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DELTA-9-TETRAHYDROCANNABINOL: A NOVEL TREATMENT FOR INFLAMMATORY DEMYELINATION R. Elkin, W.D. Lyman, C.F. Brosnan, and M.B. Bornstein. Albert Einstein College of Medicine, Bronx, NY 10461.

Delta-9-tetrahydrocannabinol (THC) has anti-inflammatory and immunosuppressive effects. Experimental autoimmune encephalomyelitis (EAE), an inflammatory demyelinating disease of the central nervous system (CNS), serves as a model for multiple sclerosis (MS). Previous studies showed that THC administered prophylactically is effective in preventing the full-development of EAE. We now report that THC suppresses EAE if given after sensitization and that it significantly reduces CNS edema and inflammation. Rats inoculated for EAE were treated with either THC emulsified in sesame oil and water (vehicle) or with vehicle alone, commencing 1, 3, 5, 7, or 9 days post-inoculation (DPI). Animals were evaluated neurologically daily. To study edema, ^{125}I-labelled albumin was injected 10, 11, or 12 DPI. These animals were sacrificed 24 hours later and edema was determined by quantitating exudation of radiolabel. Inflammation was quantitated by light microscopy. Results showed that THC treatment commencing as late as 7 DPI: (1) delays the onset of clinical signs of EAE (Vehicle 9.5 and THC 12.5 DPI); (2) reduces clinical severity (Vehicle 5.2 and THC 1.8 clinical score); and (3), suppresses edema and inflammation. Further studies will focus on the mechanism by which THC suppresses inflammatory demyelination. (Supported by NS 11920)

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CHARACTERIZATION OF ABERRANT T CELL REGULATION OF THE AUTOLOGOUS MIXED LYMPHOCYTE RESPONSE IN MULTIPLE SCLEROSIS. J.I. Greenstein and D.T. Crisp (SPON: W.S. Ceglowski). Dept of Neurology, Temple Univ. School of Medicine, Philadelphia, Pa. 19140

T-cells primed with autologous non T-cells suppress the response of a fresh autologous mixed lymphocyte response (AMLR) in normal individuals, neurologic disease controls and patients with stable multiple sclerosis (MS). In contrast, active and acute MS AMLR-primed T-cells failed to suppress the AMLR. Here, enhancement rather than suppression of the AMLR occurs. Mixed lymphocyte culture (MLR)-primed T-cells suppress the fresh MLR against the original stimulator cells. The kinetics of the fresh AMLR with and without the addition of primed T-cells was studied. Normal or stable MS AMLR-primed T-cells suppress the response after 3 days in culture. In contrast, active and acute MS AMLR-primed T-cells enhanced the fresh AMLR at all times. Affinity-plated AMLR-primed CD4+ and CD8+ subsets were then added to the fresh AMLR. Both AMLR-primed subsets suppressed the fresh AMLR in normals. In all MS cases, AMLR-primed CD8+ cells were suppressive. The fresh AMLR was not significantly suppressed by CD4+ cells in MS. Further, AMLR-primed CD4+ cells in active and acute MS enhanced the response. Interleukin 2 (IL2) levels were elevated in the active acute MS cultures. Exogenous IL2 in the priming culture induced the regulatory abnormality in stable MS. Therefore, there is a selective and phasic immunoregulatory abnormality of CD4+ T-cells to self major histocompatibility antigens in MS. IL2 may play a role in the induction of this abnormality. Similar mechanisms may also be involved in the immune response to myelin antigens in MS.

Supported in part by NIH grants NS00878, RR05417, RR00349

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DETECTION OF OLIGOCLONAL IgG IN MS CSF BY IMMUNOBLOTTING, B.A. Patrick, P. D. Mehta, and J. Black. New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314 and Isolab Inc., Akron, OH. 44321

We previously analyzed unconcentrated CSF from patients with MS and other neurologic diseases by isoelectric focusing (IEF) in agarose gel. IEF followed by silver staining was completed in about 4 hours. In order to identify oligoclonal bands immunofixation was routinely carried out. We have now developed a rapid and sensitive immunoblot method for detection of oligoclonal IgG bands in unconcentrated MS CSF. After focusing was completed, protein transfer from agarose IEF gels to nitro- cellulose papers was performed by simple diffussion. Immunostaining of the nitro-cellulose membranes, was accomplished by using rabbit anti human IgG as the primary antibody followed by incubation with goat anti-rabbit IgG-horseradish peroxidase conjugate and development in 4-chloro-1-naphthol. Only 4 hours were required for immunoblotting after the completion of IEF on agarose. An excellent resolution of the oligoclonal IgG bands in MS CSF was seen after immunostaining.

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MODULATION OF LYMPHOCYTE PROLIFERATIVE RESPONSES USING IgG ISOLATED FROM MULTIPLE SCLEROSIS PATIENT SERA E.E. Litchfield*, R.S. Bray*, J.M. Killian*, and J.M. Plummer*. Immunetech Pharmaceuticals, 11045 Roselle Street, San Diego, CA 92121

IgG was isolated to >95% purity from sera by ammonium sulfate precipitation and DEAE ion-exchange chromatography. Fourteen sera were obtained from patients in exacerbating or remitting phases of multiple sclerosis (MS). IgG fractions from all patients were incubated with mononuclear cells from normal donors or after combining cells with allogenic cells during a mixed lymphocyte reaction for 7 days at 37°C in 5% CO_2. IgG from 10 relapsing patients inhibited two way MLRs and spontaneous proliferation from 30 to 99% at 6.25 to 400 µg/ml. IgG from all 4 exacerbating patients stimulated lymphocyte proliferation from 20 to 600%. Fab fragments from MS patients had similar modulatory activity as did native IgG. IgG from normal donors did not modulate lymphocyte proliferation. These results demonstrate that IgG from multiple sclerosis patients can modulate proliferation of normal lymphocytes. Modulation appeared to be dependent upon the disease state of the patient which may provide a novel assay for monitoring multiple sclerosis.

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CHARACTERIZATION OF POLYCLONAL AND MONOCLONAL ANTI-IDIOTYPIC ANTIBODIES DIRECTED AGAINST HUMAN ANTI-DNA IgG. R. Suenaga, P. Munoz, S. Bright, M. Evans, and N. I. Abdou. Kansas Univ. Med. Ctr., Kansas City, KS 66103.

Idiotypic repertoire of anti-DNA autoantibodies has been characterized by polyclonal and monoclonal reagents. Three rabbit anti-idiotypes (R-anti-Id) and one murine monoclonal anti-idiotype (M-anti-Id) against 3 different affinity purified polyclonal human anti-DNA IgGs were prepared. Specificity was examined by direct binding to Id, lack of binding to non-anti-DNA and unrelated Ig, and inhibition of binding of Id to DNA by ELISA. R-anti-Ids seem to recognize framework sites, and partly antigen-binding sites (% inhibition 29-55). M-anti-Id recognized framework sites only (0% inhibition). Binding of M-anti-Id to the Id was inhibited (62%) by R-anti-Id. Binding of R-anti-Id to Id was inhibited (8%) by M-anti-Id suggesting that M-anti-Id binds to similar idiotope recognized by R-anti-Id. Inhibition of binding of anti-Id to homologous Id by 9 SLE sera showed that 3 R-anti-Id reacted with the homologous serum (71-85% inhibition) and not with unrelated sera (0-9% inhibition). M-anti-Id reacted with homologous lupus serum (49% inhibition). R-anti-Ids and M-anti-Id detect private idiotypes. Polyclonal anti-Ids have different binding characteristics from those of M-anti-Id. Polyclonal and monoclonal anti-Id reagents are recommended for studies of Id repertoire. (Supported by PHS grant AM29674 & BRSG SO7RR05373)

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EVIDENCE FOR GERMLINE V GENE SELECTIVITY IN AUTOANTIBODY PRODUCTION. R. Spitzer and A. Stitzel. SUNY Health Science Center, Syracuse, NY 13210.

Anti-idiotypic antibody (AIA) to C3 Nephritic Factor (C3NeF) was demonstrated in multiple sera from 5 patients with glomerulonephritis and nine normal individuals. All specimens bound to the same preparation of highly purified Fab II C3NeF indicating the presence of the same idiotope (Id^{NeF}) in all 14 individuals. In addition, mononuclear cells from 3 of the normal individuals, 3 of the patients with nephritis, and 8 unrelated newborns (umbilical cord cells) were cultured with PWM. IgG isolated from all of the cultures contained AIA to Id^{NeF}. Further, AIA isolated from 3 patients and 2 normals blocked the binding of that C3NeF to EC3bBb confirming that Id^{NeF} resides in the V region of the autoantibody. These AIA also inhibited the activity of 4/5 C3NeF preparations purified from 5 other patients (1 with partial lipodystrophy and 4 with glomerulonephritis), further demonstrating the commonality of Id^{NeF}. Thus Id^{NeF} was demonstrated in 26 individuals, both with and without auto-immune disease, and was present on 5/6 different C3NeF preparations. These data support the concept that unmutated germline V genes are expressed by pre-B cells as autoantibodies. The sharing of Id^{NeF} by autoantibodies from different individuals suggests that the responsible gene may be purposively selected for during somatic recombination.