P13.08

PERIPHERAL NEUROPATHY ORCHESTRATED BY NONNEURAL-SPECIFIC T LYMPHOCYTES.

G.K. Harvey*, R. Gold, K.V. Toyka and H.-P. Hartung
Neurologische Universitätsklinik, Julius-Maximilians Universität, Würzburg, Germany. *Institute of Clinical Neurosciences, University of Sydney, Australia.

Neural-specific T cells are held to play a pivotal role in the Guillain-Barré syndrome, and experimental allergic neuritis (EAN). Here, the effects of intraneural accumulation of nonneural-specific T cell on blood-nerve barrier permeability and peripheral nerve function were assessed.

Rat ovalbumin (OA)-specific T cells were activated in vitro and on day 0 intravenously transferred to female adult Lewis rats. Rats were then given intraneural injections of OA or casein into left and right tibial nerves respectively. On days 3 and 4, selected rats also received intravenous purified immunoglobulin from rabbits with myelin-induced EAN.

Rapid accumulation of α/β T cells and $ED1^+$ macrophages and marked increases in blood-nerve barrier permeability in OA but not casein injected nerves followed transfer of $2x10^6$ T cells. $5x10^6$ T cells induced decreases in proximal/distal CMAP amplitude ratios but also severe reductions in distal CMAP amplitudes and Wallerian degeneration in OA nerves. Demyelination was occasionally observed in nerves proximal to sites of OA injection. $5x10^5$ T cells also induced decreases in amplitude ratio but with only minor axonal degeneration and reductions in distal amplitudes. Conduction block and demyelination were considerably augmented in animals also receiving anti-myelin antibody.

Intraneural accumulation of nonneural-specific T cells can orchestrate demyelination, axonal degeneration, or both.

P06.07

LIMITED RESTRICTION IN αβTCR USAGE OF T CELL CLONES SPECIFIC FOR MBP (a.a. 84-102) AND 65kD HSP (a.a. 3-13) PEPTIDES WITHIN TWINS AND MHC IDENTICAL INDIVIDUALS.

Hawes, G., L. Struyk, B. Godhelp, and P. van den Elsen
Dept. of Immunohematology and Bloodbank, University Hospital Leiden, P.O. Box 9600, 2300 RC Leiden, The Netherlands.

In order to study the TCR repertoire in response to a given specific peptide/MHC complex, we have taken advantage of a panel of pairs of HLA indentical individuals having various levels of relation ranging from monozygotic twins to unrelated individuals. These have been previously defined by PCR analysis showing the direct correlation between the level of relatedness and the concordance of the corresponding peripheral αβTCR repertoires. By limiting dilution we have generated a panel of T cell clones specific for either MBP a.a. 84-102 or 65kD hsp a.a. 3-13. The overall repertoires between, as well as within, individuals were diverse in the V/J region usage and the composition of the CDR3 regions. However, within particular individuals there appeared to be some intra-individual limited restriction. This is illustrated by occurance of the same V and J genes being used by multiple clones from one, or a pair of individuals which are not found or are very limited in the other pairs. On the whole, there was a limited conservation in the response to the different peptides: A high frequency of Vβ2, 4, and 7 responded to MBP, whereas these V regions were not found in the hsp clones. Also, some similarities could be seen in the a.a. composition in the CDR3's of cells sharing V region usage and peptide specificity, regardless of the individual from which they were isolated. This suggests that there is a selection for these particular CDR3 regions in combination with certain V regions, and that they probably share some structural similarity in the manner of recognition allowing these a.a. to contact the peptide/MHC complex in a similar conformation.

P13.09

B CELL ACTIVITY IN IMMUNE-MEDIATED NEUROPATHIES: CELLULAR REQUIREMENTS AND CYTOKINE EFFECTS FOR SYNTHESIS OF ANTI-GM1 ANTIBODIES

E. Heidenreich and R. Ewerhart
Department of Neurology, Heinrich-Heine-University Düsseldorf, Germany

Introduction: We have previously reported on pokeweed mitogen (PWM) induced synthesis of anti-ganglioside GM1 antibodies (anti-GM1) by peripheral blood mononuclear cells (PBMC) from patients with the acute Guillain-Barré syndrome (GBS) and with multifocal motor neuropathy (MMN) (Heidenreich et al., J. Neuroimmunol. (1994) 49:97-108). We have now aimed to further characterize the immune mechanisms involved in the activation of GM1-specific B cells.

Methods: PBMC were depleted of $CD3^+$ T cells and $CD5^+$ B cells by magnetic cell separation. Anti-GM1 synthesis in culture supernatants was quantified by ELISA. PBMC were also stimulated by a monoclonal antibody to CD3 (CD3-mab) or enterotoxin T cell superantigens with or without interleukins (Il) 2, 4, 5 and 6.

Results: PWM induced anti-GM1 synthesis was inhibited by depletion of T cells from PBMC, but could be restored in a remix assay requiring cell contact of T and B cells. Anti-GM1 synthesis after depletion of $CD5^+$ B cells was not reduced as compared to unseparated cells. In 5 patients anti-GM1 secretion was greatly stimulated by the CD3-mab, while enterotoxin superantigens had only a weak effect. Interleukins alone or in combination did not stimulate anti-GM1 synthesis but enhanced stimulation by the CD3-mab considerably with the strongest synergistic effects exerted by Il 2 and combinations of Il 4, Il 5 and Il6.

Conclusions: These results demonstrate requirement of activated T cells for in vitro synthesis of anti-GM1 and are in agreement with the hypothesis of non-cognate (bystander) T cell help to ganglioside GM1 specific B cells in MMN and GBS.

W11.03

PHENOTYPIC AND FUNCTIONAL PROPERTIES OF $CD8^+$ T-LYMPHOCYTES FROM THE CNS OF RATS WITH CORONAVIRUS-INDUCED ENCEPHALOMYELITIS

A. Hein[1], H. Imrich[1], S. Sopper[1], S. Schwender[2] and R. Dörries[1].
[1]Inst. f. Virologie und Immunbiologie and [2]Zentrallabor der Medizinischen Klinik der Universität Würzburg, Germany

Intracerebral infection of Lewis (LEW) rats with the murine coronavirus JHM (JHMV) typically results in a demyelinating encephalomyelitis accompanied by a monophasic, paralytic disease. In contrast, no clinical signs can be observed in JHMV infected Brown Norway (BN) rats. In both rat strains CD8+ T-lymphocytes contribute significantly to the inflammatory infiltrate in virus-infected CNS lesions.

Phenotypic and functional properties of these CD8+ T cells were characterised by flow cytometry and determination of virus-specific cell mediated cytotoxicity at different times past infection. In LEW rats, in average 10times more CD8+ T lymphocytes were recovered from the CNS compared to the BN rat population. Nevertheless, in both rat strains the majority of these cells is characterised by the loss of the CD45RC molecule, indicating a primed or activated state. In LEW rats maximal infiltration of these lymphocytes as well as maximal cytotoxic activity coincides with the climax of clinical symptoms. In BN rats, however, the overall killing capacity of CNS extracted leukocytes is considerably lower compared to LEW rats throughout the infection.

From these data we conclude that CD8+ T-cells could contribute to neurological disease by lysis of virus-infected targets in the CNS of LEW rats. This idea is further supported by experiments using γ irradiated LEW rats that were reconstituted by a purified fraction of naive CD8+ T-lymphocytes before infection. These animals developed enhanced neurological disorders and succumbed earlier to the infection compared to irradiated but not reconstituted animals.

P04.05

BRAIN ANTIBODIES IN MS-PATIENTS - AN ACTIVITY PARAMETER FOR IMMUNOSUPPRESSIVE THERAPY ?

A.E.Henneberg, J.Pfau, P.Hartmann, H.Bittmann, D.Link, K.H.Wollinsky[1]
Dpt. of Neurology, University of Ulm, Schillerstr. 15, D - 89070 Ulm
[1]Dpt. of Intensive Care, RKU,Oberer Eselsberg, D - 89080 Ulm

Introduction: For the immunosuppressive treatment of multiple sclerosis (MS) an activity parameter is urgently needed. Since we had found antibrain antibodies in patients with chronic progressive, but not with relapsing-remitting form of MS, we were interested in confirming earlier results and in looking, whether the brain antibodies might be used as a tool for foreseeing an ungoing deteoration.

Materials and Methods: Sera and cerebrospinal fluids (csf's) of 93 MS patients were tested on normal human pons tissue and other CNS tissues using an indirect immunofluorescence assay. The csf's were concentrated 1:40 before use.

Results: 1. Sera of patients with relapsing-remitting disease were antibody-negative also on other CNS tissues. 2. Sera of patients with chronic progressive MS were antibody-positive in about 80%, showing a tendency for antibody-increase before the symptoms worsened. 3. Csf's of patients with relapsing-remitting and chronic progressive MS showed antibody-binding in about 50%. The binding was caused by IgG-antibodies (in the sera by IgM). 4. Sera and csf's of the same patients showed differences in antibody-binding; after a "mix-up" of the csf's by liquorphereses in vivo, former negative csf's became positive like the sera of the same patient.

Conclusions: Chronic progressive MS patients show antibody binding to pons tissue, while relapsing-remitting patients do not. These antibodies might be useful for being aware of a soon deterioration of the patient. These antibodies cannot be found in csf won by lumbar puncture. After "mixing" the csf's by several courses of liquorpheresis, the antibodies suddenly appear in the csf's of sera-positive patients. Lumbar puncture might be wrong for getting an activity parameter in MS.

W16.04

INTERLEUKIN-4 ENHANCES *IN VITRO* T-CELL RECRUITMENT IN GLIOBLASTOMA-BEARING PATIENTS

E. TERAO[1], Ch. FABER[2] and P. HEUSCHLING[2]
[1] Laboratoire de Biologie Cellulaire, UCL, Louvain-la-Neuve, Belgium
[2] Neuroimmunologie & Inflammation, CRP-Santé, Luxembourg

Introduction: Glioblastoma-bearing patients frequently display a severe immunodepression, due to the release, by these tumor cells, of glial cell-derived immunosuppressive agents. The resulting impairment in immune functions constitutes a major obstacle to an eventual immmunotherapy. We wanted to know whether low concentrations of interleukin (IL)-4 are able to enhance the responsiveness of peripheral blood lymphocytes (PBL) from these patients.

Materials and Methods: Primary cultures of surgically removed glioblastomas were co-cultured with syngeneic or allogeneic PBL in the presence of IL-2 (40 U/ml) and in the presence or absence of IL-4 (5 U/ml). The lymphocyte precursor frequency was estimated using the limiting dilution method. Proliferation was measured by [3H]-thymidine incorporation. The phenotype was analysed by flow cytometry.

Results: IL-4 greatly enhances the proliferation rate of specifically-stimulated PBL when compared to a stimulation in the presence of IL-2 alone. The CD8/CD4 ratio is very low for these patients, but IL-4 seems to increase this ratio. IL-4 also significantly increases the autologous glioblastoma cell-responding lymphocyte precursor frequency.

Conclusion: IL-4 enhances some of the impaired functions of the cellular immune system of glioblastoma-patients. The IL-4-induced increase in the lymphocyte population is mediated by a direct effect on the recruitment of precursor cells, as well as a mitogenic effect on the proliferating lymphocytes. Our results indicate that an IL-4 treatment might improve the conditions for glioblastoma immunotherapy