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Research Paper

Tumor necrosis factor-inducible gene 6 protein: A novel neuroprotective factor against inflammation-induced developmental brain injury

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ABSTRACT

Inflammation is an important factor contributing to developmental brain injury in preterm infants. Although tumor necrosis factor-inducible gene 6 protein (TSG-6) has immunomodulatory effects in several inflammatory conditions of adult animals, nothing is currently known about the role of TSG-6 in the developing brain, its impact on perinatal inflammation and its therapeutic potential. The aim of the current work was 1) to characterize the developmental expression of TSG-6 in the newborn rat brain, 2) to evaluate the impact of LPS exposure on TSG-6 expression and 3) to assess the therapeutic potential of exogenous TSG-6 administration. Brain hemispheres of healthy Wistar rats (postnatal day 1–postnatal day 15 (P1–P15)) were evaluated with regard to the physiological expression of TSG-6. LPS-treated rats (0.25 mg/kg LPS i.p. on P3) were analyzed for inflammation-induced changes in TSG-6 and cytokine expression. To evaluate whether exogenous recombinant human (rh)TSG-6 affects inflammation-induced brain injury, newborn Wistar rats, exposed to LPS on P3, were treated with rhTSG-6 i.p. (four repetitive doses of 2.25 mg/kg every 12 h, first dose 3 h before LPS injection). PCR, Western blotting and multiplex ELISA were performed according to standard protocols. TSG-6 is physiologically expressed in the developing brain with a linear increase in expression from P1 to P15 at the mRNA level. At P6, regional differences in TSG-6 expression in the cortex, thalamus and striatum were detected at mRNA and protein level. Furthermore, TSG-6 gene expression was significantly increased by inflammation (induced by LPS treatment). Combined treatment with LPS and TSG-6 vs. LPS exposure alone, resulted in significant downregulation of cleaved caspase-3, a marker of apoptosis and neuronal plasticity. In addition, several inflammatory serum markers were decreased after TSG-6 treatment. Finally, TSG-6 is physiologically expressed in the developing brain. Changes of TSG-6 expression associated with inflammation suggest a role of TSG-6 in neuroinflammation. Reduction of cleaved caspase-3 by TSG-6 treatment demonstrates the putative neuroprotective potential of exogenous TSG-6 administration in inflammation-induced developmental brain injury.

1. Background

The report for disability-adjusted life years, based on data from the Global Burden of Disease Study 2010, ranks preterm birth complications at #8 worldwide, which is an improvement compared to #3 in 1990. Statistics for years of life lost show similar results with a change from #3 in 1990 to #7 in 2010 (Murray et al., 2012). Thus, modern therapeutic strategies and social programs, most prominently the millennium development goals, have achieved significant improvements. Nevertheless, the impact of preterm birth complications on global health is still very high and requires further improvements. Even though the rate of preterm births is increasing among neonatal mortalities, overall mortality has declined over the past three decades. But with increasing survival rates of immature neonates, the absolute number of babies suffering morbidity is rising (Wen et al., 2004; Saigal and Doyle, 2008). The organs most affected by prematurity are the brain (Rees and Inder, 2005) and the lung (Jobe and Bancalari, 2001). Most important for long-term prognosis of brain involvement is the Encephalopathy of Prematurity (Volpe, 2009), which comprises two severe neurological disorders: periventricular leukomalacia and neuronal/axonal disease. Long-term sequels are motoric deficits up to cerebral palsy, sensory impairment of the auditory and visual system and mental disability (Larroque et al., 2008; Saigal and Doyle, 2008). The etiology of Encephalopathy of Prematurity is multi-factorial with known reasons being: drugs, hypoxia & ischemia, hyperoxia and infection/inflammation. In this study, we focused on infection/inflammation.

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Current treatment aims at prevention, because a therapeutic approach to specifically tackle the pathophysiology of inflammation-induced developmental brain injury is not yet available (Favrais et al., 2014). Interestingly mesenchymal stem cells (MSCs) have been proposed to be beneficial in many inflammatory diseases, including developmental brain injury (Castillo-Melendez et al., 2013). MSCs secrete the anti-inflammatory protein TSG-6, which has been suggested to be responsible for a significant part of the beneficial effects of MSCs. For example, this was shown for models of myocardial infarction (Lee et al., 2009), acute lung injury (Danchuk et al., 2011) and skin wound healing (Qi et al., 2014).

Multiple functions of TSG-6 have been implied, including modulation of the extracellular matrix (Wisniewski et al., 1996; Baranova et al., 2011; Sanggaard et al., 2010), as well as direct cell interactions (Choi et al., 2011). Nevertheless, current knowledge about the physiological function of TSG-6 is still limited. It has been demonstrated to be essential during ovulation (Fülöp et al., 1997; Fülöp et al., 2003; Richards, 2005) and to be important in maintaining the physiological architecture of skin (Tan et al., 2011). Furthermore TSG-6 has strong anti-inflammatory properties, which have been intensively investigated in models of rheumatoid arthritis (Bárdos et al., 2001; Mindrescu et al., 2002). To date the role of TSG-6 in the developing brain remains unclear.

The aim of the current study was to evaluate 1) the developmental expression of TSG-6 in the neonatal rat brain, 2) the role of TSG-6 in perinatal inflammation with special focus on neuroinflammation, and 3) the effect of exogenous administration of TSG-6 on inflammation-induced brain injury using an established rat model (Prager et al., 2013; Brehmer et al., 2012).

2. Methods

All animal experiments were performed in accordance with international guidelines for good laboratory practice and institutional guidelines of the University Hospital Essen and were approved by the animal welfare committees of North Rhine Westphalia.

2.1. Animal treatment

Wistar rats, provided by the central animal facility of the University Hospital Essen, were used for all animal experiments. Developmental analysis was performed on healthy rats, decapitated on P1, P3, P5, P7, P9, P11, P13 or P15. Analysis of the impact of inflammation on neonatal brain development was performed on rats, treated on P3 with 0.25 mg/kg of LPS (Sigma-Aldrich) i.p. and decapitated on P3 + 0–24 h, P6 and P11. To investigate the pharmacological effects of recombinant human TSG-6 (rhTSG-6), rats were treated with four repetitive doses of 2.25 mg/kg rhTSG-6 every 12 h beginning on P3 (first dose 3 h prior to LPS injection) and decapitated on P3 + 6 h and P5.

2.2. Tissue collection

Animals received an overdose of chloral hydrate (200 mg of chloral hydrate per kg body weight in 0.1 ml injection volume per 10 g body weight) and were transcardially perfused with sterile PBS (except animals treated with LPS and decapitated on P3 + 0–24 h). Blood was collected from the right atrium before perfusion. Serum was prepared after clotting in uncoated tubes by centrifuging at 3000*g* for 5 min. Brain tissue was obtained by careful opening of the cranial cavity with subsequent brain extraction. Hemispheres or micro-dissected cortex, thalamus and striatum were isolated, snap-frozen in liquid nitrogen and stored at −80°C.

2.3. Western blotting

Protein from snap-frozen tissue was isolated by dissolving in RIPA-buffer (Sigma Life Science), containing PMFS (Sigma Life Science) and cOmplete Mini, Protease inhibitor cocktail (Roche). Cell compartments were centrifuged at 3000*g* for 10 min to separate the nuclei fraction and supernatant was further centrifuged at 17,000*g* for 20 min to separate the mitochondrial fraction. Cytosolic fraction was diluted in Laemmli buffer and denaturated at 95°C for 10 min. Proteins were separated by electrophoresis using 10–15% polyacrylamide gradient gels and transferred onto nitrocellulose membranes. Primary antibodies are listed below: TSG-6: goat-anti-hTSG-6 (1:80, R&D Systems, RRID: AB_2240677), cleaved caspase-3: rabbit-anti-caspase-3 (1:1000, Cell Signaling, RRID: AB_2070042), IBA1: rabbit-anti-IBA1 (1:1000, Wako Pure Chemical Industries). For quantitative analysis, HRP-linked secondary antibodies against the specific host species were used, visualized using Amersham ECL Plus (GE Healthcare) and detected by ChemiDoc XRS + System. Data were evaluated densitometrically using ImageLab software (Bio-Rad).

2.4. Multiplex ELISAs

Bioplex rat cytokine analysis was performed on serum and brain tissue. Data were acquired using Luminex 200 with IS2.3/xPONENT3.1 software and further processed with the R package nCal (Fong and Sebestyen, 2013). Standard curves were fitted by logistic regression (Ritz and Streibig, 2005) to estimate absolute concentration of analytes.

2.5. Real-time PCR

Total cellular RNA was extracted using TRIzol reagent (Invitrogen, Life Technologies). Further processing included DNAse I reaction of 250–1000 ng RNA per batch and sample (Invitrogen, Life Technologies) and reverse transcription to cDNA using SuperScript II enzymes (Invitrogen, Life Technologies). The resulting cDNA was diluted using equivalent amounts of sterile distilled water. Relative quantification of cDNA amounts was performed using real-time PCR (StepOnePlus, Applied Biosystems) and the $2^{-\Delta\Delta C}{}_T$ (Livak and Schmittgen, 2001) method. The sequence of the TSG-6 forward primer was GTA GGA AGA TAC TGC GGT GAT GAA, the sequence of the TSG-6 reverse primer was GAC GGA CGC ATC ACT CAG AA and 6-FAM – TCC AGA AGA CAT CAT CAG CAC AGG AAA TGT – BHQ1 for the probe (BioTeZ).

2.6. Statistics

Statistical computing was done using R statistical environment (Team and R Core Team, 2012) and the dedicated integrated development environment RStudio. Additional packages used were gdata (Warnes, and with contributions from Ben Bolker, Gorjanc, G., Grothendieck, G., Korosec, A., Lumley, T., MacQueen, D., Magnusson, A., Rogers, J., Others, 2012), ggplot2 (Wickham, 2009), stringr (Wickham, 2012) and nCal (Fong and Sebestyen, 2013; Ritz and Streibig, 2005). Most data were $\log_2$ transformed to improve standard distribution of data. *p*-Values were calculated using analysis of variance models fitted into Tukey's Honest Significant Difference method as well as pairwise t-test using the Holm-Bonferroni method for multiple testing (#: $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$). Graphical presentation was generated using ggplot2.

3. Results

3.1. Developmental regulation of TSG-6 in the neonatal brain

We detected TSG-6 in the healthy neonatal rat brain at both mRNA and protein level by real-time PCR (Fig. 1) and Western blotting (data

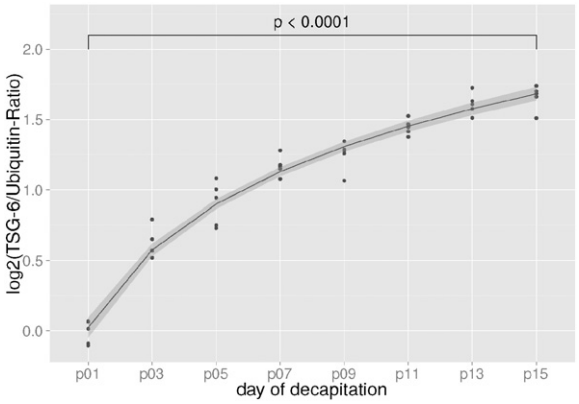

Fig. 1. Developmental expression of TSG-6 mRNA in newborn rat brains. TSG-6 mRNA is linearly up-regulated from P1 to P15 by about 3-fold. *Method*: rtPCR, *samples*: hemispheres/n = 4–5, *treatment*: decapitation 1–15 days (P1–P15) after birth, *graphics*: points ± CI, statistics: linear regression (y = log2(x)) / coefficient of determination = 0.9675.

not shown). At the mRNA level, TSG-6 expression was up-regulated more than 3-fold from P1 to P15. Calculating linear regression revealed a strong association between TSG-6 mRNA expression and day of life. At the protein level, TSG-6 expression showed high statistical variability and only weak association with the day of life. TSG-6 expression in selected brain regions on P6 revealed significant differences. The pattern of local TSG-6 expression was similar at mRNA and protein level, although differences in mRNA levels were larger (Fig. 2). In the cortex, expression was lower than in whole hemispheres with −17% at protein level and −12% at the mRNA level. Lower expression was also detected in the thalamus showing −30% at protein level and −63% at the mRNA level. Striatum showed ambivalent expression with +9% at protein level and −45% at the mRNA level. All differences between mRNA expressions in any brain region were statistically significant (Table 1), while only the differences between

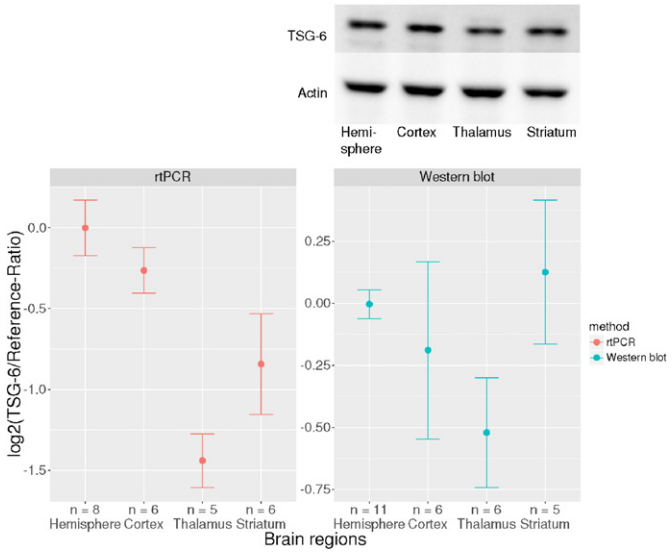

Fig. 2. Expression of TSG-6 mRNA/protein in different brain regions. TSG-6 mRNA and protein expression varies between different brain regions. It is lower in thalamus, cortex and striatum than in whole hemispheres with the exception of striatum at protein level. *Method*: rtPCR / Western blot, *samples*: brain regions/n = 5–11, *treatment*: injection of sodium chloride 0.9% on P3/decapitation on P6, *graphics*: errorplots ± CI, *statistics*: pairwise t-test/*p*-values displayed in Tables 1 and 2

Table 1
p-Values of TSG-6 mRNA expression in different brain regions.

	Cortex	Hemisphere	Striatum
Hemisphere	0.02756	–	–
Striatum	0.00025	8.1×10^{-7}	–
Thalamus	2.8×10^{-8}	3.0×10^{-10}	0.00025

TSG-6 protein expression in thalamus and in hemispheres/striatum reached statistical significance (Table 2).

3.2. Regulation of TSG-6 under inflammatory conditions in the neonatal brain

Administration of LPS to newborn Wistar pups induced an extensive, systemic inflammatory response (Fig. 3), including up-regulation of pro-inflammatory cytokines, anti-inflammatory cytokines and chemokines. The immune response peaked between 2 and 8 h after i.p. LPS administration. Interestingly, after 48 h (Fig. 4), the immune response reversed to lower levels than in sham-treated animals, with serum Interferon-γ and IL-6 being reduced the most to levels of less than 30% of the controls. In the brain, cytokine levels were below the detection limit (data not shown). As TSG-6, known for pronounced anti-inflammatory activities, is expressed in the developing brain, we investigated whether TSG-6 levels in the brain were up-regulated after i.p. LPS administration. Fig. 5 shows the TSG-6 expression 0–24 h after LPS stimulus i.p. on P3. At the mRNA level, TSG-6 expression in the brain was significantly up-regulated after LPS exposure. The maximum expression was reached 4 h after LPS administration with +52% and returned to normal levels after 12 h. At the protein level, statistical variability was higher than at the mRNA level and no significant differences in expression levels were detected (data not shown). Later timepoints (P6 + P11 for hemisphere, P6 for brain regions) did not exhibit significant differences of TSG-6 expression in hemispheres, cortex, thalamus and striatum at the mRNA level between LPS and sham-treated animals (data not shown).

3.3. Systemic and local effects of repetitive TSG-6 application on preterm rats under inflammatory conditions

Pups which received LPS on P3 and TSG-6 on P3 + P4 were evaluated on P5 for cleaved caspase-3 and microglia activation in the brain and for cytokine levels in serum. Fig. 6 shows the presence of cleaved caspase-3 in the different treatment groups. LPS exposure increased levels of cleaved caspase-3 by 3-fold, but additional TSG-6 administration decreased this up-regulation by about 30%. The same samples were evaluated for IBA1 (Fig. 7), a marker for activated microglia (Boche et al., 2013). Its expression was significantly up-regulated by about 50% after LPS and remained unaffected by additional treatment with TSG-6. Fig. 8 shows the expression of selected cytokines in serum in the different treatment groups. Interferon-γ, IL-1β and MIP-3α were highly up-regulated by LPS treatment, but down-regulated by additional TSG-6 treatment (Interferon-γ: back to initial value, IL-1β: down-regulated by 25%, MIP-3α: down-regulated by 70%).

Table 2
p-Values of TSG-6 protein expression in different brain regions.

	Cortex	Hemisphere	Striatum
Hemisphere	0.19932	–	–
Striatum	0.06828	0.27350	–
Thalamus	0.05096	0.00037	0.00025

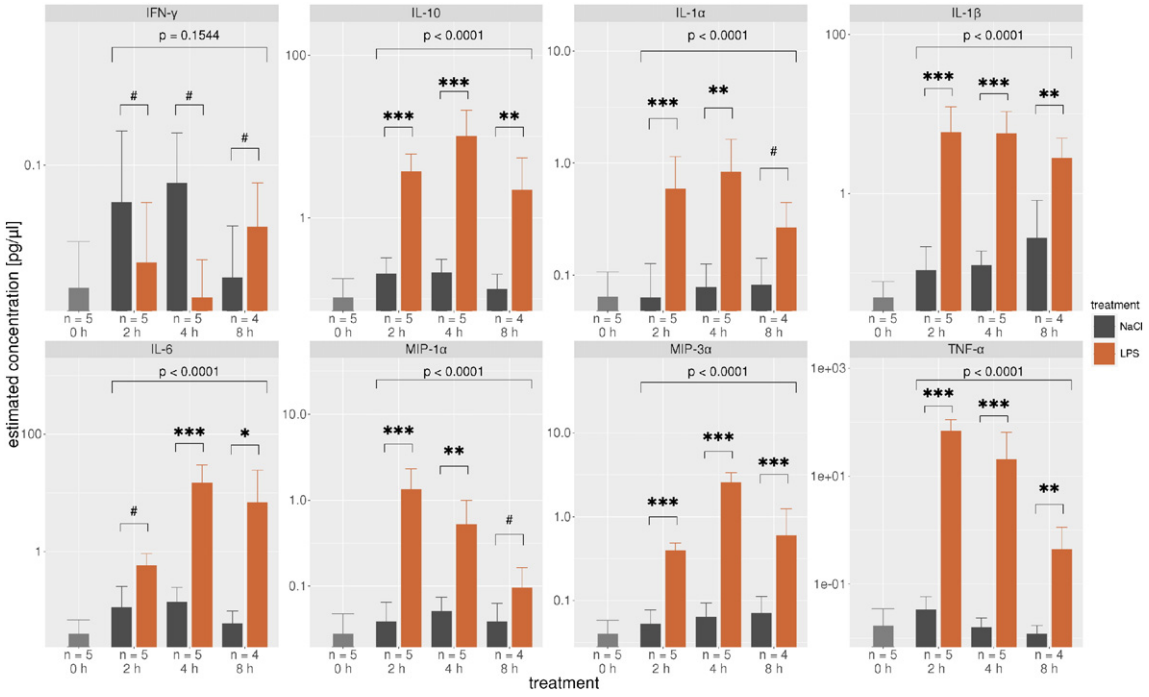

Fig. 3. Systemic inflammatory response in the first 8 h after inflammatory stimulus. Cytokine expression is elevated in serum 2–8 h after inflammatory stimulus indicating a massive systemic response. *Method*: multiplex ELISA, *samples*: serum/n = 4–5, *treatment*: NaCl = sodium chloride 0.9% [P3]/LPS = lipopolysaccharide 0.25 mg/kg [P3]/decapitation on P5, *graphics*: barplots + CI, *statistics*: Tukey's honest significance test (#: $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

4. Discussion

4.1. Role of TSG-6 in the developing brain

TSG-6 expression in the first postnatal days of brain development at both mRNA and protein levels (Fig. 1, Fig. 2) suggests that TSG-6 may be important during brain maturation. The linear up-regulation of TSG-6 mRNA between P1 and P15 in the hemispheres and the different levels of expression in hemispheres, cortex, thalamus and striatum implicate a highly regulated mechanism. In contrast, TSG-6 protein levels show profound variability during development from P1 to P15, but TSG-6 protein is certainly expressed in the neonatal brain. One possible mechanism of TSG-6 to influence brain development is its capability to modify the extracellular matrix. Baranova et al. (2011) revealed that TSG-6 oligomers cross-link and condense hyaluronan matrices in vitro under in vivo like conditions. Uniaxial stretching has been postulated to transform these matrices to fiber-like structures. Such fiber-like hyaluronan structures have been found along migratory pathways of

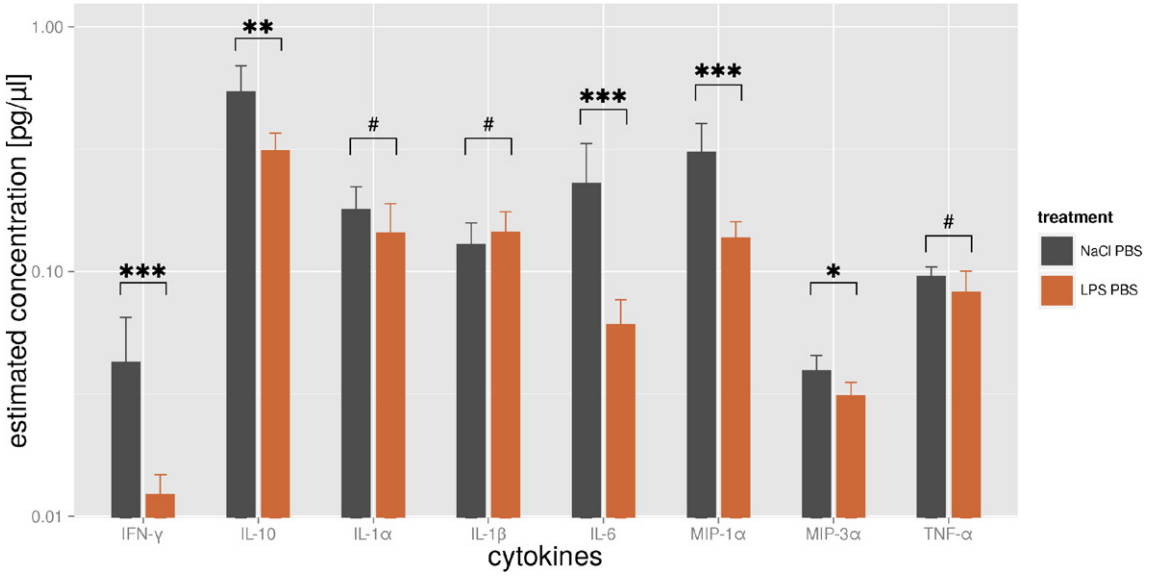

Fig. 4. Systemic inflammatory response 48 h after inflammatory stimulus. Cytokine expression is decreased in serum 48 h after inflammatory stimulus. This could contribute to the preconditioning effect of LPS on second insults. *Method*: multiplex ELISA, *samples*: serum/n = 12–15, *treatment*: NaCl = sodium chloride 0.9% [P3]/LPS = lipopolysaccharide 0.25 mg/kg [P3]/PBS = phosphate buffered saline 2×/d [P3 + P4]/decapitation on P5, *graphics*: barplots + CI, *statistics*: pairwise t-test (#: $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

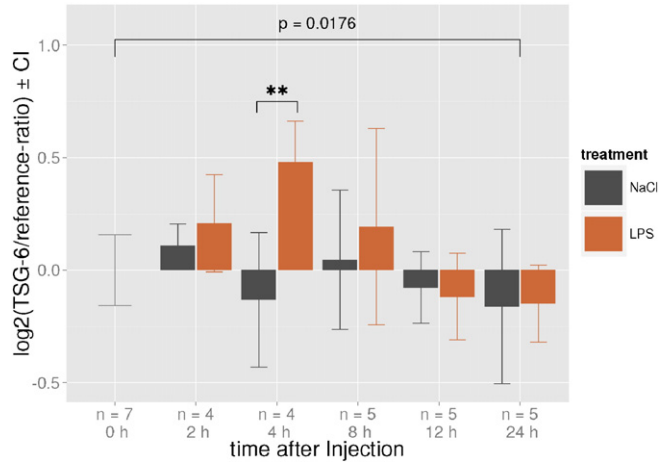

Fig. 5. Expression of TSG-6 mRNA in brain hemispheres after inflammatory stimulus. TSG-6 mRNA expression is increased in brain hemispheres 4 h after LPS injection and therefore matching the peak of the systemic inflammatory response. *Method*: rtPCR, *samples*: hemispheres/n = 4–7, *treatment*: NaCl = sodium chloride 0.9% [P3]/LPS = lipopolysaccharide 0.25 mg/kg [P3]/decapitation on P3 + 0–24 h, *graphics*: barplots ± CI, *statistics*: Tukey's honest significance test (**: $p < 0.01$).

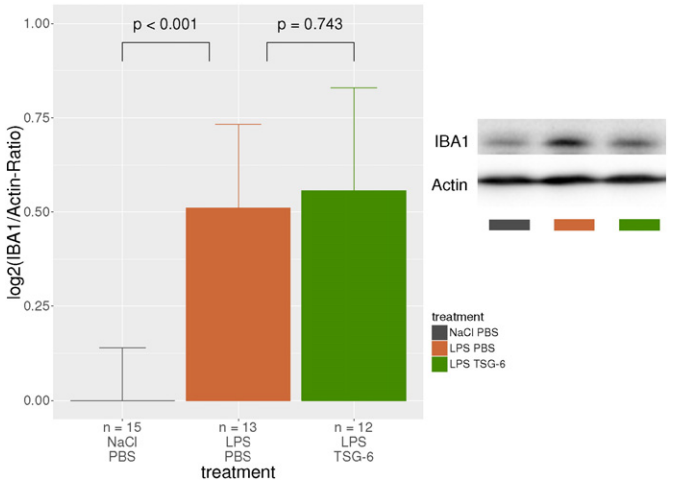

Fig. 7. Expression of IBA1 under inflammatory conditions and TSG-6 treatment. IBA1 expression in newborn rat brains, a marker for microglia activation, is increased after LPS injection. Additional TSG-6 treatment does not alter the level of expression. *Method*: Western blot, *samples*: hemispheres/n = 12–15, *treatment*: NaCl = sodium chloride 0.9% [P3]/LPS = lipopolysaccharide 0.25 mg/kg [P3]/TSG-6 = TSG-6 2.25 mg/kg 2×/d [P3 + P4]/PBS = phosphate buffered saline 2×/d [P3 + P4]/decapitation on P5, *graphics*: barplots + CI, *statistics*: pairwise t-test

neuronal precursor cells and in a pericellular pattern around oligodendrocyte precursor cells (Baier et al., 2007; Gerlach et al., 2010), suggesting a role of TSG-6 during migration of neuronal precursor cells and maturation of oligodendrocytes. Proving colocalization of TSG-6 with neuronal or oligodendrocyte precursor cells would support this hypothesis, but we did not achieve a convincing immunohistochemical staining of TSG-6.

4.2. Role of TSG-6 in developmental inflammatory brain injury

Treatment of neonatal Wistar rats with LPS induces pronounced systemic inflammation with activation of a large number of cytokines, peaking after 2–8 h (Fig. 3). Although in-vitro studies of oligodendrocyte-microglia-co-cultures have shown elevated cytokine gene expression after LPS stimulation (Brehmer et al., 2012) and in-vivo studies of brain tissue demonstrated elevated cytokine gene and protein expression after IL-1β stimulation (Adén et al., 2010), we did not detect cytokines in the brain at levels above the detection limit of our multiplex ELISA. However, we were able to show increased microglia activation by detecting elevated IBA1 levels (Fig. 7, (Boche et al., 2013)). Therefore the lack of cytokine detection in the brain is most likely due to limited sensitivity of our multiplex ELISA technique. Later on, systemic inflammation is followed by a global decrease of cytokines beginning at about 48 h after the inflammatory stimulus (Fig. 4). LPS is known to have a preconditioning effect on second insults like ischemia/hypoxia in the neonatal brain at certain doses and time intervals between the two insults (Mallard and Hagberg, 2007). A reduction in the expression of cytokines after an inflammatory stimulus could contribute to the preconditioning effect. As TSG-6 is an endogenous, anti-inflammatory protein which is expressed upon inflammation (Wisniewski et al., 1993; Bayliss et al., 2001; Al'Qteishat et al., 2006), we analyzed if TSG-6 is also up-regulated in the brain under inflammatory conditions. Fig. 5 shows that TSG-6 gene expression is significantly up-regulated after 4 h and therefore matches the peak of the systemic cytokine response. In comparison to an adult model of cerebral ischemia, the up-regulation of TSG-6 in our model is lower (Lin et al., 2013). This could either be a characteristic of our model of LPS-induced neuroinflammation or the result of a weaker response due to the immaturity of the neonatal immune system and therefore a characteristic of neonatal neuroinflammation.

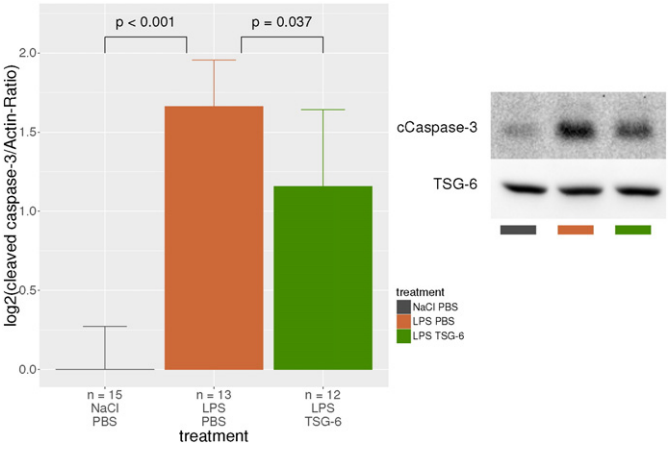

Fig. 6. Levels of cleaved caspase-3 under inflammatory conditions and TSG-6 treatment. Cleaved caspase-3 levels in newborn rat brains are increased after LPS injection. Additional TSG-6 treatment reduces this elevation by about 30%. *Method*: Western blot, *samples*: hemispheres/n = 12–15, *treatment*: NaCl = sodium chloride 0.9% [P3]/LPS = lipopolysaccharide 0.25 mg/kg [P3]/TSG-6 = TSG-6 2.25 mg/kg 2×/d [P3 + P4]/PBS = phosphate buffered saline 2×/d [P3 + P4]/decapitation on P5, *graphics*: barplots + CI, *statistics*: pairwise t-test

4.3. Therapeutic potential of TSG-6 in developmental inflammatory brain injury

Cleaved caspase-3 is a downstream effector of apoptosis, with caspase-3 being the final target of several apoptosis activating pathways. In the brain, it is not only responsible for apoptosis but also plays a crucial role in reorganizing axonal and dendritic structures, contributes to long term depression (Li et al., 2010) and inhibits long-term potentiation (Jo et al., 2011). While apoptosis is triggered by widespread cleavage and activation of caspase-3 in the whole cell, neuronal plasticity is promoted by limited activation of caspase-3 in dendritic regions (Ertürk et al., 2014). Disturbances in these processes can lead to an extensive malfunction of the CNS, especially during development (Hyman and Yuan, 2012; Lakhani et al., 2006; Kuida et al., 1996). Therefore, reduced activation of caspase-3 after administration of LPS and TSG-6, compared to sole LPS-treatment (Fig. 6) provides first evidence for the neuroprotective potential of TSG-6, raising the question of the

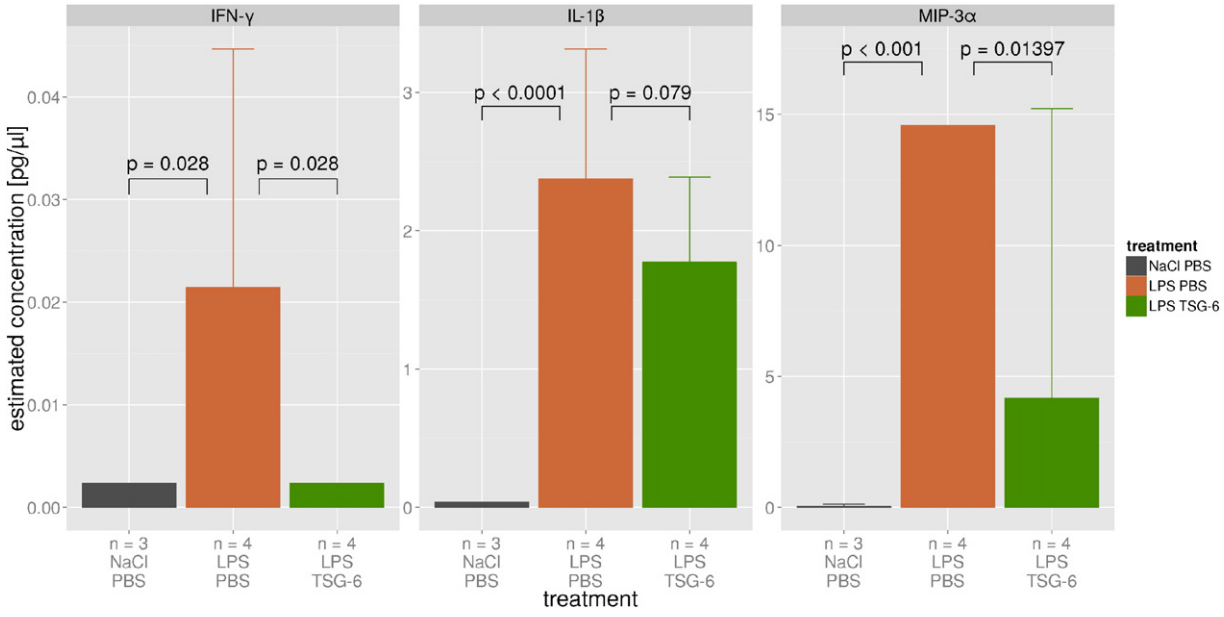

Fig. 8. Systemic inflammatory response after inflammatory stimulus and TSG-6 treatment. Cytokine expression is elevated in serum 6 h after inflammatory stimulus. Additional TSG-6 treatment reduces the elevated levels. *Method*: multiplex ELISA, *samples*: serum/n = 3–4, *treatment*: NaCl = sodium chloride 0.9% [P3]/LPS = lipopolysaccharide 0.25 mg/kg [P3]/TSG-6 = TSG-6 2.25 mg/kg 2×/d [P3 + P4]/PBS = phosphate buffered saline 2×/d [P3 + P4]/decapitation on P3 + 6 h, *graphics*: barplots + CI, *statistics*: pairwise t-test

underlying mechanism. In principle, a neuroprotective effect could be achieved at multiple time points, starting by inhibiting the initiation of the inflammatory cascade via TLR-4 (Pålsson-McDermott and O'Neill, 2004), up to modulating local inflammatory effects in the brain. In order to get a first indication where TSG-6 might alter our model of inflammatory brain damage, we investigated the systemic inflammatory response. It is substantially reduced after TSG-6 treatment exhibiting significantly reduced amounts of pro-inflammatory cytokines (IFN-γ, MIP-3α) in serum (Fig. 8). Therefore TSG-6 reduces the inflammatory cascade before it actually reaches the brain. As TSG-6 might as well have local effects, we investigated the expression of IBA1, which is a marker for activated microglia, in the brain (Fig. 7). We detected an increased expression of IBA1 after LPS treatment which was not affected by additional TSG-6 administration. Still, the consequences of this increased microglia activation remain elusive as IBA1 is just a general marker of activation and does not provide information about the actual phenotype of microglia: M1 (classical activation) and M2 (sometimes subdivided into alternative activation and acquired deactivation) (Boche et al., 2013; Ito et al., 1998).

5. Conclusion

TSG-6 expression is spatiotemporally regulated in the neonatal brain. It is up-regulated from P1 to P15 and shows varying expression in different brain regions. Therefore, according to its functional characteristics, TSG-6 may play a role in oligodendrocyte maturation and neuronal precursor cell migration. Furthermore, TSG-6 is up-regulated in the neonatal brain under inflammatory conditions. Exogenous administration of rhTSG-6 decreases the systemic inflammation and reduces apoptosis in the brain, indicating a neuroprotective effect.

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