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Microglia activation during neuroregeneration in the adult vertebrate brain

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ABSTRACT

Brain injury and neuronal loss leads to an inflammatory response, which is initiated by the innate immune system. To what extent this immune response is beneficial or detrimental for neurogenesis and regeneration is unclear. We addressed this question during regeneration of dopamine neurons in the adult salamander brain. In contrast to mammals, ablation of dopamine neurons evokes robust neurogenesis leading to complete histological and functional regeneration within four weeks in salamanders. Here we show that similarly to mammals, ablation of dopamine neurons causes microglia activation and an increase in microglia numbers in the ablated areas. Furthermore, microglia numbers remain elevated compared to the uninjured brain at least six weeks after ablation. Suppression of the microglia response results in enhanced regeneration, concomitant with reduced death of dopamine neurons during the regeneration phase. Thus neuroregeneration is not dependent on the absence of an innate immune response, but the suppression of this response may be a means to promote neurogenesis in the adult vertebrate brain.

Injury in the brain elicits an inflammatory response and activation of the resident microglia population, which may orchestrate neuronal replacement [29]. However, how the innate immune system relates to brain injury and neurodegenerative diseases remains uncertain. Acute inflammation after brain injury can result in increased neuronal loss and reduced neurogenesis [6,9,13,14,17,21]. On the other hand, growing evidence suggest that the effect of the inflammatory response is context dependent, and inflammation may support neurogenesis and recovery by for example promoting expression of neurotrophic factors or facilitating the migration of progenitors to lesion sites [1,2,10,16,18,27,28].

Naturally occurring regeneration in non-mammalian model organisms, such as in teleost fish and newts provides an opportunity to understand the role of the immune system and restoration of brain tissue. A microglia response was observed in teleost fish during neuronal regeneration in the corpus cerebelli and spinal cord [3,30]. Microglia have been identified in the normal and regenerating salamander CNS [22,26]. However the effect of the microglia response on regeneration has remained unclear.

To start addressing the relationship between immune cells and the regeneration of individual neuronal subtypes in the adult brain, we examined the dynamics of microglia activation during regeneration of dopamine neurons in the brain of the adult aquatic salamander, the red spotted newt. The newt brain responds uniquely among adult vertebrates to 6-OHDA-mediated ablation, as it regenerates all the lost neurons within four weeks [4,5,23,25]. Regeneration in newts leads to complete restoration of tissue architecture and locomotor performance, and is fuelled by the activation of quiescent ependymoglia cells, which divide and subsequently produce neurons [4,23].

Here we reveal that ablation of dopamine neurons causes a specific activation of microglia in the regions of neuronal loss, and the number of microglia remain elevated compared to the non-ablated brain throughout the entire regenerative phase. Inhibition of the microglia response by administrating dexamethasone, a glucocorticoid and a potent anti-inflammatory agent [6–8,11,17], caused enhanced neuronal regeneration as defined by the increase in the number of tyrosine hydroxylase (TH) positive neurons, which was paralleled with reduced death of TH+ neurons.

Adult red spotted newts *Notophthalmus viridescens* were maintained as described earlier [4].

Immunostainings and TH quantification were performed as described earlier [4], for detecting the ionized calcium binding adaptor molecule 1 (IBA1), and galectin 3/MAC2 (MAC2) a rabbit anti-IBA1 (1:1000, Wako) and rat anti-MAC2–biotin (1:200 Cedarlane) were used, respectively. TUNEL staining was performed as described earlier [23]. Microglia cells were quantified in the ventricular zone and in the parenchyma within a 200 μm radius from the TH+ cell populations. All images were acquired on a LSM 510 Meta laser microscope with LSM 5 Image Browser software (both Carl Zeiss MicroImaging Inc.). Projections of z series were processed using LSM 5 Image Browser software.

Abbreviations: TH, tyrosine hydroxylase; IBA1, ionized calcium binding adaptor molecule 1; MAC2, Galectin-3/Mac-2; PCNA, proliferating cell nuclear antigen.

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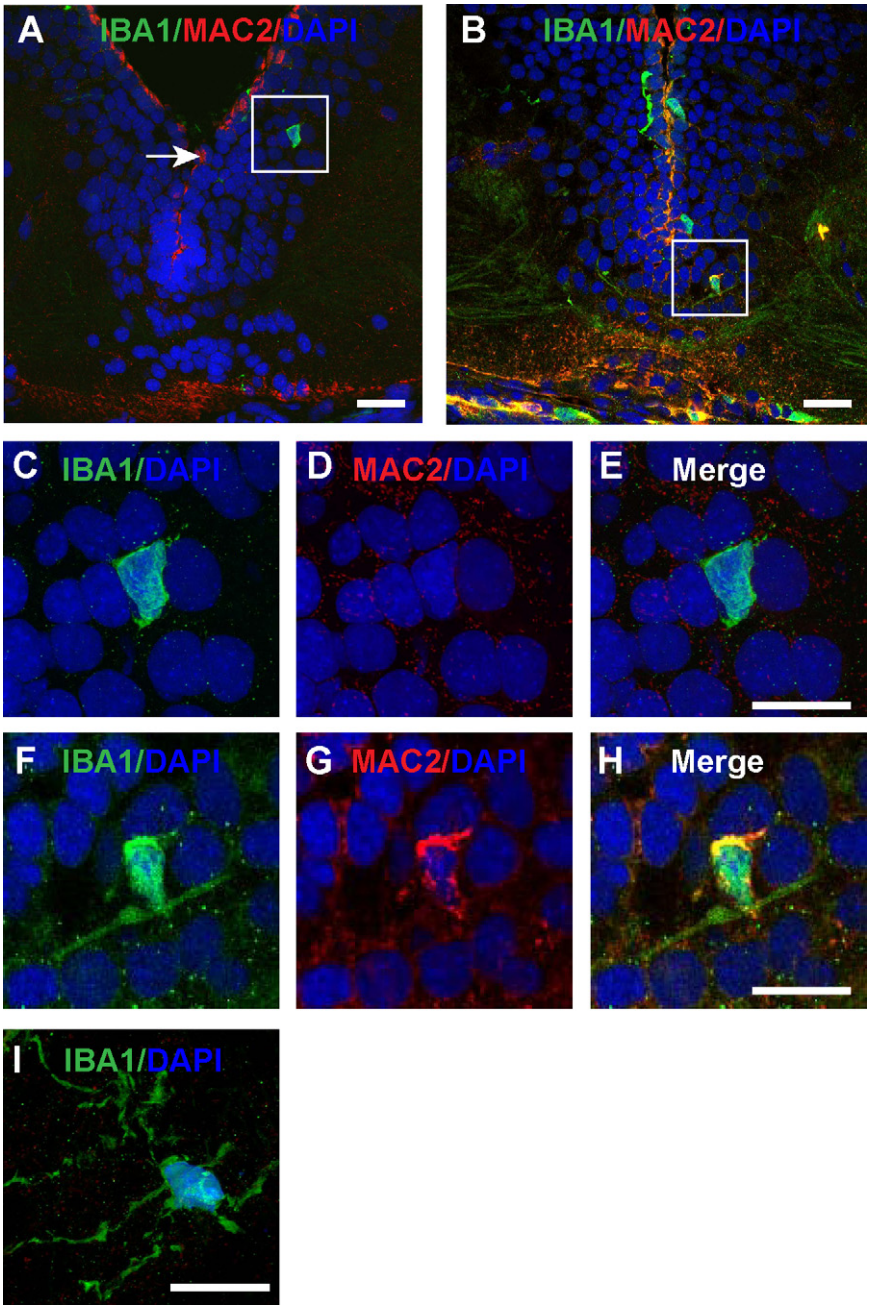

Fig. 1. Morphology and localisation of microglia in the uninjured newt brain. Majority of microglia in uninjured tissue are IBA1+ (green) and MAC2− (red) (A), with an irregular morphology and no protrusions. Box indicates region shown in (C)–(E). Arrow indicates non-specific ventricular labelling. A fraction of microglia are IBA1+ (green)/MAC2+ (red) (B), box indicates region shown in (F)–(H). Less frequently highly ramified IBA1+ (green) microglia were observed (I). Panels (C)–(E) and (I) are projections of confocal z series Scale bar 50 microm.

Ablations were performed as described earlier [23].

Dexamethasone (Sigma, 2 mg/kg) was dissolved in 10% ETOH. 100 μl dexamethasone or vehicle was injected intraperitoneally once daily between day 3 to day 14 or day 3 to day 7 after either 6-OHDA injection or sham injection. 50 μl BrdU (Sigma 20 mg/kg) was administered intraperitoneally at 12 h intervals, between day 3 to day 14.

First we identified microglia in the newt brain using antibodies against the IBA1 and MAC2. IBA1 marks resting as well as activated microglia, whereas MAC2 marks activated microglia [15,18]. We detected cells expressing IBA1 (Fig. 1A and C–E), which normally were devoid of MAC2-expression. Except for one animal, in which most IBA1+ cells with a range of morphologies expressed MAC2 (Fig. 1B and F–H), in all other cases ($n = 5$) IBA1+ cells were devoid of MAC2 expression. A majority of the IBA1+ cells displayed irregular morphology, however a subset of IBA1+ showed highly ramified extensions, characteristic of resting microglia (Fig. 1I) [19].

Next we determined the dynamics of microglia number and activation after ablation of dopamine neurons in the dien-and mesencephalon. Fig. 2A–C shows the ablation and regeneration of TH+ neurons following 6-OHDA-injection. The lowest number of TH+ cells was observed 3 days after 6-OHDA-administration (degeneration phase; Fig. 2D), after which the number of TH+ cells gradually increased and reached normal levels within 4 weeks (regeneration phase; Fig. 2D) [23]. We found that ablation of dopamine neurons caused a persistent increase in the number of microglia cells compared to sham ablation (Fig. 2E). This increase was due to local proliferation of microglia as revealed by double immuno-