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Meeting Report

First Gordon Research Conference on Neurovirology and Second International NeuroVirology Symposium 1999

Colby-Sawyer College, New Hampshire, USA

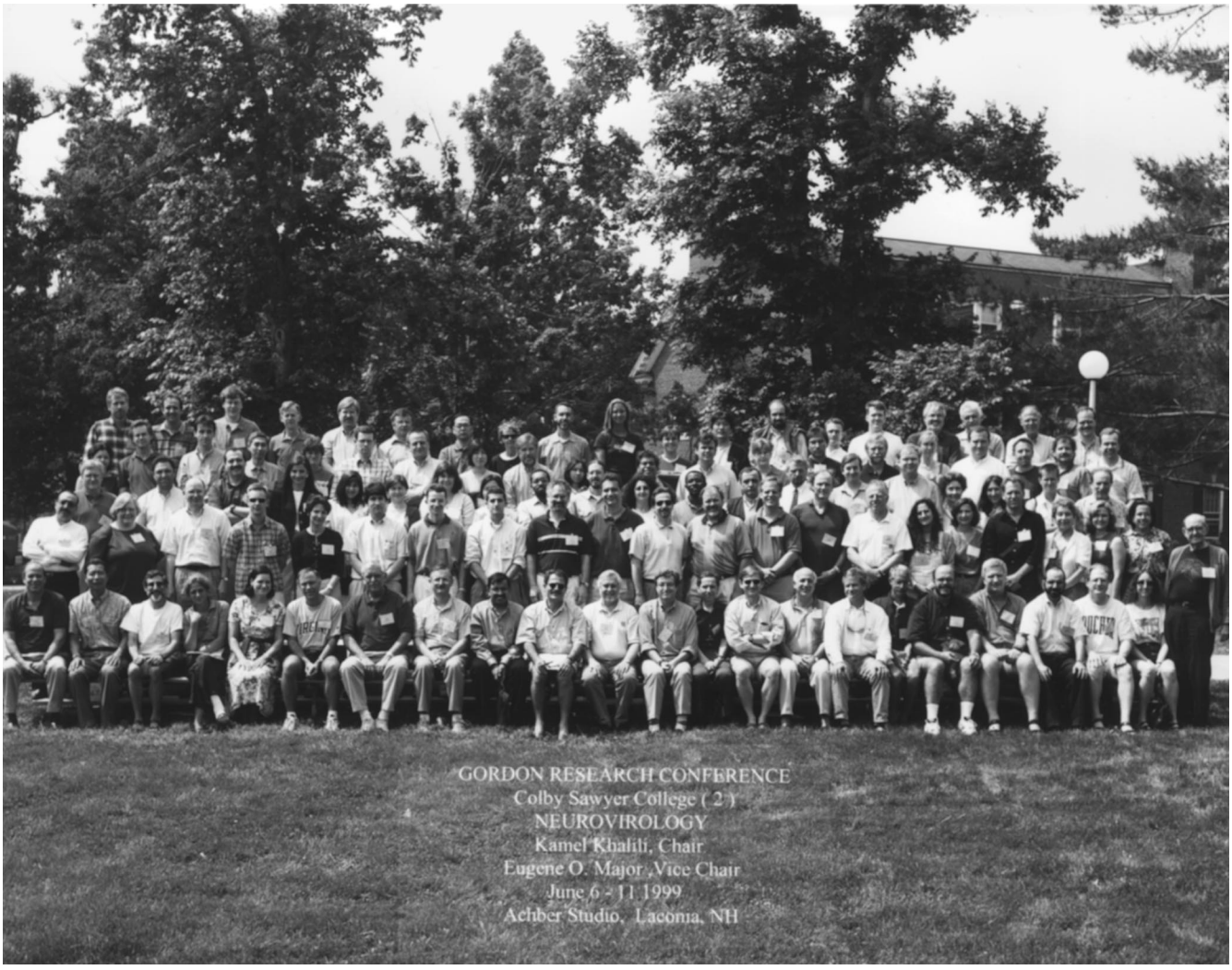
More than 120 internationally renowned scientists, postdoctoral fellows and students participated in the first Gordon Conference in Neurovirology which was held in Colby-Sawyer College, New Hampshire between June 6–11, 1999. The Conference was composed of seven sessions in addition to the opening session where Dr Richard Johnson provided a compelling history and overview of the field of neurovirology, and Dr Charles Weissman delivered the keynote speech on prions.

The first session on Monday morning began with a presentation from Dr David Volsky who described the effects of HIV-1 infection or exposure to viral envelope glycoprotein on L-glutamate transport by human fetal astrocytes *in vitro*. Dr Volsky found that HIV-1 and gp120 induced significant and long-lasting impairment of glutamate uptake by astrocytes and that this defect correlates with down-modulation of the glutamate transporter GLT-1. Dr Lynn Pulliam presented a possible monocyte phenotype that causes neurotoxicity via an apoptotic mechanism and is associated with patients with AIDS and possibly Alzheimer's dementias. Mechanisms for this neural cell killing and dysfunction were discussed. Dr Janice Clements described the SIV model for HIV infection of the brain. She found that the CSF HIV viral load was not consistent with the brain viral load. The β chemokine MCP-1, synthesized by astrocytes, was shown to attract monocyte/macrophages in the CNS. Dr Howard Gendelman reviewed the role of CD40 ligand in enhancing chemokine production, in particular MIP-1α, and inducing macrophage activation. He also discussed the involvement of the chemokine receptor CXCR4 found on neurons and SDF-1 expressed on astrocytes and their ability to facilitate neuronal cell killing by macrophage supernatants. Finally, Dr Christopher Power reported that HIV envelope sequences V1, V2 and V3 control viral tropism and that tat from HIV strains isolated from patients with AIDS dementia caused behavior changes in SCID mice. He also provided an update on the FIV neuropathogenesis model.

Session II was devoted to latency/viral persistency. First, Dr Nigel Fraser introduced the concept of the mouse as a model for studying HSV infections and pathogenesis. Although it was clear that the mouse was a valuable model in the molecular virological understanding of the mechanism of HSV latency and reactivation, there are clear limitations in using this model. For example, in studying the immune response, some viral genes do not interact with the mouse system in the same way that they do with human counterparts. This problem is also evident when studying reactivation, where the mouse rarely if ever spontaneously reactivates to show recrudescent lesions, whereas the human host does.

In the second talk, Dr Stephen Straus discussed 'Viral and Host Factors that Regulate the Latency of Reactivation of Herpes Simplex Virus'. Two viral factors and a number of possible host factors were reviewed. As indicated earlier by Nigel Fraser, when HSV is latent it expresses only one of its 80 or so genes, leading to the accumulation in neurons of a single family of latency-associated transcripts (LATs). Strauss reviewed experimental data showing that the LATs are not required for the establishment or maintenance of latency, but they modulate the rate at which the virus will recur. Deleting the LAT region from the HSV genome yields a virus that is modestly impaired for reactivation. The quantity of latent virus appears more important in defining reactivation rates that the LATs, as demonstrated by a series of studies in mice and in guinea pigs. In addition, the role of cytokines and immunoregulators in primary infection and latency was discussed.

Next, Dr Jay Nelson addressed reactivation of latent human cytomegalovirus (HCMV) in allogeneically stimulated monocyte-derived macrophages (Allo MDM). Reactivation only occurred in macrophages produced by allogeneic but not mitogenic stimulation (Con A MDM). Dr Nelson described the cellular and cytokine components, which are essentially for HCMV replication and reactivation in Allo MDM. The importance of both CD4 and CD8+ T cells in the generation of HCMV permissive Allo MDM was demonstrated by negative selection of blocking experiments using antibodies directed against both HLA class I and HLA class II molecules. Examination of the cytokines essential for the generation of HCMV permissive Allo MDM identified IFN-g but not IL-1, IL-2, TNF-a or GM-CSF as



Gordon Research Conference, Colby-Sawyer College June 6–11 1999

Row 1: H Lipton, R Fujinami, H Geller, L Mannelidis, L Pulliam, S Jacobson, R Johnson, N Fraser, R Mahalingam, K Khalili, E Major, D Volsky, PG Kennedy, N Lipkin, D Fink, H Gelbard, S Perlman, M Buchmeier, J Nelson, H Gendelman, S Strauss, S Harrold, W Tourtellotte.
Row 2: A Jurado, N Berman, J Gow, S Keir, J Clements, J Blaho, I Koralnik, A Berger, S Croul, M Tardieu, F Krebs, B Wigdahl, P Massa, B Chesebro, C Weissmann, R Klein, A Brideau, J Lokensgard, R Itzhaki, K Jordan-Sciutto, M Jones.
Row 3: T Weber, M Safak, B Sawaya, B Krynska, T Sweet, M Lafon, CJ Welsh, N Chen, C Messam, P Jensen, F Sabri-z, A Phillips, P Ferrante, K Goodkin, A Jackson, G Zu-Rhein, M McCarthy, R Carp.
Row 4: S Maddocks, B Brew, G Guillemin, Y Ohara, C Speth, S Niewiesk, R Dorries, Y Okada, B Owe-Larsson, S Coilua, C Ramos, D Purcell, J Laguardia, R Cohrs, T White, M Burgoon, G Owens, R Frisque, C Power.
Row 5: H Rempel, W Atwood, S Frye, G Stoner, D Hooper, B Dietzschold, K Morimoto, MG Cusi, D Mock, L Boven, C Pereira, H Sawa, T Michiels, J McAllister, D McGavern, D McPhee, P Cheney.

critical components in the generation of these macrophages. The presence of dendritic (CD 1a and CD83) and macrophage (CD14 and CD64) cell markers on some of the Allo MDM which reactivate HCMV suggested that the cells were related to dendritic cells. However, dendritic cells obtained by stimulation of monocytes with IL-4 and GM-CSF followed by treatment with IFN-g and TNF-a could not reactivate nor be productively infected by HCMV.

In the last presentation of the session, Dr Michael Buchmeier described the pathogenesis of mouse hepatitis virus-induced demyelination. Infection of C57BL/6 mice with mouse hepatitis virus (MHV) results in an acute encephalomyelitis followed by a demyelinating disease characterized by mononuclear cell infiltration and white matter destruction, which resembles clinical and histologic features of the human demyelinating disease multiple sclerosis (MS). Studies were undertaken to evaluate the

contribution of CD4+ and CD8+ T cells to MHV-induced demyelination. CD4 and CD8 'knock-out' (ko) mice reached higher viral titers in the brain and delayed clearance compared to syngeneic C57BL/6 mice. Significantly less severe inflammation and demyelination was observed in CD4 ko mice as compared to CD8 ko and C57BL/6 at all time points examined. Furthermore, CD4 ko had markedly lower levels of mRNA transcripts and protein for the C-C chemokine RANTES when compared to levels found in CD8 ko and C57BL/6. Immunophenotyping of the CNS infiltrates revealed that CD4 ko mice had a significant reduction in the number of activated macrophage/microglia as compared to CD8 ko and C57BL/6 mice, indicating a role for these cells in myelin destruction. These data indicate the CD4+ T cells have a pivotal role in amplification of inflammation and demyelination following MHV infection of the CNS. CD4+ T cells may act by directly or indirectly controlling the expression of RANTES which directs the trafficking of macrophages into the CNS, leading to myelin destruction.

Signal transduction was the focus of the third session. First Dr Howard Federoff addressed the role of hypoxic stress on neuronal death. Under hypoxic or hypoglycemic conditions, mammalian tissues express adaptive gene products to satisfy metabolic demands. At the cellular level, hypoxia is sensed by an iron-containing moiety resulting in heterodimerization and nuclear translocation of the Per-Arnt-Sim (PAS) transcription factors hypoxia-inducible-factor-1α (HIF-1α) and aryl hydrocarbon nuclear translocator (ARNT). This HIF-1 complex promotes the expression of genes such as erythropoietin (EPO) by binding to hypoxia-responsive enhanced elements (HREs). The necessity for these specific PAS family members to engender the adaptive response has been established through gene disruption. Loss of either HIF-1α or ARNT in embryonic stem cells rendered them largely incapable of transactivating hypoxia and hypoglycemia responsive targets.

Under conditions of extreme hypoxia, the tumor suppressor p53 promotes growth arrest of dividing cells and apoptosis through the transactivation of genes such as $p21^{Waf1/Cip1}$ and *bax*. Conversely, human tumors harboring null or mutated p53 allele(s) exhibit reduced apoptosis under these conditions. Restoration of p53 function restrains cell growth and often re-establishes apoptotic potential. Aside from its pivotal role in the control of rapidly dividing tumor cells, p53 has also been implicated in the pathologic response to ischemia exhibited by post-neurons. p53 induction occurs within neurons following an ischemia insult and temporally precedes cell death. Finally, mice deficient in p53 exhibit reduced infarct volumes following middle cerebral artery occulsion, further illustrating the functional role of p53 in ischemic neuronal death.

Recent observations provide evidence for a linkage between p53 and HIF-1α in tumor cell lines. First, treatment with hypoxia, or hypoxic-mimetic agents, stabilizes p53 and HIF-1α protein, resulting in enhanced target gene transcription. Second, removal of HIF-1α from embryonic stem cells blocks the induction of p53 levels following extreme hypoxic exposure and attenuates stress induced cell death. Although a direct association between these proteins has not been proven, these data suggest that HIF-1α, either acting through a PAS partner or with p53, is involved in both adaptive and pathologic transcriptional responses to stress. Dr Federoff demonstrated that in neurons that HIF-α signaling participates in, hypoxia-induced delayed death. Evidently, in cortical neurons, HIF-1 signaling activates delayed death in a p53-dependent manner, thus defining a new and important node of regulation of ischemic cell death.

Dr Harris Gelbard elaborated on signaling pathways in the HIV-1 infected brain which may lead to death of neurons by apoptosis. He described the possible role of pro-apoptotic gene product caspase 3 in neurons of pediatric patients with HIV-1 associated neurologic disease. Because HIV-1 infection of the CNS results in apoptosis of vulnerable neurons, using *in vitro* system, they initially investigated the role of the pro-inflammatory cytokine tumor necrosis factor alpha (TNFa), produced by HIV-1 infected macrophages and microglia, because levels of TNFa in vulnerable brain regions, correlate with neurologic disease in HIV-1 infected patients. In this regard, the role of oxidative stress, excitotoxic activation of the AMPA subtype of glutamate receptors and down-regulation of high affinity glutamate uptake sites in astrocytes, and the involvement of Tat was discussed. In addition, Dr Gelbard also discussed the role of another neurotoxin, the pro-inflammatory phospholipid mediator platelet activating factor (PAF), which is produced by HIV-1-infected, antigenically stimulated macrophages, and activates the NMDA subtype of glutamate receptors in inducing neuronal apoptosis.

Also, it was demonstrated that TNFα induces PAF release from macrophages, and that the majority of neuronal apoptosis that occurs after exposure to neurotoxins secreted by antigenically stimulated HIV-1-infected macrophages can be blocked by catabolism of PAF, suggesting that PAF receptor activation may be crucial for HIV-1 neuropathogenesis.

Finally, Dr Gelbard showed that PAF activates glycogen synthase kinase 3 beta (GSK-3b) in mature neurons which is a critical step in activation of pro-apoptotic pathways. Because lithium is a specific inhibitor of GSK-3b, potential therapeutic interventions that target this enzyme

may be important in ameliorating neuronal dysfunction and death from HIV-1 infection of the brain.

Dr Herbert Geller demonstrated that neuronal apoptosis due to DNA damage by the anticancer agent camptothecin is signaled through the same p53-dependent pathway as found in dividing cells. Camptothecin induces up regulation of p53. Neurons from p53 knockout mice are resistant to camptothecin toxicity. DNA damage to neurons also stimulates an increase in the activity of cyclin dependent kinases (cdks) 4 and 6 and an increase in phosphorylation of pRb. These factors normally stimulate cell cycle progression. Inhibition of cdk activity and Rb phosphorylation by the anticancer agent flavopiridol or the expression of endogenous cyclin dependent kinase inhibitors reduces cell death. Rb phosphorylation normally releases the E2F/DP1 transcription factors, which also stimulate cell cycle progression. Dr Geller also showed that inhibition of the transcriptional activity of E2F reduces cell death in response to camptothecin. Thus p53 activation (which would normally stop cell cycle progression) and activation of cell cycle regulatory machinery are positive signals for neuronal apoptosis in response to DNA damage.

On the session devoted to Transmissible Encephalopathies, despite the continuing debate about the molecular nature of the infectious agent, several common themes were reinforced by the speakers, including a growing appreciation of (1) distinct agent strains, (2) the enormous latency between infection and symptomatic disease (sometimes 30–40 years), and (3) the need to define more reliable and sensitive agent-specific markers to prevent the spread of these lethal and potentially epidemic infections.

Dr Thomas Weber first introduced the clinical spectrum of the human spongiform encephalopathies. He then reviewed several clinically relevant changes such as periodic sharp wave EEG complexes and the presence of 14-3-3 protein in cerebrospinal fluid, noting however that these changes were not entirely specific for Creutzfeldt-Jakob Disease (CJD). While much of the older neuropathology literature has emphasized many distinctive clinical syndromes in CJD (that might potentially relate to variant agents), some of these differences have now become more important. Dr Weber indicated skepticism for the classification of sporadic fatal insomnia as a new form of CJD. However, BSE-linked human infections (vCJD) are certainly distinctive, and caused by a new agent strain that rapidly spread in cows of the British Isles. While vCJD is currently limited to very few symptomatic cases (n=36), the typical incubation time may be longer than yet elapsed, and iatrogenic propagation from blood, transplants and other human tissue products has the potential to amplify case numbers significantly.

Dr John Collinge brought up the last case of kuru (40 years after the cessation of ritual cannibalism) as a way to emphasize his concern about epidemic human vCJD infections that can be clinically silent for many years. There are several ways of recognizing infection by different agent strains such as vCJD. Dr Collinge reviewed data from the study of many CJD cases and showed four basic band patterns of host prion protein (PrP) after limited proteinase K digestion of brain homogenates. PrP resistance can be informative in human CJD, while most agent strains in other mammals produce no unique PrP profiles. These band patterns are thought to reflect variant folding or conformations of PrP. In human CJD two of these profiles are closely related, as the PrP band differences were abolished by removing the divalent ions Cu^{2+} and Zn^{2+} (see *Nature Cell Biol.* 1:55, 1999) Wadsworth *et al*, 1999). The ability of metal ions to influence PrP conformation was suggested as a potential molecular mechanism for strain variation that may be experimentally tested. The two other patterns of resistant PrP (types III and IV) were not similarly altered by chelating agents, and the type IV pattern is found in BSE-linked vCJD. Interestingly, the brain and tonsil contain different glycoforms of PrP in vCJD (Hill *et al*, 1999) (*Lancet*, 353:183, 1999) although the agent derived from each tissue is the same. Nevertheless, PrP band profiles from tonsils are adequate for identifying the BSE-linked strain. Furthermore, because other CJD strains, as those associated with sporadic CJD for example, do not induce pathologic PrP in tonsils, analysis of tonsilar biopsies can be a useful diagnostic test. Hence, large studies are now underway to assess the extent of asymptomatic vCJD infections in England. The PrP band phenomena are not well understood, and the central question is whether the variant PrP conformations encode strain-specific information, or alternatively, if they are a by-product of infection.

Dr Laura Manuelidis suggested that PrP in its various guises could result from the imprint of a separate infectious particle or strain. Indeed, agent interactions with host PrP may induce a cascade of self-perpetuating or 'seeded' amyloid changes, some of which can be tissue-specific. Such a view reconciles data showing that pathologic PrP separates from infectivity in a variety of settings, and that tissues with different PrP profiles yield the same agent strain. Recombinant or transgenic forms of PrP also show no convincing infectivity, suggesting an essential agent element is missing. Because these agents have many virus-like structural and biologic properties, it is likely that a critical nucleic acid has been overlooked. The peripheral residence and replicating site of the agent is also not entirely clear. Where does the agent hide in natural oral infections as vCJD? Macrophages in Peyer's patches

can act as reservoirs of quiescent but persistent infections. Upon activation, these cells have the capacity to deliver infectious particles to the brain as well as to induce major pathologic changes, i.e., some strains can elicit an autoinflammatory disease. Nevertheless there are agent properties that are separate from host responses. Two distinct CJD agents could be completely discriminated without confounding host or PrP conformation variables by inoculation into a single mouse. An attenuated 'slow' CJD strain completely suppressed superinfection by a more virulent 'fast' strain in 16 of 18 mice (*Proc. Natl. Acad. Sci.* 95:2520, 1998) (Manuelidis *et al*, 1998). This remarkable interference may rest on cryptic host defenses to the invading agent (other than those associated with PrP modification), or may involve defective interfering particles made by the slow agent. When reisolated from doubly infected mice, each strain maintained its own unmodified identity, and there was no evidence for conversion of one strain to another.

Dr Bruce Chesebro continued to discuss the prion and viral theories, and noted his own difficulties with the concept of spontaneous generation of infectivity in the prion theory, and the fact that a variety of other amyloids are unrelated to transmissible disease. He then talked about the ramifications of manipulating several features of PrP with respect to 'familial' disease. An octapeptide insertion mutant of PrP increased the resistance of PrP to proteinase K (PrP-res) and also enhanced its glycolipid membrane attachment in transgenic mice. These scrapie-like PrP features were associated with a cerebellar disorder, and tests of infectivity are being pursued. A synthetic PrP peptide from 119–136 (identical in sequence in hamsters, humans and mice) bound to PrP and was able to block the formation of PrP-res. This was of particular interest for prevention of disease. Finally, productive infection has often been assumed to take place in neurons, although older studies have demonstrated low levels of replicating agent in astroglia and other cultured cells. To further understand the cellular requirements for infection, hamster PrP was placed under the astrocyte specific GFAP promoter and propagated transgenically. Non-transgenic mice are resistant to hamster 263k scrapie. In contrast, the GFAP-PrP mice (when bred on a PrP null background) became sick ~230 days after intracerebral inoculation. Further assay of these brains showed very high levels of infectivity (*EMBO J* 16: 6057, 1997) (Raeber *et al*, 1997). Thus astrocytic PrP is sufficient for robust agent replication. Reasonably high levels of infection were also found in GFAP-PrP mice bred on a normal mouse PrP+ background. However, clinical disease was completely prevented in these mice. It is not known if this effect is analogous to that seen with the 119–136 PrP peptide. Finally, in the clinically ill GFAP-PrP/null mice the pathologic picture of neuronal vacuolization was indistinguishable from that seen in scrapie infected mice that were normal, or that expressed only neuronal PrP (under the NSE promoter). This is yet another piece of evidence that the disease may be largely inflammatory, and the pathologic footprints may not necessarily reflect the wanderings of the agent.

Session V focused on demyelinating diseases. One of the major reasons for the interest in virus-induced demylinating diseases, both in humans and in animal models, is that their analysis may provide insight into the cause of the most common and puzzling human demyelinating disease, multiple sclerosis (MS). Recent findings were presented on two relevant experimental animal models for MS, Theiler's virus and mouse hepatitis virus infections in mice, and on JCV virus infection in humans, which causes progressive multifocal demyelination (PML) in the context of immunosuppressive illness. In both of the experimental viral models, demyelination is immune-mediated. Dr Howard Lipton developed the theme of an etiological dichotomy of MS either caused by any number of acute virus infections that produce their effects by triggering autoimmunity as opposed to a single viral pathogen causing disease by persistent infection, perhaps by immunopathological means.

Dr Lipton summarized the pathogenesis of Theiler's virus infection in mice, highlighting the controversy regarding the role of CD4+ and CD8+ T cells in demyelination, and showing a strong correlation between demyelination and clinical disease in this model system, which has been disputed in the literature. Dr Robert Fujinami showed that mutations critically placed in VP1 loop 1 residues, e.g. VP1081 Thr, of the DA strain of Theiler's virus attenuate viral persistence and demyelination. The exact mechanism for attenuation remains unclear but could be due to modulation of virion receptor binding. Fujinami also presented evidence for peripheral myelin protein, PO, as a candidate receptor protein for Theiler's virus.

Dr Stanley Perlman showed that in the development of clinical disease, mouse hepatitis virus is selected such that the predominant cytotoxic CD8 T cell epitope is mutated. These mutations cause a profound lack of recognition by virus-specific CD8 T cells, and introduction of mutant virus into naïve animals results in increased morbidity and mortality. This selection of CTKL escape mutants occurs in the presence of polyclonal, monofunctional T cell response directed at the epitope. Dr Perlman also showed that no demyelination is observed in mice defective in T and B cell function; however, transfer of splenocytes from mice previously immunized to the virus results in demyelination within 6–7 days, with activation and recruitment of macrophages and microglia.

Finally, Dr John Henson focused on human demyelinating diseases, progressive multifocal leukoencephalopathy, PML. First, recent events in the PML field were reviewed. Dr EP Richardson, whose neuropathology laboratory described PML in 1958, died on November 30, 1998, after a long battle against lymphoma. Dr Henson mentioned the work by Dr Gerald Stoner who has characterized unique viral strains worldwide and has assembled a phylogenetic tree. At Brown University, Dr Walter Atwood has identified an N-linked glycoprotein on glial cells that serves as a receptor for JC virus binding. Assembled JC virus coat proteins can function as efficient vehicles for transfection, as recently shown by Dr Thomas Weber. The laboratory of Dr Eugene Major has demonstrated JC virus in human tonsil tissue, suggesting that tonsil could serve as a site of initial viral entry. Dr Igor Koralnik has categorized peripheral blood lymphocyte subsets that are associated with JC virus. Dr Kamel Khalili has shown that large T-antigen deregulates glial cell cycle pathways and impairs glial cell differentiation. JC virus DNA and large T-antigen expression has been detected in the childhood brain tumor, medulloblastoma, by the laboratory of Dr Khalili. Dr Henson described the focus of his laboratory on the cell specificity of the JC virus early promoter, and the role of T-antigen in this event. In DNaseI footprint analysis, T-antigen binding to site LTa II was more extensive than expected, based on homologies with SV40, and nuclear proteins protected several regions of the proximal promoter region in a cell-specific manner. Further, mutagenesis study revealed that T-antigen-mediated activation required the JC virus TATA box sequence, a pentanucleotide repeat immediately upstream of the TATA box and an Sp1 binding site downstream of the TATA box. When T-antigen footprints were compared between wild type and mutant promoters which blocked T-antigen-induced transactivation, no change in binding was observed. These results suggest that T-antigen activates the JC virus basal promoter in non-glial cells by interactions with transcription initiation complexes.

In the session devoted to emerging neurotropic viruses, Dr Steven Jacobson explored the possibility that a ubiquitous human herpes virus (HHV-6) may be associated with the pathogenesis of MS. His laboratory initially investigated HIV-6 serum IgM and IgG responses to HHV-6. Serum from 102 individuals with and without MS were screened in a blinded manner for IgM and IgG antibodies to the HIV-6 p41/38 affinity purified antigen using an EIA assay (33). As HHV-6 is ubiquitous and thought to be latent in approximately 90% of the adult population, it was not surprising that no significant difference in the anti HHV-6 IgG response to HHV-6 p41/38 early antigen was found. By contrast, there was a highly statistically significant difference in the anti-HHV-5 IgM response to the HHV-6 p41/38 early antigen, particularly between the RRMS group compared with normal controls ($P > 0.0011$). No differences were demonstrated in MS serum IgM to EBV and CMV. They have extended these serological studies to detect active virus in patients sera by extraction and amplification of HHV-6 DNA by nested PCR. No HHV-6 DNA was amplified from the serum of 47 non-MS, however, positive HHV-6 DNA signals were demonstrated in 21 of 77 (27%) MS patients ($P > 0.0001$). In addition, they have demonstrated increased proliferative responses in MS PBL specific for the HHV-6 strain. A variant (stimulation indices of infected lysates *versus* uninfected lysates as great as 7.0) while no differences between MS patients (n=15) and controls (healthy, normal individuals n=17) were observed to either the HHV-6B strain of HHV-7.

More recently, Dr Jacobson's laboratory had the opportunity to explore further the relationship between HHV-6 and MS by a detailed neuropathological and immunohistochemical analysis of CNS autopsy material from a patient with secondary progressive MS. To assist in identification of MS lesions, T2-weighted MRI images were obtained during the early postmortem period. HHV-6 protein was detected in actively demyelinating MS lesions and not in chronic plaques. HHV-6 viral protein was absent from normal white matter adjacent to MS lesions and was not detected in 13 other control brains. Other herpes viruses included HSV-1, CMV, and EBV could not be detected in MS lesions. Analysis of CNS tissue demonstrated the presence of MS lesion-specific HHV-6 viral protein in GFAP positive astrocytic glial cells and other CNS cells. HHV-6 viral expression correlated with lesion age and severity. Whether HHV-6 is a causative agent of MS, or the presence of HHV-6 serum DNS and elevated antibody titers is epiphenomenal, has yet to be determined.

In the next talk, Dr Ruth Itzhaki using PCR, provided evidence that a high proportion of brains of AD patients and of age-matched normals harbor latent HSV1. They found that the combination of HSV1 in brain and carriage of the type 4 allele of the apolipoprotein E gene (apoE-ε4) is a strong risk factor for AD, and that neither HSV1 nor apoE-ε4 alone is a risk. Examination of brain from further cases has produced exactly similar results. Dr Itzhaki also found, in striking parallelism in the PNS, that apoE-ε4 is a risk factor for herpes labialis. She suggested that the combination of virus and apoE-ε4 is particularly harmful in the nervous system and that the virus in brain reactivates periodically, causing more damage – eventually AD in – apoE-ε4 carriers. In contrast, in another

herpes virus disease – of the cornea, herpes simplex keratitis – she showed that the ε4 allele frequency is similar to the population value.

In studies on HSV1-infected mice, Dr Itzhaki described that vaccination (with mixed viral glycoproteins) prevents establishment of latent HSV1 infection in the brain. Thus, development of a human vaccine might be feasible.

Dr Sidney Croul's talk was focused on the association of JCV with medulloblastomas. Medulloblastoma represents greater than 25% of childhood intracranial neoplasms and is considered a highly malignant tumor. This tumor, which arises predominantly in the cerebellar vermis, preferentially affects children between the ages of 5 and 15. While the etiology of medulloblastomas in humans remains unknown, results from several experiments have indicated that the human neurotropic JC virus (JCV) is able to induce cerebellar neoplasms in rodents which exhibit a phenotype similar to that of human medulloblastomas. JCV is a human neurotropic polyomavirus which is widespread in the human population with infection occurring most frequently in early childhood. Dr Croul provided evidence for the possible association of JCV with human medulloblastomas. By utilizing techniques he demonstrated that 11 out of 23 samples of tumor tissue contain DNA sequences corresponding to three difference regions of the JCV genome. He demonstrated that the presence of DNA sequences encoding the N- and C-terminal regions of the JCV oncogenic protein, T-antigen, in 11 out of 23 samples and the production of T-antigen in the nuclei of four samples of tumor tissue. These observations provided evidence for a possible association of JCV with human medulloblastomas.

Dr Ian Lipkin reviewed the current understanding of Borna Disease Virus (BDV). BDV is a nonsegmented, negative strand RNA virus that has tropism for limbic system and dopamine circuits. This virus infects a broad variety of vertebrate hosts ranging from birds to primates to cause a complex syndrome of behavioral disturbances resembling some aspects of such human psychiatric diseases as bipolar depression, schizophrenia and autism. Dr Lipkin is studying the molecular genetics of BDV, elucidating the molecular and cellular basis for BDV neurotropism and determining whether BDV or a similar virus can be implicated in the pathogenesis of human neurosychiatric illness.

Finally, the last session, chaired by Dr Gene Major, was devoted to molecular and cellular therapeutics. Dr Major provided an introduction and description of methods of delivery of viral and cell vectors to the CNS using several examples of delivery methods of Adeno Associated Virus to the brain parenchyma. This was followed by a presentation by Dr Antonio Chiocca who described the use of herpes virus vector for oncolytic effect of gliomas in rodent models; Dr David Fink on altered herpes virus genomes for long-term gene expression in the peripheral and central nervous system using the viral latency gene; and Dr Darwin Prockop on cell transplantation of mesenchymal or stromal cells engineered to produce collagen for treatment of osteogenesis imperfecta.

The session explored the use of cell and viral vectors to deliver and express genes whose products are neuroprotective or restorative for nervous system functions. Vectors which are useful in the CNS also have potential for other organ systems as well. The session opened with an overview of the field, emphasizing the importance of delivery mechanisms as a critical step in gene therapy for the CNS. For example, stereotactic injection of the neurotrophin GDNF, glial derived neurotrophic factor, was compared with CED, convection enhanced delivery, of GDNF. The convection delivery was able to deliver GDNF throughout the striatum compared with only a few millimeters from the injection site. CED was also used to distribute an AAV expression vector for DDC, dopamine decarboxylase which is the enzyme that converts 1-dopa to dopamine. Distribution of AAV and consequent infection occurred throughout the hemisphere from the infusion site in the putamen. A cell line derived from human fetal brain was also described containing a mixed population of differentiated as well as progenitor and stem cells of the human CNS. The cells differentiate into either glial or neuronal phenotypes depending upon metabolic stimulation provided in the growth medium.

Use of herpes virus vectors was also discussed in delivering anti-tumor agents into the brain. Modified HSV, HSV-RR, vectors that cannot replicate could be delivered to experimental models of gliomas in rodents through intracarotid injections. The altered HSV could infect the tumor cells, express the viral thymidine kinase gene and then kill the cell in the presence of gangcyclovir. HSV vectors could also deliver anti-tumor drugs such as cyclophosphamide. Other uses of HSV vectors was described which could take advantage of long-term expression in neurons since these viruses can establish latency, associated with a specific viral mRNA.

Bone marrow contains cells which are osteogenic and can be isolated from the marrow and grown in culture. Such cells either constitutively produce or can be genetically engineered to produce molecules such as collagen to correct genetic defects. Transplantation of these cells or

stromal cells in the marrow are being used in clinical trials of diseases such as osteogenesis imperfecta in children.

In addition to these platform presentations, more than 65 posters were presented by the participants of the meeting.

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Contributors to the report

Sidney Croul, Howard Federoff, Nigel Fraser, John Henson, Ruth Itzhaki, Steven Jacobson, Kamel Khalili (Chair), Ian Lipkin, Howard Lipton, Eugene Major (Co-Chair), Laura Manuelidis, Lynn Pulliam, David Volsky and Thomas Weber.

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