

Whitaker



CHIPS, A VISUAL MRI RATING SCALE OF WHITE MATTER HYPERINTENSITIES  
IN MULTIPLE SCLEROSIS

**O**bjective: In multiple sclerosis (MS), 40-65% of patients have cognitive dysfunction. MRI predictors of cognitive impairment are based on sophisticated computer-generated analyses that are difficult to apply in clinical settings. This study investigated whether a new visual rating scale, the Cholinergic Pathways Hyperintensities Scale (CHIPS), devised to quantify specifically lesions affecting cholinergic pathways, can be a clinically useful tool in determining cognitive impairment in MS.

**Design:** Forty clinically definite MS patients underwent a brain MRI. Lesions were assessed with the CHIPS. Cholinergic pathway hyperintensities were measured in 10 regions on 4 axial slices and classified according to a three-point rating scale. In addition, computerized lesion volumes were obtained. All subjects underwent cognitive screening with the Brief Repeatable Neuropsychological Battery. "Low" and "High" lesion score groups were computed based on the mean of the total CHIPS score. A receiver operator characteristic (ROC)-curve was created to establish optimal sensitivity and specificity of the total CHIPS score in detecting cognitive impairment.

**Results:** "Low" and "High" lesion score groups had similar demographic, disease-related, major depression and premorbid IQ profiles. Those with "High" lesion score performed significantly worse on verbal ( $p=0.007$ ) and visuospatial ( $p=0.02$ ) memory and on global index of cognitive functioning ( $p=0.001$ ). The area under the ROC curve was 0.879 (95% CL=0.769-0.989) with a cut-off point of 18 yielding 82% sensitivity, 83% specificity and a likelihood ratio of 4.75 for cognitive impairment. There was a high correlation ( $r=0.82$ ,  $p<0.0001$ ) between total CHIPS score and total hyperintense lesion load obtained by automated computer analyses.

**Conclusion:** CHIPS is an easy, fast, affordable and clinically useful tool in detecting cognitive impairment in patients with multiple sclerosis.

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**Background:** Micro-array studies from PBMC's show a decreased expression of p53 in multiple sclerosis (MS) patients when compared to healthy controls. Gamma irradiation (IR) leads to double stranded DNA breaks, and the stabilization of P53 which in turn is dependent on the activation of two kinases, ATM and CHK2. Increase in cellular expression of P53 leads to cell death. **Objective:** We examined the effect of IR on cell viability of PBMC's in MS patients when compared to HC and determined if an increase in cell viability is associated with a decrease in the stabilization of P53. **Methods:** PBMC's were isolated from 32 MS patients and 16 healthy controls (HC) and exposed to 10Gy. Cell viability was determined by flow cytometry. Western blotting of cell lysates were performed to measure levels of P53 and phospho-CHK2. **Results:** Cell viability in PBMC's following IR was increased in MS patients when compared with HC. At 48 hours, viability was 42±3.9% in HC versus 58±4.6% in MS patients ( $p<0.05$ ), and at 72h viability was 27±5.0% in HC as compared to 43±5.9% in MS patients ( $p<0.05$ ). We examined if enhanced viability in MS patients was related to defects in stabilization of p53. In 8 of 32 MS patients but none of 16 HC, there was a lack of stabilization of p53 following IR ( $p<0.01$ ). The lack of stabilization of p53 in MS patients, was associated with a decrease in the activation of CHK2 kinase, as measured by phosphorylation of CHK2 at T68. P53 expression levels correlated with activation of CHK2 at T68 ( $R=0.82$ ,  $p<0.0001$ ). **Conclusion:** Following IR, a subset of MS patients show a resistance to death which is due to impaired activation of CHK2 and stabilization of p53. These underlying defects may render autoreactive cells more resistant to death and worsen autoimmune disease.

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ANALYSIS OF INTRATHECAL ANTIBODY PRODUCTION  
IN A MOUSE MODEL OF NEUROBORRELIOSIS

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Intrathecal antibody production (ITAb) is found in arguably all patients with MS, and remains the most consistent feature of the disease, yet very little is known about the mechanisms behind the production in the CNS of large quantities of immunoglobulin. In order to address this need, we developed a mouse model of ITAb.

Since ITAb is also a feature of Lyme neuroborreliosis (LNB), we reasoned that ITAb could be induced in a mouse model of LNB. We injected a variety of strains of *B. burgdorferi*, including American *sensu stricto* and European *garrinii* strains, by the intracerebral route into a variety of mouse strains, both in- and outbred, and determined the antibody index at various times after infection by measuring total and specific antibody in the serum and CSF. We also measured spirochetal infection of the brain and other tissues, including deep cervical lymph nodes, by 16S rRNA RT-PCR as well as CXCL13 and IgG expression by RT-PCR.

ITAb was readily produced, but was not a feature of all mouse strains and all spirochete strains. A necessary but not sufficient condition for ITAb was persistent spirochetal infection of the brain. Data on correlation of ITAb with CXCL13 and IgG expression will be presented. The data is consistent with the hypothesis that B-lymphoblasts from the deep cervical lymph nodes enter the brain, and differentiate within the brain to plasma cells, where they secrete the antibody measured in the CSF.

This work demonstrates that the mouse model of LNB provides an excellent vehicle for testing hypotheses related to the mechanisms of ITAb.

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**I**n multiple sclerosis (MS), subsequent to demyelination, oligodendrocytes initially retain the capacity to remyelinate which diminishes as the lesion ages. Factors controlling oligodendrocyte behaviour in the MS lesion remain to be fully elucidated. In the rat, a role for the CXC/a chemokine, Gro-a, has been shown in oligodendrocyte proliferation and positioning. Here we investigate expression of CXC/a chemokines and their receptors in MS. Chemokines were detected by immunohistochemistry with antibodies against IL 8, Gro a and IP 10, and their receptors CXCR1, CXCR2 and CXCR3, respectively. For *in vitro* studies, astrocytes and oligodendrocytes were cultured from 18-22 week human fetal spinal cords. Chemokine levels were measured by sandwich ELISAs in astrocyte supernates after stimulation with cytokines or LPS, RNA by northern blots, and chemokine receptor expression on oligodendrocytes was detected by immunofluorescence. IL 8, Gro a and IP 10 were found to be strongly induced on hypertrophic astrocytes at the edge of active MS lesions, but weakly expressed in silent lesions, and absent in controls. Similarly, both at the protein and RNA levels, IL-1b strongly induced production of IL 8 and Gro a by astrocytes, in a time and concentration dependent manner, while IFN-g induced IP 10. Astrocytes maintained in culture media alone produced limited amounts of IL 8, Gro a and IP 10. Constitutive expression of CXCR1, CXCR2 and CXCR3 was found on oligodendrocytes in MS and non-MS tissue. In culture, immature (A2B5+) and more mature (CNPase+) oligodendrocytes displayed surface expression of all three chemokine receptors. Based on the concurrent expression of the CXC/a chemokine, Gro-a, on hypertrophic astrocytes, and CXCR2 on oligodendrocytes at lesion margins, we propose that as in rodents, these chemokines may play an important role in the recruitment of oligodendrocytes to lesioned areas in MS. Ongoing experiments, *in vitro*, are investigating the effects of CXC/a chemokines on human oligodendrocytes. These observations have major implications for remyelination in MS.

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## PERCEIVED STRESS IS ASSOCIATED WITH THE PRODUCTION OF IL-6 AND IL-10 IN MS PATIENTS

**Objective:** Individuals with Multiple Sclerosis (MS) commonly report that periods of heightened perceived stress are associated with worsening of disease. We sought to determine if perceived stress adversely modulates the production of pro-inflammatory cytokines such that individuals with MS experience increased disease symptomatology. To do so, we examined the relationships among disease symptomatology, perceived stress, and cytokine production from peripheral blood mononuclear cells in 42 outpatients with MS and 36 normative controls. **Method:** Peripheral blood was drawn from all subjects prior to the completion of a series of psychological instruments. Stress was measured using the Perceived Stress Scale and negative mood by the Profile of Mood States. Disease symptoms were measured using the Multiple Sclerosis Symptom Checklist (MSSC). Cytokine production was induced separately by lipopolysaccharide (LPS) and a combination of phytohaemagglutinin and phorbol-12-myristate-13-acetate (PHA/PMA). **Results:** In MS subjects the induced production of interleukin (IL)-6 and IL-10 positively correlated with psychological stress, mood disturbance, and disease symptomatology. The induced production of transforming growth factor beta was found to negatively correlate with perceived stress and negative mood. Perceived stress in control subjects significantly correlated with tumor necrosis factor-alpha (TNF- $\alpha$ ), and mood disturbance with tumor necrosis factor-alpha and interferon-gamma. As well, compared to controls, MS subjects exhibited a significant four-fold increase in the production of IL-12. In relationship to level of stress, MS subjects experiencing a significantly higher level of stress exhibited a significant increase in the production of TNF- $\alpha$  in response to LPS stimulation compared to low-stress MS subjects. **Conclusions:** There is in those with MS, a pattern of IL-6 and IL-10 production that correlates significantly with perceived stress and disease symptomatology.

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