Multiple system atrophy (MSA) is a sporadic neurodegenerative disease with unknown etiology, involving Parkinson’s disease, Autonomic Failure, and Olivopontocerebellar Atrophy (OPCA). To define a possible relationship between phagocytic immune cells and loss of Purkinje cells in MSA/OPCA, we measured the incidence of microglial/macrophages in close association with Purkinje cells and/or their axons and dendrites. Using immunocytochemistry methods on cerebellar sections from MSA and control brains from the New York Brain Bank at Columbia University, we identified Purkinje cells and their processes using anti-calbindin, and microglia/macrophages using anti-CD68. Calbindin labeled Purkinje cell body, axon, and dendritic profiles were counted in the Purkinje cell layer, adjacent granule cell layer, and molecular layer respectively, as were calbindin stained profiles double labeled with CD68. The relative area of calbindin labeled axons and axons double labeled with CD68 in white matter tracts adjacent to the granule cell layer was measured using computer assisted image analysis. MSA/OPCA showed significantly fewer Purkinje cell bodies (ANOVA, p<0.001), dendrites (p=0.044), and axons (granule cell layer, p=0.002; nerve tracks, p=0.027) compared to controls. A significantly greater density of microglial/macrophage staining associated with calbindin stained profiles occurred only in the nerve tracks (p=0.043) compared to controls. Thus, although MSA/OPCA shows fewer Purkinje cell related profiles in all foliar areas, no clear association with phagocytic cells is evident except in the white matter tracts. This is compatible with other work showing an association between phagocytic cells and myelin loss in fiber tracts in MSA/OPCA. The decrease in Purkinje cell profiles does not appear to be due to an autoimmune interaction with phagocytic cells aimed at Purkinje cell antigens, rather it may related to loss of myelin caused by interactions between phagocytic cells and myelinating oligodendrocytes.

Methods

ICC: Immunohistochemistry double labeling analysis with anti-calbindin (marker for Purkinje cells and their processes in cerebellar nerve tracts; DAB) and anti-CD68 (leukocyte marker expressed on activated microglia/macrophages; AP) was done on human post-mortem cerebellar tissue. Formalin -fixed, paraffin-embedded tissue sections isolated from the cerebellum of MSA and control patients, were obtained from the New York Brain Bank (NYBB) at Columbia University. Five control patients were compared to five MSA patients.

Data Analysis:

- Tissue sections were visualized using a Nikon microscope with digital SPOT camera and quantitatively analyzed for anti-calbindin and anti-CD68.
- We measured the percent area of Calb alone and double labeled with CD68 immunoreactivity within the cerebellar nerve tracts of three non-overlapping areas per slide (one tissue sample per slide) using Image J software (NIH). Computer Assisted Image Analysis (CAIA) was performed to separate tissue elements from the background staining, using Adobe Photoshop CS4.
- Texas Red and normal light photos were overlaid in Photoshop CS4, and each color profile was separated (Calb alone, CD68 alone and double labeled) making 3 separate photos. Each photo was opened in ImageJ and threshold for the appropriate color was measured for each photo.
- For quantification of calbindin and CD68, in the cerebellar nerve tracts, the percentage of the total area exhibiting positive immunoreactivity, for double and single labeling, was calculated.
- SPSS software (SPSS Inc, Chicago, IL), was used for One-Way Analysis of Variance, ANOVA, to detect percent area differences in control and MSA patients.
- Calbindin labeled Purkinje cells and their processes were quantified by counting Purkinje cell bodies, initial processes in cerebellar nerve tracts; DAB) and anti-CD68 (leukocyte marker expressed on activated microglia/macrophages).

Objective

To define a possible relationship between phagocytic immune cells and loss of Purkinje cells and their fibers in the cerebellar nerve tracts in MSA/OPCA.

Results

- The current study takes a detailed look at the involvement of microglia/macrophage-like phagocytic cells (CD68), on the degeneration of Purkinje cells and their associated dendrites and/or axons.
- MSA/OPCA patients showed significantly fewer calbindin associated Purkinje cell profiles in all foliar areas (molecular layer, granular layer and Purkinje cell layer), and significantly fewer stained calbindin fibers in the cerebellar nerve tracts.
- MSA patients had significantly higher levels of phagocytic like cells associated with calbindin fibers in the cerebellar nerve tracts and significantly higher level of phagocytic like cells present in all areas of the cerebellum.
- There was no clear association with phagocytic cells and Purkinje cell profiles in all of the foliar areas. These results are compatible with other work in our lab showing an association between phagocytic cells and myelin loss in fiber tracts in MSA/OPCA.
- Thus, the decrease in Purkinje cell profiles does not appear to be due to an autoimmune interaction with phagocytic cells aimed at Purkinje cells and there initial axons and dendrites.
- Rather the interaction between phagocytic cells and Purkinje cell axons distally in the nerve tracts, perhaps involving damage to myelinating oligodendrocytes, may underlie Purkinje cell loss in MSA.

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References

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