Attenuated Inflammatory Response in Aged Mice Brains following Stroke

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Abstract

**Background:** Increased age is a major risk factor for stroke incidence, post-ischemic mortality, and severe and long-term disability. Stroke outcome is considerably influenced by post-ischemic mechanisms. We hypothesized that the inflammatory response following an ischemic injury is altered in aged organisms.

**Methods and Results:** To that end, we analyzed the expression pattern of pro-inflammatory cytokines (TNF, IL-1α, IL-1β, IL-6), anti-inflammatory cytokines (IL-10, TGFβ1), and chemokines (Mip-1α, MCP-1, RANTES) of adult (2 months) and aged (24 months) mice brains at different reperfusion times (6 h, 12 h, 24 h, 2 d, 7 d) following transient occlusion of the middle cerebral artery. The infarct size was assessed to monitor possible consequences of an altered inflammatory response in aged mice. Our data revealed an increased neuro-inflammation with age. Above all, we found profound age-related alterations in the reaction to stroke. The response of pro-inflammatory cytokines (TNF, and IL-1β) and the level of chemokines (Mip-1α, and MCP-1) were strongly diminished in the aged post-ischemic brain tissue. IL-6 showed the strongest age-dependent decrease in its post-ischemic expression profile. Anti-inflammatory cytokines (TGFβ1, and IL-10) revealed no significant age dependency after ischemia. Aged mice brains tend to develop smaller infarcts.

**Conclusion:** The attenuated inflammatory response to stroke in aged animals may contribute to their smaller infarcts. The results presented here highlight the importance of using aged animals to investigate age-associated diseases like stroke, and should be considered as a major prerequisite in the development of age-adjusted therapeutic interventions.

Introduction

In general, the interplay between the peripheral immune system and the brain is well balanced in young organisms but becomes unfavorable with ageing [1]. Impaired immune homeostasis in aged organisms is associated with a decline in the ability to adapt to environmental stress. The consequence of this is a significant higher vulnerability for diseases and their impacts in the elderly. Stroke is the most common and most important vascular disease of the cerebral nervous system. Cerebral infarcts are the second leading cause of death worldwide and the leading cause of adult disability. Increased age is a major risk factor for stroke incidence, post-ischemic mortality, and severe and long-term disability [2,3,4]. Although stroke is an extremely important health issue, most pharmaceutical companies have terminated their research programs to that end, we analyzed the expression pattern of pro-inflammatory cytokines (TNF, IL-1α, IL-1β, IL-6, IL-10, TGFβ1) and chemokines (Mip-1α, MCP-1, RANTES) of adult (2 months) and aged (24 months) mice brains at different reperfusion times (6 h, 12 h, 24 h, 2 d, 7 d) following transient occlusion of the middle cerebral artery. The infarct size was assessed to monitor possible consequences of an altered inflammatory response in aged mice. Our data revealed an increased neuro-inflammation with age. Above all, we found profound age-related alterations in the reaction to stroke. The response of pro-inflammatory cytokines (TNF, and IL-1β) and the level of chemokines (Mip-1α, and MCP-1) were strongly diminished in the aged post-ischemic brain tissue. IL-6 showed the strongest age-dependent decrease in its post-ischemic expression profile. Anti-inflammatory cytokines (TGFβ1, and IL-10) revealed no significant age dependency after ischemia. Aged mice brains tend to develop smaller infarcts.

Following cerebral ischemia, a cascade of inflammatory mediators including cytokines (TNF, IL-1α, IL-1β, IL-6, IL-10, TGFβ1) and chemokines (MCP-1 [CCL2], Mip-1α [CCL3], RANTES [CCL5]) is initiated. Cytokines and chemokines have complex, overlapping and pleiotropic functions that may be both beneficial and deleterious. The temporal expression profile of each inflammatory mediator, its specific cell source and target, and their coordinated interaction are important for stroke recovery. The complex as well as dual role (beneficial versus deleterious) of the inflammatory response to stroke has been extensively discussed [10,11,12,13]. Ageing is associated with alterations of the cerebral inflammatory system [14,15,16,17]. In particular, TNF, IL-1, and IL-6 have been found to be increased with age [7,18]. The inflammatory reaction of aged brains to cerebral injuries has received little attention to date [19,20,21,22]. Indeed, the response to stroke is currently unknown. It is assumed that the inflammatory reaction influences the dimension of the injury [20]. Previous studies investigating the infarct size in old mice have revealed conflicting results; there are reports of increased, decreased or similar infarct sizes in aged brains [8].

We hypothesized that the extent and the progression of the immune response following an ischemic injury are altered in the
elderly and that this age-dependent inflammatory reaction is a determinant of the outcome following stroke. The aim of the present study was to systematically characterize the age-dependent changes of the infarct size and the inflammatory response in an experimental stroke model of mice. Transient occlusion of the middle cerebral artery (MCAO) — a model that closely resembles human stroke [29] — was used to induce cerebral infarction. The infarct size was analyzed at three different reperfusion times up to 7 days. The expression patterns of pro-inflammatory cytokines (TNF, IL-1β, IL-6), anti-inflammatory cytokines (IL-10, TGFβ1), and chemokines (Mip-1α, MCP-1, RANTES) were investigated at different reperfusion times (6 h, 12 h, 24 h, 2 d, 7 d).

Materials and Methods

All investigated mice were taken from a C57/BL6 inbred mouse strain, originally obtained from Jackson Laboratory, and randomly assigned to the groups (described in more detail in Supplement S1). Adult (2 months), middle-aged (9 months), and aged (15 months and 24 months) male mice were used to investigate age-related neuro-inflammation (n = 4, each). The age-dependent inflammatory response following induction of a cerebral infarct (6 h, 12 h, 24 h, 2 d, and 7 d) was studied using adult (2 months) and aged (24 months) mice that had undergone MCAO (n = 4, each). Infarct volumes were analyzed 2 h, 2 d, and 7 d after MCAO (n = 4, each). MCAO was performed as described previously [24]. Briefly, a monofilament was introduced into the internal carotid artery through an incision of the left common carotid artery. In this position, the middle cerebral artery was occluded for 30 min. Up to 7 d after reperfusion, the mortality rates were 7% and 39% in adult and aged mice, respectively. The effect of the surgical procedure was controlled using sham-operated mice (n = 4, each). These animals underwent anesthesia and surgical procedures similar to the MCAO group but without occlusion of the middle cerebral artery. In a pre-study, the cerebral blood flow (CBF) was measured by laser Doppler flowmetry (LDF) (Peri Flux System 5000, Perimed, Sweden) in adult (n = 10) and aged (n = 9) mice. Rectal temperature was measured using the DC Temperature Control System (FHC Inc., USA) in adult (n = 6) and aged (n = 8) mice. Animals were maintained with access to water and food ad libitum. All animal procedures were approved by the local government (Thuringer Landesamt für Lebensmittelsicherheit und Verbraucherschutz [TLLV], Dep.2, Gesundheitsamtlicher Verbraucherschutz, Veterinarwesen, Pharmacie, Germany, Approval ID's 02-20/05 and 02-028/10) and conformed to international guidelines on the ethical use of animals. All surgeries were performed under deep anesthesia (isoflurane).

Sample preparation

Infarct volumes were analyzed on Map2 immunostained slices [2 h, 2 d, and 7 d after reperfusion, as described in detail by us previously [25]]. Deeply anesthetized mice were fixed by perfusion through the ascending aorta with 4% paraformaldehyde. Brains were removed, cryoprotected in 0.2 M phosphate-buffered saline containing 30% sucrose, and stored at −80°C. Coronal sections (40-μm thick) were cut with a freezing microtome (MH400, Microm International GmbH).

For mRNA and protein quantification, a separate group of mice underwent MCAO. Animals were decapitated under deep anesthesia and brains were removed at 6 h, 12 h, 24 h, 2 d, and 7 d. Using a Precision Brain Slicer (BS-2000C Adult Mouse, Braintree Scientific Inc.), three coronal sections were dissected cutting +2.8 and +0.8 mm to bregma (rostral slice), +0.8 and −1.2 mm to bregma (middle slice), and −1.2 and −3.2 mm to bregma (caudal slice). The ipsilateral (ischemic tissue) and contralateral (non-ischemic tissue) hemispheres of the middle brain slice were separated and snap-frozen for details see Supplement S1). Adjacent slices were used for infarct validation (Map2 immunohistochemistry), to select mice with similar lesion size for mRNA and protein quantification.

Immunochemistry (Map2)

Free-floating sections (cerebrum, 40 μm thick) were incubated at 4°C overnight with antibody against Map2 (monoclonal mouse anti-MAP2 (2a+2b), clone AP-20, Sigma-Aldrich, 1:1,000), then further processed by the Vectastain Elite ABC Kit (Vector Laboratories) using a biotinylated secondary antibody (donkey anti-mouse, Dianova, 1:500). Immunoreactivity was developed in 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma).

qPCR

For qPCR, equal amounts of total RNA (1 μg) were transcribed in cDNA with RevertAid First Strand cDNA synthesis kit (Fermentas [27]). Amplification products were analyzed using gel electrophoresis, melting curve analysis, and sequencing to confirm the PCR product specificity. PCR was performed in a volume of 20 μl containing Brilliant® II SYBR Green® qPCR Master Mix (Stratagene), cDNA (equivalent to 25 ng reverse transcribed RNA), and specific primers (Table 1) each at a final concentration of 500 nM. Amplification was performed using the Rotor-Gene 6000 (Corbett Life Science) applying the following cycle conditions: 10 min polymerase activation, 40 amplification cycles (95°C for 30 s, 60°C for 30 s, 72°C for 30 s), and melting curve.

Cytokine bead assay (CBA)

The protein expression pattern was measured by a CBA with TNF, IL-1β, IL-6, IL-10, Mip-1α, MCP-1, and RANTES mouse Flex Sets (BD Bioscience). No antibody was available for TGFβ1. The procedure was performed according to the manufacturer’s instructions with the following modifications. To ensure a valid analysis of proteins below 10 pg/ml (the default outlined limit for quantification), (i) 300 μg whole protein was dissolved in 50 μl lysis buffer, (ii) the standard curve was constructed from 1.25–156 pg/ml (dissolved in lysis buffer), and (iii) samples as well as standards were washed twice at the end of the procedure to reduce background signals. Standards and test samples were analyzed using the Cytomics FC500 Flow Cytometry System (Beckman Coulter) with its settings optimized to ensure valid quantifications even for proteins present in very low amounts.

Data analysis

qPCR. External standard curves of purified PCR products (five 10-fold dilution series) were applied for absolute quantification, as described previously [27]. The numbers of transcripts were calculated per 1,000 transcripts of Gapdh by
including the criterion of the length of each specific amplicon. *Hmbs* was used as a second internal standard. Both *Gapdh* and *Hmbs* were stably expressed under the conditions of investigation.

**CBA.** The raw data from the Cytomics FC500 Flow Cytometry System were analyzed using FCAP Array™ software (SoftFlow). Protein concentrations of the test samples were calculated using a corresponding standard curve (supplied recombinant protein with known concentration).

### Statistical analysis

Differences between the infarct volumes of adult (2 months) and aged (24 months) mice at different time points (2 h, 2 d, and 7 d) were analyzed with ANOVA and post-hoc Tukey test. Significant differences of mRNA and protein expression values dependent on ageing per se (versus 2 months controls) were calculated with ANOVA and post-hoc Tukey test (at p ≤ 0.05). Correlation of the expression level with age (Pearson, r) was calculated for all the groups. The increased IL-6 mRNA level significantly correlated with age (Pearson, r = 0.585, p < 0.05). The expression level of IL-1α was elevated (TNF up to 61-fold, p ≤ 0.01; IL-1β up to 2.4-fold, p ≤ 0.01; Figure 2). This up-regulation was reflected in the level of protein, though it was not as pronounced (TNF 1.1-fold, n.s.; IL-1α 1.1-fold, n.s.; Figure 2).

Following ischemia in adult mice brains, the transcript expression of all the tested pro-inflammatory cytokines was clearly elevated (TNF up to 61-fold, p ≤ 0.01; IL-1α up to 13-fold, p ≤ 0.01; IL-1β up to 37-fold, p ≤ 0.001; Figure 2). This massive inflammatory response in adult ischemic brains was markedly attenuated in aged brains (by 41% for TNF; by 60% for IL-1α; by 79% for IL-1β). The up-regulation of TNF mRNA and protein peaked at 12 h after reperfusion in adult mice (mRNA 56-fold, protein 2.3-fold; p ≤ 0.001). The TNF protein level in aged mice brains following stroke showed an elevated expression but was not significantly affected. The extent of the post-ischemic response was approximately the same for IL-1α mRNA and protein, but a delayed peak expression of IL-1α protein was observed (mRNA peak at 6 h, protein peak at 12 h after reperfusion). The protein level of IL-1β could not be reliably quantified (data not shown).

### Cytokine IL-6

The increased IL-6 mRNA level significantly correlated with age (Pearson, r = 0.585, p < 0.05), though this was not reflected in its protein level (Figure 3). The post-ischemic IL-6 expression of adult mice was up-regulated by up to 69-fold for its mRNA level (peak at 12 h after reperfusion, p ≤ 0.01; IL-1α up to 13-fold, p ≤ 0.01; IL-1β up to 37-fold, p ≤ 0.001; Figure 2). The ischemic response in aged brains was attenuated by 82% at the transcript level (at 12 h) and by 85% at the protein level (at 24 h). In adult mice, the mRNA expression of IL-6 increased from 6 h to 12 h when it reached its peak expression. In aged mice, IL-6 mRNA expression peaked at 6 h and then displayed a rapid decay.

### Results

Almost all the tested inflammatory mediators showed increased expression values during ageing per se. The pro-inflammatory cytokines, particularly TNF, as well as the chemokines Mip-1α and MCP-1 showed a distinct up-regulation with age. Following stroke, the expression of almost all the tested inflammatory mediators was significantly up-regulated in ischemic tissues. However, a clearly attenuated inflammatory response was apparent in aged brains following the ischemic insult. TNF, IL-1α, IL-1β, IL-6, Mip-1α, and MCP-1 displayed an age-related decreased post-ischemic expression, with IL-6 exhibiting the strongest decrease. Aged mice brains also exhibited smaller infarcts. The sham procedure had no substantial effect on the expression of all the tested cytokines and chemokines. No significant differences of the cerebral blood flow and the body temperature were observed between adult and aged mice during or after MCAO (data not shown).

### Infarct volume

Oclusion of the middle cerebral artery for 30 min led to an ischemic injury mainly restricted to striatal regions of the mice brains. At different time points (2 h, 2 d, and 7 d) the infarct size tended to be smaller in aged mice. Taking all time points together, the mean infarct volume of aged mice was significantly smaller (Figure 1, Map2 immunohistochemistry, ANOVA with post-hoc Tukey test, p ≤ 0.05).

### Pro-inflammatory cytokines TNF, IL-1α, and IL-1β

All the tested major pro-inflammatory cytokines were significantly elevated with ageing per se regarding their mRNA level (TNF 5-fold, p ≤ 0.01; IL-1α up to 1.9-fold, p ≤ 0.05; IL-1β up to 2.4-fold, p ≤ 0.01; Figure 2). This up-regulation was reflected in the level of protein, though it was not as pronounced (TNF 1.1-fold, n.s.; IL-1α 1.1-fold, n.s.; Figure 2).

The post-ischemic response was approximately the same for IL-1α mRNA and protein, but a delayed peak expression of IL-1α protein was observed (mRNA peak at 6 h, protein peak at 12 h after reperfusion). The protein level of IL-1β could not be reliably quantified (data not shown).

### Table 1. Primer sequences.

| Primer | Sequence (5′→3′) | GenBank accession number |
|--------|-----------------|-------------------------|
| Gapdh forward | CAAACAGAACTCCCCACCTCTC | NM_008084.2 |
| Gapdh reverse | GGTCCAGGGTCTCTCTCTCTCTCT | |
| Hmbs forward | GAAATCCGTGATCCAC | NM_013551.2 |
| Hmbs reverse | GCGTTTTCTGCTCTTGGTAA | |
| TNFα forward | GTCTACACTCCCTCCGCTGCTT | NM_013693.2 |
| TNFα reverse | ATGACGGTGGTGGAGGGTTCG | |
| IL-1α forward | GCCCTATTTCTCCGGAGTCTAT | NM_010554.4 |
| IL-1α reverse | TGGATGTTGCCCTCTTCTTACA | |
| IL-1β forward | GAAAGCGCCCATCCTCTTGGA | NM_008361.3 |
| IL-1β reverse | TTCATCTCGAGCCTGATG | |
| IL-6 forward | ACAAAGCAGATCCCTCCAGAG | NM_031168.1 |
| IL-6 reverse | CATTTGGAAGTGGGTGAGGA | |
| IL-10 forward | ATGCTGTCCGCTTTCAATGCTT | NM_010548.1 |
| IL-10 reverse | AGATCTCCCTGTGGTCTCTCTC | |
| TGFβ1 forward | TGCTTCACTGCGCCACAGAA | NM_011577.1 |
| TGFβ1 reverse | TACTGTTGTCAGCAGTCCCA | |
| Mip-1α forward | TGGAACTGAAATGGCTGAGAGT | NM_011337.2 |
| Mip-1α reverse | TAGGAGATGCTGCTTAGGCTA | |
| MCP-1 forward | AGGTTGCCCACAGAGGCTGTA | NM_011333.3 |
| MCP-1 reverse | AATGTATGCTGACGACATCCC | |
| RANTES forward | CCAAGAAGAAGTGGTCGTTCAAG | NM_013653.2 |
| RANTES reverse | AAGCTGCGCTAGACTAGAGCAA | |

The GenBank accession numbers were obtained from the NCBI (March 2010). doi:10.1371/journal.pone.0026288.t001
Chemokines Mip-1α, MCP-1, and RANTES

The transcript levels of all the tested chemokines increased with ageing per se. The strongest increase was observed for Mip-1α mRNA (3.5-fold, p≤0.001; Figure 4) and MCP-1 mRNA (3.6-fold, p≤0.001; Figure 4). The protein levels of Mip-1α and RANTES correlated significantly with age (Pearson, p≤0.01 and p≤0.05, respectively; Figures 4 and 5). The protein level of MCP-1 tended to be increased; however, it failed to reach significance (Figure 4).

Mip-1α mRNA expression peaked 12 h after ischemia in adult mice (Figure 4). Aged mice displayed a significant attenuated response of Mip-1α transcript expression at 12 h after reperfusion (by 53%) and over all time points (by 33%). This post-ischemic diminished Mip-1α response was observed to be less pronounced at the protein level (by 25%, n.s.).

The transcript expression of MCP-1 peaked at 12 h after ischemia in adult mice brains (up to 102-fold, p≤0.001; Figure 4). However, this strong response was significantly attenuated in aged mice brains (up to 57%). The post-ischemic protein expression peaked at 12 h and 24 h in aged and adult mice brains, respectively (up to 9-fold, adults, p≤0.001). MCP-1 mRNA response seems also to be attenuated, in aged mice brains (by 48%, 24 h after reperfusion).

The expression of RANTES mRNA increased continuously up to 7 d following ischemia in adult brains (3.5-fold, p≤0.001; Figure 5). Aged brains showed no obvious post-ischemic increase in RANTES transcript expression in the ipsilateral hemisphere; however, the contralateral hemisphere displayed a decrease (Figure 5). Therefore, the ratio of ipsilateral versus contralateral RANTES mRNA resulted in a significant ischemic effect (Figure 5). The level of RANTES protein peaked at 24 h after reperfusion. RANTES did not display a significant age-dependent post-ischemic response (Figure 5).

Discussion

We found profound age-related alterations in the reaction to stroke. The response of pro-inflammatory cytokines and the level of chemokines were strongly diminished in the aged post-ischemic brain tissue (summarized in Figure 6). Anti-inflammatory cytokines (TGFβ1, and IL-10) revealed no significant age dependency after ischemia. In concordance, the mean infarct volume of aged mice brains was found to be smaller.

Age dependence of infarct size

Previous studies reported contradictory results on the age dependence of stroke size. Most studies found no age dependence or rather smaller infarct sizes, in experimental [28,29,30] as well as in clinical [31,32] observations. Larger stroke sizes in aged animals were reported after permanent MCAO at early times (6 h [33], 24 h [34], and 3 d [35]) while this was not observed at later times (7 d [35]), indicating that the dynamics of stroke development following permanent MCAO might be different in aged animals. Stroke size might also depend on the gender of aged animals and the hormone level. Female aged rodents had larger infarcts whereas ovariectomized rats had smaller infarcts [36,37]. Altogether, our result of a tendency to smaller infarcts in aged mice following transient MCAO is in agreement with most of the previous experimental studies [28,29,30] and in line with clinical observations [31,32].

Elevated cerebral inflammation with age

The expression levels of inflammatory mediators in the brain are low due to the immune privilege of the central nervous system
Potential reasons for the age-related attenuated post-ischemic inflammatory response

Elevated pro-inflammatory cytokine levels in aged brains may mediate a kind of preconditioning similar to the situation where administration of low levels of TNF and IL-1 leads to ischemic tolerance [44,45]. In such a scenario, greater levels of stimulation would be required to induce a comparable inflammatory response. However, if and to what extent the attenuated post-ischemic immune response is due to elevated baseline levels of pro-inflammatory cytokines in aged brains cannot be determined from our results.

IL-6 exhibited the strongest decrease in its expression profile following stroke in aged mice brains. IL-6 is a pleiotropic cytokine that coordinates inflammatory processes between the periphery and the central nervous system, and can be released by various cell types in response to injuries. Moreover, IL-6 influences the expression and function of several other inflammatory mediators. Therefore, IL-6 may be a key mediator of the reduced inflammatory reaction following stroke in aged mice.

Similar to IL-6, all the other tested inflammatory mediators interact with each other. TNFα, for instance, influences several other inflammatory mediators, e.g. IL-6 and IL-1β [46,47]. Also, IL-1β can stimulate cells to express MCP-1 [48]. Conversely, MCP-1-deficient mice express less IL-1β after permanent MCAO, indicating a signaling in both directions [49]. Therefore, a down-regulation of some of the key players of the post-stroke immune response may subsequently lead to a general reduction of the involved inflammatory mediators.

Potential functional consequences of an attenuated inflammatory response

Pro-inflammatory cytokines are generally considered to mediate detrimental effects following ischemia. Administration of TNF or IL-1 exacerbates damage [19,50], and inhibition of TNF or IL-1 as well as IL-1 deficiency lead to reduced ischemic injuries [19]. IL-6 as a pleiotropic mediator can potentially exert detrimental (early phase) or beneficial effects (late phase) following ischemia. Post-ischemic injection of recombinant IL-6 mediates neuroprotection and reduces the injury, whereas administration of anti-mouse IL-6 receptor monoclonal antibody or the use of IL-6-knockout mice increases infarct size [51].

Anti-inflammatory cytokines such as TGFβ1 and IL-10 have a beneficial function after ischemia. IL-10 deficiency exacerbates damage, whereas its over-expression reduces infarct volumes after ischemic brain injury [52]. Both of these anti-inflammatory cytokines are able to block NF-kappa B activation and thereby the expression of transcripts involved in immune and inflammatory responses [53,54].

GC chemokines or β-chemokines recruit immunocompetent cells to injured tissue [53]. Studies which inhibited MCP-1 in the rat brain or used MCP-1-deficient mice reported smaller ischemic lesions, whereas over-expression exacerbated damage [19]. Inhibition of MCP-1 and Mip-1α signaling leads to reduced infarct volumes following MCAO [56]. In concordance, the administration of Mip-1α has been found to increase the infarct volume [56]. RANTES-deficient mice exhibited decreased leukocyte adhesion, diminished blood-brain barrier permeability, and less tissue infarction [57].

The present study has revealed an attenuated response of inflammatory mediators in aged brains following stroke. Moreover, we observed smaller infarcts in aged brains. Most studies in rodents as well as in humans have reported that cerebral infarcts in aged organisms are not different from those in adults [8]. The smaller infarcts in aged brains observed here could be a consequence of the diminished pro-inflammatory response. This hypothesis is in line with the literature which reports a detrimental effect of pro-inflammatory cytokines and chemokines on the extent of injuries (as outlined above). The attenuated expression of IL-6...
Figure 3. Age- and stroke-dependent expression of IL-6, TGFβ1 and IL-10. IL-6 transcript expression significantly correlated with age; however, this correlation was not reflected in the expression of its protein. The post-ischemic IL-6 response was attenuated in aged brains (mRNA and protein). TGFβ1 mRNA significantly correlated with age. IL-10 mRNA tended to be elevated in aged brains. TGFβ1 transcript expression in the ischemic tissue increased continuously with reperfusion time at both ages. The up-regulation of TGFβ1 and IL-10 following ischemia tended to be attenuated in aged brains. Expression values are shown as mean ± SEM and ratios as geometric mean ± SEM. For details of the statistical analysis, see Figure 2’s legend or the Materials and Methods section.

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Figure 4. Age- and stroke-dependent expression of Mip-1α and MCP-1. Mip-1α and MCP-1 displayed significantly elevated expression levels with age. Mip-1α and MCP-1 transcript and protein expression increased significantly following stroke. This up-regulation was attenuated in aged brains. Expression values are shown as mean ± SEM and ratios as geometric mean ± SEM. For details of the statistical analysis, see Figure 2's legend or the Materials and Methods section.

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Figure 5. Age- and stroke-dependent expression of RANTES. The expression of RANTES significantly correlated with age. After ischemia, the level of RANTES mRNA continuously increased up to 7 d in adult brains, whereas no up-regulation was observed in aged brains. Protein levels of RANTES were up-regulated in adult and aged brains. Expression values are shown as mean ± SEM and ratios as geometric mean ± SEM. For details of the statistical analysis, see Figure 2's legend or the Materials and Methods section.

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Figure 6. Schematic diagram summarizing the post-ischemic expression profiles of all the tested inflammatory mediators. The relative expression profiles (%) of all the tested inflammatory mediators in adult (continuous line, colored area) and aged brains (dotted line, light colored area) are shown (mRNA, left panel; protein, right panel). The post-ischemic response of TNF, IL-1α, IL-1β, IL-6, Mip-1α, MCP-1, and TGFβ1 was considerably attenuated with age, with IL-6 exhibiting the strongest age-dependent effect.

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in aged post-ischemic brains may be harmful in the late ischemic phase.

Conclusions and future perspectives

From a theoretical point of view, attenuated post-lesional levels of pro-inflammatory cytokines and chemokines may be considered as a neuroprotective mechanism in aged brains. The smaller infarcts in aged brains could potentially be a consequence of this neuroprotection. However, reasons for the attenuated age-related stroke response cannot yet be specified. Increased baseline inflammation in aged brains may influence post-ischemic inflammatory processes. Modulation of the age-related neuroinflammation (e.g., by pharmacological intervention or physical activity) with subsequent stroke induction would provide an opportunity to clarify this issue. Furthermore, investigations involving aged transgenic mice with altered inflammatory brain responses are necessary to unravel the functional relevance of our findings.

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Supporting Information

Supplement S1

(DOC)

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Author Contributions

Conceived and designed the experiments: MWS RAC CF OWW. Performed the experiments: MWS. Analyzed the data: MWS. Contributed reagents/materials/analysis tools: RAC CF OWW. Wrote the paper: MWS RAC CF OWW.
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