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Newsletter Highlights

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ADVANCED TARGETING SYSTEMS

Depletion of Microglia by Mac-1-SAP in Mouse Hippocampal Slice Cultures Enhances Ischemia-Like Neurodegeneration

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Microglial cells contribute about 12% of the cells in the brain, acting as "biosensors" for homeostatic regulation in normal and pathological conditions.[3,13] Resting microglial cells in the adult brain have thin ramified processes, but transform into a non-phagocytic activated phenotype, or a phagocytic activated phenotype when activated.[14] Upon activation, structural changes towards an amoeboid appearance occur with transformation of the normal fine processes into short coarser processes[4,14] in parallel with upregulation of normal markers like Mac-1,[12] MHC class I and II molecules[5] and secretion of cytokines like interleukin-1beta (IL-1β),[2] transforming growth factor beta-1 (TGF-β1)[6] and tumor necrosis factor alpha (TNF-α).[1] The reactions to injury also include a drastic increase in the number of microglial cells at the lesion site, due to migration and local proliferation.[10] However, it is still not clear to what extent or under which conditions activated forms of microglia exert positive or negative effects on neuronal survival.[7] Experimental depletion of microglial cells, before application of a standardized neurodegenerative insult, would, however, help clarify the protective or degenerative role of microglial cells.

Depletion of microglial cells in mouse brain hippocampal slice cultures was successfully achieved by adding a microglia-targeting toxin, Mac-1-SAP (a conjugate of saporin and a CD11b antibody) to the culture medium for 7 days (Fig. 1).[8]

For experimental testing of the effects of microglia depletion on ischemia-induced neurodegeneration, Mac-1-SAP treated and corresponding untreated hippocampal slice cultures were subjected to standardized 30-min oxygen-glucose deprivation (OGD), mimicking transient cerebral ischemia and preferentially causing the more susceptible CA1 pyramidal cells to degenerate. The resulting neuronal cell death was quantified by densitometric measurements of the cellular uptake of the fluorescent intercalating agent propidium iodide (PI) in the CA1 area[11] (Fig. 2). Other Mac-1-SAP treated and control cultures were stained immunohistochemically for neuronal, astroglial and microglial markers 1, 7 or 14 days after OGD (Fig. 3).

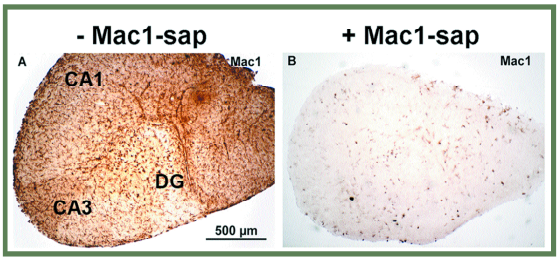

Fig. 1. A: Microglial cells in mouse hippocampal slice culture, visualized by immunohistochemical staining for Mac-1/CD11b. **B:** Immunotoxic depletion of microglial cells in mouse hippocampal slice culture treated with Mac-1-SAP for 7 days. Following this treatment almost all microglial cells are killed. Only a few remaining cells with abnormal morphology express the microglial marker Mac-1. Corresponding observations were made in slice cultures stained for other microglial markers such as tomato lectin (not shown). Scale bar valid for both A and B.

Twenty-four hours after OGD, the Mac-1-SAP treated, microglia-depleted hippocampal slice cultures displayed, in comparison to correspondingly OGD-exposed control cultures, a significant increase in CA1 pyramidal cell death, as *(continued on page 6)*