Different antigen expression and co-expression of radial glia in macrophages/microglia in rat spinal cord injury model

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Introduction: Microglia and macrophages are implicated in spinal cord injury (SCI). However, their precise role in vivo remains unclear. Recently radial glia (RG) have been recognized to be one of the neural progenitor cells.

Materials and Methods: Male SD rats (n=25) with an average body weight of 312g were used to prepare the model of SCI. After T11 and T12 laminectomy, a metal rod weighing 30 g was placed onto the dorsal spinal cord for 10 min. Animals were sacrificed at 1, 4, 8, and 12 weeks after surgery. Frozen cross-sections at 5 mm rostral were then prepared and examined. The antibody ED1 was used as a marker for macrophages. Antibody Iba1, which has been found to be highly and specifically expressed in microglia. Double immunohistochemistry was performed using ED1 and Iba1. Triple immunohistochemistry staining was performed using the above-described procedure and DAPI. Triple labeling immunohistochemistry was performed using ED1 and 3CB2 (a RG specific marker) antibody. The sections were incubated in DAPI solution. Data analysis: Image analysis was performed with Image-Pro Plus software. Semi-quantitative evaluation was performed to evaluate differences of immunoreactive ED1 and Iba1 expression.

Results: Strong activation of ED1 immunoreactivity (IR) at 4 weeks after contusion in both the ventral white matter (VWM) and the dorsal funiculus (DF) was observed. Many ED1-positive cells became round in shape with granules in the cytoplasm. Iba1 IR was detected throughout the white matter and gray matter in sham-operated rats. Iba1-positive cells showed the morphology of resting microglia. At 4 weeks after SCI, many round, big ED1-positive cells with phagocytic morphology were detected at the site of the DF. Some cells were ED1-positive but Iba1-negative, or Iba1-positive but ED1-negative. These results shows that ED1-positive macrophages and Iba1-positive microglia existing separately. Triple staining of ED1, 3CB2, and nucleus shows at 4 weeks after injury, many round and big ED1-positive cells with phagocytic morphology were detected at the site of VWM; at the same time, we also detected some ED1-positive cells with long fibrous processes. 3CB2-positive cells showed elongated radial morphology from pial side. Some ED1-positive cells with processes were co-expressed with 3CB2-positive cells.

Discussion: Damaoiseaux proved that monoclonal antibody ED1 binds specifically to a single-chain glycoprotein of activated macrophages. Ito and Imai indicate that Iba1 is expressed in microglia alone. This result may explain the results of the double-staining, indicating that some Iba1-positive cells were also ED1-positive, while others were ED1 negative. Some of ED1 positive cells observed had elongated fibrous shapes. These cells also expressed 3CB2 and showed the same radial morphology. As we have previously reported, 3CB2 positive cells are also stained by nestin antigen. Nestin positive cells might differentiate to astroglia, oligodendrocyte, and neurons at appropriate niches.

We were able to separately stain macrophages and microglia following SCI in the present study. Since some ED1-positive cells were also stained 3CB2, this may suggest that macrophages may have some lineage to RG.


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