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# Inflammasomes are neuroprotective targets for sex steroids

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ABSTRACT

Neuroinflammation in the central nervous system is triggered by toxic stimuli or degenerative events, orchestrates the interplay of brain-intrinsic immune cells and neighboring neural cells, and sequentially allows leukocyte extravasation from the periphery into the brain parenchyma. During the inflammatory cascade, immune-competent cells become activated and secrete a plethora of cytokines and chemokines which form a local inflammatory signaling network important for warding off harmful stimuli to the host but are likewise necessary to preserve damaged brain tissue. Inflammatory responses are initiated by extra- and intra-cellular pathogen and danger-associated receptors. These signals are processed by multi-protein complexes termed inflammasomes which trigger the production of biologically active interleukins-1 and 18 after the cleavage of caspase-1. Estrogens and progesterone are neuroprotective and anti-inflammatory in diverse disease models of the brain in particular under acute inflammatory conditions such as stroke and traumatic brain injury. Both steroids are able to attenuate pro-inflammatory cytokine activity. Recent literature and our own studies provide convincing evidence that the anti-inflammatory potency of these steroids result from a complex interaction with the inflammasome activation and their up-stream regulatory network of miRNAs in brain-intrinsic innate immune cells. This article examines steroid–inflammasome interactions in the brain during brain injury and illuminates the importance of regulation initial upstream events during neuroinflammation.

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## 1. Introduction

The brain is considered an "immune-privileged" organ due to the presence of a selective blood–brain barrier (BBB) that impedes the entry of foreign pathogens and immune mediators. Consequentially, in fact all neural cells and not only local microglia participate in the control of neuroinflammatory processes. The multi-protein complexes termed inflammasomes play a decisive role for the initiation and perpetuation of inflammation in the central nervous system (CNS). Inflammasome-mediated pathways are therefore under intensive investigation [1]. The activation of inflammasome components in innate brain immune cells typically reflects one of the primary and critical steps of neuroinflammation sequentially triggered by harmful stimuli and traumatic challenges through pathogen-associated molecular patterns (PAMPs) and damage-associated molecular pattern molecules (DAMPs) followed by chemokine and cytokine production and release as will be detailed later [2]. Although only rudimentary decoded, it becomes more and more evident that the stimulation of inflammasomes in the brain irrespective of the subtype of inflammasome constituent is part of the complex process of tissue protection, neuronal elimination, host defense, immune

*Abbreviations:* AIM2, absent in melanoma 2; ASC, apoptosis-associated speck-like protein containing a CARD; BBB, blood–brain barrier; CARD, caspase activation and recruitment domain; CNS, central nervous system; DAMPs, damage-associated molecular pattern; E2, 17β-estradiol; EBBP, estrogen-responsive B box protein; FDA, Food and Drug Administration; FIIND, function-to-find domain; GFAP, glial fibrillary acidic protein; IBA1, ionized calcium-binding adapter molecule 1; IL, interleukin; i.v., intravenous; Let7f, lethal gene 7f; LRR, leucine-rich repeat; miRNA, micro RNA; mt, mitochondria; NAIPs, NLR family, apoptosis inhibitory proteins; NBD, nucleotide-binding domain; NeuN, neuron specific nuclear protein; NLRC4, NLR family CARD domain-containing protein 4; NLRPs, NACHT, LRR and PYD domains-containing proteins; NLRs, NOD-like receptors; NSAIDs, non-steroidal anti-inflammatory drugs; NFkappaB, nuclear factor kappa B; P, progesterone; PAMPs, pathogen-associated molecular pattern; PRRs, pattern recognition receptors; PUFA, omega-3 polyunsaturated fatty acids; PYD, pyrin domain; RA, rheumatoid arthritis; ROS, reactive oxygen species; SERM, selective estrogen receptor modulator; TBI, traumatic brain injury; TLR, Toll-like receptors; tMCAO, transient focal middle cerebral artery occlusion; TNFa, tumor necrosis factor alpha; tMCAO, transient middle cerebral occlusion; *vs.*, versus; XIAP, X-linked inhibitor of apoptosis protein.

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coordination, and balance of steady simmering or fulminant overshooting inflammatory responses. Besides being part of the anti-viral and anti-bacterial infection cascade, an emerging body of literature point to inflammasomes also to be involved in the pathogenesis of classical neurodegenerative diseases, *i.e.*, Alzheimer's disease and Parkinson syndrome diseases, acute traumatic events including stroke, and even psychiatric illness such as major depressive disorders [1,3–6]. The importance and unique feature of inflammasomes at the beginning and during the flowchart of inflammation makes them ideal candidates as points of therapeutic applications. Neuroinflammation is a "natural" response to eliminate the initial cause of brain cell injury and necrotic cells resulting from the original injury. After an acute boost of local inflammatory responses and if brain homeostasis is not restored, neuroinflammation becomes a chronic condition that erodes the surrounding tissues and causes late sequela. Under such conditions, tissue deterioration and healing proceed simultaneously. Non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids are both considered to be anti-inflammatory agents by regulating and curbing the transcription of inflammatory molecules and inhibiting prostaglandin synthesis. They play an important role in the treatment of neuro-oncologic patients [7] but, irrespective of their anti-inflammatory potential, these drugs do obviously not alleviate brain-intrinsic inflammatory activity in distinct CNS diseases when considering acute or chronic brain degeneration [8,9] rather exacerbate tissue damage or prevent neuroprotection [10–12]. Generally and although many compounds have been tested in the past in preclinical trials, the development of anti-inflammatory drugs in the brain is beset by disappointments and setbacks and to this day, no adequate treatment regime have been established to counter brain inflammation. Gonadal steroid hormones, and in particular 17β-estradiol (E2) and progesterone (P) and their related derivatives possess a high neuroprotective and anti-inflammatory power in diverse acute and chronic brain disease models [10,13–19]. A plethora of literature is available which has documented the potency and related cellular mechanisms of these steroid hormones to protect brain cells and tissue from damage in acute toxic, hypoxic and chronic degenerative animal models. Therein, it is clearly expressed, and this holds in particular for acute brain harm models such as stroke and traumatic brain injury (TBI) that the prompt and sustained interference with the inflammatory cascade which ends up in dampening or attenuation of the transcription and activation of pro-inflammatory molecules is essential for achieving an optimal tissue protection. The available data records mainly confirm that E2 and P regulate downstream events in the glial inflammatory signaling cascade [18,20,21]. Only few studies investigated and clarified the role of these steroids on the upstream key mechanisms such as inflammasomes [13] and found them to regulate inflammasome activity *via* the estrogen-responsive B box protein (EBBP) in a cell culture model [22], in the myocardial mitochondria of rats [23], and in human renal proximal tubule epithelial cell *in vitro* [24]. Our recent studies using brain hypoxia and chronic neurodegeneration animal models now show that the inflammasomes are central targets of steroid-dependent regulation of neuroinflammation and neuroprotection [25,26]. The following chapters will deal with this topic and attempt to give a short but comprehensive overview about inflammasomes, their composition and regulation as well as cellular assignment under neuropathological challenges.

## 2. Structure of inflammasomes and sources in the brain

The innate immunity is the first line of the host defense whether sterile or non-sterile (pathogenic) inflammation occurs. PAMPs or DAMPs are sensed by different receptors on the membrane surface, like Toll-like receptors (TLR), or at the intracellular level by different NOD-like receptors (NLRs). Nevertheless, both types belonging to pattern recognition receptors (PRRs) discerning the presence of PAMPs (infectious stimuli) or DAMPs (non-infectious stimuli) to trigger an inflammatory cascade. In the brain, they are primarily expressed by glial cells such as microglia/macrophages and oligodendrocytes. Intracellular NACHT, LRR and PYD domains-containing proteins (NLRPs) are the most studied and best characterized protein complexes during inflammation, and in particular NLRP3 [27,28]. NLRP inflammasomes such as NLRP1 and NLRP3 belong to cytosolic macromolecular complexes comprising the NLRP receptor, the adaptor apoptosis-associated speck-like protein containing a CARD (ASC) containing a caspase activation and recruitment domain (CARD), precursor caspase-1, precursor caspase-11 and/or X-linked inhibitor of apoptosis protein (XIAP) [29]. Most NLRPs consist of a pyrin domain (PYD), a nucleotide-binding domain (NBD) needed for oligomerization and a carboxy-terminal leucine-rich repeat (LRR) representing the putative sensory component [30]. In contrast to NLRP3, further domains occur in NLRP1, a unique function-to-find domain (FIIND) and CARD, so that caspases can be activated without ASC recruitment. Interestingly, the PYD domain in human NLRP1 is replaced in murine NLRP1 by an NR100 domain (amino-terminal domain of rodent NLRP1 of about 100 amino acids) resulting in three homologs, NLRP1a, NLRP1b and NLRP1c [30]. Further, it was shown that in comparison to human NLRP1 which rapidly forms an active complex after stimulation [31], murine components of the NLRP1 inflammasome are preassembled before activation in the CNS [32]. NLR family CARD domain-containing protein 4 (NLRC4) has a similar structure as NLRP3, except a CARD instead of a PYD domain, so that NLRC4 can activate caspases without ASC recruitment. Absent in melanoma 2 (AIM2) belongs to the pyrin and HIN domain-containing (PYHIN) inflammasomes and consists of two major domains, a C-terminal DNA-binding HIN and an N-terminal PYD domain which interacts with ASC after sensing and binding double stranded DNA [33]. Fig. 1 summarizes the present knowledge about the composition of the different inflammasome complexes.

Tissue expression profiles suggest that the CNS is fully equipped with the components of the NLRP1 and NLRP3 inflammasomes localized in neurons and microglia, respectively [34–36]. Mouse microglia express parts of the NLRP3 and NLRC4 inflammasomes [37,38]. AIM2 appears to be expressed in neurons [39]. In a series of immunofluorescence staining, we were able to localize different types of inflammasomes to neurons, astroglia and microglia in response to hypoxic conditions in the CNS. Fig. 2 shows representative microphotographs. NLRP3 appears to be expressed by neurons and astroglia, whereas ASC is predominantly found in microglia. This supports our previous connotation that inflammatory processes in the brain can be pictured as a complex cellular network involving different neural cell types. Recently, it became evident that a novel NLRP2 is expressed in human astrocytes and might have an important role in CNS inflammatory responses similar to the other known NLRPs, since it interacts with the P2 × 7 receptor and the pannexin-1 channel [40]. Irrespectively of being involved in the inflammatory cascade, NLRP2 was recently discovered in mouse oocytes and granulosa cells during folliculogenesis and described as regulator of early embryogenesis [41]. Knockdown of NLRP2 led to an early embryonic arrest and suggest NLRP2 as a mammalian maternal effect gene required for normal embryonic development. Altogether, these data show that the inflammasomes are complex multiprotein structures consisting of a variety of subtypes and components in diversified constellations and activated under different neuropathological scenarios. Inflammasomes appear to be the centerpiece during inflammatory and pathological responses in the brain and elsewhere by sensing early

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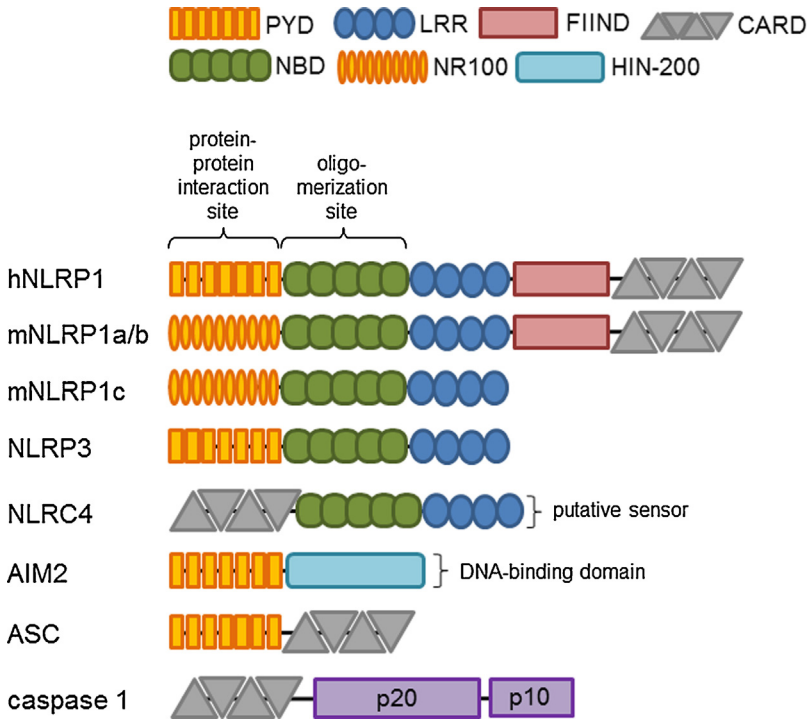


**Fig. 1.** Schematic structure of inflammasome components. Basic structural composition of the different inflammasomes reveals protein–protein interaction- and oligomerization domains. Except AIM2, the oligomerization domain NBD and the LRR domain are additional parts of the NLR inflammasomes. NLRP3 and human NLRP1 possess a PYD domain for protein interactions. In contrast, human NLRP1 differs in the interaction site from its rodent homologue. In human, a PYD domain is responsible for binding, whereas in rodents, a NR100 domain is found. NLRP1c, NLRP3, and NRLC4 lack the FIIND and CARD binding domain. Therefore, human NLRP1 is able to interact with ASC either through the PYD or CARD domain. ASC contains both, PYD and CARD, domains (the graph is adapted from 30 [30]. For abbreviations see text.

detrimental processes through TLRs and damaging intracellular signaling cascades and by controlling immune modulating cytokine release. Thus, they communicate between tattered or moribund cells and the innate immune system and are part of the primary defense line and final execution machinery.

## 3. General aspects of inflammasome regulation

Numerous activators of the inflammasomes and several different activation pathways have been described leading to a pro-inflammatory form of cell death called pyroptosis [42]. For NLRP3, the best-studied inflammasome so far, it is a well-accepted model that two-steps are required for complete activation: first, a priming step to produce pro-IL-1β [43] and second, the activation of caspase-1 and the processing of pro-IL-1β into its mature form [44]. Various stimuli lead to NLRP3 activation suggesting that this inflammasome represents a general sensor of cellular damage (Fig. 2). Several DAMPs such as ATP, reactive oxygen species (ROS) or protein aggregates are implicated in NLRP3 activation [45]. On the other hand, DAMPs can lead to NLRP3 activation in glial cells without a priming step [27]. Most recent evidence highlights the importance of canonical and non-canonical activators and co-receptors for inflammasome activation and reveals the presence of a multiplicity of such co-regulators which cannot be discussed here. We refer to a recent review article by Latz et al. [43] who summarized in detail processes and mechanisms leading to inflammasome regulation. An priming step was also demonstrated for the AIM2 inflammasome, although the responsible mechanisms are still unclear [46]. AIM2 normally senses double-stranded DNA from viral, bacterial and ectopic host origin [47], but it is unclear how between foreign and host DNA can be distinguished.

Recently, it was reported that NLRC4 activation also requires co-factors [48]. In mice, NAIPs (NLR family, apoptosis inhibitory protein) can recognize NLRC4 ligands and, in turn, activate the assembly of the NLRC4 inflammasome. Furthermore, NAIP5/NAIP6 detect flagellin and NAIP2 the type III secretion system compound PrgJ, both ligands of NLRC4 [49]. In contrast in human, only one orthologous NAIP was found sensing a type III secretion protein. It still remains unclear whether and how human NLRC4 detects flagellin without another NAIP. The involvement of NLRC4 in acute brain injury was not yet studied.

In a phase II clinical human study of an IL-1 receptor antagonist, NLRP1 was responsible for caspase-1 activation and production of mature IL-1β and IL-18 after cerebral ischemia [50]. For the activation of the human NLRP1 inflammasome, auto-proteolysis at the FIIND domain is required which can be enhanced through a single-nucleotide polymorphism (SNP) in the FIIND region or inhibited in an alternative splice variant of NLRP1 [51]. Elevated anti-apoptotic proteins B cell lymphoma-2 (BCL-2) levels also prevented NLRP1 and NLRP3 activation in cortical neurons and brain tissue following ischemia by interfering with ATP binding at the NACHT domain [52]. Further, interactions between the NLRP1 inflammasome complex, pannexin-1 and the purinergic P2 × 7 receptor was shown [36]. A third protein taking part in the regulation of NLRP1, the inhibitor of apoptosis protein XIAP may balance inflammasome expression [35]. Further regulators of inflammasomes gene expression are short endogenous noncoding RNAs termed miRNAs. This 20–23 long nucleotides regulate gene expression by complementary base pairing in the 3′ untranslated regions (3′ UTR) leading to either mRNA degradation or translation inhibition [53]. In the context of immunity, it was shown that different miRNAs play a crucial role in immune component expression [54]. In particular, miR-223 appears to play a decisive role in the regulation of NLRP3 regulation and expression [55]. A brief schematic summary of miR-223 involvement in inflammasome regulation is given in Fig. 2. Further, miR-143 was able to induce the expression of AIM2 and ASC in the Jurkat cell line which represents an immortalized line of human T lymphocyte cells [56].

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Another example is miR-155 which is expressed in several types of immune cells, including B- and T-cells, macrophages, and dendritic cells, indicating its important role in the activation of these cells [54]. This miRNA is a multifunctional regulator of native but also in adaptive immunity. Overexpression of miR-155 in rheumatoid arthritis (RA) led to the down-regulation of SHIP-1, an inhibitor of inflammation, and the overproduction of pro-inflammatory cytokines. Chronic alcohol abuse induces miR-155 in the cerebellum in a TLR4-dependent manner and regulates TNFα and MCP1 expression but not caspase-dependent IL-1β increase in neuroinflammation [54,57]. The group of Unlu and coworkers demonstrated that a specific miRNA expression signature involving miR-34c and miR-214 in mononuclear blood cells is associated with the inflammatory response to damaged/injured cells and carries implications for many acute and chronic inflammatory disorders [58]. We are just at the beginning to unravel the meaning and diversity of miRNA regulation (initiation, perpetuation, dampening, and cessation) of local inflammatory cascades but this small regulatory molecules will certainly help in future to better understand the fine tuning of inflammatory processes.

## 4. Inflammasomes in neurodegeneration and acute CNS injury

CNS injury either acute or under chronic conditions elicits immune responses resulting from activated resident immune competent cells, *i.e.*, microglia, astroglia and endothelial cells, as well as peripheral immune cell infiltrates after BBB is comprised. Inflammatory processes are widely present and described in different neurodegenerative events in the CNS. As pointed out in the previous chapter, the activation of inflammasomes by pattern recognition receptors and the sequential signaling *via* TLRs and NLRs constitute the platform for interleukin precursor processing followed by IL-1β and IL-18 secretion. In the center of the intracellular inflammatory network are inflammasomes which activate caspase-1 and coordinate different immune pathways within a cell. Accumulating evidence suggest that inflammasome-mediated inflammatory pathways are active in neurodegenerative diseases [59] such as depression [60], Alzheimer's disease [61], Parkinson's disease [62], Huntington's disease [63] or multiple sclerosis [64]. Besides these classical neurodegenerative disorders, activation and involvement of inflammasomes also play an instrumental role in psychiatric disorders including major depression and schizophrenia [1] as well as diminished cognition [65]. Although the knowledge about the contribution of neuro-inflammatory processes to the onset and/or course and perpetuation of the above chronic brain diseases is still at the beginning, it appears conceivable that brain-intrinsic immune responses and microglia as the main sentinel for CNS immunity play a calamitous role [3,30,66,67]. Besides disorders leading to chronic neuropathology, inflammasomes as must be expected take also part in inflammatory cascades following acute brain injury which can be caused by external impact leading to TBI. TBI induced the assembly of NLRP3, increased ASC and capase-1, and thereby stimulated the processing of IL-1β and IL-18 in the rat cerebral cortex [68]. Using embryonic cortical neurons, it was demonstrated that treatment of neurons with the cerebrospinal fluid (CSF) from TBI patients leads to an activation of the inflammasome AIM2 and ASC oligomerization [69]. This effect could be inhibited by the co-administration of inhibitors of the pannexin-1 channel suggesting the importance of pyroptosis during TBI. A second important component of the early innate immune response, NLRP1, is activated 24 h after TBI in rat neocortical neurons and followed by caspases-1 activation [70]. To test the influence of hypothermia on the outcome, the authors used hypothermia after TBI and describe reduced NLRP1 formation in posttraumatic hypothermia *vs*. normothermic conditions.

An intracerebral "impact" can cause acute changes in cerebral conditions characterized by a sudden decrease of blood flow to the brain tissue or a hemorrhage resulting in stroke. In elegantly

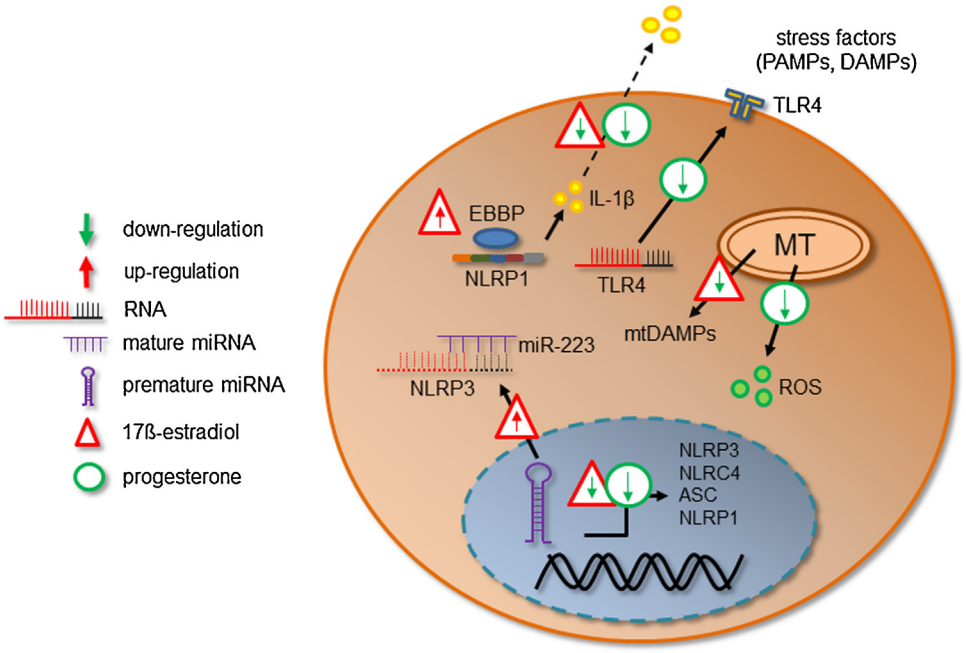


**Fig. 2.** Schematic illustration summarizing the influence of sex steroids on inflammasome activation and interference with miRNAs.Cellular stressors lead to inflammasome-mediated cytokine secretion involving the mitochondrial (mt) compartment. This causes the release of mtDAMPs and ROS which, in turn, activate the inflammasome complexes by the two-step model directly or indirectly via gene expression (see chapter 3). Sex steroids attenuate ROS formation, improve mitochondrial function and reduce DAMP action. miRNAs (i.e., miR-223) affect the translational machinery of inflammasome components. Both steroid hormones dampen gene expression of distinct inflammasome components and upstream PAMPs and DAMPs sensor TLR4 in the first step. Further, miRNA expression profiles can be changed by gonadal steroids and in particular miR-223 a strong suppressor of NLRP3 mRNA is increased. Another regulatory staring point represents EBBP which directly interacts with NLRP1. TLR4: Toll-like receptor 4, mt: mitochondria, mtDAMPs: mitochondria released DAMPs, EBBP: estrogen-responsive B box protein.

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**Table 1**
Effect of gonadal steroid hormones on the regulation of inflammasomes and related miRNAs in the CNS.

| Inflammasome component | E2 | P | Reference |
|---|---|---|---|
| NLRP1 | mRNA ↓ + protein ↑ | Protein ↑ | Unpublished data |
| NLRP3 | mRNA ↓ + protein ↑ | mRNA ↓ + protein ↑ | Unpublished data |
| NLRC4 | mRNA ↓ | mRNA | Unpublished data |
| ASC | mRNA ↓ + protein ↓ | mRNA ↓ | Unpublished data |
| miRNAs | miR-199a-3p/214 ↓ | miR-199a-3p/214 ↑ | [87] |
| | miR-223 ↑ | | [44,85] |
| Activators | ROS ↓ | ROS + NFκB ↓ | [89,90,117] |

designed studies, Fann et al. [52,71] have investigated the influence of the NLRP1 and NLRP3 inflammasomes in ischemic stroke in mice and in cultured primary cortical neurons using oxygen-glucose deprivation as hypoxic *in vitro* model. Ischemia-like conditions increased both inflammasome subtypes in the ipsilateral cortex *in vivo* and *in vitro*. Importantly, also in postmortem brain tissue samples of human patients both inflammasomes were elevated. The simultaneous intravenous (i.v.) infusion of mice with immunoglobulin (IVIg), an FDA-approved therapeutic modality for inflammatory diseases, clearly suppressed inflammasome activation and protected neurons. Similar to these observations and using a thromboembolic stroke mouse model, protein association between NLRP1, ASC, caspases-1 and XIAP was observed after ischemia and localized inflammasome proteins in neurons, astrocytes, and microglia or macrophages [72]. Additionally, NLRP3 seems to be activated in astroglia after cerebral ischemia by DAMPs ([27,72]. Keeping in mind that inflammasome participation was first associated with auto-inflammatory syndromes and defense strategies against pathogens [73], the present knowledge about the involvement of inflammasomes in neurological and psychiatric disorders, either acute or chronic, has substantially broadened in the past years, thus opening a new window for therapeutic strategies in the treatment of brain disorders.

## 5. Gonadal steroids and inflammasome regulation

It is widely accepted that E2 and P, both individually or in concert, hamper the initiation and perpetuation of innate and adaptive inflammatory responses due to their regulatory role for innate immune cells (T and B cells, microglia, macrophages) and immune-associated cells in the brain (astroglia) which carry the respective nuclear and non-nuclear steroid receptors at a variable extent and in relation to their activation status [74–79]. An intriguing and yet sketchy point however remains the question whether these steroid hormones execute their anti-inflammatory function upstream of inflammasome component gene expression also involving the potential influence of the corresponding regulatory miRNAs and/or concerns the direct activation effect on inflammasome complexes including cytokine production and signaling. We do not want to further look into the cytokine signaling aspect in this article, since there exist up to date and detailed reports regarding this topic and regulation by sex steroids mainly dealing with their role in endometrial and breast cancer, autoimmunity regulation, and dysfunctions of the musculoskeletal system [74,80,81]. Although first evidence on inflammasomes and their role in processing inflammation dates back until 2002, relative sparse information is available concerning the influence of gonadal steroid hormones on inflammasome regulation in general. This also holds true for other steroid hormones such as androgens, vitamin D, and glucocorticoids where only few data on their role in inflammasome modulation have been reported, although it is known that in particular the latter steroids are anti-inflammatory. Here, corticosteroids appear to sensitize and enhance NLRP3 activation in immune cells [82], testosterone and vitamin D increased AIM2 and NLRP3/caspases-1 expression, respectively [83,84]. Before we started our most recent studies on the influence of E2 and P on inflammasome expression and regulation in the brain, only information was available with respect to peripheral organ systems and their cellular equivalents. Using a cell culture approach, *i.e.*, COS-1 kidney fibroblasts from African green monkey, it was shown that the estrogen-responsive B box protein (EBBP) which is regulated by E2 forms a common platform with the inflammasome NLRP1 thus positively reinforcing IL-1β secretion [22]. Again in the kidney, albumin-bound free fatty acids trigger inflammasome expression and activation (NLRP3 and ASC) in human proximal tubule epithelial cells *in vitro*. This effect is inhibited by the selective estrogen receptor modulator (SERM)

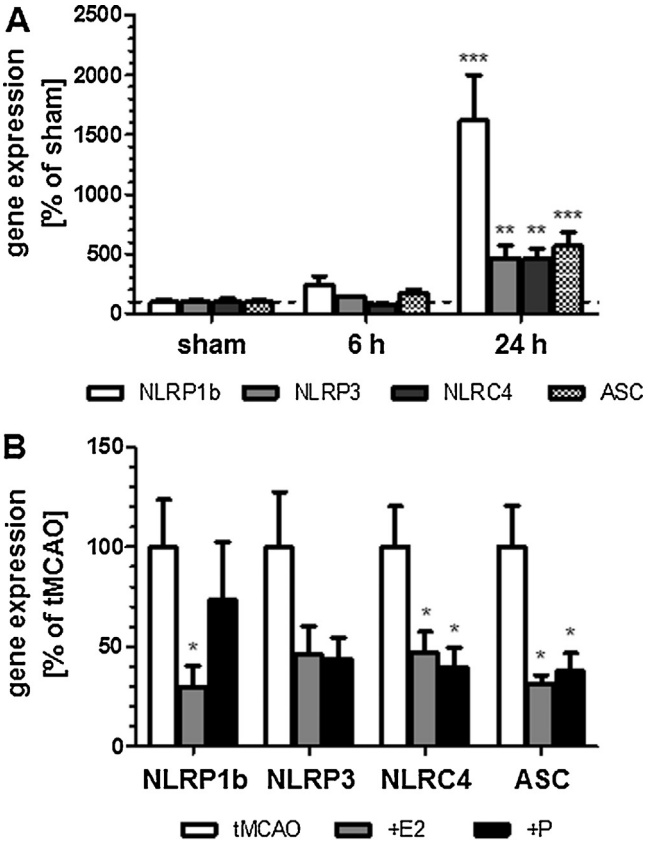


**Fig. 3.** Time course of inflammasome activation after hypoxia in the brain and regulation by sex steroids.Time course of inflammasome gene expression in the penumbra of the male rat cerebral cortex before, 6 and 24 h after the onset of tMCAO which lasted for 2 h (A), $^{**}p < 0.01$ and $^{***}p < 0.001$ *vs.* sham. Effect of E2 (25 μg per kg body weight) or P (10 mg P per kg body weight) i.v. administration 24 h after the beginning of ischemia on inflammasome expression (B). Note that E2 and P reduced the gene expression of a set of different inflammasome components. $^{*}p < 0.05$ *vs.* tMCAO.

raloxifene which has clear renoprotective effects [24]. And in the heart, where E2 has a clear cardio-protective function, this effect involves the reduction of mitochondrial-derived DAMPs, thus indirectly reducing the post burn activation of inflammasomes at the posttranscriptional level An indirect upstream regulatory role for E2 in NLRP3 regulation is also most likely, since E2 up-regulated miR-223 levels in lymphocytes [85] and this miRNA subtype is one of the strongest transcriptional repressor of NLRP3 expression (see also chapter 3) [86]. Only sporadic information about interactions of P with the inflammasomes system can be found. P enhanced uterine miR – 199a-3p and miR-214 which target inflammation-related enzymes such as cyclooxygenase-2 and others [87]. Further, treatment of peripheral blood mononuclear cells of pre-eclampsia patients with P in a dose-dependent manner inhibited TLR4 expression at the mRNA level [88]. However, P improves recovery after TBI and in neurodegenerative disorders by positively affecting ROS formation and NFkappaB signaling [89–91]. Both pathological stressors of cell physiology and function are, in turn, identified as leading stimulators and activators of the inflammasome multiprotein complexes [92,93].

Under different neuropathological conditions, an array of inflammasome components are activated [30,94] and it is evident that post-ischemic DAMPs and their inflammasome counterparts are essential of neural protection [95]. Since E2 and P are both well-known anti-inflammatory and neuroprotective effectors, it can be assumed that they tackle the inflammasome complex to manifest at least parts of their neuroprotective action. Table 1 gives a brief overview about the most recent findings on the regulation of inflammasome components in the brain by E2 and P. In brain hypoxia, NLRP1 and NLRP3 inflammasome formation occurs in neurons and local glia [26,72]. The neutralization of NLRP1 activity [71], the application of plant extracts such as chrysophanol [96], and treatment with cell-protective omega-3 polyunsaturated fatty acids (PUFA) [26] which both antagonize NLRP3 activation strongly reduce post-ischemic inflammation and damage. We have investigated in more detail the time course of activation of different inflammasome complexes in the penumbra after transient focal middle cerebral occlusion (tMCAO) in male rats and their cellular allocation (manuscript submitted). Interestingly, we observed that the different inflammasome complexes reveal a specific post stroke time course of expression and activation (Fig. 3A) which appears to be specifically inhibited by the administration of E2 or P (Fig. 3B). Further, we provide evidence by immunofluorescence double labeling that neurons, astroglia and microglia show a cell-specific expression pattern with neurons and astroglia expressing NLRP3, whereas ASC is expressed by astroglia and microglia (Fig. 4). *In vivo*, the administration of E2, P or a combinatory treatment either i.v. which was most effective or using subcutaneous oil depots reduced the infarct volume and improved behavioral recovery after 24 h and one week post

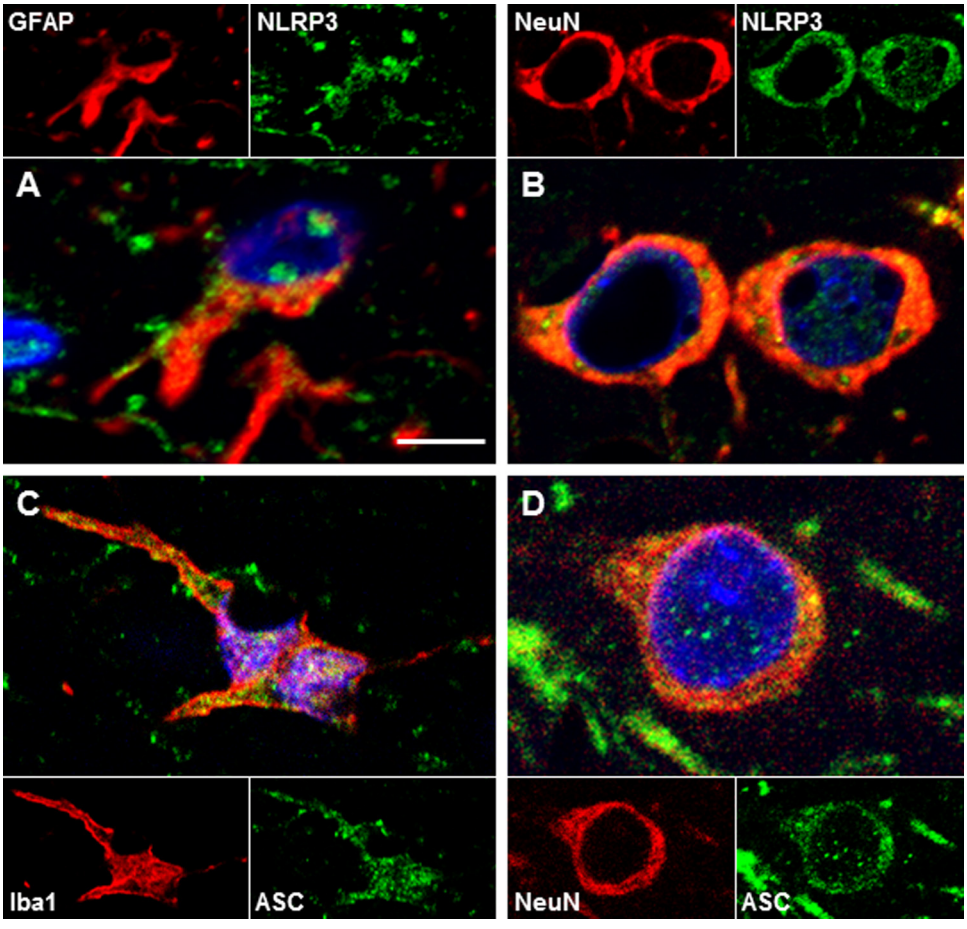


**Fig. 4.** Co-localization of inflammasome components with neural cells. Immunofluorescence triple staining of Hoechst 33342, the neuronal marker NeuN, the pan-microglial marker Iba1, and the astroglial marker GFAP with ASC and NLRP3 after tMCAO in the cerebral cortical penumbra of rats. Scale bar indicates 5 μm. Slices are counterstained with nuclear Hoechst 33342 (blue). Above and below the merged pictures (A–D), single channel images are shown. Note that NLRP3 is mainly found in neurons and astroglia (A, B) and that ASC is expressed in microglia and neurons (C, D). (A) Merged co-staining for NLRP3 and GFAP, (B) merged co-staining for NLRP3 and NeuN, (C) merged co-staining for ASC and Iba1, (D) merged co-staining for ASC and NeuN. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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ischemia [14,25,97] which is paralleled by a prevention of distinct inflammasome induction and suppression of cytokine production (Figure 4B). In particular, the sex steroids significantly inhibited the expression of NLRP1b, NLRC4 and ASC. In contrast, NLRP3 which was already active in the control brain was reduced at the protein levels. All described effects selectively occurred in the penumbra of the cerebral cortex. Concordantly, we measured a clear reduction of cortical pro-inflammatory cytokines. Using an *in vitro* approach, *i.e.*, astroglia-, microglia- and neuronal cell cultures from the mouse brain under controlled hypoxic condition, we currently analyze in greater detail the cell type specific expression and regulation pattern of the different inflammasomes as well as the receptor specificity and signaling pathways of steroid-dependent regulation. The control of upstream regulatory non-coding RNA networks such as miRNAs provides another means of varying the expression levels of inflammatory inflammasome responses. For many disorders in the brain, miRNAs have attracted significant attention and are discussed as predicative and diagnostic markers, as well as therapeutic targets for the treatment of chronic neurological and mental–psychiatric illness such as Multiple sclerosis, Parkinson's, Alzheimer's disease, as well as schizophrenia and bipolar disorders [98,99]. In this relatively young research division, only moderate progress and information were achieved. Nevertheless, there is increasing evidence that both steroids are implicated in the regulation of circulating and local miRNAs with respect to breast cancer [100,101], hepatocellular carcinoma [102], placental and ovarian dysfunction [87,103,104] and hormone replacement therapy [105]. *Vice versa*, miRNAs contribute to the regulation of steroid signaling by affecting post-transcriptional regulation of progesterone and estrogen receptors [106,107]. It becomes more and more clear that P-mediated anti-inflammatory responses are inevitably linked to miRNA regulation [108]. Thus, suppressive action of P on TLR-triggered immune responses of macrophages is mediated by miR-155 [109]. Similarly, the anti-inflammatory potency of E2 is connected with the modulation of an array of miRNAs [110,111]. In stroke and under hypoxic conditions in the brain and in the heart, where hypoxia selectively coordinates biogenesis and activity of distinct miRNAs termed "hypoxamirs" [112], recent data suggest that the gonadal steroid environment critically controls miRNA action such as lethal gene 7f (Let7f) [113], miR-151-5p [114] and others as reviewed by Ritzel et al. [115]. It seems logical that miRNAs, inflammasomes and steroid hormone action are integrated into the complex hierarchical regulatory network of neuroprotection by E2 and P and, as suggested by others, might also contribute to known sex differences after cerebral ischemia [116].

## 6. Conclusion

Recent data show that sex steroids are capable of directly or indirectly regulating neuroinflammatory cascades and the intracellular "master switch" components termed inflammasomes and related miRNA networks in the brain. Inflammasome-mediated inflammatory pathways are triggered by irritable and harmful stimuli and implicated in different chronic and pathogen-related CNS disorders also including acute traumatic events. Different inflammasome components and subtypes as well as miRNAs appear to be expressed in a cell type- and disease-specific way in the CNS. The inflammasome activation pathway turns out to be complex and requires the cooperation of brain-intrinsic and peripheral immune cells as well as locally-active diffusible immune-modulatory factors. Since microglia and astroglia are well-recognized targets for sex steroids which express subsets of nuclear and non-nuclear steroid receptors, it seems plausible that the anti-inflammatory potential of sex steroids is the result of the inflammasome regulation, or at least components of the inflammasomes, in these cell types. Further research into these mechanisms is advisable for a better understanding of sex steroid–inflammasome interactions and their role in CNS disease progression.

## Disclosure statement

The authors have nothing to disclose.

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