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COMPETITIVE PCR MEASUREMENT OF CYTOKINES IN AUTOIMMUNE ENCEPHALOMYELITIS (EAE) AND XENOGENIC NEURAL TRANSPLANTATION. N. Tanuma, Y. Matsumoto*, Y. Ohkura, Y. Aikawa, T. Shin and T. Kojima Tokyo Metropolitan Institute for Neuroscience, Fuchu, Tokyo 183, Japan

Recent in vitro studies have suggested that cytokines play a crucial role in various pathological conditions in the central nervous system (CNS). However, it was difficult to examine cytokines in situ because of the low amount of their protein and mRNA. In this study, we measured the amount of mRNA of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), transforming growth factor-β1 (TGF-β1) and interleukin-10 (IL-10) in EAE and xenogenic CNS graft rejection using the competitive PCR technique. The preliminary study revealed that mRNA of interest in total RNA ranging from 0.1 μg to 2.0 μg was quantitated well by this method. In rat acute EAE, both IFN-γ and TNF-α mRNA increased at the early stage and rapidly decreased at the peak stage of the disease. In contrast, TGF-β1 mRNA was demonstrated in all stages and its amount was correlated well with the severity of the clinical signs. The maximal expression of IL-10 mRNA by infiltrating inflammatory cells was decteted at the early stage of EAE. In the CNS graft rejection, TNF-α, IFN-γ and IL-10 mRNA expression was generally weak compared with that in EAE. In addition, perforin mRNA was up-regulated at the early and peak stages of rejection. These results suggest that TNF-α and IFN-γ play a crucial role in the induction of EAE. On the other hand, the cytolytic protein such as perforin rather than pro-inflammatory cytokines might regulate the CNS graft rejection.

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INVOLVEMENT OF γδ T CELLS DURING CHRONIC RELAPSING EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS Y.L. Gao, A. Rajan, C.F. Brosnan and C.S. Raine.* Albert Einstein College of Medicine, Bronx, NY.

Lymphocytes expressing the γδ T cell receptor (TCR) may provide a first line of defense against highly conserved antigens such as heat shock proteins (hsp), some of which have been implicated in a number of autoimmune conditions. In this study, we assessed γδ T cells in spleen and CNS tissue from mice sampled at different stages of adoptively-transferred chronic relapsing experimental allergic encephalomyelitis (EAE) using immunocytochemistry and FACS analysis. In addition, γδ TCR and hsp 60 levels in the CNS of the same animals were correlated. Striking disease-related changes in γδ T cell levels were found in inflammatory infiltrates and the numbers of γδ T cells increased in association with disease activity. Colocalization of γδ T cells with hsp 60 was evident in lesions of all ages, particularly during chronic EAE, with γδ T cells most concentrated around the lesion edge. While FACS analysis showed that the number of γδ T cells remained low at all stages in the spleen (~2%), in the CNS, they increased to approximately 12% at the height of disease and during the recovery phase, they fell to values comparable to those found at the onset of disease (~5%). γδ T cell levels rose again (~12%) in the CNS, during the chronic phase. Of these, ~ 16% also expressed the CD8 co-receptor at the height of disease and ~28% during the chronic phase. These findings show dynamic changes in γδ T cell numbers in the CNS in this autoimmune model. In combination with co-expression of hsp 60, the results support an important role for these cells in disease progression.

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A DEMYELINATING CORONAVIRUS MHV-A59 CAUSES UPREGULATION OF INTERFERON BETA GENE EXPRESSION IN ASTROCYTES. E. Lavi*, Q. Wang, and J.A. Haluskey.
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Infection of mice with coronavirus mouse hepatitis virus strain MHV-A59 causes focal acute encephalitis, hepatitis and chronic demyelinating disease. To investigate host interferon (IFN) response to viral infection within the brain, RNA was extracted from A59-infected and mock-infected mice, RT-PCR amplified with primers specific for the various IFNs, transferred to nylon membranes and hybridized with IFN specific digoxigenin-labeled probes. A59 infection caused upregulation of IFN-beta and IFN-gama RNA (but not IFN-alpha) within the brain 1-4 days after inoculation and returned to normal at day 7 post inoculation. Infection of primary astrocyte cultures from newborn mice with A59 caused upregulation of IFN-beta RNA, but not IFN-gama or IFN-alpha. Polyclonal rabbit-anti mouse IFN alpha/beta or anti IFN beta was given to groups of 4-week-old C57Bl/6 mice at a dose of 10,000 U per one I.P. treatment, 24 hours prior to I.C. inoculation of $1LD_{50}$ of MHV-2 (a non-neurotropic strain), or MHV-A59. At various intervals post inoculation virus titers from brains and livers were determined by plaque assay, and the histopathology was analyzed by H&E staining. Treatment with pre-immune rabbit serum had no effect on disease outcome in either one of the viruses. While IFN antibodies had little or no effect on the outcome of disease in MHV-A59 infection, mice treated with either anti IFN alpha/beta or anti IFN beta prior to MHV-2 infection had higher titers of virus recovered from the brain and histopathological enhancement of acute meningoencephalitis. Thus, while IFN-gama may be produced by inflammatory cells during acute encephalitis, IFN-beta upregulation is probably due to a local effect of astrocytes. IFN-beta may also have a protective role against brain invasion of the non neurotropic MHV-2 virus.

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THE INTERACTION BETWEEN APOLIPOPROTEIN E AND ALZHEIMER'S AMYLOID β-PEPTIDE IS DEPENDENT ON β-PEPTIDE CONFORMATION. **Adam A Golabek, Claudio Soto and Thomas Wisniewski* NYU Medical Center, Departement of Neurology, 550 First Avenue, New York, NY 10016**

Alzheimer disease (AD) is neuropathologically characterized by the cerebral deposition of amyloid in the form of senile plaques and amyloid angiopathy, accompanied by neurofibrillary tangles formation and neuronal loss. The major component of the amyloid is a 39 to 44 amino acid residue protein termed amyloid β (Aβ). The Aβ peptide also exists as a normal protein in biological fluids, called soluble Aβ. sAβ is thought to have a more random coil and/or α-helical structure, while Aβ in the amyloid posses a crossed β-sheet structure. The major risk factor for late-onset AD is the inheritance of the apolipoprotein (apo) E4 isotype of apo E. We and others have shown the immunohistochemical and biochemical presence of apo E within senile plaques, where it is complexed with Aβ. It has also been shown that apo E binds to Aβ and that apo E4 in particular, promotes a β-sheet structure *in vitro*. Currently, we have investigated the sequential and conformational aspects of the apo E/Aβ interaction. We show, that apo E preferentially binds to Aβ peptides with a high content of β-sheet conformation. This can in part explain the formation of complexes between Aβ and apo E within the senile plaque *in vivo*.