Depletion of Microglia by Mac-1-SAP Enhances Ischemia-Like Neurodegeneration

(continued from page 1) monitored by cellular PI uptake (Fig. 2), as well as increased astroglial reactivity. At the same time point, the OGD lesioning induced an increase in Mac-1-positive cells in the microglia-depleted cultures, meaning that the few visible Mac-1 cells remaining after the immunotoxic treatment (and possible additional ones with staining below detection level) did, as normally, respond to the ischemia-like insult by stronger staining for microglial markers (Fig. 3). Using BrdU-labeling of dividing cells we also demonstrated lesion-induced proliferation of the few remaining microglial cells.[8] At 7 and 14 days after OGD the differences between Mac-1-SAP treated and non-treated cultures remained noticeable in terms of more neuron loss in cultures deprived of microglia, while the astroglial reactivity seemed to even out. During the same period more microglial cells appeared in the originally deprived cultures at the same time as the appearance of the cells normalized (Fig. 3 G, H). Such reoccurrence over time has also been noted for depleted cultures left to survive for an additional 2-3 weeks.

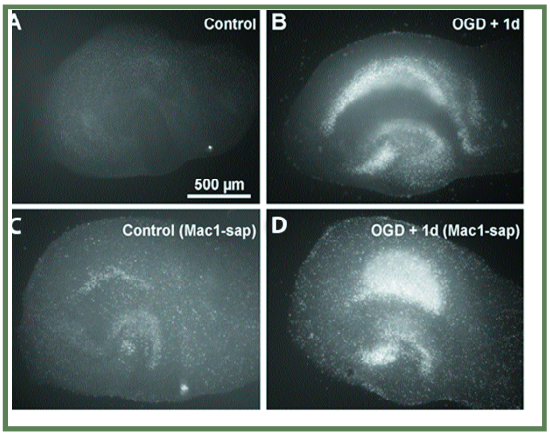
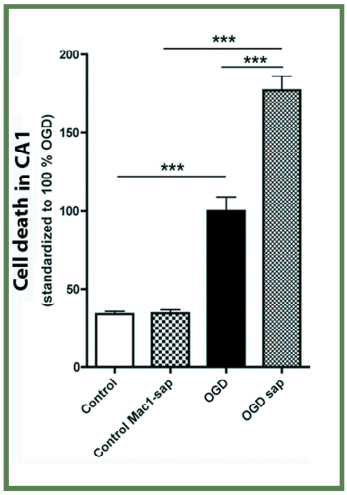


Fig. 2. Upper panel: Fluorescent micrographs of distribution and density of cellular uptake of PI in control (A, C) and OGD-lesioned mouse hippocampal slice cultures (B, D) with normal microglial content (A, B) and prelesional depletion of microglia (C, D). The OGD-induced cell death primarily affects CA1 pyramidal cells (CA1). **Lower panel:** Densitometric measurements of the cellular uptake of PI in CA1 pyramidal layer of untreated and Mac-1-SAP treated slice cultures as well as corresponding cultures 24 hours after 30 min. of OGD. Data are shown as a percentage of PI uptake standardized to OGD (n = 11-18) (*** $p < 0.001$).



Conclusions: (1) Exposure of mouse hippocampal slice cultures to Mac-1-SAP efficiently eliminates almost all microglial cells present in the cultures. (2) Mac-1-SAP treatment does not by itself induce increased neuronal cell death as monitored by cellular uptake of PI. (3) When subjected to an ischemia-like transient oxygen-glucose deprivation (OGD), Mac-1-SAP treated cultures displayed an increase in CA1 pyramidal cell death, as compared to non-treated OGD-lesioned cultures.

Perspectives: Brain slice cultures are easily accessible for microscopical inspection and addition of compounds and, as such, widely used as experimental models for CNS injury and disease and for screening of neurotoxic and neurotrophic compounds. Efficient and controlled depletion of microglial cells by Mac-1-SAP in slice cultures is a valuable tool for studies of microglial interactions with other cell types in the CNS, and microglia-mediated actions of, for example, anti-inflammatory compounds.[8]

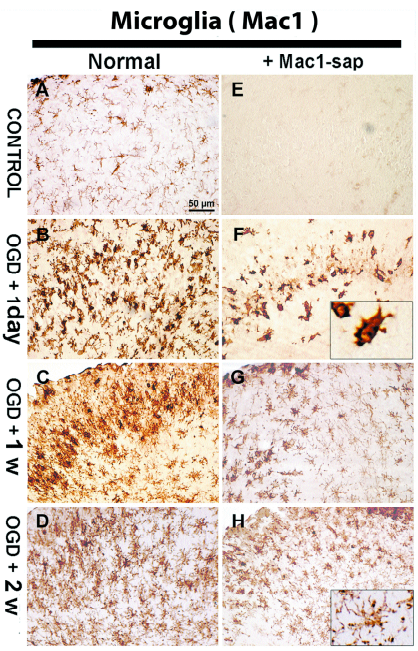


Fig. 3. Microglial depletion and reactions to 30 min OGD in CA1 area of mouse hippocampal slice cultures, visualized by Mac-1 immunohistochemistry. Treatment of 1 week old slice cultures with the Mac-1-SAP complex for 1 week dramatically reduced the number of microglial cells (A and E). One day after OGD, the number of reactive, Mac1-positive microglial cells increased in both normal (non-depleted) (B) and depleted cultures (F), followed by a normalization in morphology and staining density over the next 2 weeks, which also included the originally depleted slice cultures (E, F, G, H).

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