NEUROIMMUNOLOGY
Satellite Symposium of the VIth International Congress of Immunology

July 12-14, 1986
The University of Western Ontario
London, Ontario

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Program

Session I — **Immunological Approaches to CNS Development and Function**

Co-chairmen: Dr. John C. Roder, Mount Sinai Hospital Research Institute, Toronto
Dr. Seung U. Kim, University of British Columbia, Vancouver

Keynote Presentations

Phenotypic expression of cellular antigens by human neurons and glia in culture	Dr. S.U. Kim
Ultrastructural studies of central monoaminergic and peptidergic neurons identified by immunocytochemistry	Dr. Virginia Pickel, Cornell University Medical Center New York, New York

Session II — **Immunoregulation in CNS Autoimmunity**

Co-chairmen: Dr. Dale McFarlin, National Institute of Health, Bethesda
Dr. Cedric S. Raine, Albert Einstein College of Medicine, Bronx, New York

Keynote Presentations

Molecular isolation and characterization of neuronal receptors	Dr. Mark I. Greene, University of Pennsylvania School of Medicine, Philadelphia
THE F.R. ECCLES MEMORIAL LECTURE Regulations and deregulations of B cell proliferation and maturation	Dr. Fritz Melchers, Basel Institute of Immunology, Basel

https://doi.org/10.1017/S0317167100036891 Published online by Cambridge University Press

Session III — **Viral Infections in CNS Autoimmunity**

Chairman: Dr. Samuel Dales, University of Western Ontario, London

Keynote Presentations

The role of virus-specific and autoimmune host responses in Theiler's virus-induced demyelinating disease of mice	Dr. Howard L. Lipton, Department of Neurology, Northwestern University, Medical School, Chicago
Molecular mimicry: a mechanism for viral-induced autoimmunity	Dr. Robert Fujinami, Department of Pathology, University of California San Diego, La Jolla
Multiple causes of multiple sclerosis	Dr. Elsworth C. Alvord, Department of Pathology, University of Washington, Seattle

Session IV — **Immunopathology**

Co-chairmen: Dr. Elsworth C. Alvord Jr., University of Washington, School of Medicine, Seattle
Dr. Joseph J. Gilbert, University of Western Ontario, London

Keynote Presentations

Murine models of chronic-relapsing autoimmune demyelination — immunopathologic considerations	Dr. Cedric S. Raine, Albert Einstein College of Medicine, Bronx
KROC FOUNDATION LECTURER T lymphocyte immune reactivity within the nervous system	Dr. Hartmut Wekerle, Max Planck Institute, Wurzburg

Session V — **Immunotherapy**

Co-chairmen: Dr. George C. Ebers, University of Western Ontario, London
Dr. Byron H. Waksman, The National Multiple Sclerosis Society, New York

Keynote Presentations

Design of monoclonal antibodies for therapy in neurologic autoimmune disease	Dr. Lawrence Steinman, Stanford University Medical Center, Stanford
Immunotherapy of multiple sclerosis with anti-T-cell monoclonal antibodies and cyclophosphamide	Dr. Howard L. Weiner, Harvard Medical School, Boston

https://doi.org/10.1017/S0317167100036891 Published online by Cambridge University Press

Symposium Summary

An international neuroimmunology meeting was held July 12-14, 1986, at the University of Western Ontario (London, Ont.) as a satellite symposium of the 6th International Congress of Immunology (Toronto, Ont.). The symposium was divided into 5 half-day sessions and dealt with the following topics: 1. Immunological approaches to CNS development and function, 2. Immunoregulation in CNS autoimmunity, 3. Viral infections in CNS autoimmunity, 4. Immunopathology and 4. Immunotherapy.

There were 2-3 keynote lectures, 8-9 short (10 minute) presentations and a few posters in each session. The keynote speakers gave a broad overview of their respective fields focusing on their own investigations.

S.U. Kim (Vancouver) presented results on the immunocytochemical localization of galactocerebrosides and several classes of gangliosides on the surface of various cell types derived from dissociated cell cultures of human nervous tissue (both neurons and glial cells). The localization was made with monoclonal antibodies. A specific and characteristic set of gangliosides could be found on the cell surface of different neural cell types depending on their stage of development.

V. Pickel (New York) presented studies demonstrating that dopaminergic and cortical afferents terminate on common spiny neurons in the rat striatum. These cells also receive synaptic input from terminals with enkephalin-like immunoreactivity. The common target neurons contain GABA but apparently no choline acetyl transferase. The dual labeling (immunoautoradiography and immunoperoxidase) is equally applicable to the study of synaptic interactions between neurons in other parts of the CNS.

M. Greene (Philadelphia) reported studies on 2 receptor systems in the brain. With the help of monoclonal antibodies, multiple, structurally distinct domains were recognized on these receptors. Genes that encode these receptors were isolated and the functional aspects of these receptors in the development of the CNS were discussed.

F. Melchers (Basel) gave a general talk on autoimmune disorders with special reference to the control and deregulations in the B lymphocyte cycle in NZB, NZW and BXSB mice. He showed that there were 3 restriction points at which B cells must receive signals in order for their normal development to proceed. If signalling at any given point becomes defective or does not occur, loss of control and autoimmunity may ensue.

R. Fujinami (La Jolla) showed that there is considerable amino acid sequence homology between the encephalitogenic site responsible for Experimental Allergic Encephalomyelitis (EAE) in the rabbit and Hepatitis B virus proteins. After immunizing rabbits with a synthetic viral peptide, both anti-myelin basic protein (MBP) antibodies and *in-vitro* lymphocyte proliferation to MBP were detected. In some of the immunized animals there were histologic lesions similar to those encountered in EAE.

E.C. Alvord Jr. (Seattle) talked about the causes of Multiple Sclerosis (MS). The geographic distribution of the disease may be related to the initial contact and degree of sensitization to viruses and/or bacteria which share antigenic determinants in common with potentially-encephalitogenic peptides present in CNS myelin proteins. Many of these common epitopes in microorganisms may also possess adjuvant activity and may thus contribute to the sensitization of T cells to cross-reacting antigens (recognizing both microbial and CNS antigens).

H. Lipton (Chicago) reported on the complete nucleotide sequence of Theiler's vuris polyprotein and its deduced amino acid sequence. Current evidence suggests that myelin breakdown is mediated by MHC class II-restricted delayed hypersensitivity reactions in which chronic levels of Theiler's virus may play an inducing role. Antigenic cross-reactivity between MBP and proteolipid protein (PLP) on one hand and Theiler's virus polyprotein on the other was analyzed by searching for amino acid sequence homologies between these 3 structures and by functional T cell analyses such as delayed hypersensitivity and *in-vitro* lymphocyte proliferation. The data do not support a role for either MBP or PLP in Theiler's virus-induced demyelination.

C. Raine (New York) presented immunopathological data on the chronic-relapsing EAE (CR-EAE) in the mouse. Two forms were discussed. The actively induced-disease, i.e. by the administration of CNS tissue to susceptible strains of mice results in a destructive disease of the white matter. On the other hand induction of CR-EAE by the adoptive transfer of lymph node cells or T cell lines educated *in-vitro* to MBP determines in the recipients, a demyelinating disease which histologically is a better correlate of MS than the actively-induced one. In any case neither form is a better model of human MS than the CR-EAE actively-induced in juvenile strain-13 guinea pigs.

H. Wekerle (Wurzburg) presented work on the properties MBP-specific T cell lines maintained in long-term cultures. He showed that when exposed to astrocyte cultures, these cell lines destroy the target by direct contact and cytotoxicity despite the fact that the T cell lines are of the helper-inducer and not cytotoxic/suppressor phenotype. These results suggest that *in-vivo* the immune attack by T cells in EAE — and possibly in MS — operates by direct cytotoxicity.

L. Steinman (Stanford) discussed the advantages and potential negative consequences of suppressing EAE by the administration of anti-T cell marker antibodies.

H. Weiner (Boston) discussed strategies for immunotherapy in MS. He felt that the best chances for successful intervention would be a combined approach of chemotherapy (cyclophosphamide) and immunotherapy (administration of antibodies to Leu-4 (human T helper/inducer surface marker).

The abstracts of the 10 minute presentations and of the posters are presented on the following pages.

J.J. Gilbert
G. Strejan

Abstracts

1.

Effect of Desferrioxamine on Experimental Allergic Encephalomyelitis

N.A. BOWERN, D.O. WILLENBORG and P.C. DOHERTY
(Canberra, Australia)

Experimental allergic encephalomyelitis (EAE) is a cell-mediated autoimmune demyelinating disease of the central nervous system. It is readily induced in Lewis rats by injection of complete Freund's adjuvant (CFA), containing either guinea pig spinal cord homogenate (SCH) or myelin basic protein (BP). Treatment of SCH-CFA sensitized rats with the iron-chelating agent desferrioxamine B (DFO) suppresses both the duration and severity of disease. In addition, DFO abrogates the expression of delayed hypersensitivity responses; this may be due to the diminution of T cell proliferation and to the alteration of T cell migratory patterns. We have observed a differential effect of DFO on EAE, in that the symptoms induced by BP-CFA are not suppressed by treatment with CFO. This is not due to the amount of encephalitogen (BP) in the inoculum, since the effect was observed over a large dose-range of BP, including a dose which induced disease in only 50% of animals. The reason for this is not clear. It may be that the effect is due to a qualitative difference in the immune response, particularly in the numbers of suppressor cells induced by either injection regimen. This is currently under investigation.

2.

Detection of Soluble FcγR-like Material in Cerebrospinal Fluid of Patients with Neurological Disorders

W. De SMET, D. KARCHER, F. FRANCK and A. LOWENTHAL
(Belgium, Netherlands)

FcγR are among the most important surface structures of a number of immunocompetent cells. They have been implicated in several immune reactions. To investigate their possible involvement in the immunoregulation of MS and SSPE, we measured (i) soluble FcγR-like material in CSF by means of a solid-phase RIA, and (ii) percentage of $Fc\gamma R^+$ cells (and their staining intensity) in peripheral blood by means of FMF. Four mAb, all recognizing the FcγR on human natural killer cells and neutrophils, were used: B73.1 (a kind gift of Drs. B. Perussia and G. Trinchieri, The Wistar Institute, Philadelphia, PA), VEP13 (BMA 070, Behring), anti-Leu-11a and anti-Leu-11b (Becton-Dickinson). The optimal working conditions for our RIA were set up with protein solutions containing FcγR, on the one hand cell lysates of mononuclear and polymorphonuclear leukocytes, on the other hand plasma fractions eluted from columns consisting of aggregated IgG-Sepharose. To check the specificity of the RIA, in other words to test whether we were indeed measuring FcγR-like material, the following three controls were included: (i) binding on lysates of $Fc\gamma R^-$ cells or Daudi cells (which are $Fc\gamma R^+$ by rosetting but which possess an FcγR type that cannot be detected with the above mentioned mAb), (ii) binding with a mAb of the same sub-class but of irrelevant specificity, and (iii) total removal of reactive material by passing the fraction through a Sepharose-IgG column but only slight loss by passing it through Sepharose-F $(ab')_2$ or Sepharose-ovalbumine columns. CSF from 35 MS patients, 20 SSPE patients and 65 patients with other neurological diseases were investigated. In most MS CSF the presence of FcγR remained within the average limit with a few exceptions corresponding to exacerbations. SSPE patients had a significantly increased amount of FcγR. By FMF, 4 MS and 1 SSPE patient were studied. No marked differences in the number of $Fc\gamma R^+$ cells (in both lymphocyte and neutrophil preparations) or their staining intensity was observed. We conclude that soluble cell-free FcγR-like material might play a major role in the regulation of the immune response in SSPE and MS with exacerbations.

3.

Targeting of Virus-Immune T Cells to the Central Nervous System

P.C. DOHERTY and J.E. ALLAN (Canberra, Australia)

The severe inflammation of the central nervous system (CNS) which results from infection of adult mice with lymphocytic choriomeningitis virus (LCMV) can also be induced by transfer of class I MHC compatible T cells from immune donors to immunosuppressed, virus-infected recipient mice. We have used this model to examine which stages of this immunopathological disease are controlled by the MHC. The recipients for the adoptive transfer studies were chimeras prepared by transferring F1(kxb) bone marrow into irradiated mice of either the b or k phenotype. Eight weeks later these chimeras were immunosuppressed, LCMV-infected and injected with LCMV-immune T cells from either b or k donors. The presence of LCMV-specific cytotoxic T cells in the spleen of both sets of chimeras indicated that a substantial proportion of the lymphoid tissue had originated from the bone marrow donor.

However, examination of the number of cells in the cerebrospinal fluid (CSF) showed that although b immune cells were present in both sets of chimeras they would only cause meningitis in recipients in which the radiation-resistant cells were also of the b phenotype. This suggests that it is necessary for immune T cells to recognize radiation-resistant MHC compatible cells at the blood-CSF barrier before they can enter the central nervous system. In contrast, k immune cells induced severe meningitis in both sets of chimeras which may indicate that bone-marrow derived cells contribute to the barrier and have been replaced more extensively in the (kxb→ b) chimeras.

4.

LN-1 as a Marker for Microglia in Paraffin Sections

J.M. MILES and S.M. CHOU (Cleveland, Ohio)

While the cytogeneology of microglia has been unsettled it has recently been suggested that they arise from glial cells and not from blood monocytes (J Neuropath Exp Neurol *45*:1, 1986). Several monoclonal and polyclonal markers for monocytic/lymphocytic lineages were tested to see if a microglial marker which shares antigenicity with lymphocytic/monocytic lineages might be identified. Sixteen formalin-fixed paraffin-embedded autopsied brains with and without neurologic conditions were studied with monoclonal and polyclonal bodies to LN-1, LN-2, Leu-M1, GFAP, and chymotrypsin using the immunoperoxidase technique with avidin-biotin complex (ABC). Neuropathological diagnoses at autopsy included: multiple sclerosis (5 cases) cerebral infarct (2 cases), malignant glioma (2 cases), generalized cerebral edema (2 cases)

https://doi.org/10.1017/S0317167100036891 Published online by Cambridge University Press

and amyotrophic lateral sclerosis (1 case). Four cases had no CNS lesions. Patients ranged in age from 2.5 to 69 years, with 9 females and 7 males. Eleven cases were positive for LN-1, a B-lymphocyte antigen, only and were negative for the rest of the markers used. Immunostaining for LN-1 was confined to cells with elongated rod-shaped nuclei and bipolar cytoplasmic processes and recapitulated cytological features characteristic of silver-impregnated microglia. LN-1 positive microglia were noted in the gray matter, usually near the gray-white junction. The distribution of LN-1 positive microglia was independent of sites for histopathologic processes. Both cases of malignant glioma had a history of radiation therapy to the CNS and were negative for LN-1 positive cells. Two cases of multiple sclerosis were negative for unknown reason. The expression of LN-1 by microglia suggests a functional role in the regulation of the immune system. The recent data which indicates that microglia arise from glial cells and not from blood monocytes may be pertinent since reactive astrocytes are known to "internalize" IgG and other immunoglobulins, secret interleukin 1 or interleukin 3 *in vitro*, and are capable of expressing Ia antigen (class II MHC antigen) in active MS lesions (J Neuroimmunol *8*:1, 1985). It may not be farfetched to assume then that microglia may be activated and converted to become astrocytes.

5.

Antibodies to Acetylcholine Receptor in Myasthenia Gravis: Comparison Between Human and Fetal Calf Antigen

J. OGER, R. KAUFMAN and T. AZIZ (Vancouver, British Columbia)

We have modified the assay described by Lindstrom to test for the presence of antibodies to the acetylcholine receptor (AchR) in serum of patients with myasthenia gravis (MG) and controls as well as to compare AchR of human and fetal calf origins. AchR in Triton-X extract was labelled with $^{125}I-$ alpha bungarotoxin. 10 ul of serum was added and incubated overnight at 4°C. Cpm precipitated by Staph A were counted. Values over 2 SD above the mean of the controls were considered positive. Using human AchR, we found 30/31 (97%) generalized MG and 12/19 ocular MG (63%) to be positive. 71/73 (97%) non MG as well as 10/12 (83%) MG in remission were negative. 1/2 positive non MG was a case of botulism (out of 6 tested). We have found that 6/42 (14%) of the MG sera positive with the human antigen were negative with the fetal calf antigen. These results partially confirm those of Gotti *et al* who found only 3/52 (6%) false negatives using fetal calf antigen. Among 73 sera negative for human antigen only one was positive for fetal calf antigen. This patient had generalized MG. AchR from fetal calf muscle cannot replace human AchR but could be useful as an antigen tested in parallel.

6.

Characterization of Canine Distemper Infected Brain Cells *In Vitro*

S. PEARCE-KELLING, B. SUMMERS, W.J. MITCHELL and M. APPEL (Ithaca, New York)

Canine Distemper Virus (CDV), a morbillivirus closely related to measles virus, induces central nervous system (CNS) disease in dogs. Neuropathological changes include gray matter injury (polioencephalomyelitis) and white matter injury (demyelination), depending largely on viral strain. We are testing which brain cells are permissive to CDV infection and how this varies with strain differences. We have grown mixed brain cells *in vitro* from neonatal dogs and have infected them with both virulent and attenuated virus strains observing them for up to sixty-five days post infection. We have characterized astrocytes, oligodendrocytes and neurons by indirect immunoflourescent stains using primary antibodies directed against glial fibrillary acidic protein, galactocerebroside and neurofilament protein respectively. Simultaneously, we have stained the cultures with fluorescein labelled anti-CDV antibodies allowing us to quantify virus infected cell types. We have found that, *in vitro*, both glial cells and neurons are susceptible to CDV infection. It has also been observed that the number of infected astrocytes varies with the strain of CDV used. In the strain which produces delayed CNS injury (A75-17) the percent of infected astrocytes remains low until at least four weeks post infection. In the Snyder Hill strain which induces a more acute disease the astrocytes are nearly 100% infected by two weeks post infection. Persistent infection is seen in these cells for up to sixty-five days post infection with minimal cytopathic effect. In contrast the attenuated strain, Rockborn, produces a highly cytopathic and rapid infection of astrocytes. Strain dependent variations of CDV infected brain cells *in vitro* may help us to understand the varied types of encephalitis induced by CDV in the dog.

7.

The *In Situ* Cellular Immune Response in Acute Viral Encephalitis

R.A. SOBEL, A.B. COLLINS, R.B. COLVIN and A.K. BHAN (Boston, Massachussetts)

To characterize the *in situ* cellular immune response in acute viral encephalitis (VE) we stained frozen cerebral cortical biopsy specimens from 26 patients and 8 controls with a panel of monoclonal antibodies to identify inflammatory cell subsets and expression of activation markers and major histocompatibility complex (MHC) molecules. Nineteen patients had herpes simplex encephalitis, proven by immunohistochemical staining for herpes simplex antigens and/or culture. Other patients had mumps encephalitis (1), Eastern equine encephalitis (1) or meningoencephalitis of presumed viral etiology (5). Four controls were from histologically normal biopsies and 4 were from normal autopsy temporal cortex. Parenchymal and meningeal inflammatory infiltrates in VE were composed of T cells, with fewer natural killer and B cells, and variable numbers of granulocytes and macrophages. T4+ and T8+ cells were present in nearly equal numbers. Inflammatory cells expressed the interleukin-2 receptor, MHC class I (HLA-α chain, β-2 microglobulin) and Class II (HLA-DR, HLA-DQ) molecules. Many parenchymal cells, including neurons and vascular endothelial cells, also expressed MHC molecules in VE ($p<.05$ vs. controls). Vascular expression of HLA-DR in VE was increased over controls ($p<.05$) and was greater than that of HLA-DQ ($p<.01$). No correlations between the magnitude of immune parameters with amount of corticosteroid therapy or duration of neurologic illness prior to biopsy, or outcome were found, although patients with VE not shown to be due to herpes simplex generally had more benign courses and less marked immune responses in their biopsies. These findings suggest that T cell-mediated cytotoxic and delayed hypersensitivity mechanisms may contribute to tissue injury in viral encephalitis, particularly that due to herpes simplex.

Supported by NIH NS-00858 and HL-18646.

8.

Interleukin-2 Receptor Expression on Peripheral Blood Lymphocytes in Multiple Sclerosis Patients

K. SELMAJ, C. PLATER-ZYBERK, K.A. ROCKETT, R.N. MAINI, R. ALAM, G.D. PERKIN and F.C. ROSE (Lodz, Poland; London, United Kingdom)

Interleukin-2 (IL-2) receptor expression on peripheral blood lymphocytes (PBL) was measured, using anti-Tac monoclonal antibody, in multiple sclerosis (MS) patients in phases of acute relapse and subsequent remission, other neurological diseases (OND) patients and healthy subjects. The percentage of IL-2 receptor positive cells in MS patients in acute relapse showed a modest but significant increase over the same

patients in remission and from control groups. The IL-2 receptor expression on PBL of MS patients stimulated with PHA was within the same range as of healthy controls, suggesting that there is no general defect of IL-2 secretion and receptor expression on T lymphocytes in this disease. The Il-2 receptor expression on PBL stimulated with human myelin basic protein (MBP) was slightly higher in MS patients. However, similar increase in IL-2 receptor expression upon MBP stimulation was observed in healthy subjects. The results suggest the occurrence of small subpopulation of activated T lymphocytes in peripheral blood of MS patients in acute relapse.

9.

A Cell ELISA to Measure MBP and GalC in Glial Cell Cultures

I.N. MONTGOMERY, S. COLA and H.C. RAUCH (Detroit, Michigan)

Cell ELISAs have been reported[1,2] that can measure various cell surface markers. Using the method of McCarthy and deVellis[3] we have established glial cell cultures. For purposes of this assay they are grown in 96 well tissue culture plates. The cell ELISA is based upon that of Cobbold and Waldmann[1] modified by the substitution of a B-galactosidase conjugated second antibody and *o*-nitrophenyl-B-D-galactopyranoside as the substrate, to avoid the problem of a high background due to endogenous alkaline phosphatase and peroxidase in these cells. Additionally saponin is used in the myelin basic protein (MBP) assay to enhance the permeability of the cell membranes to antibody. The cell cultures are fixed with 4% paraformaldehyde at the start of the assay. MBP and galactocerebroside (GalC) cell ELISA assays have been used to monitor the development of oligodendroglia in cultures using both the media originally described[3] and a defined media without fetal calf serum which appears to facilitate the differentiation of precursor cells into oligodendroglia.

[1]Cobbold, SP and Waldmann, H, 1981. J Immunol Methods *44*:125.
[2]Morris, RE, Thomas, PT and Hong R, 1982. Human Immunol *5*:1.
[3]McCarthy, KD and deVellis, J, 1980. J Cell Biol *85*:890.
Supported in part by NIH Grant NS 18898.

10.

Interactions Between Cerebral Vascular Endothelial Cells and Myelin Basic Protein-Sensitized T Cells

R.M. McCARRON, M. SPATZ, O. KEMPSKI and D.E. McFARLIN (Bethesda, Maryland)

Lymphocyte migration across the blood-brain barrier (BBB) into the central nervous system has been implicated in the pathogenesis of several neurological disorders. The potential interaction of lymphocytes with cerebral vascular endothelial cells (EC), which are a principal component of the BBB, imply that EC may be involved in the pathogenesis of such disorders. To study this possibility lymph node cells (LNC) were removed from SJL mice which had been immunized 10 days earlier by subcutaneous injection with guinea pig myelin basic protein (MBP) in complete Freund's adjuvant. Single cell suspensions were prepared by passing LNC through mesh and B-cell depleted suspensions were prepared by panning on anti-mouse IgG coated plates. Macrophages-monocytes (MO) were depleted by two successive incubations on plastic Petri dishes followed by passage on Sephadex G-10 columns. Lymphocyte proliferation was assayed by culturing cells for 96 h at 37° C in humidified air plus 5% CO_2 and pulsing with 1 uCi of [methyl-^{3}H]thymidine. Whole LNC preparations proliferate *in vitro* to MBP and these cells are also able to transfer experimental allergic encephalomyelitis (EAE) to normal syngeneic mice. The proliferative response is abolished by depletion of MO but can be reconstituted by the addition of EC freshly isolated from syngeneic mice with adoptively transferred acute EAE. Reconstitution by EC from mice with EAE can be blocked by pretreatment of EC with syngeneic anti-I-A antisera. Freshly isolated EC from normal syngeneic mice do not restore responsiveness but can be induced to present antigen by culture with murine recombinant gamma-interferon or supernatants from a variety of immune cell cultures.

These results are consistent with the hypothesis that immune cells release interferon and/or other soluble factors which induce I-A molecules on EC which subsequently acquire the capacity to present antigen. The implications of these findings relate to the migration of cells across the BBB and are of importance to the understanding of the pathogenesis of several neurological disorders.

11.

Analysis of Astrocyte Ia Regulation in Rat Strains Differing in Susceptibility to Autoimmune Demyelination

P.T. MASSA, H.-J. HÄDRICH, R. DÖRRIES, H. WEGE and V. ter MEULEN (Hannover, F.R.G.)

Experimental allergic encephalomyelitis (EAE) in the rat involves T-lymphocyte mediated immune reactions directed toward myelin basic protein. Demyelinating lesions within the white matter involve T-lymphocytes and macrophage infiltrations characteristic of delayed type hypersensitivity reactions (DTH). Helper T-lymphocyte involvement in DTH is restricted by class II antigens (Ia) encoded within the RT-1 locus of the rat. The role of astrocyte Ia expression in EAE has been proposed. Susceptibility to EAE is related to the RT-1 locus, however, at least one other gene outside the RT-1 locus is involved. Lewis rats are highly susceptible to EAE while Brown-Norway (BN) rats are resistant. Congenic Lewis.BN rats have intermediate susceptibility. The susceptibility to murine corona virus (JHM) induced EAE-like disease developed in this laboratory follows a similar pattern. We propose that differences in astrocyte Ia regulation correlates with susceptibility and resistance to these demyelinating diseases. To analyse Ia regulation we utilize primary glial cultures from Lewis, BN, congenic Lewis.BN and numerous recombinant inbred rat strains produced from Lewis x BN. Cultures are treated with either gamma interferon (IFN) or JHM virus which are both known inducers of astrocyte Ia. We show that Lewis, BN and congenic Lewis.BN astrocytes are equally induced to express Ia by gamma IFN. However, only Lewis and Lewis.BN rat astrocytes are induced to express Ia by JHM virus and not the BN rat. Moreover, the recombinant inbred rat strains show distinct differences in Ia inducibility by JHM virus. The results suggest that a Lewis specific gene located outside the RT-1 locus is responsible for hyperinduction of astrocyte Ia and possibly the severity of autoimmune demyelination.

12.

Anti-Idiotypic Antibodies of Myelin Basic Protein (MBP) Antibodies from Guinea Pigs with Chronic Relapsing (R-EAE)

P.D. MEHTA and H.M. WISNIEWSKI (Staten Island, New York)

Clinical and morphological findings in chronic R-EAE guinea pigs injected with spinal cord in CFA are consistent with those of multiple sclerosis. Our recent immunologic studies showed high titer antibodies to MBP and presence of oligoclonal IgG bands with antibody activity to MBP in CSF and serum from R-EAE animals. The purification of specific anti-MBP antibodies from sera of guinea pigs with R-EAE was achieved by affinity chromatography using an immunoabsorbent column containing purified MBP. The specific antibody was eluted with acidic buffer and their isoelectric focusing profiles revealed multiple oligoclonal IgG bands with widely different electrophoretic mobility. Anti-idiotypic antibodies were raised in a rabbit using anti-MBP anti-

body eluted from serum of a single guinea pig with R-EAE. Cross reacting idiotypes were present in the different bleedings of the homologous animal and in some heterologous sera while these were absent in others.

Supported in part by the NIH Grant #NS14406.

13.

Multiple Sclerosis: Relationship Between Suppressor Cell Function, IgG Secretion *In Vitro* and the Attacks of Multiple Sclerosis (MS) as Studied by Serial Clinical and MRI Examinations

J. OGER, L. KASTRUKOFF and D. PATY
(Vancouver, British Columbia)

Seven relapsing-remitting MS patients were examined monthly and had MRI of the head. Peripheral blood lymphocytes were also tested monthly in MS and paired controls by Con A induced suppression (Con A S) and *in vitro* pokeweed mitogen induced IgG secretion (IgG Sec). Three patients had 5 clinical attacks. Con A S was reduced ($-16\% \pm 18$) before the attacks (2 to 21 days) compared to the same day controls ($34.6\% \pm 8.8$, $p<.05$) and to other measures in the same patients ($29.2\% \pm 7.1$, $t = 2.34$, $p<.05$). IgG Sec was also reduced.

Two patients developed major MRI lesions not correlated with clinical attacks. When both had small new lesions appearing on MRI IgG Sec was high and Con A S was normal. When both MRI lesions became large Con A S dropped from $15.8\% \pm 10.8$ (5 measures) to -68% and -83% and IgG Sec from 1337 ng $\pm$ 120 to 649 and 169 ng. On following assays, Con A S averaged $33.2\% \pm 10.6$ (6 measures). IgG Sec remained low. We conclude that Con A S and IgG Sec are depressed before clinical attacks; depression coincides with the peak of large MRI lesions. These abnormalities are not present as new MRI lesions appear on MRI. Reduced Con A S and IgG Sec may be secondary to the size of the lesions and do not seem to precede new lesions.

14.

Effector Cell Activation in Autoimmune Encephalomyelitis

R.H. SWANBORG and N.S. HAYOSH (Detroit, Michigan)

Lewis rats develop experimental autoimmune encephalomyelitis (EAE) following immunization with myelin basic protein (BP) + adjuvant. Adoptive transfer of EAE is readily achieved with spleen cells (SpC) from immunized donors provided that the SpC are first activated *in vitro*. Activating substances include BP and Con A. Recently we found that SpC are also activated to transfer EAE during mixed lymphocyte reactions (MLR) against allogeneic Brown Norway rat cells, suggesting that autoimmunity may be initiated in bystander fashion during responses to third-party antigens. MLR-induced activation of SpC is associated with interleukin-2 (IL-2) production. Moreover, BP-induced activation of SpC is inhibited by monoclonal antibody W3/25, which reportedly blocks IL-2 production by helper T cells; this inhibition is abrogated by T cell growth factor (TCGF) which contains high levels of IL-2. TCGF alone stimulates SpC to proliferate, but it does *not* activate these cells to transfer EAE.

Thus, effector cell activation appears complex, and is not simply initiated by lymphokines. It is believed that T cell activation first requires triggering via antigen receptors, followed by expression of IL-2 receptors. Why, then, does a MLR vs. Brown Norway cells trigger the autoreactive effector cells of EAE? Resolution of this question may increase our understanding of mechanisms governing the induction of autoimmunity.

Supported by NIH NS06985-19, and National Multiple Sclerosis Society 1073 C6.

15.

Activation of an Encephalitogenic T Lymphocyte Line with a Cell Free Supernatant

A.A. VANDENBARK, P. TEAL and H. OFFNER (Portland, Oregon)

Activation of a myelin basic protein specific T lymphocyte line (BP-1) requires presentation of epitope(s) surrounding residue 79 of guinea pig basic protein (GP-BP) by accessory cells which express major histocompatibility complex (MHC) gene products. To determine if the T cell activation signal was APC associated or was shed into the medium, supernatants from a thymic APC population pulsed with GP-BP were collected after a 24 hr incubation, centrifuged at 400 x g to remove cells, and added to resting T cell lines. Supernatants containing GP-BP but not Bovine-BP or PPD induced highly significant proliferation of the BP-1 line cells, and transfer of these supernantant activated cells produced clinical signs of experimental autoimmune encephalomyelitis and delayed type hypersensitivity reactions to GP-BP. The GP-BP component was not inhibited by either of two monoclonal antibodies directed at determinants on either side of the epitope(s) recognized by BP-1 cells. However, activity was inhibited by an anti-I-A but not an anti-I-E monoclonal antibody, suggesting the involvement of the I-A Class II MHC gene product in T cell activation. Subcellular components which can activate encephalitogenic T cells may be of particular importance in the CNS where BP or BP fragments could associate with membrane vescicles which express Class II MHC antigens shed from various accessory cells.

16.

Specificity of the "Recovery Associated" Suppressor Cells Found in EAE of Lewis Rats: Studies with T Cell Lines and a Synthetic Encephalitogenic Peptide (EP)

S. VARRIALE, D. BRANDLI, J. BARBARIA, E. BERAUD, M.M. GOLDSTEIN and D. BERNARD (Marseille, France)

A T cell line anti-MBP (Myelin Basic Protein) and a T cell line anti-PPD, as control, were stimulated *in vitro* with the mitogen ConA or with anti-genes (MBPS, PPD or the synthetic peptide 71-84 which represent the minimal encephalitogenic sequence), in the presence of spleen or thymus cells taken out from rats 15 days after either an encephalitogenic challenge (MBP in CFA), or a control injection (saline) in CFA), or nothing. In most experiments, normal accessory cells were also present. We have previously found, at the time of recovery, a transitory occurrence of suppressor cells inhibiting, *in vivo*, the induction of EAE (E.J.I., 1982; 12, 926-930).

We found that thymus cells of the recovering rats inhibit the proliferative response of the anti-MBP T-line to MBP, but neither to ConA nor to EP. Moreover, they do not inhibit the responses of the anti-PPD T line to ConA or to PPD. Saline/CFA control animals do not possess such specific thymic suppressor cells. On the contrary, in the spleen of recovering rats and of CFA controls rats we found non-specific suppressor cells acting on both T lines whatever the stimulus be. Splenic as well as thymic suppressor cells are radio-resistant. Coculture experiments in the presence of normal accessory cells showed that the effect is a true suppressor phenomenon and not the result of an inadequate presentation of the antigen.

These results strongly suggest that the thymic "recovery associated" suppressor cells are not directed at the T cell receptors of the encephalitogenic T line cells used as target, contrarily to what seems to be the case of suppressor cells found later, in already cured animals: A. Ben Nun and I.R. Cohen, J Immunol, 1982; *128*: 1450. They clearly are antigen (MBP)-specific but appear not to recognize the same small EP (71-84) as the T line cells they suppress. Whether it is a larger or an

entirely different determinant is not yet known. Therefore the "recovery associated" suppressor cells are probably different from that found in already cured animals.

17.

Experimental Allergic Encephalomyelitis Induced by Proteolipid Apoprotein in Lewis Rats

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Proteolipid, a major constituent of CNS myelin, was suggested to be an antigen of EAE in the 1950s, but its encephalitogenicity has been debatable for a long time. Recently, chronic relapsing or chronic EAE associated with demyelination was induced by purified proteolipid apoprotein (PLP) in outbred animals. Here, we report the successful induction of EAE in the inbred Lewis rats by sensitization with bovine PLP and the successful transfer of clinical and/or histologic illness by PLP-sensitized lymphoid cells. The levels of contamination of myelin basic protein (MBP) in our PLP are less than 0.014%. Eighteen to 61 days after a single injection of 100 μg of bovine PLP emulsified in complete Freund's adjuvant, 12 of 31 Lewis rats (39%) developed clinical EAE and 18 of 23 (78%) showed histologic EAE with significant demyelination mainly seen in the subpial regions in the spinal cord. Lymphocyte proliferative responses and antibodies to PLP were elevated but did not correlate with the clinical or histologic state (J Neuroimmunol *in press*). Eleven days after sensitization, lymph nodes or spleens were removed from sensitized rats and single cell suspensions were prepared. The lymphoid cells were cultured for 2 - 4 days in the presence of PLP or Con A. Then, the cultured cells were passively transferred into naive or irradiated (400 R) recipients. Four of 5 irradiated recipients developed clinical EAE by passive transfer of 2 - 2.5 x 10^8 precultured cells. Histologic EAE could be transferred by as few as 5 x 10^7 cells into naive rats. A large number of cells pre-incubated with MBP did not transfer EAE (Cell Immunol *submitted*). This is the first demonstration of passive EAE by PLP-sensitized lymphoid cells and suggests the pathogenetic importance of cell-mediated immunity to PLP. The present inbred model will contribute to the study of autoimmune CNS inflammation and demyelination.

18.

Transmission of Transient Tolerance to Experimental Allergic Encephalomyelitis (EAE) During Lactation

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The effect of pregnancy on the development of EAE in female outbred rats and the susceptibility of their offspring to EAE-induction was tested. The incidence of EAE in pregnant rats sensitized to spinal cord + CFA + Bordetella pertussis was similar to control non-pregnant animals (78% vs 81%). Sensitized mothers transferred a transient resistance to induction of EAE in challenged offspring. Only 38% of the sensitized young rats (30 days old), born to immunized mothers, developed mild EAE with a short duration. Whereas 83% of the normal age-matched counterparts, born to non-immunized mothers, developed severe EAE. The intensity of protection to EAE in offspring was not influenced by the occurrence of phenotypic clinical signs in the mothers. By switching offsprings and nursing mothers we found that the resistance was transferred during the whole lactation period until weaning and not during the pregnancy term. Litters born to non-immunized mothers and nursed by immunized female rats acquired a resistance to induction of EAE (up to 30 days after birth). On the other hand litters born to immunized mothers and nursed by non-sensitized female rats were able to develop EAE. The resistance to induction of EAE correlated directly with the level of circulating anti-MBP antibodies in offspring. Using a solid phase RIA, anti-MBP antibodies were detected in offspring born to non-immunized mothers and nursed by immunized mothers. The antibodies reached high levels during the lactation period and decreased gradually to background levels after 10 weeks. We believe that such transfer of resistance can be used as a model for the study of milk-transmitted immunocompetent factors, as well as a model to study the mechanisms involved in development of resistance to autoimmune diseases.

19.

The Induction of Unresponsiveness to Disease Challenge in the Murine EAE Model

I.N. MONTGOMERY, A. KHATANA and H.C. RAUCH (Detroit, Michigan)

The murine model of experimental allergic encephalomyelitis (EAE) differs in several significant aspects from the guinea pig and rat models: disease induction (challenge) with myelin basic protein (MBP), adoptive transfer of disease following culture with MBP, and antigen-induced unresponsiveness to challenge (protection). Challenge in the murine model requires at least 200 μg of MBP[1] while 5 μg is adequate for the guinea pig and 20 μg for the rat. MBP concentrations in the culture transfer of EAE in the murine model[1] are also higher, 100 μg/ml versus 5 μg/ml for the rat model.[2] Finally, we have been unable to induce unresponsiveness to challenge in the murine model using protocols that are successful in the rat and guinea pig model. We report here additional protocols that explore this difference between the murine and other species models. Mice were immunized with either IFA, IFA/MBP, IFA/MBP + GC or IFA/whole CNS tissue. They received one *id* injection per week for three weeks followed one week later by disease challenge. All the groups developed disease, and in several cases disease onset was earlier or more severe clinically. When this series of protection protocols included an *ip* injection of cyclophosphamide (CY) 24 hours after each protection inoculation, experimental animals had either a delayed onset of disease or remained well. We have previously described an intrinsic suppressor cell population in mice[3] that must be overcome for both challenge and adoptive transfer; classic regimens of protection do not appear to induce an antigen-specific suppressor cell population to complement the intrinsic population. It appears, rather, that these protection protocols in mice induce primary sensitization of the antigen-specific effector cells, augmenting disease induction. In contrast CY reduces the effector cell population resulting in unresponsiveness to later challenge.

[1]Pettinelli, C and McFarlin, D, 1981. J Immunol *127*:1420.
[2]Swierkosz, J and Swanborg, R, 1977. J Immunol *115*:631.
[3]Montgomery, IN and Rauch, H, 1984. Neurochem Research 9:1399.
Supported in part by NIH Grant NS 18898.

20.

Effects of Anti I-A and I-E Antibodies on Encephalitogenic T Lymphocyte Functions

H. OFFNER, A.A. VANDENBARK and S. BROSTOFF (Portland, Oregon; Charleston, South Carolina)

Two monoclonal antibodies, OX-6 and OX-17, were used to evaluate respectively the role of I-A and I-E MHC Class II gene products in the *in vitro* activation and subsequent function of encephalitogenic T cell lines. Activation of the T cell lines with GP-BP presented by accessory cells (APC) resulted in an increase in the number of blasts and was reflected by increased uptake of ^{3}H-Tdy. However, proliferation in the

presence of OX-6 was reduced by 90%, but to a much lesser extent with OX-17. OX-6 but not OX-17 appeared to block T cell activation primarily by inhibiting APC function, since pre-incubation of APC but not T cells with OX-6 before stimulation resulted in complete inhibition of the cultures. After activation, the BP-1 T cell line or D-9 clone transferred severe paralysis to normal recipient rats. Recipients of OX-6 treated BP-1 or D-9 T cells exhibited very mild or no signs, whereas recipients of OX-17 treated cells developed only slightly less severe EAE than recipients of untreated encephalitogenic control cultures. In contrast, treatment with OX-17 but not OX-6 reduced the ability of BP reactive T cells to transfer delayed type hypersensitivity reactions. These results suggest that both the I-A and I-E gene products may contribute to the activation and subsequent function of encephalitogenic T cells.

21.

Autoimmune Disease Induced in SJL Mice Following Neurological Virus Infection

N. CASTEEL, S.A. STOHLMAN and L.P. WEINER (Los Angeles, California)

C57BL/6 and SJL mice were injected with a neurotropic strain of mouse hepatitis virus to determine whether either strain developed relapsing autoimmune encephalomyelitis subsequent to viral infection. A small plaque variant of JHM virus caused mild encephalomyelitis and demyelinating lesions in 100% of surviving SJL and C57BL/6 mice when injected intracranially at 6 weeks of age. Typically 80-90% of the deaths occur from days 9-12 post-infection (p.i.). Large amounts of viral antigen are found in the brain and spinal cords of both strains of acutely-infected mice when sacrificed at day 8 p.i. SJL mice which recover have intermittant evidence of neurological disease over the ensuing 2-3 months, while B6 mice show no clinical symptoms. Brain tissue taken from SJL mice during relapse shows focal areas of inflammatory cells but no viral antigen. Spleen cells from acutely infected SJL responded to both JHM viral antigen and myelin basic protein (MBP) during *in vitro* proliferation assays. Spleen cells from recovering SJL did not give respond to JHMV, but continued to respond to MBP at a higher rate than acutely-infected or recovered mice showing no clinical symptoms. Spleen cells from acutely-infected SJL mice or those undergoing relapsing neurological disease adoptively transfer neurological disease to naive mice following *in vitro* stimulation with Con A. Histological examination of the brains of adoptively transferred mice show focal areas of lymphocytic infiltration and an absence of viral antigen. These findings indicate the autoimmune nature of the induced disease.

22.

SFV An Experimental Model of Immune Mediated Demyelination: Implications for Autoimmunity and Multiple Sclerosis

J.K. FAZAKERLEY, S. AMOR and H.E. WEBB (London, United Kingdom)

Multiple sclerosis (MS) is often considered to be a demyelinating disease of the central nervous system (CNS), with an immunopathogenesis, initiated by viral infection. SemLiki Forest virus (SFV) infection of mice is a useful experimental model of viral demyelination disease. Infection of adult mice with the avirulent A7(74) strain of SFV results in a viraemia, virus replication in CNS cells including oligodendrocytes, a transient disturbance of the blood-brain barrier, intrathecal antibody production and a demyelinating encephalomyelitis with involvement of the optic nerves and retina. Immunosuppression of infected mice by total body irradiation results in very high brain virus titres, but no demyelination. Infection of athymic nude mice produces a persistent CNS infection without demyelination. These findings and others, including adoptive transfer experiments in nude mice, clearly demonstrate that demyelination in SFV infection is dependent upon activated T-Lymphocytes though we have not yet determined the specificity of these cells. As we have proposed previously, one possible explanation of MS is that several viruses are involved and that these all act in the same way by initiating an autoimmune response to glycolipids. Immune reactivity to glycolipids in MS is well documented. The question remains, could this activity be induced by virus infection(s)? Brain cell glycolipids are incorporated into the membrane of eveloped viruses during budding. The glycolipids of the SFV envelope, for example, are known to be representative of the cell from which the virus is derived, and can be demonstrated on the viral surface by immunoelectron microscopy. We have recently shown that infection with the virus and inoculation of inactivated brain derived virus both give rise to anti-glycolipid antibodies.

23.

Multifocal Central Nervous System (CNS) Demyelination in Mice Peripherally Inoculated with Herpes Simplex Virus Type 1 (HSV1)

L.F. KASTRUKOFF, A.S. LAU and S.U. KIM (Vancouver, British Columbia)

Infectious agents have been implicated in the etiology and pathogenesis of demyelinating disease. Complimentary DNA sequences of herpes simplex virus (HSV), a common cause of latent infection of the human peripheral nervous system (PNS), have recently been identified in the central nervous system (CNS) of MS patients, but the significance of this observation to the development of demyelinating disease is unclear. Following peripheral inoculation with HSV 1, experimental animals develop a latent infection of the PNS, complimentary HSV DNA sequences in the CNS and unifocal CNS demyelination. The potential of HSV 1 to produce multifocal CNS demyelination in genetically predisposed mice was examined. The pathological appearance of the CNS in three strains, selected on the basis of natural resistance to HSV 1, varied from focal collections of inflammatory cells in C57BL/6J mice (resistant strain) and unifocal demyelinating lesions in Balb/cByJ mice (moderately resistant strain) at the trigeminal root entry zone, to multifocal demyelinating lesions throughout the brain in A/J mice (susceptible strain). The multifocal lesions in A/J mice developed later than unifocal lesions and at a time when infectious virus could not be isolated from the CNS. The results suggest that in genetically predisposed animals, HSV 1 can give rise to multifocal CNS demyelination which is immune mediated and likely "triggered" by the acute infection.

24.

Glycolipid Cross-Reactivity Between a Demyelinating Enveloped Virus and CNS Myelin. Implications for Autoimmunity

A. KHALILI-SHIRAZI and H.E. WEBB (London, United Kingdom)

Infection of adult mice with Semliki Forest virus (SFV), and RNA enveloped alphavirus, induces a T-cell mediated demyelination of the central nervous system (CNS). It is not clear whether the demyelination results from T-cells reacting against viral and or self antigens on the surface of CNS cells. To investigate the possible cross reactivity between this virus and CNS myelin a panel of monoclonal antibodies (MAbs) were produced to each of these antigens. Amongst the MAbs raised to brain tissue culture derived SFV were MAbs to the viral spike proteins (E1 and E2) and a MAb reacting against a CNS glycolipid. One of the MAbs raised against CNS myelin reacts against a glycolipid component of myelin, labels brain derived SFV by immunoelectron microscopy and reacts with this virus in an ELISA. This MAb has no

viral neutralising activity, but it inhibits neutralisation by the anti-E1/E2 MAbs. Immuno thin-layer chromatography reveals that the anti-glycolipid MAb raised against myelin and cross reacting with SFV, and the anti-glycolipid MAb raised against SFV, are both reactive with a glycolipid which runs as a band within the total ganglioside fraction. This band has not yet been identified but it is not a ganglioside nor is it identical to any of the common glycolipids. These experiments demonstrate that infection with a neurotropic enveloped virus generates antibodies cross-reactive with a glycolipid component of CNS myelin, and that this glycolipid is very closely related, or identical to, a glycolipid present in the envelope of SFV. Consideration of these findings suggests that it is possible that any of several enveloped neurotropic viruses replicating in the same CNS cell-type (e.g. oligodendrocytes) could incorporate the same host cell glycolipid (e.g. galactocerebroside) into their viral envelope. Such glycolipids, normally haptens, in association with the proteins of the viral envelope could be antigenic and in a susceptible individual could induce an anti-glycolipid autoimmune response to CNS cells, resulting in demyelination. Such a mechanism could be important in the pathogenesis of multiple sclerosis.

25.

Acute Hemorrhagic Leukoencephalitis with Localized Herpes Simplex Virus Type I (HSV) Infection of the Brain Stem

B. LACH and E.A. ATACK (Ottawa, Ontario)

The etiology and pathogenesis of acute hemorrhagic and necrotizing leukoencephalomyelitis (AHLE) in man is not known. The role of a viral infection has been frequently suspected but never documented.

We present a case of a 29 year old man with a clinically fulminating encephalomyelitis and typical morphological changes of AHLE with extensive demyelination (DM) throughout the CNS. In addition, the anterior spinal artery showed acute arteritis with thrombosis and the cervical spinal cord displayed circumscribed infarction. Immunoperoxidase method (PAP) and electronmicroscopy revealed the presence of HSV Type I in the glia, neurons and walls of a few capillaries in the spinal trigeminal tract, only in the medulla oblongata. Foci of DM were negative for HSV. Areas of demyelination showed loss of myelin basic protein (MBP) increased numbers of astrocytes strongly positive for glial fibrillary acidic protein (GFAP), numerous macrophages, and slight or no inflammatory infiltrations. Some foci of DM had central necrotic capillaries with perivascular deposits of IgG, IgM and fibrinogen. Macrophages in DM contained granules positive for IgG, IgM, IgA, MBP and GFAP.

Our results are the first to demonstrate the presence of HSV Type I in the connections of the trigeminal nerve in the human CNS, and association of this virus with AHLE. It is postulated that focal expression of the viral antigen in walls of vessels in the brain stem, precipitated acute auto-immune reaction resulting in the vascular injury and subsequent demyelination manifested by AHLE.

26.

An Autoimmune Reaction to Myelin Basic Protein (MBP) May Contribute to the Pathogenesis of Measles Virus Induced Subacute Encephalitis in the Lewis Rat

U.G. LIEBERT, C. LININGTON and V. ter MEULEN (Würzburg, F.R.G.)

Recently it has been suggested that autoimmune responses to MBP may play a role in acute measles encephalitis in man. In order to analyse this an experimental model has been established in the Lewis rat, a strain susceptible to MBP induced experimental allergic encephalitis (EAE).

A subacute measles encephalitis (SAME) is induced in the Lewis rat following intracerebral inoculation with measles virus. Histopathologically SAME is characterized by extensive mononuclear perivascular infiltrations of the CNS white and grey matter resembling to some extent experimental allergic encephalomyelitis (EAE). Infectious measles virus cannot be recovered from SAME animals due to a restriction of measles virus gene expression, as has been shown by molecular biological studies. A cell mediated immune response directed against myelin basic protein (MBP) is found in some of the SAME animals when their spleen cells are restimulated *in vitro* with MBP and ^{3}H-thymidin uptake is measured. T cell lines specific for MBP proved to be encephalitogenic for recipient rats can be recovered from clinically diseased rats.

These results indicate that autoimmune reactions may be involved in the pathogenesis of measles virus induced subacute encephalitis in the Lewis rat.

27.

Chronic Theiler's Virus Infection: MBP Appears in CSF and MBP Antibody Appears in Serum

H.C. RAUCH, I.N. MONTGOMERY, W. HARB and J.A. BENJAMINS (Detroit, Michigan)

Infection with the neurotropic strain of Theiler's murine encephalomyelitis virus (TMEV) can lead to chronic demyelination. We have described the appearance of myelin basic protein (MBP) in the cerebrospinal fluid (CSF) of mice with experimental allergic encephalomyelitis where there is acute demyelination. The presence of MBP correlates with clinical disease but the quantity of MBP present in the CSF does not regularly reflect the intensity of the pathologic reaction. We have also examined the CSF of mice following intracerebral infection with TMEV. MBP appears in the CSF coincidental with the clinical signs associated with chronic demyelination, at about 12 weeks following infection and throughout the remaining disease course. An immune response to MBP is also coincidental with chronic demyelination inasmuch as antibody to MBP appears in the sera. Since antibody directed against MBP and also antibody against TMEV appear simultaneously in some groups of infected animals, absorption studies were undertaken with TMEV or MBP indicate the non-identity of the antigens and the specificity of the antisera as measured by ELISA. Immunoblot analysis of sera confirmed the ELISA findings. The mechanism of induction of antibody directed against MBP and its role in TMEV-associated demyelination remains to be determined.

Supported, in part, by NIH Grant NS 18898.

28.

The Effect of T-Cell Functions on JHM Virus Infections of the Rat CNS

O. SORENSEN and S. DALES (London, Ontario)

Rats rapidly develop resistance to intracerebral challenge with JHM virus (JHMV). *In vitro* studies have shown that rat oligodendrocytes develop resistance to JHMV infection with maturation. It is not known, however, whether this cellular resistance is sufficient to produce the *in vivo* resistance of rats. Circulating antibody levels do not influence the disease process but the role of the cell mediated immune response has yet to be determined. Two approaches were used to test for the influence of T-cell responses on the resistance of rats to JHMV infections of the CNS. First, cyclosporin A immunosuppression of resistant rats followed by JHMV challenge assessed the effect of a decrease in helper cell functions. Secondly, athymic nude rats were challenged at various ages to assess whether or not the animals developed resistance. Cyclosporin A immunosuppression was, to a limited extent, able to

abrogate the age related resistance. Nude rats displayed some evidence of increased resistance with age but were still infectable at all ages tested. Thus it appears that the development of cellular resistance alone is insufficient to account for the resistance observed *in vivo*.

29.

Immunological Studies on Thieler's Induced Demyelination

C.J.R. WELSH, P. TONKS and A.A. Nash (Cambridge, United Kingdom)

Following intracranial injection of Theiler's murine encephalomyelitis virus (TMEV) susceptible mice develop demyelinating disease analagous to multiple sclerosis in man. Current evidence favours the participation of both the virus and the immune system in the demyelinating process. However, the precise contribution of the immune response to the disease process is poorly understood.

To investigate the nature of the T and B cell responses, mice were selectively depleted *in vivo* of L3T4 positive cells or Lyt2 positive cells using specific rat monoclonal antibodies (Cobbold *et al*, 1984, Nature, *312*, 548). B cells were depleted by anti-IgM antibody injections from birth. Serological and virological studies were conducted in serial samples from infected mice using ELISA assays and plaque formation of BHK cells respectively. Mice were sacrificed at intervals to check for histological signs of neurological damage.

The B cell depleted and L3T4 depleted mice suffered from early polio-like symptoms, failed to produce antibody responses and developed increased viral titres in the CNS. Depletion of Lyt2 positive cells resulted in increased viral titres in the CNS despite the production of specific antibody responses to TMEV. Specific depletion of T cells in the late stage of demyelination results in alleviation of clinical symptoms compared to control infected mice.

In conclusion, both T and B cell responses are important in controlling the spread of virus in the early stages of TMEV infection. T cell responses also appear to contribute to the pathogenesis of late stage demyelination.

30.

Cell Surface Interactions During the Transfer of EAE in the Rat

S.W. BROSTOFF and D.W. MASON (Charleston, South Carolina; Oxford, United Kingdom)

EAE can be transferred by splenocytes from sensitized Lewis rats incubated in culture with myelin basic protein (MBP) prior to injection into naive recipients. T helper cells (CD4+) and MHC class II cells (I-A phenotype) are required for transfer since incubation of splenocytes with monoclonal antibodies against either phenotype will inhibit transfer. We report here the use of anti CD4 and anti I-A antibodies in the transfer model of EAE to investigate the role of cells bearing these phenotypes in the disease process. Using these antibodies, transfer of EAE can be prevented by depletion of either CD4+ cells or I-A+ cells prior to incubation in culture. Although I-A is needed for antigen presentation, little if any I-A is expressed in the nervous system of normal, unsensitized rats. We therefore sought to determine whether activated T cells could serve also as antigen presenting cells in this system since some activated cells are known to express class II MHC. Transfer of EAE could be restored by incubation of the I-A depleted cells with splenocytes from normal, unsensitized Lewis rats. In one experiment, when cells depleted of CD4 were incubated with the I-A depleted cells, transfer of EAE was also restored. Neither depleted population transferred disease by itself. It is clear from the above experiments that the activated cells are not serving as antigen presenting cells (APC) in this system. Although it was always possible to prevent transfer of EAE by depleting the I-A cells, we were not able to consistently deplete all of the activated CD4+ cells since the CD4 depleted population still was able to transfer disease in several additional experiments. Investigation of the level of expression of CD4 on cells suggested that there were fewer CD4 molecules present on activated than on normal cells. Cells activated with Concanavalin A in culture appeared to have fewer CD4 molecules than normal cells as measured by FACS fluorescence after labelling with CD4 antibody or by maximum binding of CD4 antibody as measured by ELISA. This decrease in the number of CD4 molecules on the surface of the activated cells could explain our inability to consistently prevent disease transfer with CD4 depleted cells. These results also suggest the possibility that the CD4 molecule plays a role in T cell activation.

This work was supported in part by NIH grant No. NS-11867.

31.

Circulating Encephalitogen-sensitised Lymphocytes in Chronic Relapsing EAE: Implications for Multiple Sclerosis

P. GLYNN, D. WEEDON and M.L. CUZNER (London, United Kingdom)

The autoimmune status of multiple sclerosis (MS) is equivocal. This is partly because several studies could not detect peripheral blood lymphocytes (pbl) sensitised to myelin basic protein (MBP). To clarify this question we determined the frequency of detectable MBP-sensitised pbl in an animal model of MS with a definite autoimmune aetiology.

Chronic relapsing experimental autoimmune encephalomyelitis (CREAE) was induced by immunising juvenile guinea pigs with homologous spinal cord homogenate in complete adjuvant. After recovering from an acute attack by 1 month post-immunisation (pi), animals generally suffered at least 2 neurological relapses during the following 5 months. Pleocytosis and serum proteins in cerebrospinal fluid (CSF), inflammation and demyelination in spinal cord sections, and pbl proliferative responses to MBP and its encephalitogenic nonapeptide (NP) were determined in animals killed at various stages of CREAE. Up to 3 months pi, the onset of relapses was usually accompanied by marked CSF pleocytosis; this parallelled a transient appearance in the blood of MBP-NP-responsive lymphocytes which became undetectable within 1-2 days. By 3-6 months pi the predominant neuropathological feature was demyelination. Intrathecal IgG secretion was also evident. Neurological exacerbations were now associated with far less CSF pleocytosis and MBP/NP-responsive pbl could not be detected even on the day of onset.

In summary, early inflammatory relapses in CREAE may be initiated by circulating encephalitogenic lymphocytes. However, by the later chronic phase, the immune reaction seems to be sequestered within the central nervous system (CNS); neurological exacerbations may now reflect temporary conduction block in partially-demyelinated fibres. Like the latter CREAE group, most MS patients have marked demyelination, intrathecal IgG synthesis and low levels of CSF pleocytosis. Thus, in established MS, evidence of autoimmune sensitisation should be sought in the CNS itself.

32.

Analysis of T-cell Receptor Beta Chain Gene Rearrangements in T-cells Cloned Directly From Active MS Plaques

D.A. HAFLER, A.D. DUBY, J.G. SEIDMAN and H.L. WEINER (Boston, Massachusetts)

Multiple sclerosis (MS) is an inflammatory CNS disease in which T-cells and macrophages are found in active lesions. Although peripheral blood and CSF T-cells have been extensively studied, there is no

functional data regarding T-lymphocytes at the site of inflammation in the brain itself. Here, we report the isolation and characterization of a series of IL-2 dependent T-cell clones derived from active MS plaques and blood obtained immediately post mortem. Plaques were excised and tissue disrupted by gentle teasing. T-cells were cloned by serially diluting and then directly plating the brain suspension prior to expanding with mitogen and IL-2. Thus, the clones obtained represented the original lymphocyte population. None of the 63 clones from the brain proliferated to myelin basic protein or proteolipid protein. Southern blot analysis of DNA samples prepared from 13 clones demonstrated a common rearrangement of the T-cell receptor β-chain in 2 of 7 clones from the blood and 1 of 6 clones from the brain. The results suggest there may be selective expansion of a common T-cell clonotype in MS.

33.

NMR Studies of CNS and MBP Induced Acute EAE in the Hartley Guinea Pig

S.J. KARLIK, J.J. GILBERT and J.H. NOSEWORTHY (London, Ontario)

OBJECTIVES: We are attempting to understand the tissue properties of the lesions identified by magnetic resonance imaging (MRI) in Multiple Sclerosis (MS) patients through a series of studies on the EAE animal models of MS in the guinea pig. One can determine how the pathological changes seen in EAE (edema, inflammation, cellular infiltration, demyelination) influence NMR relaxation parameters by studying the different stages of EAE in these several models. **METHODS:** Acute-EAE was induced in juvenile Hartley guinea pigs with MBP-CFA and CNS-CFA (CFA-treated animals served as controls). Clinical scores were recorded daily, paramagnetic contrast agents (Gd-DTPA and Gd-Deferoxamine) were administered to some animals prior to sacrifice. The CNS was dissected and divided into 5 coronal sections of spinal cord and 7 sections of brain. T1 and T2 relaxation times were determined on a Praxis NMR spectrometer at 10.7 MHz at 20°. Tissue specific gravity was measured by microgravimetry and hemoglobin concentration was measured spectrophotometrically. These same tissues were scored for the degree of pathological change by a blinded neuropathologist (JJG). **RESULTS:** 1. In CNS induced acute-EAE, T1 is increased with perivascular and meningeal cellular infiltration. T2 is increased if demyelination accompanies these changes. Relaxation times are normalized with extensive cellular penetration of parenchyma (myelitis). 2. Changes in NMR relaxation times (particularly T2 increase in spinal cord) precede clinical and pathological events in EAE perhaps reflecting increased blood-brain barrier permeability. Paramagnetic contrast agents enhance T1 and T2 relaxation rates to a similar degree in control and study animals. **CONCLUSION:** Comparative *in-vitro* NMR and histopathological studies should permit definition of the tissue characteristics of the MRI-detected lesions in MS. MRI studies are in progress to extend these observations.

34.

Demyelination *In Vivo* Mediated by a Monoclonal Antibody Specific for a Minor Myelin Glycoprotein

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Putative target antigens for antibody mediated demyelination of the CNS should fulfill certain criteria: they should be (i) specific for CNS myelin and the oligodendrocyte membrane, (ii) exposed at the external surface of the myelin membrane, (iii) provoke an autoimmune response and (iv) passive transfer of specific antibody under the appropriate conditions should be able to initiate demyelination *in vivo*. We describe a mouse monoclonal antibody (mab) that defines a minor glycoprotein component of CNS myelin that fulfills all these criteria. The monoclonal antibody, 8-18C5 is specific for a 54kD myelin glycoprotein which immunohistochemical and Western blotting experiments have shown to be restricted to the CNS myelin of a number of mammalian species, including rat, guinea pig and man. No positive reaction was seen with PNS material. Immunoelectron microscopy of developing rat CNS confirmed the surface localisation of the antigen on both myelin and oligodendrocyte membranes. In addition antigen was detected within the oligodendroglial Golgi apparatus. The demyelinating activity of the antibody was demonstrated following the injection of 25 μg purified 8-18C5 with guinea pig complement (total volume, 50 μl) into the lumbal-sacral CSF space of 200 g Sprague-Dawley rats. After 48 hours animals were perfused with paraformaldehyde and post-fixed in glutaraldehyde and osmium tetroxide. Demyelination was evaluated on plastic embedded sections by both light and electron microscopy. In contrast to rats injected with sera from guinea pigs with CREAE, which gave a mixed picture of both CNS and/or PNS demyelination, the antibody 8-18C5 initiated extensive demyelination that was restricted to the CNS. Controls including polyclonal mouse IgG, mouse mab's of matched isotype, but irrelevant antigen specificity and complement alone proved negative. The target antigen has now been isolated. ELISA results suggest that this antigen accounts for less than 0.04 w/w% of the myelin membrane. Yet, guinea pigs with chronic relapsing EAE mount a substantial antibody response to this component. The possible role of this and other myelin antigens in the pathogenesis of demyelinating lesions in CREAE and MS will be discussed.

35.

Vascular Permeability Changes in the Central and Peripheral Nervous System of Rabbits with EAE

R.D. SIMMONS, T.M. BUZBEE and D.S. LINTHICUM (Houston, Texas)

Experimental autoimmune encephalomyelitis (EAE) is a putative animal analog of multiple sclerosis (MS). In both EAE and MS, increasing interest has focussed on ascertaining the precise mechanisms, presently unknown, by which leukocytes, proteins and other blood constituents pass through an impaired blood-brain (spinal cord)-barrier. In a preliminary study, rabbits in early stages of acute EAE (induced with homologous spinal cord homogenate in Freund's adjuvant) were injected i.v. with fluoresceinated dextrans of graded molecular size, in an attempt to determine the properties of very early vascular permeability change in both the central and peripheral nervous system. Thirty minutes after injection of tracer, rabbits were killed by i.v. injection of euthanasia solution, then the spinal cord, nerve roots and dorsal root ganglia were quickly removed and frozen in liquid nitrogen prior to processing for fluorescence microscopy. Adjacent serial sections were immuno-stained with monoclonal antibodies to determine the nature and degree of cellular infiltrates. Results indicate that, at least in rabbits immunized with homologous spinal cord, the earliest and most dramatic leakage of tracer and mononuclear cells occurs in the peripheral nervous system, particularly the dorsal root ganglia.

36.

Magnetic Resonance Imaging (MRI) of Experimental Allergic Encephalomyelitis (EAE) in Primates

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The potential use of quantitative MRI to characterize MS lesions *in vivo* is being investigated. Studies are being carried out on post mortem

human tissue and on experimental models. EAE has been induced in 4 *Macaca fascicularis* monkeys, and the development of the disease followed using quantitative MRI. MRI data were collected on a Picker International NMR Cryogenic 2000 system, operating at a field strength of 0.15 Tesla (proton resonance frequency 6.4 MHz). Serial scans were recorded daily, starting on day 9 after inoculation. Lesions were detected before the onset of clinical signs, due to an elevation in the spin-lattice relaxation time (T_1) and the spin-spin relaxation time (T_2). Once the disease was detected, T_1 and T_2 measurements were obtained from the brain every 10 hours. The T_1 and T_2 values of lesions increased over time, indicative of progressive change at a molecular level. The appearance of any new lesions and any changes in existing ones were noted. This allowed dating of the lesions post mortem. Pathological correlation showed the long T_1 and T_2 values to be associated with the presence of inflammation, demyelination and haemorrhagic necrosis. Microscopically similar lesions were shown to have the same MR characteristics. In addition, these studies show the oldest lesions to be the most haemorrhagic. This is contrary to the belief that haemorrhage is a secondary event in EAE. The results indicate that it should be possible to use quantitative MRI to distinguish acute inflammatory lesions from more chronic demyelinating ones. A chronic EAE model is being used to test this hypothesis.

37.

Acute and Relapsing EAE in Balb/c Mice Sensitized with DM-20

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Proteolipid apoprotein (PLP) is a major protein component of CNS myelin. It shows two bands on sodium dodesyl sulfate-polyacrylamide gel electrophoresis, DM-20 and major PLP. This is the first demonstration of acute and relapsing EAE in mice with DM-20.

PLP was separated from bovine spinal cord by Folch's method. It was delipidated and separated by gel filtration with a methylated Sephadex G-100 column. DM-20 and major PLP were separated by ion exchange column chromatography with a CM-trisacryl column. Balb/c mice were immunized with 100 μg of DM-20 or major PLP in complete Freund's adjuvant. Nine of 15 animals (60%) given a single injection of DM-20 developed clinical signs of EAE 16 to 27 days after sensitization (mean 21.3 days). Part of animals relapsed 2 to 6 weeks after the first attack. Pathologically classical histologic features of EAE including perivascular and intraparenchymal infiltration of mononuclear cells and macrophages with significant demyelination were observed. Five of seven animals given a booster injection of DM-20 developed clinical signs of EAE. None of 5 animals given a single injection of major PLP developed clinical signs of EAE, but 3 of 5 animals given a booster injection developed tail atony and paraplegia with a mean onset of 21 days without relapse.

Antibody titers to DM-20 in the serum were measured by ELISA but mean titers were not different between the groups with and without evidences of EAE. Balb/c nu/nu mice were reconstituted with either T cells or non T cells which were separated from spleen cells of naive Balb/c mice and were immunized with DM-20 in complete Freund's adjuvant. Five of 11 animals reconstituted with T cells developed EAE with a mean onset of 33 days but none of 8 nude mice that received non T cells developed clonical or histologic signs of EAE.

These observations suggest that DM-20 is an encephalitogen in Balb/c mice and T cells are required for the induction of this EAE.

38.

IgG Uptake in Cultured Human Non-neoplastic Astrocytes: Enhancement by Mononuclear Leukocyte Products

B.P. BARNA, S.M. CHOU and B. JACOBS (Cleveland, Ohio)

We reported previously that DNA synthesis and proliferation of cultured adult human non-neoplastic astrocytes (HNA) was enhanced by cytokine-containing supernatant fluids (SF) from mitogen-stimulated human mononuclear leukocytes (MNL) or T lymphocytes (J Neuroimmunol 10:151, 1985). In this study, we examined effects of MNL SF on human IgG uptake in cultured HNA (defined by the presence of glial fibrillary acidic protein) derived from biopsies of epileptogenic foci, CCF-STTG1 grade IV astrocytoma cells, and as a non-astrocytic control, CAKI renal carcinoma cells. By immunoperoxidase staining, cytoplasmic IgG was detected in all cultures exposed to IgG without SF although patterns and distribution of reaction products differed. IgG uptake was quantitated in cells cultured for various times with ^{125}I-IgG in the presence or absence of MNL SF at a concentration (0.8%) shown to enhance HNA (but not CCF-STTG1 or CAKI) DNA synthesis. In two experiments, HNA accumulated significantly ($p<.025$) more pg ^{125}I-IgG/mg cell protein without SF than either CCF-STTG1 or CAKI. Efficiency of protein uptake at 24 hours without SF in a typical experiment was in the order: HNA (1717.1 ± 43.6 SD pg ^{125}I-IgG/mg cell protein) > CCF-STTG1 (482.8 ± 23.8) > CAKI (243.4 ± 26.9). SF exposure significantly ($p<.01$) enhanced 24 hour IgG uptake only in HNA (2195.6 ± 163.3) and did not affect either CCF-STTG1 (431 ± 12.2) or CAKI (282.4 ± 49.4). The presence of a thousand-fold excess of unlabelled IgG in both experiments significantly ($p<.05$) blocked 31% of ^{125}I-IgG uptake in HNA and 46% in CCF-STTG1, indicating partial specificity of IgG uptake. Unlabelled IgG had no effect on uptake in CAKI cells. These data indicate that cytokines enhance IgG uptake as well as other activities of HNA, and that IgG uptake in astrocytic cells appears to involve some specificity. Results suggest that cytokine exposure may be one mechanism by which astrocytes accumulate cytoplasmic IgG in multiple sclerosis and other inflammatory CNS diseases. The contribution of such astrocyte activity to the pathophysiology of these disorders remains to be defined.

39.

Experimental Autoimmune Encephalomyelitis Induced by Encephalitogenic Antigens and Anti-basic Protein T-cell Line. A Clinico-Pathological Correlation

E. BERAUD, A. CARNINO and A.J. ZAMORA (Marseille, France)

In Lewis rats, the inflammatory acute phase of experimental autoimmune encephalomyelitis (EAE) is followed by a quick recovery. We have already shown the presence of suppressor cells at the time of recovery in rats injected with guinea pig basic protein (GPBP) in complete FREUND's adjuvant (CFA), suggesting that these suppressor cells could block the process before demyelination. To test this hypothesis, we performed a light and electronmicroscopical analysis of the spinal cord of rats where the emergence of suppressor cells had been prevented by means of adoptive transfer. Lewis rats receiving spinal cord homogenate (SCH) in CFA showed little or no demyelination; extensive mononuclear cell infiltration was observed in leptomeninges, in pericapillary spaces and in the neuropil, where fibrinous edema, proliferation of astrocytes and activation of microglia were present; in addition, some degenerating myelinated fibers were observed in the dorsolateral fasciculus. A similar picture was displayed in rats injected with synthetic encephalitogenic peptide in CFA, and also in those rats injected with lymphocytes from donors sensitized to SCH in CFA. Clinically, these animals presented a mild limb paresis. Surprisingly, all those rats injected with an anti-GPBP T-line cells developed severe paralysis in few days without showing any evidence of leptomeningeal and perivascular mononuclear infiltration. These results suggest that myelin constituents do not modify the action of the encephalitogenic peptide, and that a severe, monophasic clinical disease can be triggered — in absence of perivascular infiltration — by means of anti-GPBP T-lymphocyte line. This injury could be produced by soluble factors directly or indirectly related to the activation of anti-BPT-lymphocytes.

40.

Reactive Astrocytes in Multiple Sclerosis are Positive for Leu-M1 in Paraffin Sections

S.M. CHOU and J.M. MILES (Cleveland, Ohio)

It has been suggested that astrocytes may interact with immune cells and participate in immunopathic processes including demyelination. To test this hypothesis, several monoclonal and polyclonal antibodies directed against monocytic/histiocytic/granulocytic lineages were tested on astrocytes. Anti-Leu-M1 produced the most distinctive staining for reactive astrocytes in formalin-fixed and paraffin-embedded autopsy brains. The monoclonal antibody, anti-Leu-M1 (Becton Dickinson), specifically reacts with cells of granulocytic lineage and with Reed-Sternberg cells of Hodgkin's disease in formalin-fixed and paraffin-embedded tissue (Am J Clin Path 82:29, 1984). Sections from 5 MS brains and 10 non-MS controls with or without other neurological diseases were studied with monoclonal antibody to Leu-M1 utilizing the immunoperoxidase technique with avidin-biotin complex (ABC). Granulocytes in blood vessels served as internal positive controls in the anti-Leu-M1 reaction. Leu-M1 positive astrocytes were most numerous at the edge of MS plaques in all 5 MS brains. Lower numbers of positive astrocytes were seen within the plaque and in the adjacent white matter. No immunostaining was seen in the white and gray matter distant from the plaque. The staining pattern of astrocytes was exclusively cytoplasmic, with both perinuclear and fibrillary processes positive for Leu-M1. Hypertrophic (gemistocytic) astrocytes showed a peripheral cytoplasmic staining reaction with the central homogenous portion of the cytoplasm negative. No differential immunostaining was identified in acute, subacute or chronic MS lesions. Non-MS controls were negative for Leu-M1. Of great interest is the fact that, similar to Reed-Sternberg cells, reactive astrocytes are known to be capable of internalization of exogenous IgG and/or phagocytosis of immune complexes, and both cells share the common antigen, Leu-M1. While the phenotypic expression of monocytic/histiocytic/granulocytic lineages on reactive astrocytes at the margin of MS plaques might not be specific for MS, it tends to support the contention that astrocytes are involved in the initiation and/or perpetuation of the immunopathic demyelinating process involved in MS.

41.

Abrogation with Cyclophosphamide of the Resistance to EAE in F_1-Guinea Pigs

I. GORANOV, M. STAYKOVA, M. SVETOSLAVOVA and T. AL-IK (Sofia, Bulgaria)

F_1-guinea pigs were rendered tolerant to experimental allergic encephalomyelitis (EAE) if their mothers were treated with MBP + CFA during pregnancy or with MBP + IFA during pregnancy and/or lactation. About 80% of the offspring was proved to be EAE resistant at the age of 4 months. However, when a relatively low dose of cyclophosphamide was given 2-3 days prior to the encephalitogenic challenge this resistance was broken and the guinea pigs developed acute EAE.

42.

Vascular Function and Innervation in Experimental Allergic Encephalomyelitis

G.J. LEO, R.J. KONKOL, D.R. HARDER, W. KARPUS and J. KILLEN (Milwaukee, Wisconsin)

Increased blood-brain barrier (BBB) permeability, related to small vessel damage, is recognized as a significant factor in the pathogenesis of experimental allergic encephalomyelitis (EAE). Our study was undertaken to determine the effect of EAE upon large cerebral vessel function and innervation. Large cerebral vessels play a major role in the determination of blood flow. Recent work by Brosnan et al has demonstrated that prazosin, an alpha-1 blocker, attenuates the clinical course of EAE. A mechanism for this effect may be vascular relaxation resulting in increased cerebral blood flow. DiRocco has shown areas of ischemic hypoxia, distinct from inflammatory lesions, in brainstems of animals with EAE. These two studies offer indirect evidence of altered vascular reactivity and blood flow in EAE. We measured vascular contractility with an *in-vitro* technique in which vascular tone was determined in isolated segments of basilar artery. Increased reactivity of the EAE vessel segments was demonstrated by a heightened contractile response to serotonin (as determined by a dose-response curve) and an increased incidence of spontaneous rhythmical activity. The increase in reactivity first appears at the 10th day post-innoculation. This relatively early appearance of altered reactivity coincides or may even precede the development of increased BBB permeability as measured by Juhler and others. Utilizing a histofluorescence technique, the same vessels demonstrated an increased brightness, and possible density increase, of the adrenergic innervation at the time of maximal reactivity changes. Our studies have demonstrated altered reactivity and innervation of large cerebral vessels in EAE. Since these large vessels control cerebral blood flow to a large extent, alteration in function may play a significant role in the pathogenesis or course of EAE. Further studies will be needed to determine if these changes are due to direct or indirect effects of autoimmune inflammatory disease.

43.

Long Lasting Effect of Immunosuppressive Treatment in Patients with Multiple Sclerosis

M.J. BERNARD, W.M. NILLESEN and O.R. HOMMES

Since 1971 in our hospital patients with multiple sclerosis were treated with a short course of intensive immunosuppression (IIS) which consists of a total dose of 8 grams cyclophosphamide and 2 grams prednisone administered orally in 20 days. We examined peripheral blood (PB) and cerebrospinal fluid (CSF) of 13 patients immediately before and after IIS. Clear changes in cellular and humoral aspects of immunology were observed. We also examined PB and CSF of 21 patients who did receive only one IIS 2.5 - 13.5 years ago and no other immunosuppressive treatment afterwards indicating a benign course of the disease after IIS. In these patients many of the short-term changes can still be found. Correlations between the different parameters and the relation with the clinical findings will be reported.

44.

Treatment of EAE in the Rat with Monoclonal Antibodies

S.W. BROSTOFF and D.W. MASON (Charleston, South Carolina; Oxford, United Kingdom)

We first reported in 1984 (J Immunol 133:1938) that EAE in the rat could be treated successfully with a non-complement fixing anti-CD4 monoclonal antibody. A subsequent report (Science 227:415, 1985) made the same observation in the mouse utilizing a complement fixing anti-CD4 monoclonal antibody. We sought to compare the efficacy of using a complement fixing versus a non complement fixing anti-CD4 in the same species (rat). We report that both W3/25 (IgG_1) and OX35 (IgG_{2a}) anti-CD4 monoclonal antibodies are equally efficacious in treatment of EAE in the rat. Paralyzed animals recovered full neurologic function within 48 hours after a single injection of 1-1.5 mg of monoclonal antibody, compared to 4-5 days for recovery of untreated

controls. In contrast to W3/25, which did not appear to lower the number of circulating CD4+ cells, OX35 reduced the number of CD4+ cells in the peripheral blood by approximately 25% without any noticeable improvement in recovery times when compared to W3/25 treated rats. Dose response curves of each antibody showed similar therapeutic activity of both antibodies at similar concentrations. These findings suggest that it is sufficient to block the CD4 molecule for successful treatment rather than to destroy the CD4+ cells.

We also explored the ability of anti I-A antibody to treat EAE in the rat following the observation (J Exp Med 158:362, 1983) that such treatment was successful in the mouse model of EAE. In contrast to the results in mice, we report here that even at doses as high as 12 mg (6 mg/day for two days) little if any improvement in recovery time of treated rats was observed.

This work was supported in part by NIH grant No. NS-11867.

45.

Use of Electroimmunoblotting to Detect Oedema Induced by EAE and Neurotoxins

N.K. de ROSBO, M.V. JAGO, P.R. CARNEGIE and C.C.A. BERNARD (Bundoora and Parkville, Australia)

The electroimmunoblotting technique allows the study of a specific protein in a complex mixture. It involves the electrophoretic transfer of proteins separated by SDS-PAGE on to nitrocellulose paper which is then probed with an antibody specific for the protein. Antigen-antibody complexes are then visualized by auto-radiography of the paper following incubation with ^{125}I-Protein A. This technique has been applied quantitatively to the detection of extravasated plasma proteins into the spinal cord of rats affected with experimental autoimmune encephalomyelitis (EAE). Significant increases in the level of IgG were observed throughout the spinal cord of EAE rats and correlated with the onset of typical clinical signs. Such clinical signs (limp tail, hind limb paralysis) are also observed in young adult rats two days after injection with tunicamycin, a toxin closely related to corynetoxin, the causative agent of annual rye grass toxicity (ARGT) to sheep. Examination of the lumbar region of the spinal cord in tunicamycin-treated rats by electroimmunoblotting also indicated extensive increases in IgG when clinical signs appeared. These increases, indicative of perturbations in the blood-spinal cord barrier, offer more evidence that vasogenic oedema occurs during neurological disorders such as EAE and ARGT and could be one of the major causes of clinical impairment.

Supported by NMSSA and NH & MRC.

46.

Immunomodulatory Roles of the Cerebral Neocortex

P.J. NEVEU, P. BARNEOUD, S. VITIELLO and M. Le MOAL (Bordeaux, France)

The cerebral neocortex is known to be involved in the control of the immune system. In female C_3H/He mice in which either the right or left fronto parietal cortex was lesioned, mitogen-induced B-and-T cell proliferation with Con A, PHA and LPS was performed. In left lesioned mice, T cell proliferation was depressed by about 50% as compared to controls. In right lesioned mice, lymphocyte mitogenesis was increased by 140% as compared to controls and by 220-300% as compared to that observed in left lesioned animals. However in animals with more restricted lesions, the results are somewhat puzzling. Animals with right lesions showed depressed mitogen-induced lymphoproliferation whereas animals with bilateral lesions exhibited enhanced mitogenesis, but lower antibody production to sheep erythrocytes. Left lesions appeared not to modify lymphoproliferation induced by mitogens. Furthermore the percentage of suppressor T lymphocytes is depressed in animals with bilateral lesions as compared to any of the other groups. None of the lesions performed appeared to modify the natural killer cell activity. These results indicate that the immunomodulatory functions of the cortex depends on the various area of the latter and that some connections between left and right cortex are involved in modulation of the immune system.

47.

Treatment of Experimental Allergic Encephalomyelitis (EAE) in Primates with Anti-CD4 and Anti-HLA-DR Monoclonal Antibodies

L.M. ROSE, E.C. ALVORD JR., S. HRUBY, S. JACKEVICIUS, R. PETERSEN and E.A. CLARK (Seattle, Washington)

There is considerable evidence that multiple sclerosis (MS) is an autoimmune disease and that effective treatment of MS may require the elimination of autoreactive cells. Several research groups are attempting to eliminate or block specific populations of cells using monoclonal antibodies (MAb). Very little is known about the effects of this type of treatment on 1) the course of the disease, 2) the immune system, or 3) the central nervous system. We are attempting to study these questions in a primate model of MS, Experimental Allergic Encephalomyelitis (EAE). We are currently testing the effectiveness of anti-CD4 MAb and anti-HLA-DR MAb in the treatment of EAE. At this time, 21 animals have been induced to develop EAE by sensitization with purified autologous myelin basic protein (BP) in complete Freund's adjuvant. Once these animals exhibit clinical signs of at least ± → + severity, they have been treated with either *anti-CD4 antibody:* Leu-3a (4 animals) or OKT4a (3); *anti-HLA-DR antibody:* HB10a (4), H4 (3), or HU-30 (2); or control proteins: Leu-21 or saline. When we measure the *mean survival* (ms) of primates after the onset of EAE for each treatment (maximum = 30 days), we find the following: *Leu-3a (n = 4) ms = 20.3 days*; OKT4a (n = 3) ms = 8.3 days; total anti-CD4 (n = 7) ms = 15.1 days; total anti-HLA-DR (n = 9) ms = 8.8 days; *total control treatments (n = 5) ms = 3.0 days*. Our results indicate that anti-CD4 MAb, and in particular Leu-3a, can prolong survival and in some cases, completely reverse the disease process. However, most animals are not cured of their EAE. These results suggest that CD4+ cells are not the only cell type important to the pathogenesis of EAE and that a combined antibody therapy may be more effective in the treatment of this disease.

48.

Observation on the Intraventricular Administration of Autologous or Homologous LAK Cells and RIL-2 to Patients with Meningeal Dissemination from Brain Tumours

K. SHIMIZU, Y. OKAMOTO, Y. MIYAO, Y. MATSUI, S. NAKATANI, M. YAMADA, N. TSUDA and H. MOGAMI (Osaka, Japan)

Our *in vitro* studies demonstrated that peripheral blood lymphocytes activated with human recombinant interleukin-2 (rIL-2) generated cells that were lytic for fresh autologous tumour cells but not for normal lymphocytes or lymphoblasts. Then, we treated 5 patients with meningeal dissemination derived from some brain tumours (2 medulloblastomas, 1 germinoma, 1 cerebellar astrocytoma, and 1 metastatic) in whom standard therapy had failed. Patients received both 2 to $10x10^9$ autologous lymphokine-activated killer (LAK) cells or homologous (one-haplotype identical matching) LAK cells, generated from lymphocytes obtained through multiple leukaphereses, and rIL-2 for two to three months. 1 to $2x10^8$ LAK cells per ounce were intrathecally administered to the patients with brain tumours through Ommaya reservoirs or venticulo-peritoneal shunt valves three times a week. 500 units of rIL-2

were diluted in 50 ml of normal saline and infused intravenously over 15 minutes, or 25 to 50 units were injected intracisternally, during the period of LAK cell administration. Objective regression (no malignant cells were detected in the CSF) of brain tumours was observed in 4 of the 5 patients: complete tumour regression occurred in one patient with meningeal dissemination of tongue cancer and has been sustained for up to 10 months after therapy, and partial responses occurred in three patients with meningeal dissemination from medulloblastoma, germinoma and astrocytoma. 4 in 5 patients were also effective in reducing the clinical symptoms and signs (headache, appetite loss, abducens palsy and gait disturbance). Thus, this therapy is an attractive approach for the treatment of malignant tumours that have poor immunogenecity or are insensitive to several anticancer agents, and for patients with severe immunosuppressive conditions induced by repeated radiation therapy or chemotherapy.

49.

Antigens in the Brain Parenchyma Evoke an Immune Response in Cervical Lymph Nodes Via Nose Lymphatics

H. WIDNER, G. MÖLLER and B.B. JOHANSSON
(Stockholm, Sweden)

The brain is immunologically privileged, denoting a lowered capacity to reject grafted tissue. Claims have been made that this is due to a lack of lymphatic drainage. We have shown that there is a specific antigen response in the deep cervical lymph nodes, after an injection of 5 μl SRBC into the neostriatum (nucleus putamen) of adult CBA mice. The maximum response was on day 6, IgM757 ± 109 Plaque Forming Cells/10^6, and IgG_1 415 ± 85 PFC/10^6, measured by the protein A method. There was no response in the lymph nodes after i.v. immunization. There was a prominent splenic response both after i.v. and i.c. immunization, indicating for the i.c. injection a passage to the blood, presumably via the CSF and directly into the blood, in the damaged area.

The lymph node weight increased 2.6 times on day 5 after i.c. injection. The route of the antigens have been directly visualized with radioactive tracers, and 5-15% of the injected material can be found in the lymph nodes, within 2 hours, depending on the size of the particles.

The injections have caused a transient barrier breakdown, which was healed for macromolecular tracers passage within 4 days, as shown by the extravasation of Evans Blue labelled albumin.

The transport is extracellular, as shown by the injection of HRPeroxidase injected in the same manner. The passage is across the lamina cribriformis, and the antigens are taken up by the lymphatic vessels in the nasal mucosa.

We conclude that immunization of antigens injected into the brain parenchyma can take place in a regional lymphatic tissue, and that the blood-brain barrier is more likely to be responsible for the immunologically privileged status of the brain.

50.

Oligoclonal IgG with Anti-brucella Specificity in CSF in Chronic Brucella Meningitis with Myeloradiculopathy

E.W. WILLOUGHBY, J.A. LAMBERT and S. BRAAN-STRÖ
(Auckland, New Zealand)

We report a case of chronic brucella meningitis with myeloradiculopathy and CSF containing oligoclonal IgG bands which had anti-brucella specificity. A 40 year old slaughterman had a paraparesis, progressive over 4 months with extensor plantar responses, absent ankle jerks and impaired sensation in the sacral dermatomes. Myelography was normal. CSF: protein 4.4 g/L; glucose 0.9 umol/L; WBC 174/cmm (95% lymphocytes); IgG/total protein 19%; multiple oligoclonal IgG bands on agarose gel electrophoresis and isoelectric focussing. Faint bands were variably seen in the serum. Brucella titres were: (agglutinins/comp. fixation) CSF: 1-320/1:512 serum 1:640/1:628. The organism was not cultured from blood or CSF. The oligoclonal bands were removed from the CSF by absorption with brucella antigen while oligoclonal bands in multiple sclerosis CSF and monoclonal serum IgG bands were not absorbed.

Treatment with both antibiotics and steroids were necessary for sustained neurological improvement. The findings provide further indication that the bulk of the CSF oligoclonal bands in inflammatory neurological disorders are in most cases directed against the responsible foreign antigen. Clinical improvement may require treatment directed against both the foreign organism and the inflammatory response.

51.

Monoclonal Anti-T-cell Antibodies for *In Vivo* Treatment of Rats With Experimental Allergic Neuritis (EAN)

T. OLSSON, K. STRIGÅRD, P. LARSSON, R. HOLMDAHL and L. KLARESKOG (Stockholm, Sweden)

To see if disease course can be modulated and to elucidate lymphocyte phenotype function during EAN, rats were treated with monoclonal antibodies *in vivo*. The effects of intraperitoneal injection of 5 different anti-rat T lymphocyte antibodies were first evaluated immunohistochemically in lymphoid organs of normal rats. Single injections of 1 mg W3/13 (Pan T cell), Ox19 (Pan T cell) or W3/25 ("helper" cells) caused partial elimination of their respective target cells. 0x8 ("suppressor/cytotoxic" cells) were completely eliminated whereas 0x6 (anti Ia) did not affect Ia-expressing cells. Lymphocytes isolated from animals injected with W3/13, W3/25 or 0x8 all showed profound decreases of their respective functions tested *in vitro*. EAN was then induced in Lewis rats by immunization with bovine peripheral nerve myelin and Freund's complete adjuvant. Groups of EAN rats were injected with 1 mg of one mouse monoclonal antibodies days 0, 9 (2 days before clinical EAN signs) or 15 (at height of disease), post immunization (p.i.) degree of clinical symptoms was followed daily. W3/13 injected day 0 p.i. prevented disease, when injected day 9 p.i., disease was reduced. W3/13 injected day 15 resulted in no difference compared to controls. 0x19 injected day 9 p.i. resulted in a profound disease exaggeration. W3/25 and 0x6 injected day 9 p.i. reduced disease. 0x8 injection gave different results depending on time for injection after immunization. 0x8 injected day 0 p.i. resulted in increased EAN symptoms, injection day 9 p.i. reduced disease and day 15 p.i. increased disease. In conclusion, monclonal antibodies may be used to influence an autoimmune demyelinating disease. 0x19 reactive cells may contain a population of cells with suppressive influences. Such cells may not occur among 0x8 reactive cells, at least not during certain phases of EAN. As expected, W3/25 and 0x6 reduced disease. The anti-Ia antibody (0x6) may act by blocking Ia-expressing macrophage/dendritic cells, activated T cells or block migration of T cells to target. The complex and sometimes unexpected effects of *in vivo* treatment with monoclonal antibodies underlines the importance of experimental studies before use therapeutically in humans.