Phenotyping primary microglia from the normal and multiple sclerosis brain
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Research question and background
Immunohistochemical studies have indicated subtle changes in inflammatory and neuroprotective pathways in normal appearing white matter (NAWM) of multiple sclerosis (MS) patients, particularly in microglia. Still, little is known about the functional phenotype of microglia in MS NAWM, though it may hold valuable clues about mechanisms for lesion development. Therefore, we set out to unravel the phenotypic and functional properties of microglia in NAWM of MS patients to define their activation status in more detail.

Methods and tissues used
We studied microglia from NAWM obtained post-mortem from control subjects (n=25) and MS patients (n=21) for their phenotype ex vivo and their immune responsiveness in vitro, using a microglia isolation method that omits culture and adherence, which was recently developed in our group.¹

Results and conclusion
By flow cytometry, ex vivo microglia from MS NAWM displayed elevated CD45 levels and increased size and granularity, but were distinct from autologous choroid plexus macrophages by absent or low expression of additional markers, in particular CD206. Flow cytometric analysis of microglia from NAWM of 3 controls and 4 MS patients showed alterations in levels of Fc-gamma receptors in MS. In primary microglia from a bigger sample of subjects, analysis of Fc-gamma receptors by quantitative PCR indicated a significant increase in mRNA levels of the inhibitory CD32b isoform in MS NAWM. Despite their changed activation status, microglia from MS NAWM were unresponsive to LPS in vitro. Notably, culture with dexamethasone led to an impaired induction of the inflammation-limiting cytokine CCL18 in microglia from MS NAWM compared to those from control NAWM. Together, these data demonstrate that microglia in MS NAWM are in an alerted state, but display features of immunosuppression. Thus, the activation status of microglia in NAWM of MS patients likely reflects a response to ongoing neuroinflammation, which coincides with upregulation of immunoregulatory molecules to prevent full activation and damage to the vulnerable milieu.