TOLL-LIKE RECEPTOR 2 DEFICIENCY AFFECTS POST ISCHEMIC LESION EVOLUTION AND CELL APOPTOSIS

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Objective

Microglial activation following ischemic/reperfusion injury in central nervous system is associated with a strong induction of the innate immune receptors such as TLR2. Using in vivo imaging and the TLR2 reporter mice, we recently demonstrated that microglial activation/TLR2 response may persist several months after initial stroke. At present, the role of TLR2 in brain response to ischemic injury is not well understood. Therefore, the aim of this study was to investigate the effects of microglial activation and the innate immune response, in particular Toll-like receptor 2 on infarction size and post-ischemic cell apoptosis in time dependent manner.

Results

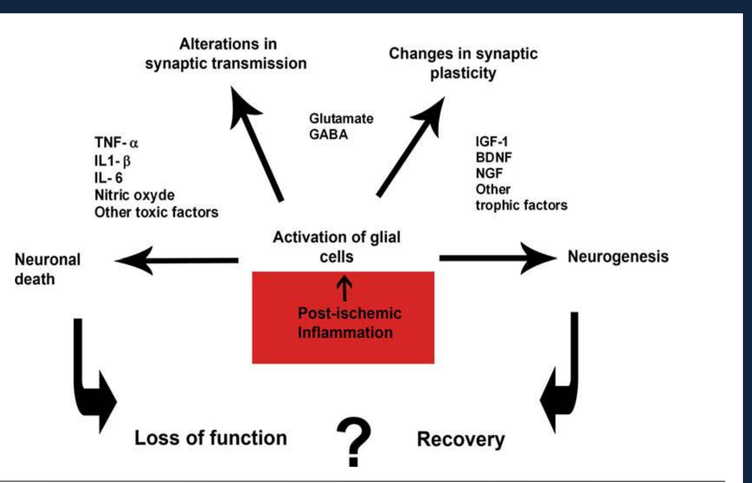
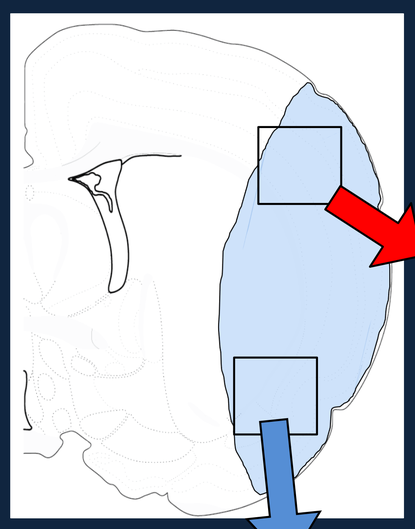


Figure 1:
Simplified presentation of the key players and potential modulators of post-ischemic inflammation and the brain plasticity and recovery



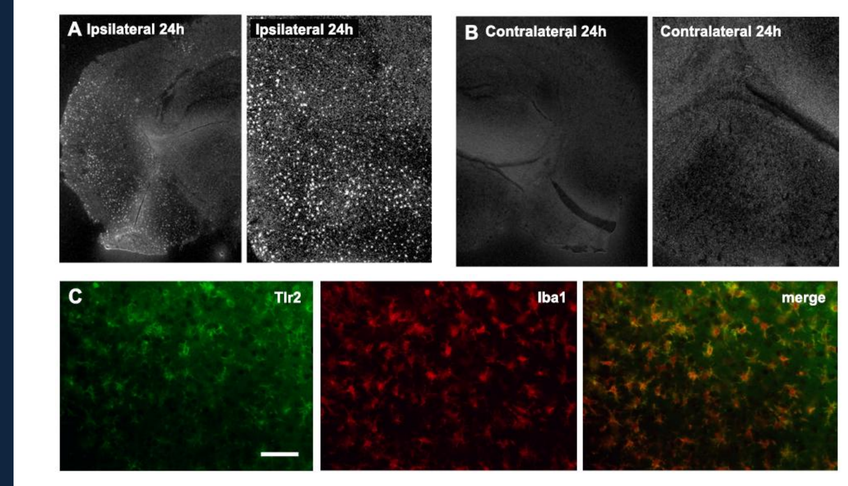


Figure 3:
(A,B) Marked induction of TLR2 signal in ischemic area was observed 24h after transient MCAO, compared to contralateral, non-ischemic area where no TLR2 signal was observed. (C) Almost all of the TLR2 positive cells were colocalizing with Iba1 staining, suggesting that TLR2 receptors are mainly expressed on microglial cells.
Scale bar: 50 μm

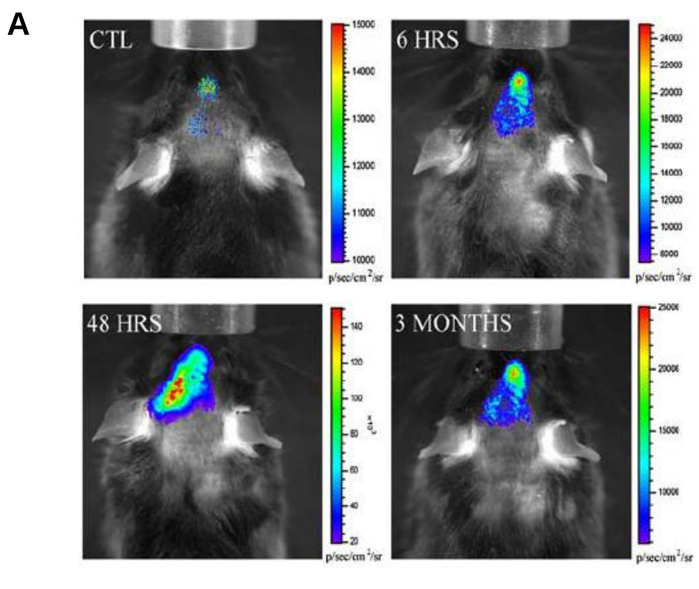
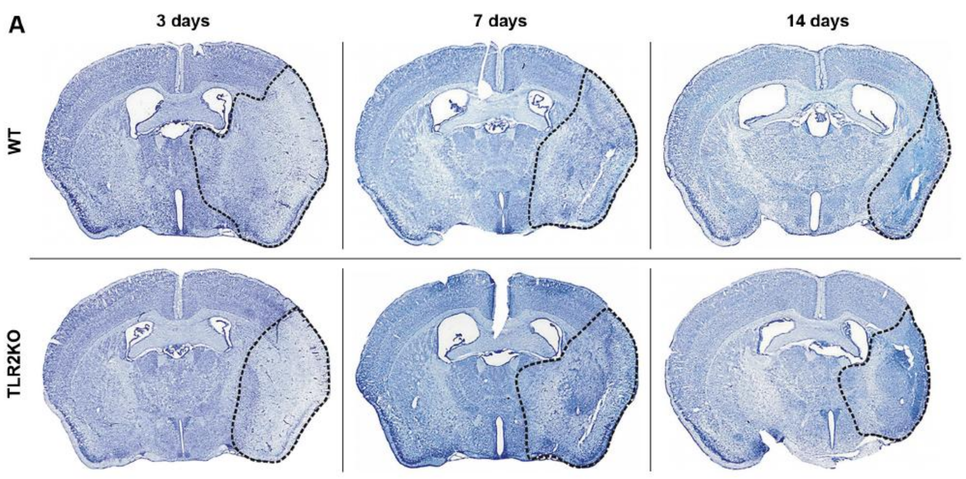


Figure 2:
(A) Representative photographs of single transgenic TLR2-luc/gfp reporter mouse imaged at different time points following 1-h transient MCAO. Real-time imaging of TLR2 response/microglial activation reveals a long-term induction of inflammatory signals (up to 3-months post-ischemia).
(B) Plot of the data obtained by measuring the luciferase activity at the site of ischaemic lesion (in photon per second, p/s). The solid blue line shows the TLR2 induction after MCAO: note the strong induction of the promoter in the first week after MCAO with a peak of expression at 48h, as well as smaller peak of expression at 1 month.



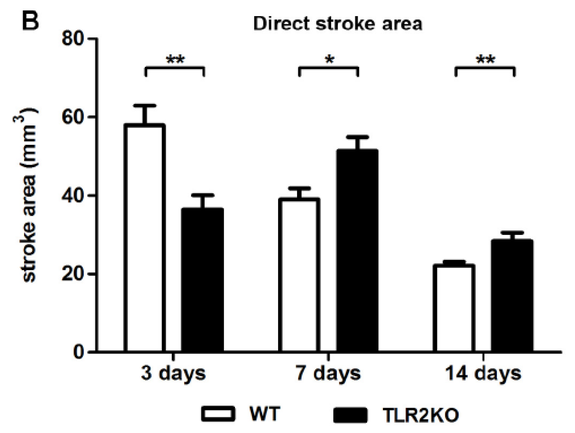
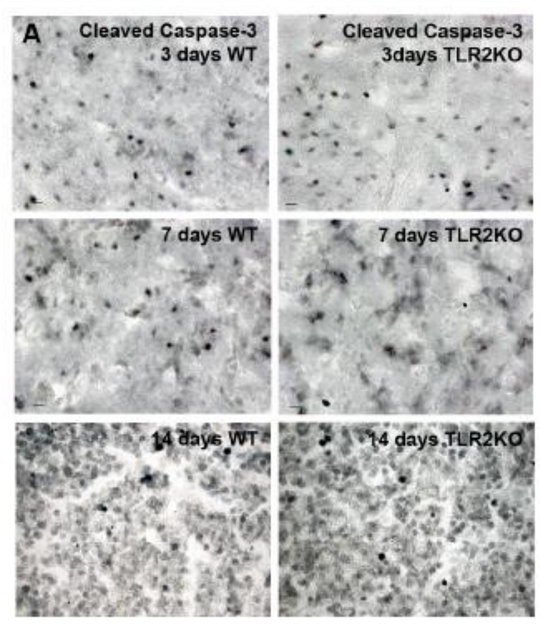


Figure 4:
(A,B) Analysis of the size of ischemic lesion (cresyl violet staining) showed marked difference in size of stroke area between groups of WT and TLR2 deficient mice. Compared to WT mice, size of direct stroke area in TLR2 deficinet mice was reversed, showing smaller stroke area in early time point (3d) and larger stroke area in later time points. Note the larger stroke area in TLR2 -/- mice in 7and 14 days timepoints compared to group of WT mice.



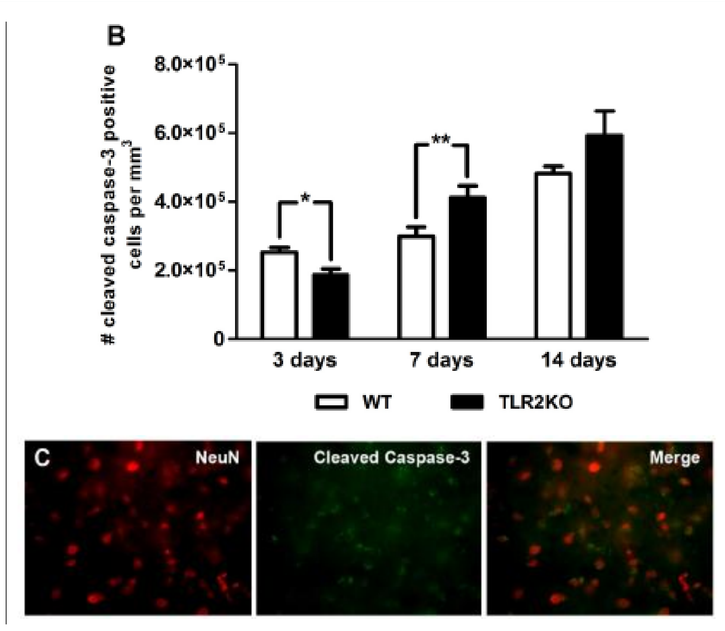


Figure 5:
(A,B) Analysis of the cell death with anti-caspase-3 antibody.
In early time point (72hrs) higher number of apoptotic cells in WT compared to TLR2 deficient mice.
Interestingly, in later time points (7 and 14 days), statistically higher number of caspase-3 positive cells was observed in group of TLR2 mice compared to group of WT mice.
Bar = 10μm
(B)*p < 0,05, **p<0.01

Conclusion

Altogether, these results show that evolution of ischemic lesion and processes of cell death are TLR2 dependant. Importantly, these processes act in time-dependent manner, which must be considered in future planning of experiments which will try to explain effects of TLR2 deficiency on processes of post-ischemic inflammation.

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