Altered Human Pancreatic Ribonuclease in Multiple Sclerosis

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INTRODUCTION

In a previous proteomic analysis of cerebrospinal fluid (CSF), we found that human pancreatic ribonulcease (RNase 1) levels were decreased in multiple sclerosis (MS). RNase 1 is a protein capable of degrading both single- and double-stranded RNA and thus may enhance viral clearance. In addition, RNase 1 induces dendritic cell maturation and activation. In this study we further characterized RNase 1 activity in MS CSF and brain tissue.

DESIGN AND METHODS

CSF Sample Collection

CSF was obtained from a total of 42 patients, comprising 15 primary progressive (PPMS), 15 secondary progressive (SPMS) and 12 control subjects. All samples were obtained with informed consent from patient volunteers at the International Multiple Sclerosis Management Practice (IMSMP) through an IRB-approved protocol. CSF was obtained via aspiration of the access port of implanted pumps (Medtronic) or by standard lumbar puncture using aseptic precautions. All samples were coded and CSF aliquots frozen at -70°C until analysis.

RNase 1 Protein Western Blot Analysis

CSF was concentrated 15-fold by centrifugation using centrifugal filter units with a molecular weight cut-off of 3 kDa (Centricon, Millipore). Concentrated CSF proteins were separated by SDS-PAGE in a reducing environment and Western Blotted. A standard curve was generated for each blot using human recombinant RNase 1 (Bachem). Blots were probed using a RNase 1 polyclonal antibody (IgTech) followed by a peroxidase-conjugated rabbit antibody (Sigma). Bands were visualized using ECL Advance Western Blotting Detection Kit (GE Healthcare). Intensity of band staining was analyzed by densitometry using Kodak Molecular Imaging Software.

Brain Tissue

Four MS brain sections, each with a lesion, were immunostained and examined for RNase 1 expression. Brain tissue specimens were acquired from the Human Brain and Spinal Fluid Resource Center sponsored by NINDS/NIMH, National Multiple Sclerosis Society, Department of Veterans Affairs (Los Angeles, CA).

Immunohistochemistry

Immunohistochemical staining was performed using the avidin/biotin method on frozen and paraffin-embedded tissue sections (5μm). Briefly, after deparaffinization and rehydration, sections were blocked in 1X PBS/10% horse serum for 1 hour at room temperature and incubated with an RNase 1 antibody (IgTech) for 16 hours at 4°C. A biotinylated secondary antibody coupled with streptavidin-horseradish peroxidase was then used with 3,3-diaminobenzidine tetrahydrochloride (DAB) as a substrate. For myelin basic protein (MBP, Chemicon International) staining, appropriate anti-human and anti-mouse antibodies were used. Positive and negative controls were included with each experiment.

RNase 1 positive cells were counted for the four MS brain samples each containing a lesion. For each brain section, three images were analyzed from a lesion area and three images were analyzed from a normal appearing white matter region, with the images captured serially at 100 μm apart. Lesion areas were confirmed by anti-MBP immunostaining.

Statistical Analysis

The Mann-Whitney test was used for all statistical analyses, except for the Western Blot analysis comparing PPMS, SPMS and control groups, for which ANOVA was used. All analyses were conducted using GraphPad Software.

RESULTS

CSF RNase 1 Quantitation

CSF RNase 1 levels were extrapolated off a standard curve using densitometric analysis as shown for a representative gel in Figure 1.

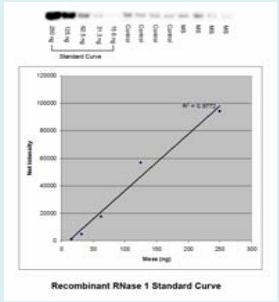

Figure 1. Western Blot of cerebrospinal fluid RNase 1 levels with quantification using known standard curve values.

CSF RNase 1 Results

MS patients (n=30) had significantly decreased CSF RNase 1 levels (p=0.0011) in comparison to control patients (n=12). Subgroup analysis of CSF RNase 1 levels also showed significant differences between PPMS, SPMS and control patients (p=0.002). The decrease in CSF RNase 1 levels was most evident in PPMS versus controls (p=0.0012), and less so in SPMS versus controls (p=0.0205). Although PPMS RNase 1 CSF levels were lower than SPMS levels the results did not attain statistical significance (Figure 2).

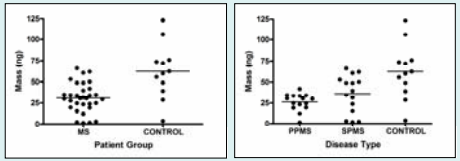

Figure 2. Scatter plot graph showing RNase 1 protein levels measured by quantitative Western Blot are decreased in the CSF of MS patients (p=0.0011) as analyzed by Mann-Whitney and significantly different in PPMS, SPMS and control subjects (p=0.002) as analyzed by the Kruskal Wallis test.

Human MS Brain RNase 1 Immunoreactivity

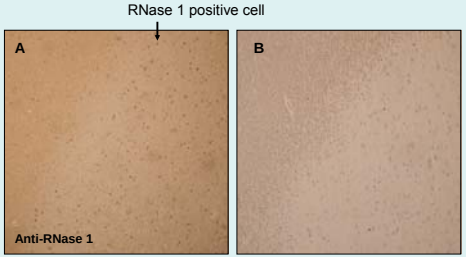

Figure 3. Increased intracellular RNase 1 immunostaining in areas of demyelination in MS brain as viewed by 100X light microscopy.

By immunohistochemistry, RNase 1 positive cells were increased in MS plaques. The intracellular increase of RNase 1 (Figure 3A) coincided with areas of demyelination (Figure 3B).
RNase 1 in normal appearing white and grey matter of MS brains did not differ from control brains (not shown).

There was a significant difference between the number of RNase 1 positive cells in areas of demyelination as compared to non-demyelinated white matter (p=0.0142) (Figure 4).

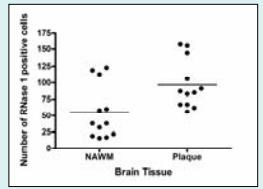

Figure 4. Significant increase in intracellular RNase 1 immunostaining in areas of demyelination in MS brain versus normal appearing white matter areas (p=0.0142) as analyzed by Mann-Whitney.

CONCLUSIONS

1. RNase 1 appears decreased in CSF of progressive MS patients as determined by previous proteomic analysis and recent quantitative Western Blot analysis.
2. PPMS patients exhibit a more profound decrease of RNase 1 in CSF than SPMS patients.
3. The number of RNase 1 positive cells are increased in areas of demyelination as shown by immunohisto-chemistry.
4. Future studies may determine whether this CSF decrease in RNase 1 allows for CNS viral persistence or activates dendritic cells in MS.

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