However, the molecular mechanism underlying the down-regulation of Foxp3 is not understood yet. Because IL-12p40 homodimer (p40-2) is markedly up-regulated in response to various inflammatory stimuli, the present study was undertaken to explore the role of p402 in the regulation of Foxp3 in naïve mouse splenocytes. IL-12p40-2 dose-dependently inhibited the expression of Foxp3 and CD25, but not CD4. Interestingly, this inhibition was absent in splenocytes of IL-12Rβ1 (−/−), but not IL-12Rβ2 (−/−), mice. Moreover, suppression of Foxp3 in wild type and IL-12Rβ2 (−/−) splenocytes was accompanied by production of NO. Consistently, L-NIL, an inhibitor of iNOS, and PTIO, a scavenger of NO, restored the expression of Foxp3 and CD25 in p40-2-stimulated splenocytes and p40-2 was unable to down-regulate Foxp3 and CD25 in splenocytes from iNOS(−/−) mice. Furthermore, NO, but not p40-2, was able to inhibit Foxp3 in purified CD4 + CD25 + T cells in the absence of iNOS-expressing cells. Hence, our results clearly demonstrate that p40-2 induces NO production via IL-12Rβ1 and that NO subsequently suppresses Tregs in naïve mouse splenocytes. This study, therefore, delineates an unprecedented biological function of p40-2 in the regulation of Foxp3 via IL-12Rβ1-mediated NO production. Supported by grants from NIH (NS39940 and NS48923).

PTW05-07

CUPRIZONE INDUCED-DEMYELINATION IN MICE ALTERS BRAIN EXPRESSION OF GENES INVOLVED IN ARACHIDONIC ACID METABOLISM

Bosetti, F., Palumbo, S., Toscano, C.D.

Molecular Neuroscience Unit, Brain Physiology and Metabolism Section, National Institute on Aging, NIH, Bethesda, MD, USA

Chronic feeding with the copper chelator cuprizone in mice causes oligodendrocyte death and subsequent reversible demyelination. Although the mechanism of demyelination is unknown, activation of glia is integral to the process. Since metabolism of arachidonic acid (AA) is involved in glial activation, we hypothesized that cuprizone exposure would alter expression of AA cascade genes. Mice were fed 0.2 % cuprizone in the diet for 6 weeks and then returned to a normal diet. Histochemistry with the myelin stains Black Gold and Fluoromyelin demonstrated that frank demyelination and influx of glial cells into the corpus collosum begins at week 3 and peaks at week 5. A decrease in myelin and oligodendrocyte markers, accompanied by increased expression of markers of microglia (CD11b) and astrocytes (glial acidic fibrillary protein), was evident at week one. Gene expression of cyclooxygenase-2 and 15-lipoxygenase (LOX) was also changed at week one, suggesting that these genes are either involved in or respond to early demyelination. Expression of 5-LOX was not changed during early demyelination but it peaked during week 5, when glial markers and frank demyelination also reached their peak, suggesting that 5-LOX expression is a consequence of the massive influx of inflammatory cells into the area of demyelination. Our study is the first to demonstrate that multiple enzymes involved in arachidonic acid metabolism are altered in the cuprizone model of demyelination and remyelination. These data may help to develop new therapeutic targets to treat human demyelinating diseases, such as multiple sclerosis.

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PTW05-08

CALPAIN INHIBITION OF MBP-SPECIFIC T CELLS BEFORE ADOPTIVE TRANSFER AMELIORATES RELAPSING-REMITTING EAE IN SJL/J MICE

Guyton, M.K.[1], Das, A.[2], Inoue, J.[3], Azuma, M.[3], Ray, S.K.[4], Brammachari, S.[5], Pahan, K.[5], Banik, N.L.[2]

[1]Medical University of South Carolina (MUSC), Dept. of Microbiology and Immunology, Charleston, SC, USA
[2]MUSC, Dept. of Neurosciences, Charleston, SC, USA
[3]Senju Pharmaceutical Co., Hyogo, Japan
[4]USC School of Medicine, Dept. of Pathology, Microbiology and Immunology, Columbia, SC, USA
[5]Rush University, Dept. of Neurological Sciences, Chicago, IL, USA

Multiple sclerosis (MS) is an autoimmune disease of the CNS. MS has both immune and neurodegenerative arms. Adoptive transfer (AT) of myelin basic protein (MBP)-specific T cells into naïve SJL/J mice results in a relapsing-remitting (RR) form of experimental autoimmune encephalomyelitis (EAE) that mimics the RR subtype in 85% of MS patients. Blocking mechanisms by which MBP-specific T cells are activated to induce disease may help dissect immune arm of the disease and offer novel targets for therapy. One such target is calpain since calpain is involved in T cell activation, migration of immune cells into the CNS, and degradation of axonal and myelin proteins. We tested the hypothesis that incubating MBP-specific T cells with the calpain inhibitor SJA6017 before AT would diminish ability of these T cells to elicit inflammatory response. EAE mice that received MBP-specific T cells incubated without SJA6017 (ES-0) developed classic symptoms of disease, while EAE mice that received MBP-specific T cells incubated with 10 to 100 μM SJA6017 (ES-10 to ES-100) exhibited dose-dependent reduction in clinical symptoms and relapse rate. These reductions correlated with decreases in demyelination, inflammation, axonal damage, and loss of neurons and oligodendrocytes. DNA laddering, calpain:calpastatin and Bax:Bcl-2 ratios, tBid production, and activities of calpain and caspases. Thus, these data further suggest calpain as a target for treating EAE and MS. This study was supported in part by the R01 grants from the NINDS.