Purkinje Cell Association with Microglia/Macrophages in Degenerating Cerebellum of Multiple System Atrophy Patients

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GLEAMS Great Lakes Environmental and Molecular Science Center

Abstract

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 tracts, p=0.027) compared to controls. A significantly greater density of microglia/macrophage staining associated with calbindin stained profiles occurred only in the nerve tracts (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.

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.

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/macrophage cells; 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 overlayed in Photoshop CS4, and each color profile was separated (CaBP 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 axonal segments and/or dendritic processes. The expression of microglia/macrophage-like cells was measured by counting the number of calbindin positive Purkinje cell profiles directly associated with CD68 in the Purkinje cell, molecular and granular layers.

Results

Figure 1

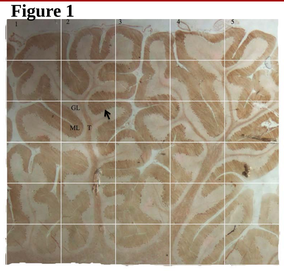

Figure 1: Control cerebellar tissue stained using ICC methods. Numbered grids were used for randomized selection of representative areas; granular layer (GL), molecular layer (ML), cerebellar nerve tracts (T) and black arrow indicates Purkinje cell layer.

Results

Figure 2

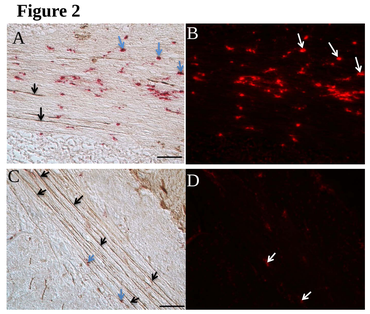

FIGURE 2: Both bright light and Texas Red photos of CaBP and CD68 show a decrease in CaBP fibers (A; black arrows) and an increase in double labeled CD68 cells (A; blue arrows, B; white arrows), in the cerebellar nerve tracks of a MSA patient. Similar photo from a control patient showing a significant increase in CaBP fibers (C; black arrows) and a significant decrease in double labeled CD68 cells (C; blue arrow, D; white arrows); bar equals 40µm.

Table 1

Antibodies	N	Control Mean	MSA Mean	P Value
CaBP	5	10.09±2.05	3.77± 1.13	0.027
CaBP;CD68	5	0.63± 0.13	1.62 ± 0.39	0.043
Total CD68	5	1.06± 0.24	2.44±0.49	0.035

TABLE 1: Mean average % area of total stain (N=5). Abbreviations CaBP= calbindin; CD68= microglia/microphage-like cells. Control patients had significantly higher levels of CaBP and MSA patients had significantly higher levels of CD68 double labeled with CaBP in the cerebellar nerve tracks; MSA patients had significantly higher levels of total CD68 present in the cerebellar nerve tracks, granular, molecular and Purkinje cell layers.

Figure 3

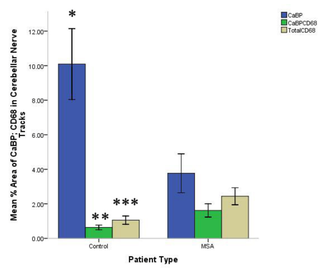

FIGURE 3: A quantitative analysis of Calbindin alone (CaBP; blue bar); microglia/macrophage CD68:CaBP double labeled profiles (green bar); total CD68 present in MSA vs. control patients (tan bar). Measurements were taken in the cerebellar nerve tracts (N=5). Control patients had significantly more CaBP (*), where MSA patients had significantly more CD68:CaBP profiles (**); and significantly higher levels of total CD68 (***) (tan bar) in the cerebellum.

Figure 4

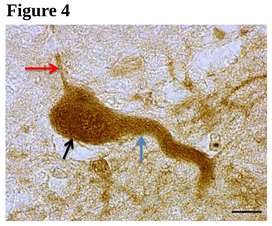

FIGURE 4: ICC staining of a cerebellar Purkinje cell body and immediate processes positive for CaBP, axon (red arrow), cell body (black arrow) and dendrite (blue arrow); bar equals 40µm.

Table 2: Purkinje Cell Profile Counts in the Cerebella of MSA and Control Patients

ICC Test analysis	N	Control Mean	MSA Mean	P Value
Purkinje Cell Body	5	122 ± 10.27	53.4± 9.178	0.001
Purkinje Cell Body & Dendrite	5	70.6±8.95	41±8.58	0.044
Purkinje Cell Body & Axon	5	17±2.47	3.8±1.5	0.002
Purkinje Cell Body; CD68	5	3.2±0.73	4.8±2	0.476
Purkinje Cell Body & Axon; CD68	5	0.6±0.4	1.2±0.8	0.521
Purkinje Cell & Dendrite; CD68	5	1±0.44	2±0.89	0.347

TABLE 2: Control vs. MSA ICC analysis of CaBP and CD68 in the granular layer, molecular layer and Purkinje cell layer. Mean values represents the average number of Purkinje cell profiles. (N=5)

Results

Figure 5

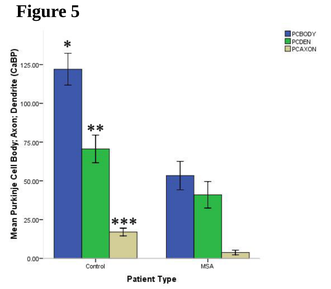

FIGURE 5: CaBP and CD68 ICC analysis in the cerebellum of control vs. MSA patients. The mean number of Purkinje cell bodies (blue bar) was significantly (*) higher in control patients compared to MSA; in addition, control patients had significantly (**) higher numbers of initial dendritic segments (green bar) and significantly (***) higher initial axonal segments (tan bar) in the molecular and granular layer, respectively, compared to MSA (N=5; *p≤.05).

Figure 6

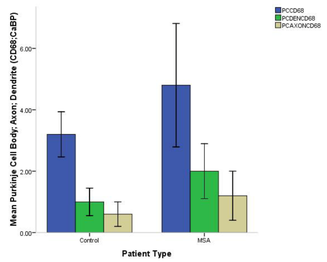

FIGURE 6: The mean number of Purkinje cell bodies (blue bar), initial axonal (tan bar) and dendritic segments (green bar) directly associated with CD68 was insignificant ($p \geq 0.05; N = 5$) in control patients compared to MSA. Different from the association of CD68 with CaBP fibers in the cerebellar nerve tracks, there was no significant association of CD68:CaBP in the cerebellar folia.

Conclusions

- The current study takes a detailed look at the involvement of microglia/macrophage-like phagocytic cells (CD68), on the degenertaion 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.

Acknowledgements

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References

1. Langerveld *et al.* (2007). Gene expression changes in postmortem tissue from the rostral pons of multiple system atrophy patients. *Movement Disorders* 22(6): 766-777