



INCREASED FETUIN-A LEVELS IN ACUTE DEMYELINATING LESIONS IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS AND MULTIPLE SCLEROSIS

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INTRODUCTION

Fetuin-A (Alpha2-Hermans-Schmid glycoprotein, AHSG) is a biomarker of interest in multiple sclerosis based on proteomic analysis of cerebrospinal fluid (CSF). Fetuin-A is a negative acute phase protein, and is involved in a variety of biological functions such as regulation of calcium metabolism and osteogenesis, opsonization, and immune regulatory function. In the CNS, functionally, fetuin-A is implicated in increasing blood-brain barrier (BBB) permeability by activating matrix metalloproteinases. Indeed in patients with active MS, significantly higher levels of CSF fetuin-A are seen in comparison to levels in patients with inactive disease. To determine whether these CSF fetuin-A abnormalities could be extended to pathological finding, we investigated the distribution of fetuin-A in MS brain lesions and in experiment autoimmune encephalomyelitis (EAE), an animal model for MS.

METHODS

Induction and Evaluation of EAE

The protocol for induction of EAE in 20 C57BL/6 mice with myelin oligodendrocyte glycoprotein (MOG) peptide fragment 35-55 (Synthetic Biomolecules) was followed as described in Stromnes, IM, and Goverman, JM. EAE clinical score was assessed on a 0 to 5 scale as follows: grade 0, normal; grade 1, tail paralysis; grade 2, tail paralysis and hind-limb weakness (waddling gait); grade 3, hind limb paralysis; grade 4, hind limb plus forelimb paralysis; grade 5, moribund state. Spinal cords of C57BL/6 mice were obtained at day 28 after EAE induction at the peak of disease when the EAE score was between 3 and 4 and used for immunohistochemical staining. Five control mice were studied for comparison.

MS and Normal Brain Tissue

Fetuin-A protein expression was examined in 7 active and inactive MS brain samples in plaques, and from areas of normal appearing white and grey matter. Ten control brains were similarly examined. Brain tissue specimen was 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

We immunostained for fetuin-A in regions of acute inflammation in the spinal cord of 20 mice induced to have EAE and compared for intensity of staining in non-inflamed regions of the same spinal cord and in 5 normal mice spinal cord. In addition, 7 active and inactive brain lesions of MS were immunostained for fetuin-A. 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 polyclonal anti-human (R&D Systems and Biovondor Laboratories) or anti-mouse Fetuin-A antibody (Santa Cruz Biotechnology Inc.) 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) staining, appropriate anti-human and anti-mouse antibodies were used. Luxol fast blue (LFB) staining using standard protocol was used in frozen and paraffin-embedded human and mouse tissue. Hematoxylin and eosin (H&E) were used as routine nuclear and cytoplasmic counterstains. Positive and negative controls were included with each experiment.

RESULTS

Fetuin-A Immunostaining in EAE

Fetuin-A immunostaining in EAE mice spinal cords correlated strongly with all areas of acute cellular infiltration and demyelination (Figure 1,2). In other spinal cord regions of EAE mice many of small interneuron's and large motor neurons strongly immunostained with fetuin-A (Figure 3). By contrast, immunostaining for fetuin-A was not detected in unaffected spinal cord tissue except for neurons of EAE mice; in control mice spinal cord only scattered and light neurons immunostaining with fetuin-A was observed (Figure 3).

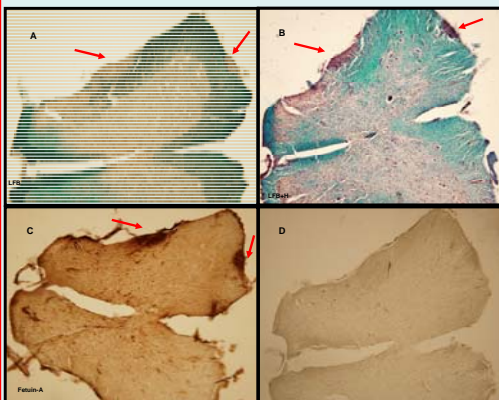


Figure 1: Increased fetuin-A immunostaining in active EAE plaques in flash frozen sections as viewed by 10X light microscopy. A) Demyelination; B) Cellular Infiltrates; C) Fetuin-A; and D) Control staining

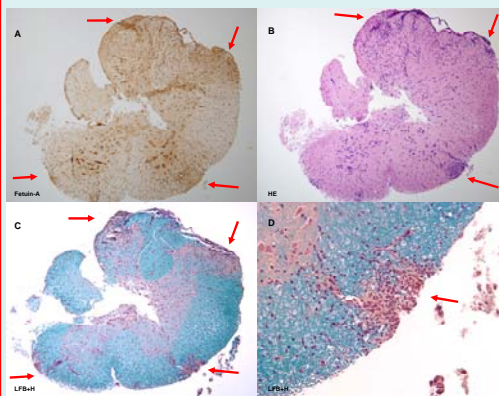


Figure 2: Increased fetuin-A immunostaining in active EAE plaques in paraffin sections. A, B, C 10X, D 40X.

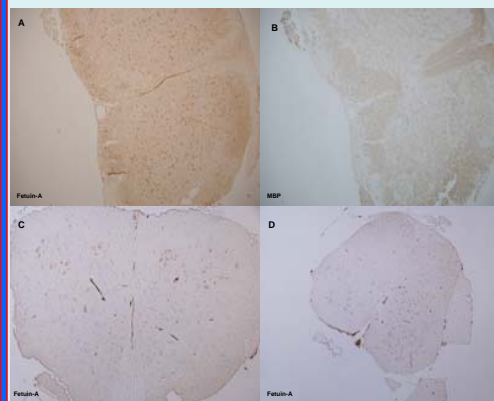


Figure 3: A) increased fetuin-A immunostaining in active EAE plaques and many neurons; B) adjacent section A staining for MBP. C and D normal mice spinal cord staining for fetuin-A 10X.

Human MS Brain Fetuin-A Immunostaining

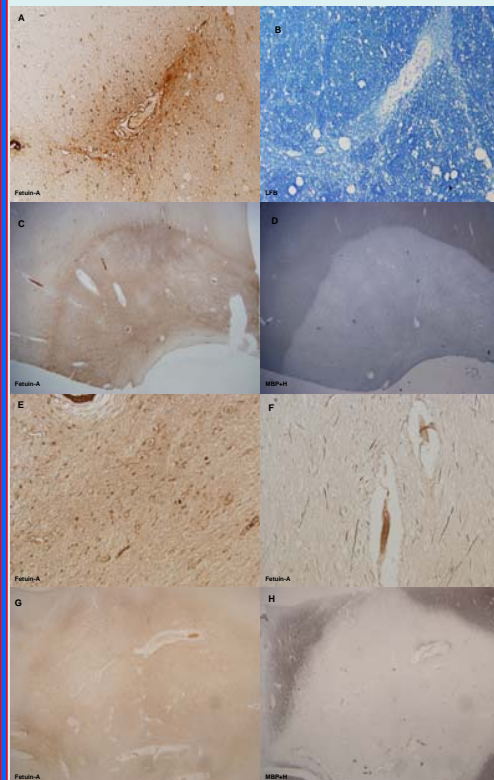


Figure 4: Increased fetuin-A in active MS patients brain.

Human MS Brain Fetuin-A Immunostaining

In MS human brain, fetuin-A expression was increased in all MS plaques examined (Figures 4 and 5). The areas of increased fetuin-A coincided with the areas of demyelination. Fetuin-A immunostaining in human brains was most prominent in active peri-vascular lesions characterized by lymphocytic infiltration and demyelination. By comparison, fetuin-A immunostaining was consistently less intense in demyelinated chronic lesions (Figure 5). In non-plaque areas, the most notable immunostaining for fetuin-A was seen in the Purkinje cells of the cerebellum in MS brains. Fetuin-A in normal appearing white and grey matter of MS brains as well as in control brains, was not increased above background levels.

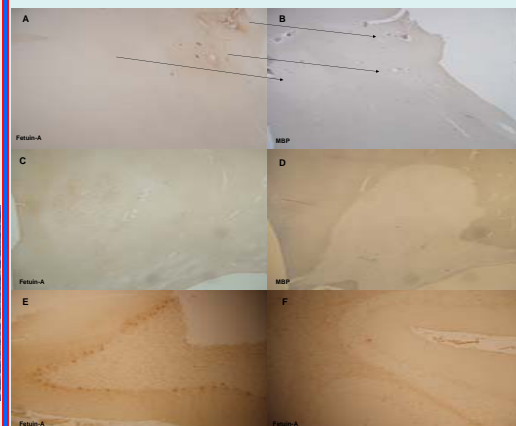


Figure 5: Fetuin-A in chronic and active patient plaques (A-D) and cerebellum (E), compared with normal human cerebellum (F). 10X

CONCLUSIONS

- Previously, CSF fetuin-A levels are significantly elevated in patients with active disease by comparison to the levels seen in stable MS patients.
- By immunohistochemistry, in EAE mice, fetuin-A is uniformly and abundantly present in all areas of acute cellular infiltration and demyelination.
- In other spinal cord regions of EAE mice, fetuin-A expression levels were dramatically increased by many neurons, compared with normal mice spinal cord.
- In MS brains, fetuin-A is specifically increased in all MS plaques and in many Purkinje cells. Similarly with EAE, fetuin-A positive-staining in human brain most prominent in active lesions.
- These novel pathological findings support a role for fetuin-A in the development of new lesions in demyelinating diseases and may represent a biomarker of active disease.
- The pathophysiological significance of our findings remains to be determined. Fetuin-A is known to activate metalloproteinases and may result in enhanced matrix turnover and increased blood-brain barrier permeability. Also, fetuin-A is an antagonist of TGF-β and thus may promote inflammatory activity.

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