Combined Genetic Deletion of IL (Interleukin)-4, IL-5, IL-9, and IL-13 Does Not Affect Ischemic Brain Injury in Mice

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Background and Purpose—After ischemic injury, microglia and infiltrated macrophages may acquire different polarization phenotypes promoting inflammation and injury (M1) or repair and protection (M2). There is evidence that immunomodulation, via type 2 helper T-cells (Th2) cytokines, exerts neuroprotection after ischemia. We investigated the consequences of simultaneous genetic deletion of Th2 cytokines (IL [interleukin]-4, IL-5, IL-9, IL-13) on the histopathologic outcome, microglia and infiltrated macrophages markers, and ischemic microenvironment at different time points after ischemic injury in mice subjected to permanent occlusion of the middle cerebral artery.

Methods—Wild-type and Th2 cytokine-deficient mice (4KO) were subjected to permanent occlusion of the middle cerebral artery by electrocoagulation and followed up to 5 weeks after permanent occlusion of the middle cerebral artery. Neuropathologic outcome was assessed at 24 hours (n=6), 7 days (n=6), and 5 weeks (n=6–7) by examination of the ischemic lesion, neuronal count, microglia and infiltrated macrophages markers, brain atrophy, collagen deposition, and GFAP (glial fibrillary acidic protein) immunohistochemistry. Selected gene expression was investigated at 7 days (n=6).

Results—4KO mice showed no difference in lesion and neuronal count 7 days and up to 5 weeks after permanent occlusion of the middle cerebral artery compared with wild type. Ischemic 4KO mice had lower CD16/32 expression at 24 hours, lower CD11b and CD16/32 expression at 7 days than wild type. They had higher CD206 expression at 24 hours, higher CD206 and arginase1 at 7 days, and increased mRNA for CXCL9 (chemokine [C-X-C motif] ligand 9) compared with wild type. Additional histopathologic analysis, including brain atrophy, gliotic scar, and collagenous scar confirmed no difference between genotypes at 5 weeks.

Conclusions—This study casts light on the proposed neuroprotective function of Th2 cytokines, showing that combined IL-4, IL-5, IL-9, IL-13 deletion does not affect the neuropathologic response to ischemic stroke in the subacute and chronic phases. Our findings indicate that Th2 cytokines are not an essential neuroimmunological cue able to drive the brain's ischemic outcome.

Visual Overview—An online visual overview is available for this article. **(*Stroke*. 2019;50:2207-2215. DOI: 10.1161/STROKEAHA.119.025196.)**

Key Words: brain ischemia ■ cytokines ■ inflammation ■ mice ■ microglia

Microglia and infiltrated macrophages (M/M) are the main cellular contributors to the postinjury inflammatory response.[1] In response to environmental stimuli, they polarize into diverse phenotypes (M1-like and M2-like)[2–4] classified on the basis of the polarization of type 1 helper T-cells (Th1) and type 2 helper T-cells (Th2).[5–9] However, the M1-like to M2-like paradigm is an oversimplification of the in vivo activation because a heterogeneous mixture of M/M phenotypes populates specific microenvironments.[4,10,11] In animal models of stroke, traumatic brain injury, and spinal cord injury, M/M co-expressing M1 and M2 markers have been described at the lesion site.[2,12–14] An M/M activation profile toward the M1-like phenotype is associated with worsening of the lesion[2,15,16] whereas the switch to M2 like-phenotype is involved in protective/proregenerative changes of the lesion environment.[13,17–19] Furthermore, drugs of different classes provide neuroprotection after stroke by reducing the M1/M2 ratio.[20–24] These data support the idea that skewing M/M toward M2 balance may limit the detrimental phase of acute brain injury and indicate M/M as legitimate targets for therapeutic intervention.[3,25]

There is evidence that Th2 cytokines can affect the ischemic outcome. IL (interleukin)-4 treatment upregulates arginase1, FIZZ1 (found in inflammatory zone 1), CD206, chemokine ligand 22, reduces the Th1/Th2 ratio, and exerts neuroprotection.[3,26–28] In models of acute brain injury, IL-4, IL-5, and IL-13 are associated with neuroprotection.[26–30] IL-9/IL-9 receptor signaling has been suggested as an endogenous antiapoptotic

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mechanism for cortical neurons.[31] In patients with stroke, the acute serum levels of IL-9 negatively correlate with the Fatigue Severity Scale[32] while whether IL-9 plays a role in an experimental stroke model is awaiting further investigation.[33]

We, therefore, investigated whether an overall Th2 response might be a factor affecting the nature and outcome of the injured environment after ischemic stroke. We used mice with combined deletion of IL-4, IL-5, IL-9, IL-13[34] (4KO) to address the response to ischemia in the acute, subacute, and chronic phases after injury. We also focused on M/M activation and on the local ischemic microenvironment. 4KO mice showed modified temporal pattern of M/M phenotype markers in the brain with no change in the neuropathologic outcome up to 5 weeks after ischemic stroke. These data show that the brain undergoing ischemic injury can cope with a complete lack of Th2 cytokines and suggest that the reported associations of type 2 responses with neuroprotective effects are not the result of a direct causative mechanism.

Methods

This article adheres to the American Heart Association Journals implementation of the Transparency and Openness Promotion Guidelines. The detailed description of procedures can be found in the online-only Data Supplement. The datasets generated and analyzed during the current study are available in the Figshare repository 10.6084/m9.figshare.7599242

Animals

We used IL-4, IL-5, IL-9, IL-13 deleted mice[34] developed on BALB/c mice (bred in our animal facility as homozygotes), and BALB/c mice, 9 weeks of age (wild type [WT], used as the control strain) purchased from Envigo Laboratories (Udine, Italy). Both genotypes were bred and housed in certified specific pathogen-free vivaria. Mice of both genotypes were co-housed for at least 2 weeks before surgery and co-housing was maintained also after stroke surgery. Ethical approval to animal procedures was obtained from the Italian Ministry of Health (authorization number 224/2016-PR) and conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The experiments were designed, performed, and reported according to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines (https://www.nc3rs.org.uk/arrive-guidelines, check list provided in the online-only Data Supplement).

Focal Cerebral Ischemia

Mice (total number n=99) were randomized (https://www.random.org/lists/) and permanent occlusion of the middle cerebral artery (pMCAo) was conducted as previously described.[2,35] Briefly, anesthesia was induced by 3% isoflurane inhalation in a N_2O/O_2 (70%/30%) mixture and maintained by 1% to 1.5% isoflurane inhalation in a N_2O/O_2 (70%/30%) mixture. A vertical midline incision was made between the right orbit and tragus. The temporal muscle was excised, and the right middle cerebral artery was exposed through a small burr hole in the right temporal bone and the middle cerebral artery permanently occluded by electrocoagulation just proximal to the origin of the olfactory branch. pMCAo was confirmed visually (equal to laser doppler flowmetry; Figure 1) before closing the wound with sutures. Mortality was 0%. Sham-operated mice received the identical anesthesia and surgical procedure without artery occlusion. No animals were excluded from the analysis.

Physiology and Susceptibility of WT and 4KO Mice to Middle Cerebral Artery Occlusion

Vascular anatomy, blood pressure, heart rate, and cerebral blood flow before and after pMCAo were analyzed in WT and 4KO mice as previously described[36,37] (see the online-only Data Supplement).

Analysis of Infarct Size and Neuropathologic Damage

We analyzed infarct size, neuronal cell count, poststroke atrophy on coronal brain sections stained with cresyl violet. The infarcted area was delineated by tracing the pale area of histological staining on a video screen, and infarct volumes were calculated by integrating the infarcted areas on each brain slice after correction for the percentage of brain swelling because of edema (determined by subtracting the area of the ipsilateral from that of the contralateral hemisphere). Quantification of collagen deposition was performed on coronal sections stained with Sirius Red.

Immunohistochemistry

Staining of brain coronal sections was performed as detailed in the online-only Data Supplement.

Real-Time Polymerase Chain Reaction

Total RNA extraction from tissues and immunophenotyping were performed as described by Garetto et al[38] and detailed in the online-only Data Supplement.

Statistical Analysis

Data are presented as box and whiskers with line at mean and min-to-max. Group size was defined pre hoc using the formula: $n=2\sigma^2 f(\alpha,\beta)/\Delta^2$ (SD in groups=σ, type 1 error α=0.02, type II error β=0.2, percentage difference between groups Δ=30). In line with literature data,[2,12,35,39] the variability of this stroke model in our hands has a SD of 14.85 %. Using data from previous experiments, when the ischemic volume at 24 hours after pMCAo was set as primary outcome, the pre hoc definition of group size (using the formula: $n=2\sigma^2 f(\alpha,\beta)/\Delta^2$ [SD in groups=σ, type 1 error α=0.05, type II error β=0.2, percentage difference between groups Δ=30]) is n=6 as number of mice to be used. Standard software packages GraphPad Prism (GraphPad Software, Inc, San Diego, CA, version 7.0) were used. Groups were compared using 1-way ANOVA or 2-way ANOVA followed by an appropriate post hoc test. *P* lower than 0.05 were considered statistically significant. Description of the test used is provided in the figure legends. Physiological parameters were analyzed using 2-tailed *t* test.

Results

Physiological Parameters and Susceptibility of WT and 4KO Mice to Middle Cerebral Artery Occlusion Injury

Blood pressure and heart rate as well as cerebral vascular anatomy were not different in the 2 genotypes (Table; Figure 1A). Similarly, cerebral blood flow drop after middle cerebral artery occlusion were not different in the 2 genotypes (Figure 1B).

Genetic Deletion Modifies Histological Outcome at Acute (24 Hours) but Not at Subacute (7 Days) Phases After pMCAo

pMCAo induced an infarct area in the ipsilateral cortex (Figure 1C). At 24 hours after pMCAo, WT mice had higher ischemic lesion compared with 7 days. Compared with WT mice, 4KO mice had a significantly smaller ischemic volume at 24 hours but no difference at 7 days after pMCAo (Figure 1C).

Neuronal count in the ischemic area of mice 7 days after pMCAo indicated 90% neuronal loss (see online-only Data Supplement; Figure 1D) compared with the uninjured contralateral area with no differences between genotypes (Figure 1D).

Genetic Deletion Modifies the Temporal Pattern of M/M Markers in the Ischemic Area

We then assessed whether the genetic deletion was associated with different M/M activation after pMCAo by quantitative immunohistochemical analysis in the ischemic area (Figure 2). CD11b, a pan marker for M/M, was present in sham-operated mice, with no difference between genotypes (Figure 2A).

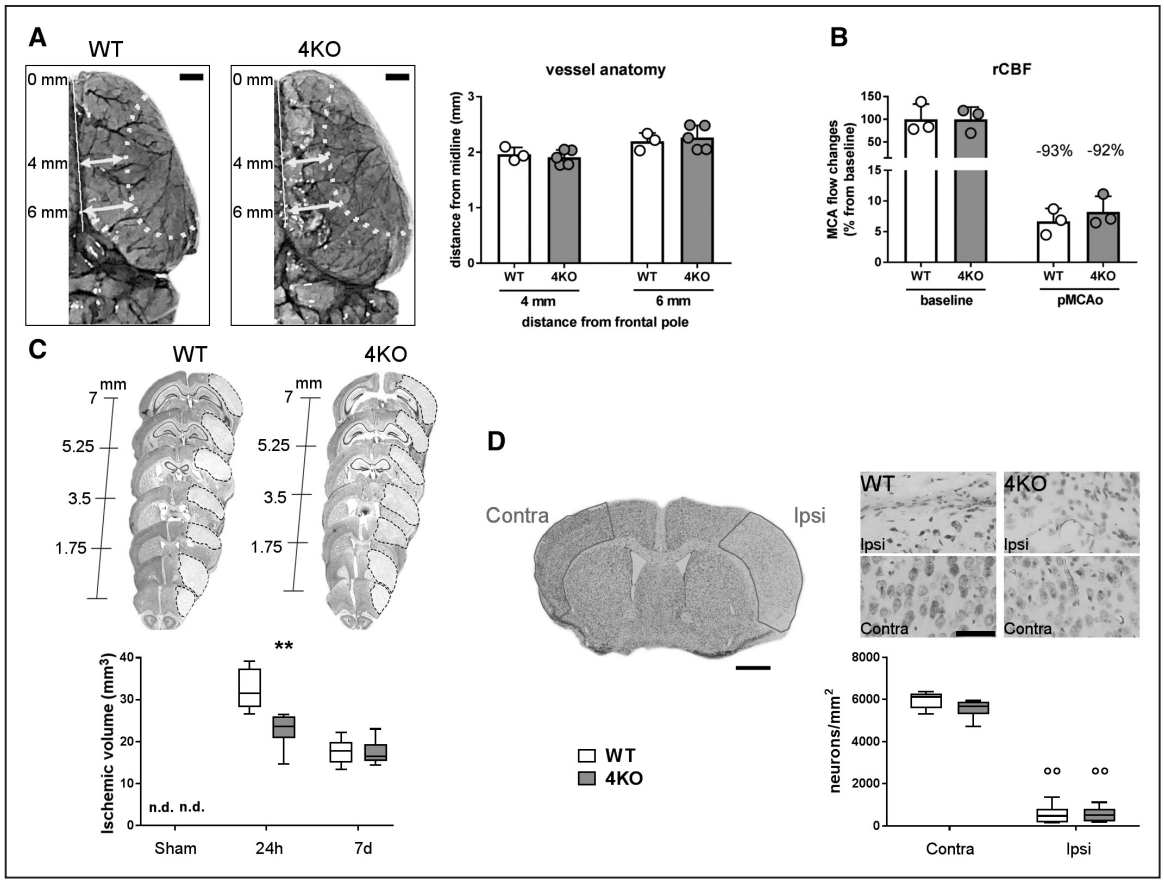

Figure 1. Vascular anatomy, cerebral blood flow, and brain ischemic lesion. **A**, **left**; anastomotic line (yellow) connecting the terminal branches of the middle cerebral artery and the anterior cerebral artery of wild-type (WT) and 4KO mice. Bar, 1 mm. **A**, **right**; distance (millimeters) of the anastomotic line to the midline at 4 and 6 mm from the frontal pole in the brains of WT and 4KO mice, n=3–5. Bar, 1 mm. **B**, Cerebral blood flow (CBF) recorded by laser doppler flowmetry in the middle cerebral artery territory before surgery (baseline) and after permanent occlusion of the middle cerebral artery (pMCAo) in WT and 4KO mice, n=3. **C**, **upper**; distribution of the ischemic lesion 24 h after pMCAo in WT and 4KO mice. Pale demarcarted areas indicate the ischemic lesion. **C**, **lower**; quantification of the ischemic volume 24 h and 7 d after pMCAo. **D**, **left**; representative microphotograph of cresyl violet staining. The ipsilateral cortex was quantified in the traced region of interest, identified as the cortical area involved in the lesion, and compared with the relative contralateral cortex (bar, 1 mm). **D**, **right**; neuronal count in the cortical ischemic lesion 7 after pMCAo. Two-way ANOVA F int (1, 20)=8.654; P=0.0081 for ischemic lesion. $^{**}P<0.01$ vs WT; $^{\circ\circ}P<0.01$ vs contralateral, Sidak test. n=6 mice. Bar, 50 μm.

After pMCAo, CD11b immunostaining greatly increased in WT at 24 hours and 7 days compared with sham mice. 4KO mice had lower CD11b staining at 7 days after pMCAo but no difference at 24 hours (Figure 2A). CD68 staining was undetectable in sham-operated mice (Figure 2B). After pMCAo, CD68-positive cells showed similar temporal increases in WT and 4KO mice (Figure 2B). No $CD45^{high}$-positive cells could be detected in sham-operated mice or in the contralateral hemisphere of ischemic mice (Figure 2C). $CD45^{high}$-positive cells had a rounded morphology and corresponded to recruited macrophages, neutrophils, and lymphocytes (see the online-only Data Supplement). $CD45^{high}$ cells were evident in the ischemic area, with no differences between genotypes.

We investigated M/M phenotypes by quantifying Ym1, CD206, arginase1, and CD16/32 (Figure 3). Ym1 was undetectable in sham-operated mice (Figure 3A). After pMCAo, Ym1-positive cells showed similar increases in 4KO and WT mice at 24 hours, whereas Ym1 was undetectable in the ischemic area of both genotypes at 7 days.

CD206 was present in sham-operated mice. After pMCAo, CD206-positive cells significantly increased in 4KO mice compared with WT at 24 hours and at 7 days (Figure 3B). Arginase1 was undetectable in sham-operated mice (Figure 3C), but positive cells showed similar increases in 4KO and WT mice at 24 hours while in 4KO mice they were significantly increased for 7 days. 4KO sham-operated mice had lower expression of CD16/32 (Figure 3D). After pMCAo, CD16/32-positive cells increased less in 4KO mice than WT at 24 hours and at 7 days.

Deletion Modifies Gene Expression in the Ischemic Area in the Subacute Phase After pMCAo

To characterize the ischemic microenvironment better, we examined cytokine and chemokine gene expression changes

Table. Physiological Data in WT and 4KO Mice

	WT	4KO	P Value (t Test)
SAP (mm Hg)	107±12	100±9	0.31
HR (beats/min)	482±26	495±18	0.35

Data are mean±SD (n=6). HR indicates heart rate; SAP, systolic arterial pressure; and WT, wild type.

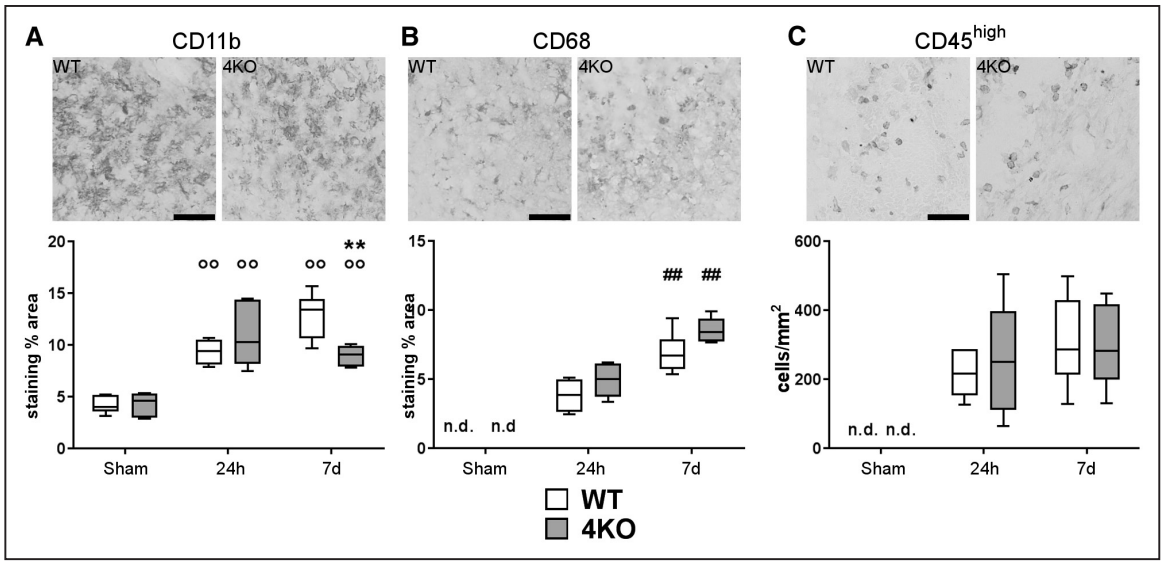

Figure 2. Representative micrographs of immunostaining and quantitative analysis in the cortical ischemic lesion 24 h and 7 d after permanent occlusion of the middle cerebral artery. CD11b (**A**), CD68 (**B**), and leukocyte-like CD45^{high}+ cells (**C**). n=6 mice. Two-way ANOVA *F* int (2, 30)=7.976; *P*=0.0001 for CD11b. n.d. indicates not detectable. Bar, 50 µm. ***P*<0.01 vs wild type (WT); °°*P*<0.01 vs sham-operated mice; ##*P*<0.01 vs 24 h, Sidak test.

by real-time polymerase chain reaction in the cortical ischemic area. Induction of regulatory T-cells was analyzed by Foxp3 gene expression (Figure 4).

In WT mice, pMCAo upregulated the mRNA expression of factors such as CXCL9 (chemokine [C-X-C motif] ligand 9), CXCL10 (chemokine [C-X-C motif] ligand 10), TNF-α (tumor necrosis factor-α), IFN-γ (interferon-γ), IL-6, IL-17a, IL-1-β, IL-12a, TGF-β, and IL-10 compared with sham-operated mice. pMCAo in 4KO mice further increased CXCL9 mRNA expression compared with ischemic WT mice. Ischemic 4KO and WT mice had similar mRNA expression of CXCL10, TNF-α, IFN-γ, IL-6, IL-17a, IL-1β, IL-12a, TGF-β, and IL-10. Ischemia slightly downregulated Foxp3 gene expression in WT and 4KO mice with no significant differences between genotypes.

Genetic Deletion Does Not Modify Neuropathologic Outcome in the Chronic Phase (5 Weeks) After pMCAo

The cell count in the whole ischemic cortex of WT mice (see the online-only Data Supplement; Figure 5A) indicated 45% neuronal loss compared with the uninjured contralateral cortex (Figure 5B). 4KO mice had a similar reduction in neurons with no significant difference between genotypes. GFAP (glial fibrillary acidic protein) immunstaining in the perilesional cortex showed that gliotic scar formation in 4KO mice was similar to WT (Figure 5B). No significant difference in the collagenous scar was observed in the perilesional cortex between WT and 4KO mice (Figure 5C). pMCAo induced significant atrophy in the injured hemisphere compared with the contralateral one (Figure 5D). Quantification of brain atrophy showed no significant difference between WT and 4KO mice.

Discussion

The major finding of our study is that combined genetic deletion of Th2 cytokines, namely IL-4, IL-5, IL-9, and IL-13, does not affect the neuropathologic response to ischemia in the subacute and chronic phases. This deletion was associated with an increase in the mRNA for type 1-associated chemokine CXCL9 confirming the shift to a type 1 helper T-cells polarization. Intriguingly, the deletion of Th2 cytokines was also associated with an apparent upregulation of M2-like M/M phenotype markers such as CD206 and arginase1 and with a downregulation of the M1-like marker CD16/32 in the ischemic area. These data suggest that the brain responds to the lack of Th2 cytokines with a shift toward M1 responses. Yet, we also observed an upregulation of markers commonly used to identify M2 responses in mice that cannot form M2 responses as the deleted cytokines are required for the signals leading to polarization commitment. The latter may signify that either these commonly used markers are not accurate indicators of type 2 polarization state or that the compensation of type 2 response absence triggers a more complex myeloid cell response in the brain.

We used a mouse model that induces a moderate-sized cortical lesion and a pronounced inflammatory reaction.[12,35] Infarcts modeled by the pMCAO in mice mimic human stroke lesions in the cortical middle cerebral artery territory, especially those observed in the large number of patients with stroke having insufficient reperfusion, thus highlighting the clinical relevance of this model.[40,41] Unfortunately, one limitation of the model is the weak sensitivity of behavioral tests to detect neurological dysfunction, thus preventing a sound assessment.[35]

The observation that this difference in infarct size does not persists at later time points suggests that the evolution of the ischemic lesion in the 4KO mice has a different progression than that in WT mice. Notably, the lesion outcome, including infarct size and neuronal count at subacute and at the chronic phases is similar between genotypes (eg, 7 days and 5 weeks), indicating that the actual impact of the 4 genetic deletions on this injury is overall negligible. We then investigated the brain's inflammatory response after pMCAo. We did not dissect a specific role for microglia or

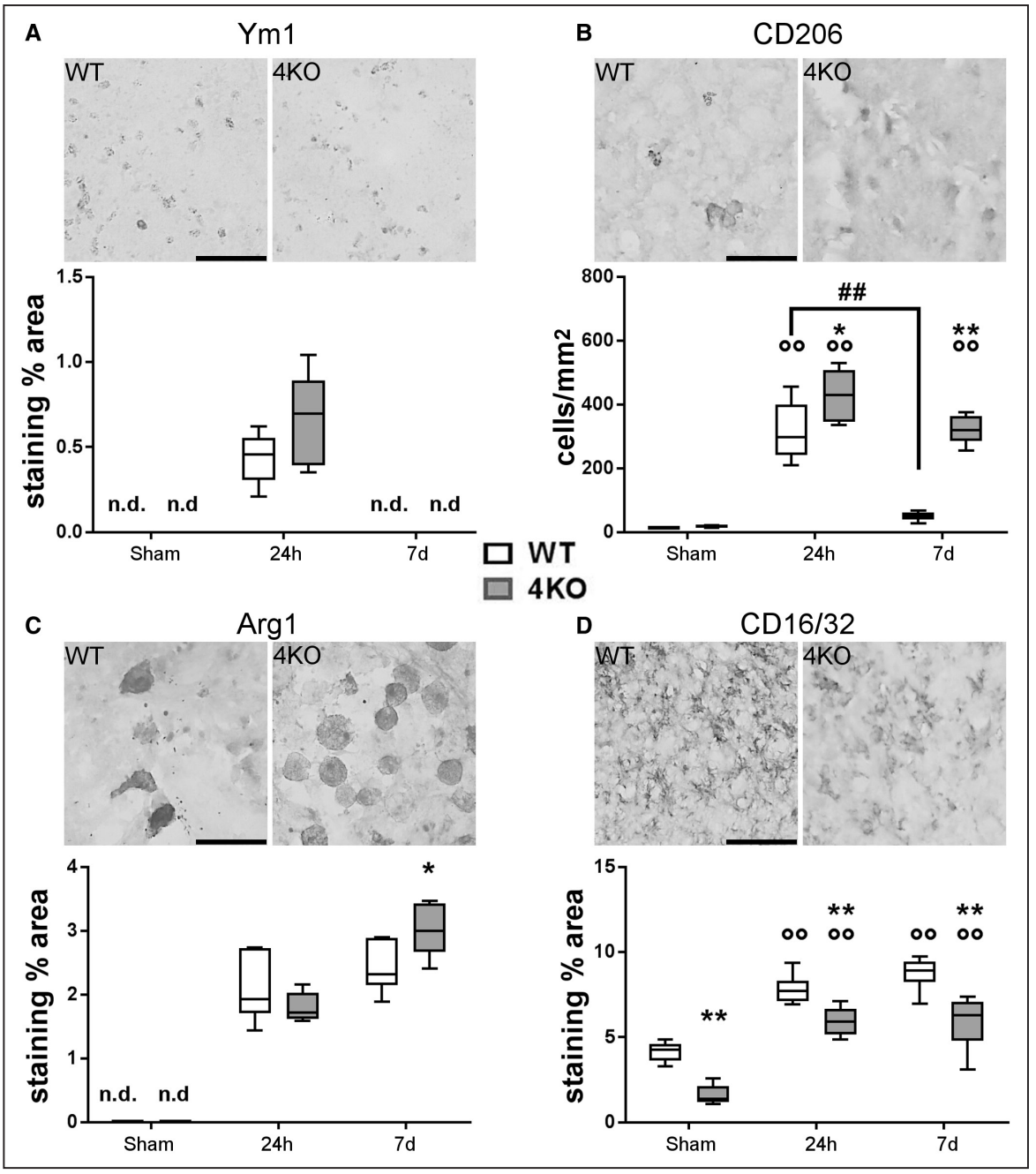

Figure 3. Representative micrographs of immunostainings and quantitative analysis in the cortical ischemic lesion 24 h and 7 d after permanent occlusion of the middle cerebral artery. Ym1 (**A**), CD206 (**B**), arginase1 (**C**), and CD16/32 (**D**). Two-way ANOVA *F* int (2, 28)=16.79, for CD206, *P*=0.0001; *F* int (2, 29)=5.71, *P*=0.01 for arginase1. n.d. indicates not detectable. Bar, 50 μm. **P*<0.05; ***P*<0.01 vs wild type (WT); °°*P*<0.01 vs sham-operated mice; ##*P*<0.01 vs 24 h, Sidak test. n=6 mice.

macrophages but focused on myeloid cells as a whole, considering the ischemic environment as a major determinant of their activation state and effector functions.[1] In line with our previous data,[2,12] the results show intense M/M activation after pMCAo with characteristics of mixed phenotypes and the temporal kinetics of M/M polarization in the ischemic area. 4KO mice had reduced M/M activation (7 days), an early increase (24 hours) in CD206 immunoreactivity and increased CD206 and arginase1 immunoreactivity at 7 days compared with WT mice. Surprisingly, we observed the presence and upregulation of markers commonly used to identify M2 responses in 4KO mice, which are deleted from genes encoding cytokines leading to M2-like commitment,[5–8] suggesting that the brain could have a unique ability to modulate the immune response.

In 4KO mice, cytokine deletion did not alter the recruitment of peripheral immune cells (CD45^{high}-positive cells) or the expression of the phagocytic marker CD68 as compared with WT mice. As immune cell recruitment and phagocytosis are key contributors to the ischemic lesion development and evolution,[1,2,42] these results are in line with the unchanged neuropathologic outcome after pMCAo.

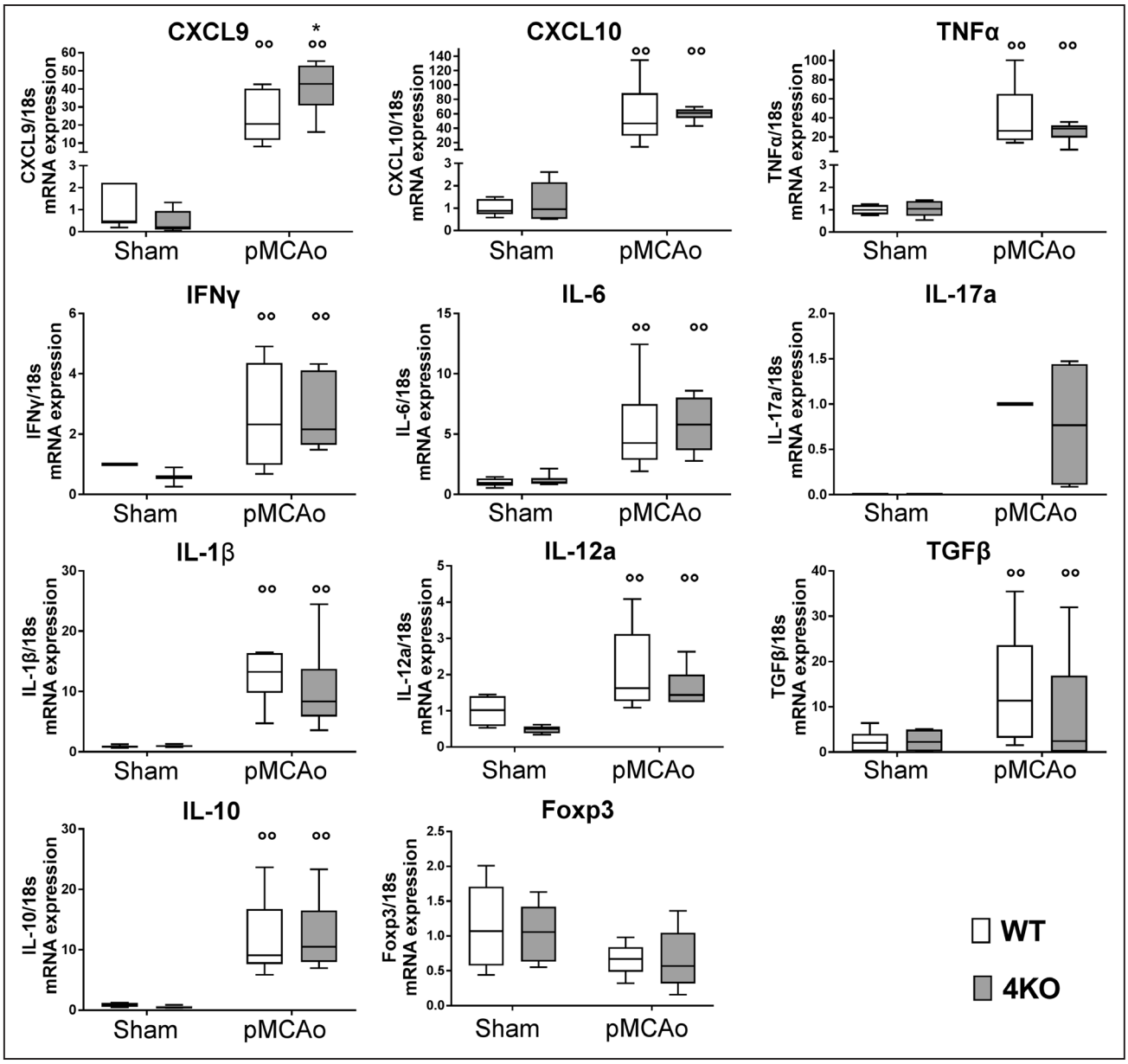

Figure 4. Relative gene expression of chemokines and cytokines in the cortical ischemic area 7 d after permanent occlusion of the middle cerebral artery (pMCAo). Two-way ANOVA, *F* int (1, 20)=4.485, *P*=0.0475 for CXCL9 (chemokine [C-X-C motif] ligand 9). Data are expressed as fold induction compared with the wild-type (WT) sham-operated group (n=6). IFN-γ indicates interferon-γ; IL, interleukin; TGFβ, transforming growth factor; and TNF-α, tumor necrosis factor-α. **P*<0.05 vs WT; °°*P*<0.01 vs sham-operated mice, Sidak multiple comparisons test.

We found that CD16/32 was downregulated in sham 4KO mice as well as after pMCAo.

As the main difference in M/M phenotype markers was evident 7 days after pMCAo, at this time point, we investigated the ischemic environment by gene expression analysis for cytokines and chemokines associated with pro- or anti-inflammatory states and different polarizations.[7,8] Ischemia in 4KO mice led to a mixed activation profile of both pro- and anti-inflammatory chemokine and cytokine expression such as TNF-α, IL-12a, and TGF-β as well as an increase in type 1-associated proinflammatory CXCL9 mRNA expression compared with WT mice. In addition, in 4KO mice, there was no difference in the mRNA expression of IL-10, CXCL10, IFN-γ, IL-6, IL-17a, IL1-β compared with WT mice. These results show that the increased expression of CD206 and arginase1 in 4KO mice 7 days after ischemia did not accurately reflect the expected shift toward a type 1-polarized state in the ischemic microenvironment at the same time.

The lack of major differences in the immune responses present in the ischemic microenvironment in 4KO versus WT mice was further substantiated by assessment of IL-10, a canonical anti-inflammatory cytokine that plays a critical role in the control of immune response.[5,7,8,43] Many preclinical studies have investigated its role in acute brain injury.[44] IL-10 is expressed in response to acute ischemic injury and is thought to facilitate the resolution of postischemic inflammation through its protective effect on the vascular endothelium and attenuation of the inflammatory cascade.[44]

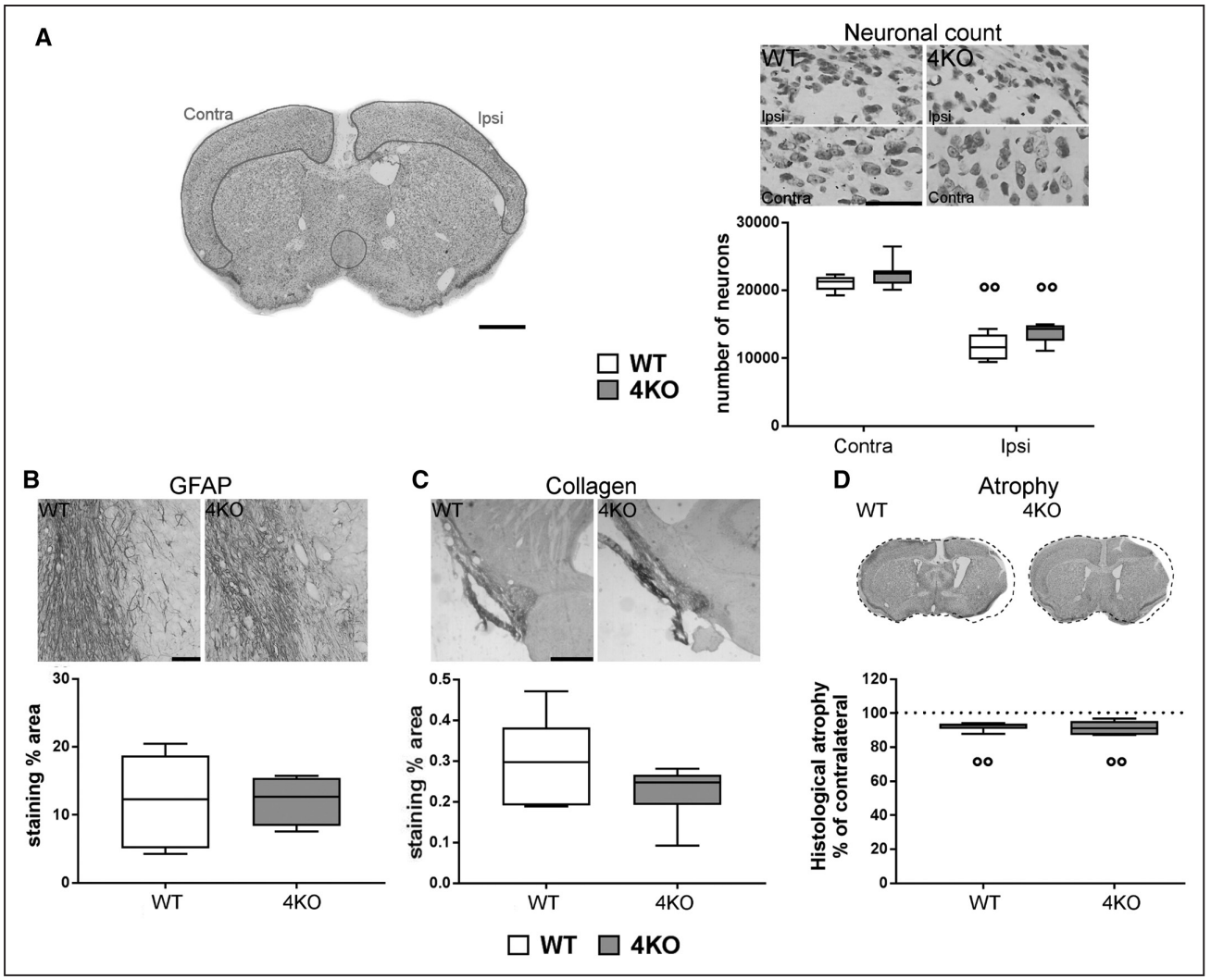

Figure 5. Histopathologic findings at 5 wks after permanent occlusion of the middle cerebral artery (pMCAo). **A**, **left**; position of selected cortical regions of interest (traced line) for neuronal quantification, bar, 1 mm. **A**, **right**; microphotographs of cresyl violet staining and neuronal count in the entire cortex of ipsilateral and contralateral hemispheres; bar, 50 μm. **B**, Representative immunoreactivity for GFAP (glial fibrillary acidic protein) in the perilesional region and quantification of the stained area; bar, 50 μm. **C**, Microphotographs of collagen staining in the perilesional region and quantification of stained area; bar, 500 μm. **D**, Representative micrographs and quantification of typical brain atrophy after pMCAo. Two-way ANOVA followed by Tukey post hoc test for neuronal count. WT indicates wild type. °°P<0.01 vs contralateral. Mann-Whitney U test for histological atrophy, °°P<0.01 vs contralateral (100%). n=6–7 mice.

Moreover, regulatory T-cells are capable of modulating neuroinflammation in stroke also through IL-10 secretion.[45] We investigated the presence of regulatory T-cells (Foxp3+ cells) in the ischemic area by gene expression analysis. Seven days after pMCAo, IL-10 was enhanced by ischemia but was not affected by genetic deletion. We also found that ischemia downregulated Foxp3 mRNA expression with no difference between genotypes, suggesting that IL-10 expression and recruitment of regulatory T-cells in the ischemic area were not affected by the absence of type 2 responses.

Last, we examined the consequences of the absence of IL-4, IL-5, IL-9, IL-13 on the neuropathologic outcome at a late chronic phase after pMCAo. 4KO and WT mice had similar neuronal loss up to 5 weeks after pMCAo. As these cytokines have healing profibrotic functions,[34,46] we also investigated collagenous fibrotic scar formation in the perilesional tissue and the typical poststroke brain atrophy at 5 weeks. Among cells of the glial scar, activated astrocytes surround the lesion and take residence in the lesion border, resulting in a possible barrier to endogenous repair.[47,48] Astrocytic activation and collagen deposition in the perilesional area were comparable, and there was similar ischemia-induced brain atrophy in 4KO and WT mice. These data suggest that the different temporal patterns of M2 phenotype markers displayed by 4KO mice in the subacute phase (7 days) indeed do not affect the lesion evolution and neuronal viability in the chronic stage (5 weeks) after ischemic injury.

This study casts light on the proposed neuroprotective function of Th2 cytokines, showing that elimination of the Th2 response by combined IL-4, IL-5, IL-9, IL-13 genetic deletion does not affect the outcome of ischemic stroke up to 5 weeks post-injury. Our findings indicate that Th2 cytokines are not an essential neuroimmunological cue able to drive the brain's ischemic outcome. Thus, immunomodulation in the brain still needs to be further investigated to establish how and when it may offer therapeutic opportunities.

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Disclosures

None.

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