A novel method for rapid isolation of “untouched” neurons by immunomagnetic depletion of non-neuronal cells

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Introduction

Dissociation of neural tissue results in cell suspensions that comprise a wide variety of different cell types. Selective targeting and purification of a particular cell type is often the prerequisite for experimental *in vitro* studies. A straightforward and gentle method for the isolation of a desired cell type is magnetic cell separation by MACS® Technology. This method is based on MicroBeads, i.e., superparamagnetic particles conjugated to highly specific antibodies against a particular cell surface antigen. To facilitate the isolation of neurons from neural tissue by MACS Technology, we have screened for markers which are either exclusively expressed or absent on neuronal cells, and identified a cell surface antigen that is expressed on the majority of non-neuronal cells but not on neurons. An antibody specific for the new marker allowed the depletion of non-neuronal cells and thus the isolation of “untouched” neurons from mouse brain with high purity.

Results

1 Expression of the new non-neuronal cell surface marker by different neural subpopulations

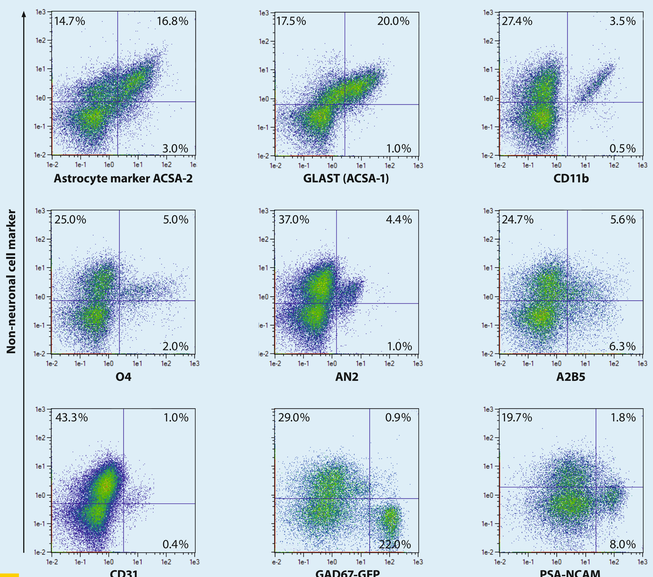


Figure 1

Expression of the non-neuronal cell–specific antigen on different neural cell types was analyzed by flow cytometry. Mouse brain tissue derived from P1 or P2 mice was dissociated with the Neural Tissue Dissociation Kit (P) or (T). Single-cell suspensions were stained with an antibody detecting the new non-neuronal cell marker and antibodies specific for astrocytes (ACSA-2 and GLAST (ACSA-1)), microglia (CD11b), oligodendrocytes (O4), oligodendrocyte progenitors (AN2), glial precursor cells (A2B5), endothelial cells (CD31), and neuronal progenitor cells (PSA-NCAM).

GABAergic neurons were identified by their GFP expression in brains derived from GAD67-GFP mice.[1] No staining of the non-neuronal cell marker was observed on PSA-NCAM$^+$ neuronal progenitors or GAD67-GFP$^+$ GABAergic neurons. In contrast, astrocytes, oligodendrocytes, a fraction of glial precursors as well as microglia were clearly detected by the antibody specific for the new marker, providing evidence that the marker is specifically expressed on non-neuronal cells (fig. 1).

2 “Untouched” isolation of neurons by magnetic cell separation

Whole brain tissue or different brain regions obtained from early postnatal mice were dissociated into single-cell suspensions using the Neural Tissue Dissociation Kit – Postnatal Neurons in combination with the gentleMACS™ Dissociator. Non-neuronal cells within the cell suspension were magnetically labeled using a biotinylated antibody specific for the new cell marker in combination with Anti-Biotin MicroBeads (Neuron Isolation Kit, mouse). Magnetically labeled non-target cells were retained within a MACS Column placed in a MACS Separator and depleted, while the highly enriched “untouched” neuronal cells were collected in the flow-through (fig. 2). The original cell fraction (fig. 3, left panel) as well as the neuronal cells (fig. 3, middle panel) and the depleted non-neuronal cells (fig. 3, right panel) were stained with an antibody specific for the non-neuronal cell marker to determine the purity of the unlabeled target cells. The neuronal cell fraction showed a high purity (98.6–99.9%) and only a low percentage of contaminating non-neuronal cells. High purities were obtained regardless of which brain region was used for cell isolation, e.g., cortical hemispheres, cerebellum, hippocampus, midbrain/diencephalon, and olfactory bulb – all comprising varying percentages of non-neuronal cells. The separation yielded 1 – 1.8 × 10^6 neuronal cells per P1-4 whole mouse brain (n=8). Target cells showed a viability rate of >90%.

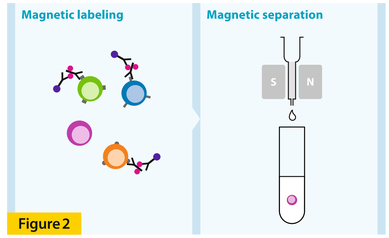


Figure 2

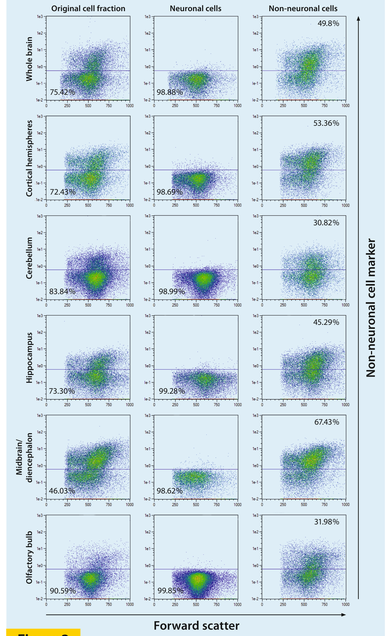


Figure 3

3 Expression of the new non-neuronal cell marker during embryonic and postnatal development of the mouse brain

Percentages of cells expressing the non-neuronal cell marker and markers specific for astrocytes (GLAST (ACSA-1)), oligodendrocytes (O4), and microglia (CD11b) were determined during embryonic and postnatal development. Single-cell suspensions obtained from dissociated embryonic as well as early postnatal whole mouse brain tissue were stained with the respective fluorochrome-conjugated antibodies and analyzed by flow cytometry. As shown in figure 4, the percentage of cells expressing the non-neuronal cell marker was very low until E16, and then increased in parallel to the glial markers GLAST (ACSA-1), O4, and CD11b. This indicates that the non-neuronal cell marker was not down-regulated during neurogenesis, but up-regulated when cells differentiated into the glial lineages. Thus, the new marker can be used to detect non-neuronal cells during mouse embryonic and postnatal development, thereby identifying the neuronal cells.

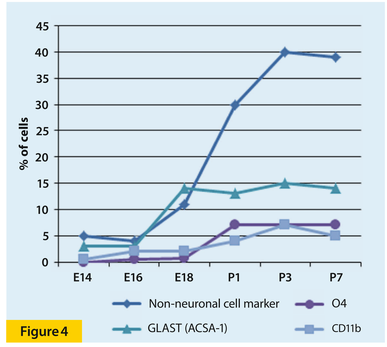


Figure 4

4 Cultivation of neurons isolated by MACS® Technology

Cortical hemispheres and hippocampi obtained from postnatal-day-1 mice were dissociated into single-cell suspensions by using the Neural Tissue Dissociation Kit – Postnatal Neurons. Non-neuronal cells were depleted as described above. Isolated neurons and non-neuronal cells were cultured in MACS Neuro Medium supplemented with MACS NeuroBrew-21. After 2 days *in vitro* cells were fixed and stained with antibodies detecting different neural cell types to determine the purity of cultured neuronal cells. The cultured neuronal cell fraction showed mainly neurons as determined by using MAP2- and NeuN-specific antibodies in case of cortical (fig. 5A–D) and hippocampal cells (fig. 5E–H). Almost no contaminating GFAP$^+$ and GLAST (ACSA-1)$^+$ astrocytes (fig. 5A, E and fig. 5B, F, respectively), or MBP$^+$ oligodendrocytes (fig. 5C, G) were detected. In contrast, the non-neuronal cell fraction showed a high number of glial cells (fig. 5I–K, M–O) and only few neurons (fig. 5L, P). The scale bar represents 20 µm.

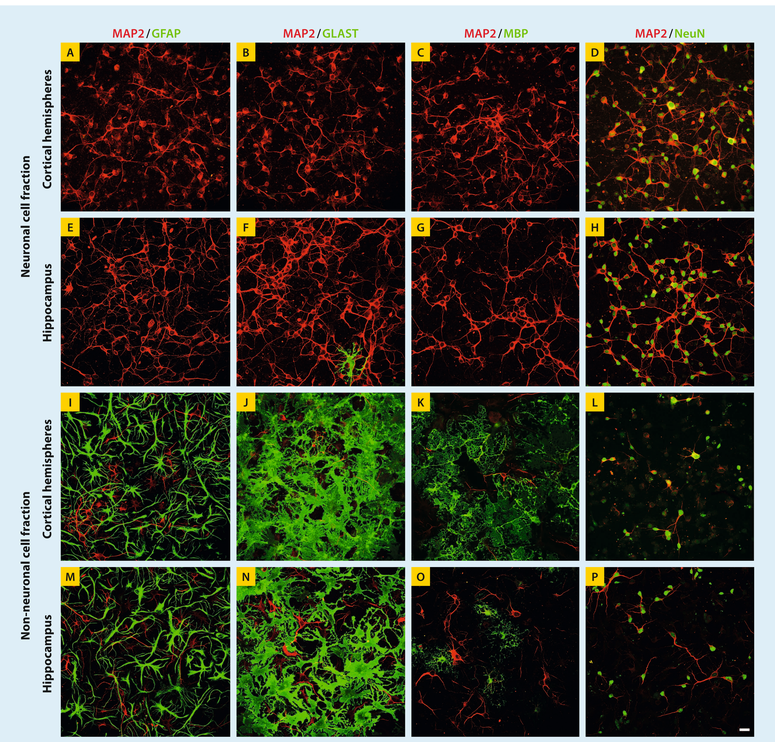


Figure 5

Conclusion

- We identified a new cell surface marker that is specifically expressed on non-neuronal cells, including astrocytes, oligodendrocytes, microglia, endothelial cells, and fibroblasts, but not on neurons.
- Untouched neurons can be isolated from whole mouse brain or different mouse brain regions by depletion of non-neuronal cells with an antibody specific for the new non-neuronal cell surface marker.
- Depletion of non-neuronal cells using the Neuron Isolation Kit, mouse is a straightforward, reliable, and fast method for the isolation of neurons without the need for transgenic mice.
- Isolated neurons were cultured in MACS Neuro Medium supplemented with MACS NeuroBrew-21. Cultures showed only minimal contamination by non-neuronal cells.
- Further analyses will reveal the nature and function of the antigen in the context of its exclusive expression on non-neuronal cells.

References

1. Tamamaki, N. *et al.* (2003) J. Comp. Neurol. 467: 60–79.