Immune cells in the diffusely abnormal white matter of multiple sclerosis

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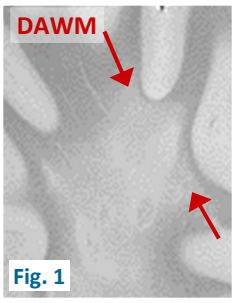
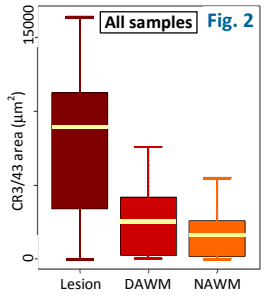


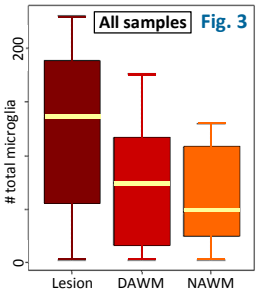
BACKGROUND: Multiple sclerosis (MS) diffusely abnormal white matter (DAWM) is mildly hyperintense and typically periventricular with a poorly defined boundary[1] (Fig. 1). DAWM is present in ~20% of MS cases. Post-mortem histological studies have reported reduced myelin and axons and gliosis[2-5] in DAWM. However, it is not known whether the pathological process leading to DAWM is similar in nature to lesions with an immunopathogenic etiology, or if it is instead a neurodegenerative process. Microglia are activated by even small pathological changes. During activation microglia initially upregulate Class II major histocompatibility complex (MHC), a molecule that is normally not detectable in the central nervous system (CNS) and has an important role in the presentation of antigen to T-cells during immune and autoimmune responses. If the activating stimulus results in CNS damage microglia also undergo a change in shape from small cell bodies with long branching processes ("ramified microglia") to larger ameboid-shaped macrophages.

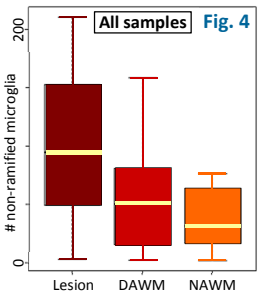
PURPOSE: Given the potential prognostic importance of non-lesional white matter abnormalities in disability and clinical progression, further histological studies are warranted to fully elucidate the pathology of DAWM. To determine if the immune system may be important in the pathogenesis of DAWM we examined T-cells, B-cells and activated microglia in MS post-mortem brain tissue.

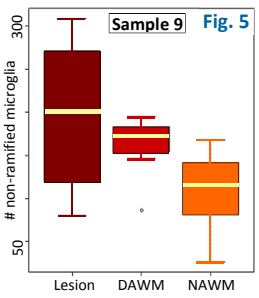
METHODS: MR Experiments & Analysis: Twelve slices of formalin-fixed brain from 8 MS cases (mean age = 60 yrs (35-76 yrs); 5F/ 3M; 3 primary progressive/5 secondary progressive, mean disease duration = 30yrs (14-38 yrs) were examined with a 32-echo T_2 relaxation measurement at either 1.5T (n=8, GE, TR head coil, TR/TE=3000/10ms, matrix = 256x256, 3mm thick, in plane resolution = 586µm x 586µm) or 7T (n=4, Bruker, TR/TE=1500/6.673ms, matrix = 256x256, field of view=6 cm, 1mm thick, in plane resolution = 234µm x 234µm). Regions of interest (ROIs) were outlined in 82 lesions, 86 DAWM and 190 normal appearing white matter (NAWM) areas on the first echo of the T_2 relaxation data for each brain sample. **Histological Staining & Analysis:** Brain slices were paraffin-embedded, sectioned at 10 µm-thickness, and stained immunohistochemically for CD3 for T-cells, CD20 for B-cells and Class II MHC (CR3/43 antibody) for activated microglia. ROIs were mapped onto histology sections and five high resolution fields were photographed per ROI for each of the 3 histochemical stains (40x objective on a Leica DM4000B brightfield microscope with a Leica DFC408 camera). CD3 and CD20 cells were counted with manual tagging. CR3/43 stained area was quantified using automatic segmentation followed by manual correction. Microglia were counted and classified as "ameboid macrophage", "ramified" or "intermediate" (for those which had features of both) with manual tagging. Image Pro Plus 5.1 was used for all image analysis. **Statistical Analysis:** Tissues were compared using a t-test for each histologically quantified marker across all samples and within individuals samples ($p<0.05$).

RESULTS: CD3 T-cells: On average, no significant differences were observed for CD3 count between any tissue type (mean count across samples (standard error, SE) for lesion =7.9 (2.1), DAWM=3.4 (0.6), NAWM=3.0 (0.6) with p value = 0.08 (lesion vs. NAWM), 0.10 (lesion vs DAWM), 0.66 (DAWM vs. NAWM)). 2 samples showed significantly increased CD3 count in lesions relative to NAWM. **CD20 B-cells:** No significant differences were observed for CD20 count between any tissue type, in any sample (mean count (SE) for lesion =1.1 (0.2), DAWM=0.7 (0.1), NAWM=0.77 (0.08) with p value = 0.13 (lesion vs. NAWM), 0.09 (lesion vs. DAWM), 0.81 (DAWM vs. NAWM)). **CR3/43 Microglia:** On average, CR3/43 positively stained area was significantly higher in lesions relative to DAWM and NAWM ($p=0.01$ and 0.005), but no significant difference between DAWM and NAWM was observed ($p=0.4$) (Fig. 2). However, variation across samples was observed, with 3 samples showing significantly more staining in DAWM than NAWM. On average, the total number of microglia and total number of non-ramified microglia (round/ameboid plus intermediate) was significantly higher in lesions than NAWM ($p=0.04$, $p=0.02$), but no group differences were observed between DAWM and NAWM, or lesion and DAWM (Figs. 3,4). Three samples showed significantly higher total and non-ramified microglia in DAWM than NAWM (Fig. 5).Visual inspection revealed morphological differences in microglia shape between lesion, DAWM and NAWM for some cases (Fig. 6).







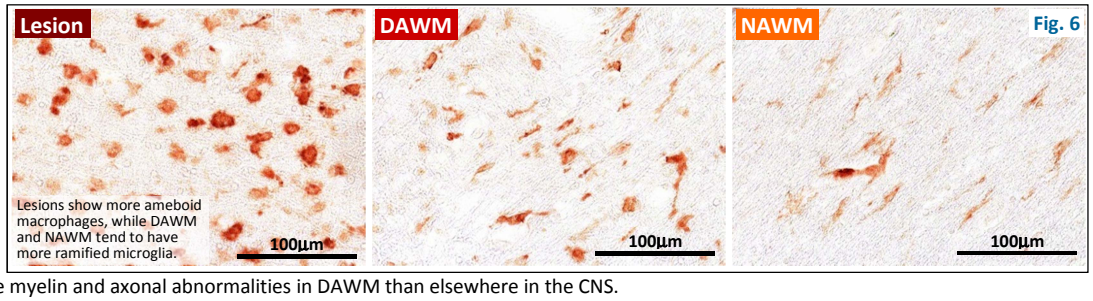


DISCUSSION

In general, similar levels of microglial activation, B-cells and T-cells were observed in DAWM and NAWM. While previous studies have reported myelin and axonal abnormalities in DAWM relative to NAWM, these abnormalities appear not to correlate with the degree of microglia activation or the presence of B or T-cells.

CONCLUSION

The presence of T-cells, B-cells and activated microglia does not discriminate between DAWM and other white matter regions in MS. The findings are consistent with an immune/autoimmune process affecting all areas of MS white matter and suggest, at least based on the presence of its cellular components, that the immune response does not have a more important role in the pathogenesis of the myelin and axonal abnormalities in DAWM than elsewhere in the CNS.



ACKNOWLEDGEMENTS: MS subjects & their families. Funding support from the MS Society of Canada, Women Against MS, the endMS Research & Training Network.

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