Subject Name: [redacted] **Date: June 4, 2013**

Serum Test for Neuronal and Glial Autoantibodies

Report

Sera obtained from [redacted] and from healthy adults (controls) were assayed for the level of autoantibodies against nervous system-specific proteins associated with:

1. Neurogenesis, i.e., neurofilament protein (NFP), microtubule associated protein-2 (MAP-2), tau proteins; and tubulin, a protein present in all tissues, including the nervous system.
2. Myelinogensis, i.e., myelin basic protein (MBP); and
3. Gliogenesis, i.e., glial fibrillary acidic protein (GFAP) and S-100, the glial calcium-binding protein; these two proteins are specific markers for injury to the central nervous system.

Increased levels of serum autoantibodies against neuronal and/or glial proteins are indicative of injury to the nervous system.

RESULTS

Percentage change of the subject's sera autoantibodies against neuronal and glial proteins compared to healthy subjects are listed in Table 1. The results that are expressed as percentage (%) of healthy controls representing the mean values of triplicate assays of optical density arbitrary units normalized to albumin optical density level in each human serum, following Western Blotting assay of autoantibodies at 1:50 serum dilution.

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Levels of serum autoantibodies levels against all neuronal proteins; neuronal neurofilament proteins (NFP), tau proteins, myelin basic proteins (MBP), and microtubule associated proteins-2 (MAP-2), exhibited highly significant increase over controls. Also, autoantibodies against the protein associated with gliogenesis, the glial protein, GFAP and S-100 exhibited highly significant increase over controls.

DISCUSSION

Alterations of the cytoskeletal structure are prominent features in some neurological diseases and chemically induced neurological disorders. Neurofilament, tubulin and Tau proteins are major constituents of the axon and MAP-2 is mostly present in neuronal dendrites. Increased autoantibodies of these proteins in human sera are indicative of axonal degeneration. Also, increased autoantibodies against MBP are consistent with axonal demyelination. Many neurotoxicants, such as organophosphorus esters such as insecticides, as well as other insecticides, solvents and heavy metals cause neuronal cell death and axonal degeneration and over-expression of GFAP (approximately 2-fold increase), with subsequent release of neuronal, myelin, and glial proteins into circulation, followed by the formation of autoantibodies against these proteins.

Table 1. Percentage Change in Autoantibodies Compared to Healthy Subjects

Brain-Specific Protein	Neurological function	%	Significance	Location of Tissue Injury	Associated Neurological Deficits
Neurofilament protein (NFP)	Neurogenesis	555	**	Axonal degeneration	**1. Cerebral Cortex** Weakness, Deficits in: posture, locomotion and deficits in movements of fingers, speech, and facial expression
TAU Proteins (TAU)	Axonal development and axonal transport	1,673	**		
Tubulin	Axonal Transport Present in other tissues	859	**	Axonal degeneration and damage to other tissues	
Myelin basic Protein (MBP)	Myelinogensis Myelin Development	763	**	Demyelination	**2. Limbic System** Learning, memory deficits
Microtubule Associated Proteins-2 (MAP-2)	Neurogenesis Dendrite Development 0f nerve cell	2,237	**	Dendrite Degeneration	**Purkinje Cells (Cerebellum):** Incoordination, staggering ataxia
Glial Fibrillary Acidic Protein (GFAP)	Gliogenesis Forms scar in injured axons	1,772	**	Axonal Injury	Chronic axonal injury
S-100 Protein	From astrocytes in acute injury	1,306	**	Acute, traumatic Brain injury	Acute axonal injury

The values from subjects were compared to the control group using a paired t-test.
A p value < 0.05 was accepted as statistically significant. *p < 0.5, Not Significant (NS)
*p > 0.05, Significant, **p > 0.01, Highly Significant

The results show increased levels of the subject's serum autoantibodies against all of the neuronal and glial proteins that correlate well with complaints of neurological disorders. Highly significant increased autoantibodies against NFP, tau, tubulin, and MBP, and microtubule associated-2 (MAP-2) that are biomarkers for axonal degeneration in various regions of the brain, suggest severe neuronal deficits. Injury to cerebral cortex results in weakness and deficits in posture, locomotion and movements of fingers and facial expression. Damage to the hippocampal circuitry leads to cognition, learning and memory deficits. Neuronal degeneration of the limbic system and central motor system (associated with mood, judgment, emotion, posture, locomotion, and skilled movements) results in psychiatric disorders. Increased autoantibodies against the neuronal dendrite protein, MAP-2 suggest degenerative alteration of the dendrite-rich Purkinje cells of the cerebellum, resulting in gait, coordination abnormalities and staggering ataxia.

GFAP and S-100, both of which are secreted by the astrocytes, are the only two antigens studied that are not present in the peripheral nervous system, but reflect effect on the central nervous system consistent with brain injury. The finding of highly significant increase in autoantibodies against GFAP is consistent with previous reports that individuals with neuropsychiatric disorders have elevated levels of GFAP and is also consistent with axonal degeneration. S-100, a small calcium-binding protein is produced mostly by astroglial cells of the central nervous system, exerts both detrimental and neutrophic effects, depending on its concentration in brain tissues. Traumatic acute injury results in great destruction of astrocytes leading to massive release of S100 protein into plasma, whereas its levels in psychiatric disorders were slightly higher in patients competed to controls, correlating well with its neuroprotective action. Such finding was documented in neurological disorders. The high increase in autoantibodies to S-100 is consistent with recent brain injury and/or trauma that results in extensive destruction of astrocytes leading to a significant release of S-100.

CONCLUSIONS

While not diagnostic for specific disease, the presence of circulating autoantibodies against neuronal and glial proteins, at higher levels in patients who had been exposed to neurotoxic chemicals and developed neurological deficits, over that of controls, can be used as further confirmation for chemical-induced nervous system injury. The patient's serum profile of highly significant increased autoantibodies against nervous system-specific proteins of NFP, myelin basic protein (MBP), MAP-2, tubulin and GFAP, are consistent with the presence of severe nervous system injury.

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