-Hebert M, Gowing G, Simard A, Weng YC, Kriz J (2007) Selective ablation of proliferating microglial cells exacerbates ischemic injury in the brain. J Neurosci 27:2596–2605
17. Kitamura Y, Takata K, Inden M, Tsuchiya D, Yanagisawa D, Nakata J, Taniguchi T (2004) Intracerebroventricular injection of microglia protects against focal brain ischemia. J Pharmacol Sci 94: 203–206
18. Hanisch UK (2002) Microglia as a source and target of cytokines. Glia 40:140–155
19. Wan S, Cheng Y, Jin H, Guo D, Hua Y, Keep RF, Xi G (2016) Microglia activation and polarization after intracerebral hemorrhage in mice: the role of protease-activated receptor-1. Transl Stroke Res 7(6):478–487
20. Perego C, Fumagalli S, Zanier ER, Carlino E, Panini N, Erba E, De Simoni MG (2016) Macrophages are essential for maintaining a M2 protective response early after ischemic brain injury. Neurobiol Dis 96:284–293
21. Wang G, Zhang J, Hu X, Zhang L, Mao L, Jiang X, Liou AK, Leak RK et al (2013) Microglia/macrophage polarization dynamics in white matter after traumatic brain injury. J Cereb Blood Flow Metab 33:1864–1874
22. Kroner A, Greenhalgh AD, Zarruk JG, Passos Dos Santos R, Gaestel M, David S (2014) TNF and increased intracellular iron alter macrophage polarization to a detrimental M1 phenotype in the injured spinal cord. Neuron 83:1098–1116
23. Boddaert J, Bielen K, Jongers B, Manocha E, Yperzeele L, Cras P, Pirici D, Kumar-Singh S (2018) CD8 signaling in microglia/macrophage M1 polarization in a rat model of cerebral ischemia. PLoS One 13(1):e0186937
24. Fumagalli S, Perego C, Ortolano F, De Simoni MG (2013) CX3CR1 deficiency induces an early protective inflammatory environment in ischemic mice. Glia 61(6):827–842
25. Petersen MA, Dailey ME (2004) Diverse microglial motility behaviors during clearance of dead cells in hippocampal slices. Glia 46(2):195–206
26. Kreutzberg GW (1996) Microglia: a sensor for pathological events in the CNS. Trends Neurosci 19(8):312–318
27. Michels M, Vieira AS, Vuolo F, Zapelini HG, Mendonça B, Mina F, Dominguini D, Steckert A et al (2014) The role of microglia activation in the development of sepsis-induced long-term cognitive impairment. Brain Behav Immun 43:54–59
28. Sharshar T, Bozza F, Chrétien F (2014) Neuropathological processes in sepsis. Lancet Neurol 13(6):534–536
29. Michels M, Sonai B, Dal-Pizzol F (2017) Polarization of microglia and its role in bacterial sepsis. J Neuroimmunol 303:90–98
30. Michels M, Danielski LG, Dal-Pizzol F, Petronilho F (2014) Neuroinflammation: microglial activation during sepsis. Curr Neurovasc Res 11(3):262–267
31. Michels M, Steckert AV, Quevedo J, Barichello T, Dal-Pizzol F (2015) Mechanisms of long-term cognitive dysfunction of sepsis: from blood-borne leukocytes to glial cells. Intensive Care Med Exp 3(1):30
32. Chen T, Chen C, Zhang Z, Zou Y, Peng M, Wang Y (2016) Toll-like receptor 4 knockout ameliorates neuroinflammation due to lung-brain interaction in mechanically ventilated mice. Brain Behav Immun 56:42–55
33. Gonzalvo R, Martí-Sistac O, Blanch L, López-Aguilar J (2007) Bench-to-bedside review: brain-lung interaction in the critically ill a pending issue revisited. Crit Care 11(3): 216.
34. Reardon C (2016) Neuro-immune interactions in the cholinergic anti-inflammatory reflex. Immunol Lett 178:92–96
35. Liu Z, Wang Y, Wang Y, Ning Q, Zhang Y, Gong C, Zhao W, Jing G et al (2016) Dexmedetomidine attenuates inflammatory reaction in the lung tissues of septic mice by activating cholinergic anti-inflammatory pathway. Int Immunopharmacol 35:210–216
36. Wang YR, Mao XF, Wu HY, Wang YX (2018) Liposome-encapsulated clodronate specifically depletes spinal microglia and reduces initial neuropathic pain. Biochem Biophys Res Commun 499(3):499–505
37. Fink MP, Heard SO (1990) Laboratory models of sepsis and septic shock. J Surg Res 49(2):186–196
38. Faustino J, Wang X, Johnson CE, Klibanov A, Derugin N, Wendland MF, Vexler ZS (2011) Microglial cells contribute to endogenous brain defenses after acute neonatal focal stroke. J Neurosci 31(36):12992–13001
39. Rice RA, Spangenberg EE, Yamate-Morgan H, Lee RJ, Arora RP, Hernandez MX, Tenner AJ, West BL et al (2015) Elimination of microglia improves functional outcomes following extensive neuronal loss in the hippocampus. J Neurosci 35(27):9977–9989
40. Sanchez-Mejias E, Navarro V, Jimenez S, Sanchez-Mico M, Sanchez-Varo R, Nuñez-Diaz C, Trujillo-Estrada L, Davila JC et al (2016) Soluble phospho-tau from Alzheimer's disease hippocampus drives microglial degeneration. Acta Neuropathol 132(6): 897–916
41. Ragu-Varman D, Macedo-Mendoza M, Labrada-Moncada FE, Reyes-Ortega P, Morales T, Martínez-Torres A, Reyes-Haro D (2019) Anorexia increases microglial density and cytokine expression in the hippocampus of young female rats. Behav Brain Res 363:118–125
42. Hug A, Dalpke A, Wieczorek N, Giese T, Lorenz A, Auffarth G, Liesz A, Veltkamp R (2009) Infarct volume is a major determiner of

post-stroke immune cell function and susceptibility to infection. Stroke 40:3226–3232
43. Wong CH, Jenne CN, Lee WY, Le'ger C, Kubes P (2011) Functional innervation of hepatic iNKT cells is immunosuppressive following stroke. Science 334:101–105
44. Andersson U, Tracey KJ (2012) Neural reflexes in inflammation and immunity. J Exp Med 209:1057–1068
45. Fu Y, Liu Q, Anrather J, Shi FD (2015) Immune interventions in stroke. Nat Rev Neurol 11:524–535
46. Audebert HJ, Rott MM, Eck T, Haberl RL (2004) Systemic inflammatory response depends on initial stroke severity but is attenuated by successful thrombolysis. Stroke. 35(9):2128–2133
47. Yoshimoto Y, Tanaka Y, Hoya K (2001) Acute systemic inflammatory response syndrome in subarachnoid hemorrhage. Stroke. 32(9):1989–1993
48. Kalita J, Bastia J, Bhoi SK, Misra UK (2015) Systemic inflammatory response syndrome predicts severity of stroke and outcome. J Stroke Cerebrovasc Dis 24(7):1640–1648
49. Laterza C, Wattananit S, Uoshima N, Ge R, Pekny R, Tornero D, Monni E, Lindvall O et al (2017) Monocyte depletion early after stroke promotes neurogenesis from endogenous neural stem cells in adult brain. Exp Neurol 297:129–137
50. Neumann J, Riek-Burchardt M, Herz J, Doeppner TR, König R, Hütten H, Etemire E, Männ L et al (2015) Very-late-antigen-4 (VLA-4)-mediated brain invasion by neutrophils leads to interactions with microglia, increased ischemic injury and impaired behavior in experimental stroke. Acta Neuropathol 129(2):259–277
51. Shechter R, London A, Varol C, Raposo C, Cusimano M, Yovel G, Rolls A, Mack M et al (2009) Infiltrating blood-derived macrophages are vital cells playing an anti-inflammatory role in recovery from spinal cord injury in mice. PLoS Med 6(7):e1000113
52. Jin WN, Shi SX, Li Z, Li M, Wood K, Gonzales RJ, Liu Q (2017) Depletion of microglia exacerbates postischemic inflammation and brain injury. J Cereb Blood Flow Metab 37(6):2224–2236
53. Jantzie LL, Todd KG (2010) Doxycycline inhibits proinflammatory cytokines but not acute cerebral cytogenesis after hypoxia-ischemia in neonatal rats. J Psychiatry Neurosci 35(1):20–32
54. Schwalm MT, Pasquali M, Miguel SP, Dos Santos JP, Vuolo F, Comim CM, Petronilho F, Quevedo J et al (2014) Acute brain inflammation and oxidative damage are related to long-term cognitive deficits and markers of neurodegeneration in sepsis-survivor rats. Mol Neurobiol 49(1):380–385
55. Comim CM, Cassol OJ Jr, Constantino LS, Felisberto F, Petronilho F, Rezin GT, Scaini G, Daufenbach JF et al (2011) Alterations in inflammatory mediators, oxidative stress parameters and energetic metabolism in the brain of sepsis survivor rats. Neurochem Res 36(2):304–311
56. Lazzarini M, Martin S, Mitkovski M, Vozari RR, Stühmer W, Bel ED (2013) Doxycycline restrains glia and confers neuroprotection in a 6-OHDA Parkinson model. Glia 61(7):1084–1100
57. Santa-Cecília FV, Socias B, Ouidja MO, Sepulveda-Diaz JE, Acuña L, Silva RL, Michel PP, Del-Bel E et al (2016) Doxycycline suppresses microglial activation by inhibiting the p38 MAPK and NF-kB signaling pathways. Neurotox Res 29(4):447–459
58. Rice RA, Pham J, Lee RJ, Najafi AR, West BL, Green KN (2015) Microglial repopulation resolves inflammation and promotes brain recovery after injury. Glia 65(6):931–944
59. Michels M, Danieslki LG, Vieira A, Florentino D, Dall'Igna D, Galant L, Sonai B, Vuolo F et al (2015) CD40-CD40 ligand pathway is a major component of acute neuroinflammation and contributes to long-term cognitive dysfunction after sepsis. Mol Med 21: 219–226
60. Tanaka M, Ishihara Y, Mizuno S, Ishida A, Vogel CF, Tsuji M, Yamazaki T, Itoh K (2018) Progression of vasogenic edema induced by activated microglia under permanent middle cerebral artery occlusion. Biochem Biophys Res Commun 496:582–587
61. Lu Y, Xiao G, Luo W (2016) Minocycline suppresses NLRP3 inflammasome activation in experimental ischemic stroke. Neuroimmunomodulation 23(4):230–238
62. Lull ME, Block ML (2010) Microglial activation and chronic neurodegeneration. Neurotherapeutics 7(4):354–365
63. Paolicelli RC, Ferretti MT (2017) Function and dysfunction of microglia during brain development: consequences for synapses and neural circuits. Front Synaptic Neurosci 10(9):9
64. Karve IP, Taylor JM, Crack PJ (2016) The contribution of astrocytes and microglia to traumatic brain injury. Br J Pharmacol 173(4):692–702
65. Lund H, Pieber M, Harris RA (2017) Lessons learned about neurodegeneration from microglia and monocyte depletion studies. Front Aging Neurosci 9:234
66. Fouda AY, Newsome AS, Spellicy S, Waller JL, Zhi W, Hess DC, Ergul A, Edwards DJ et al (2017) Minocycline in acute cerebral hemorrhage: an early phase randomized trial. Stroke 48(10):2885–2887
67. Esalatmanesh S, Abrishami Z, Zeinoddini A, Rahiminejad F, Sadeghi M, Najarzadegan MR, Shalbafan MR, Akhondzadeh S (2016) Minocycline combination therapy with fluvoxamine in moderate-to-severe obsessive-compulsive disorder: a placebo-controlled, double-blind, randomized trial. Psychiatry Clin Neurosci 70(11):517–526
68. Husain MI, Chaudhry IB, Rahman RR, Hamirani MM, Qurashi I, Khoso AB, Deakin JF, Husain N et al (2015) Minocycline as an adjunct for treatment-resistant depressive symptoms: study protocol for a pilot randomised controlled trial. Trials 16:410
69. Elmore MR, Najafi AR, Koike MA, Dagher NN, Spangenberg EE, Rice RA, Kitazawa M, Matusow B et al (2014) Colony-stimulating factor 1 receptor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain. Neuron 82(2):380–397
70. Fleming SD, Leenen PJ, Freed JH, Campbell PA (1999) Surface interleukin-10 inhibits listericidal activity by primary macrophages. J Leukoc Biol 66(6):961–967
71. Fleming SD, Campbell PA (1996) Macrophages have cell surface IL-10 that regulates macrophage bactericidal activity. J Immunol 156(3):1143–1150
72. Gonzalez P, Burgaya F, Acarin L, Peluffo H, Castellano B, Gonzalez B (2009) Interleukin-10 and interleukin-10 receptor-I are upregulated in glial cells after an excitotoxic injury to the postnatal rat brain. J Neuropathol Exp Neurol 68(4):391–403