Journal of NeuroVirology (1995) 1, 323–325

Guest editorial

Coronaviruses

Coronaviruses are ubiquitous in nature, and infection of many avian and mammalian species by these positive sense RNA viruses results in important veterinary diseases. In humans, coronaviruses are typically associated with upper respiratory tract infections such as the common cold. Most murine coronaviruses cause hepatitis, with the result that this group of viruses is usually referred to as mouse hepatitis viruses (MHV). However, a number of MHV strains, such as strain A59 or strain JHM, are very neurotropic. These neurotropic MHVs cause interesting natural and experimental diseases in the central nervous system (CNS) of rodents. Under defined conditions, these viruses cause paralysis and primary demyelination which model multiple sclerosis (Kyuwa and Stohlman, 1990). Also, several reports have linked coronaviruses to multiple sclerosis tissue directly, although this remains an area of current controversy and investigation (Murray *et al*, 1992; Stewart *et al*, 1992).

In this issue of the *Journal of NeuroVirology* two interesting papers explore different aspects of coronaviruses pathogenesis. The paper by Yokomori and colleagues explores the role of viral-specific factors in pathogenesis. In particular, this study contrasted the neuropathogenicity of two MHV isolates, JHM(2) and JHM(3). These isolates differ primarily in the copy number of a pentanucleotide sequence (UCUAA) in the leader of the viral genomic RNA. JHM(2), which has two copies of this pentanucleotide, expresses large amounts of the hemagglutinin-esterase (HE) protein. By contrast, the three copies of the pentanucleotide sequence in the leader of JHM(3), through unknown mechanisms, leads to the expression of very little HE. Polyacrylamide gel electrophoresis of viral structural proteins and sequencing of the viral spike or S protein suggest that the principle difference between JHM(2) and JHM(3) is the level of HE expression, although subtle point mutations and alterations of non-structural proteins cannot be entirely ruled out.

Studies in mice reveal an interesting contrast between the pattern of infection of the two JHM strains. Early in infection the pathological changes induced by the isolates are indistinguishable; however, later in infection, on days 5–7 post-inoculation (P.I.), JHM(2) is found predominantly in neurons and causes a clinically evident encephalitis with early death. By contrast, at days 5–7 P.I. JHM(3) is found predominantly in glial cells of the white matter, although by day 14 P.I. JHM(3) ultimately infects neurons and causes a severe encephalitis. Despite these differences, the total viral yield from the central nervous system of mice infected with JHM(2) and JHM(3) is roughly equivalent at any given time, indicating that the observed difference in pathogenesis is more a matter of the relative distribution of viral infection than the absolute magnitude of viral load.

Thus, increased expression of the HE protein seen in JHM(2) is associated either with increased intrinsic tropism of the virus for neurons or more rapid spread to neurons. This difference is not absolute, however, since JHM(3) ultimately does infect neurons. Similar results have been found in antibody escape mutants of JHM which have marked or exclusive tropism for the glial cells of white matter in immunocompetent mice, but ultimately will infect neurons in immunosuppressed mice (Fleming *et al*, 1987 and unpublished observations). These results may perhaps best be conceptualized as the results of a 'race' in the CNS between the virus and the host immune system. Glial cells outnumber neurons by a factor of ten to fifty times in the vertebrate central nervous system (Kandel *et al*, 1991), and this, coupled with other unknown factors, may make them the 'default' cell of choice for attenuated MHVs. Ordinarily, an effective level of immunity occurs at the stage of glial infection, and the virus is extinguished or suppressed before neurons are infected. By contrast, those MHVs which have increased efficiency of infection or altered tropism by virtue of surface protein expression are more likely to infect neurons early in the disease, before the immune system is sufficiently activated to prevent fatal encephalitis.

Of the two factors — efficiency of spread and altered cellular tropism — the authors favor the later, principally because of altered growth in mixed glial cultures and primary astrocytic cultures *in vitro*. These studies show that after 10 days in culture the yield of extracellular and intracellular virus is substantially higher with JHM(3) than it is with JHM(2). However, examination of these data shows that at earlier time points, (which may be more relevant to the situation *in vivo*) the yield of the two viruses in these cultures is virtually indistinguishable; a difference in yield is only significant at the last time point, when the titer of JHM(2) begins to decline. Clearly, future studies will be necessary to resolve this interesting issue. For the time being, however, what matters is that Yokomori *et al* have shown for the first time that the level of

HE protein expression contributes to the effective viral tropism of JHM and thus the sort of disease caused by this virus.

The paper by Hein and colleagues also focuses on coronavirus JHM. In contrast to the paper of Yokomori *et al*, these authors concentrate on the influence of host factors in pathogenesis. To do this, they exploited the differential response of Lewis (LEW) inbred rats and Brown Norway (BN) inbred rats to JHM. It has been known for some time that the LEW rats are susceptible to JHM-induced paralysis and demyelination, while the BN rats are relatively resistant to JHM-induced disease. The authors have previously shown that during the acute phase of infection with JHM, both rat strains have lymphocytic infiltrates in the central nervous system which are predominantly of the $CD8^+$ T lymphocyte set and presumably cytotoxic lymphocytes (CTL). In order to characterize these cells functionally, assays for virus specific cytotoxic activity of CNS derived lymphocytes were performed after short term *in vitro* stimulation of leukocytes isolated from the brains of the two rat strains. Surprisingly, CTL activity was found in leukocytes of both rat strains, with roughly comparable kinetics, although the absolute level of CTL activity was higher in LEW than in BN rats by a factor of four.

These intriguing results lead to an apparent paradox: although cellular immune responses in general and CTL responses specifically have been suspected of contributing to paralysis and demyelination in this model, the minor difference which was observed in CTL activity between the two strains did not parallel the dramatic difference in pathogenicity. The resolution of this paradox probably lies in previous observations by the authors which indicate that BN rats have a very robust humoral response to the virus early in disease, with neutralization titers that are at least thirty times higher in magnitude than those of the LEW rats. Thus, an early, effective humoral response probably limits viral spread, and thereby diminishes both the stimulation for CTL production and the ultimate target for activated CTL. Hein *et al* also raise the interesting possibility that $CD8^+$ T cells which are prominent in tissues during disease may limit virus and cause immunopathology through cytokine expression rather than direct cytotoxicity. Again, further studies will be required to unravel this and other aspects of pathogenesis such as the role of $CD4^+$ T cells as regulators of the different arms of the immune system.

The studies of Yokomori *et al* and Hein *et al* are of interest because they show how rigorous study of experimental viral infections of the CNS of rodents can lead to interesting insights with relevance to basic mechanisms of pathogenesis and perhaps with relevance to human diseases as well. What other lessons can we take from these papers? I think both papers remind us of the complexity and multifactorial nature of viral pathogenesis *in vivo*. For example, most studies of viral factors in MHV pathogenesis have concentrated on S, the major surface glycoprotein of MHVs. In distinct contrast, the studies of Yokomori and colleagues show that the HE protein, usually considered a minor and dispensable component of the virus, especially *in vitro*, can nevertheless have important influences on the behavior of the virus *in vivo*. Similarly, the studies of Hein and colleagues focus on cellular immunity as the most important host factor determining the outcome of pathogenesis. In this, they are in the mainstream of temporary coronavirus research. Unexpectedly, however, they show that a major difference in cellular immune response in two rat strains likely is secondary to an early difference in humoral immune response to the virus. This early, robust humoral response reduces the viral load and indirectly abrogates subsequent immunopathology mediated by cellular immunity. The important observation of both papers is that a 'minor' component of pathogenesis, usually thought to be of little or no consequence, can, under certain experimental conditions, have a decisive influence on the outcome of disease.

By necessity, most scientific investigations must take a reductionistic approach which focuses on a small number of crucial variables which can be identified and rigorously investigated. However, the studies above remind us of the complexity and interrelatedness of all factors contributing to pathogenesis during infection, especially in tissues as intricate as those of the CNS. These papers also imply that the results of a reductionistic analysis must be related to or understood in terms of the whole picture of viral CNS infection, a situation which is obviously influenced by numerous viral and host factors. For example, it is currently fashionable to look for the particular nucleotides which may define viral promotors or to search for point mutations which alter viral virulence. Against this, however, are contemporary theorists such as Eigen (1992) and Kauffman (1993) who argue that all genomes, including viral genomes, should be thought of as ensembles of interacting genetic elements, and that evolution and selection depend upon the combinatorial optimization of the whole ensemble. Thus, the efficiency and pathogenic potential of MHV strains ultimately depends upon how well the HE, S, and other gene products interact in a self-organizing unit. In a similar vein, theoreticians of the immune system have emphasized the importance of networks and regulatory cycles involving all aspects of the immune apparatus in response to challenge with microbes such as viruses (Cohen, 1992). This is particularly well-illustrated in the observations of Hein and colleagues, who show the dependence of cellular immune responses on prior humoral immune responses. Finally, recent studies of virus-host pathology, such as that of

Novak and colleagues (1995) on the interaction of HIV-1 with the immune system, indicate that these interactions must be understood in dynamic and quantitative terms.

Taken together, the two papers make interesting and unexpected observations with regard to viral and host factors during pathogenesis. Perhaps of equal or greater importance is the corrective effect they have in showing the shortcomings of studies which exclusively focus on a reductionistic analysis of a putative major or crucial determinant of pathogenesis. Both papers remind us how complex pathogenesis is in reality and how exciting its study can be. They do this by counterintuitive observations which show that supposedly minor components of a virus or host may have a major impact on the outcome of disease.

John O Fleming
Department of Neurology
Department of Medical Microbiology and Immunology
University of Wisconsin Medical School
600 Highland Avenue
Madison WI 53792, USA

References

Cohen IR (1992). The cognitive paradigm and the immunological homunculus. *Immunology Today* **13**: 490–494.

Eigen M (1992). *Steps Toward Life: A Perspective on Evolution*. Oxford University Press: Oxford, pp 1–173.

Fleming JO, Kachuck NJ, Stohlman SA, Weiner LP (1987). Viral neurotropism may be altered by immunosuppression. *Neurology* **37** (Suppl 1): 343.

Kandel ER, Schwartz JH, Jessell TM (1991). *Principles of Neural Science*. Elsevier: New York, p. 22.

Kauffman SA (1993): *The Origins of Order: Self-organization and Selection in Evolution*. Oxford University Press: New York, pp 1–709.

Kyuwa S, Stohlman SA (1990). Pathogenesis of a neurotropic murine coronavirus, strain JHM in the central nervous system of mice. *Semin Virol* **1**: 273–280.

Murray RS, Brown B, Brian D, Cabirac GF (1992). Detection of coronavirus RNA and antigen in multiple sclerosis brain. *Ann Neurol* **31**: 525–533.

Nowak MA, May RM, Phillips RE, Rowland-Jones S, Lalloo DG, McAdam S, Klenerman P, Koppe B, Sigmund K, Bangham CRM, McMichael AJ (1995). Antigenic occillations and shifting immunodominance in HIV-1 infections. *Nature* **375**: 606–611.

Stewart JN, Mounir S, Talbot PJ (1992). Human coronavirus gene expression in the brains of multiple sclerosis patients. *Virology* **191**: 502–505.