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# Virus – cell interactions in the nervous system and the role of the immune response

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The outcome of a viral infection within the nervous system depends on a complex interplay between the virus, its target cell and the immune system. Recent research has elucidated a variety of mechanisms involved in these interactions and their role in the production of disease.

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## Introduction

The inter-relationship between viruses and the nervous system is complex and diverse. Whereas some viruses may cause a lethal encephalitis, others, within the immunoprivileged environment of the central nervous system (CNS), are able to establish lifelong infections. Anatomically the CNS is protected by the blood–brain barrier, which not only serves to limit the entry of infectious agents, but also limits the influx of serum and cells. However, many viruses have evolved sophisticated ways to breach these barriers, either by entering from the bloodstream via infection of endothelial cells or inside haemopoietic cells (Trojan Horse effect), or by infecting nerve endings and then travelling retrogradely inside neurons. The ensuing immune response, whilst being a necessary response to the invader, may itself contribute towards pathology. This review will focus on the interaction of virus and cell, and the interplay between the infected cell and the immune response leading to nervous system disease.

## Cell – virus interactions within the nervous system

The outcome of a viral infection in the nervous system — in terms of whether it causes death of the infected cell, or establishes a persistent or latent infection — is governed, in part, by the cellular environment in which the virus finds itself. Viral behaviour may be influenced by the differentiation state of the host cell in the nervous system. For example, the behaviour of Japanese encephalitis virus within the brain of Fischer rats is determined by the maturity of its hosts' neurons [1]. Animals infected prior to age 13 days die, but those infected after age 14 days

do not. Furthermore, kinetic studies showed that viral antigen disappears from the cerebral cortex as neuronal maturation occurs. Similarly, Sindbis, an alphavirus, causes cytolysis of most dividing cells, but establishes a persistent infection in post-mitotic neurons. Levine *et al.* [2••] have shown that Sindbis virus induces apoptosis in a number of different cell types in culture. Bcl-2, a proto-oncogene, is able to block apoptosis in neurons and there is a correlation between levels of Bcl-2 mRNA and resistance to Sindbis. Transfection of a non-neuronal cell line with a Bcl-2-expressing plasmid is able to prevent Sindbis-induced death, but the virus is then able to persist and replicate [2••]. Thus, by counteracting cell apoptosis, Bcl-2 activity may be involved in the persistence of some viruses within the CNS.

The role of the cell in controlling the behaviour of a virus is illustrated by herpes simplex virus (HSV), which establishes a truly latent infection in dorsal root ganglia; the only RNA's produced in this state are the latency-associated transcripts (LATS), but no viral proteins are expressed [3]. The virus may subsequently reactivate with the possibility of spread to a new host. The immune system clearly plays a role in the control of the primary infection and reactivation [4], but during the latent phase, the virus expresses no protein and so it is invisible to the immune system. Therefore, control of expression during latency and the initial signal to reactivation must fundamentally lie with the transcription factor environment of the neuron. This is reflected in the fact that in humans the virus can reactivate spontaneously despite well developed immunity.

Conversely, viruses can alter cellular function in a non-lethal manner. Lymphocytic choriomeningitis virus (LCMV), if injected intracerebrally into adult mice will result in death mediated by CD8<sup>+</sup> T cells. In neonatal mice, however, a state is established in which the virus is able to replicate without apparent ill effect. Some of

## Abbreviations

ADCC—antibody-dependent cell-mediated cytotoxicity; CNS—central nervous system; CSF—cerebrospinal fluid; CTL—cytotoxic T lymphocytes; HIV—human immunodeficiency virus; HSV—herpes simplex virus; IL—interleukin; LCMV—lymphocytic choriomeningitis virus; MHC—major histocompatibility complex; SCID—severe combined immune deficiency; TGF—transforming growth factor; TNF—tumor necrosis factor.

the infected neonatal mice do show, however, a growth retardation syndrome that is linked to a persistent infection of the pituitary gland [5]. De la Torre and Oldstone [6•], using a rat pituitary cell line that produces growth hormone and prolactin, have demonstrated that these pituitary cells support LCMV replication without structural damage, but show a fivefold reduction in growth hormone mRNA, and, furthermore, that continued viral replication was needed to maintain this effect. This raises the possibility that similar persistent viral infections may be involved in producing neurological disease in humans without producing changes that would be detectable on even the most careful histopathological examination.

### Immune effector mechanisms in the CNS

The requirement to avoid immune-mediated destruction in tissue in which a key cell population, namely the neurons, is terminally differentiated and irreplaceable, places certain constraints on the immune response for controlling infection at this site. The immunologically privileged status of the nervous system may be one reason why viruses are able to persist at this site. Even so, the immune response engendered by the virus may cause damage to the CNS, as in the case of Theiler's virus, a picornavirus that establishes a persistent infection in the nervous system [7]. The response to HSV type 1 has also been described as causing a (late phase) multifocal demyelination within the CNS, the pattern of the disease varying according to the genetic background of mouse used [8]. Hence, the immune response within the nervous system provides examples both of rather specialized effector functions, and of novel pathologies that arise from the interaction between virus and the host defence system.

For a virus to elicit an immune response within the brain, foreign antigens must be clearly perceived by T lymphocytes. Major histocompatibility complex (MHC) proteins are expressed at comparatively low levels within the CNS. The lack of MHC expression on neurons, in particular, has been invoked to explain the ability of a number of viruses to persist in these cells and the inability of MHC class 1-restricted T cells to recognize and kill virally infected neurons [9]. Joly *et al.* [10••] conclude that neuronal cells do not express sufficient MHC molecules to act as targets for cytotoxic T lymphocytes (CTL), but that this can be overcome by adding interferon gamma, which upregulates MHC expression. This failure of neurons to present antigens in association with MHC appears to result from a down-regulation of peptide-transporting proteins Tap 1 and 2 (or Ham 1 and 2) the levels of which can also be increased by interferon gamma [10••]. This may be a means by which, except in very cytopathic infections, neurons harbouring viral antigen escape CTL destruction. Although protection of these irreplaceable cells may be seen to be of value for the host, the avoidance of immune eradication may thus contribute to viral persistence [11].

MHC class 1-restricted CD8<sup>+</sup>T cells are generally thought to kill their target cell through release of perforins and granzymes, which induce apoptosis. There is evidence, however, that they may control viral infection without inducing death of the target cell. Simmons and Tschärke [12•], in a quantitative study on the effect of depleting CD8<sup>+</sup> T cells on HSV infection of dorsal root ganglia, show that these cells limit the spread of HSV in the peripheral nervous system. They demonstrated that the number of neuronal profiles showing antigen positivity greatly exceeds the number destroyed, and that anti-CD8<sup>+</sup> antibody administration (to deplete CD8<sup>+</sup> T cells) leads to increased neuronal destruction. The authors therefore propose a non-lytic role for CD8<sup>+</sup> T cells in clearing herpes simplex infection in the nervous system. In a study on the effect of CD8<sup>+</sup> T cells on herpes simplex infected fibroblasts, Martz and Gamble [13] found that inhibition of viral replication could not be accounted for by the percentage of cells killed, and so they also proposed a non-cytotoxic role for CD8<sup>+</sup> T cells in the reduction of viral gene expression. In a recent review, Ramsay, Ruby and Ramshaw [14] propose that the antiviral activity of CTLs is mediated by the direct antiviral action of cytokines such as interferon gamma.

In certain viral infections, such as human immunodeficiency virus (HIV), the pathological effects seen in the CNS may result from the (appropriate) release of soluble mediators. The neuronal loss seen in a murine model of retrovirus-induced spongiform encephalopathy, is not due to direct neuronal infection, but arises secondarily to infection of non-neuronal cells [15]. The selective tropism of HIV in the brain does not, directly, explain the neuropathologies seen in this condition. In the late stages of acquired immune deficiency syndrome (AIDS) there appears to be a state of immune activation within the CNS, with the production of interferon gamma, interleukin (IL)-1, IL6 and tumor necrosis factor (TNF)- $\alpha$ , mainly distributed in endothelium and stellate parenchymal cells, probably microglia [16]. In addition, elevated levels of TNF- $\alpha$  have been detected in the cerebrospinal fluid (CSF) of HIV-positive patients with neurological disorders [17]. It is possible that these cytokines could play a role in inducing the brain pathology. However, *in vitro* evidence suggests that the release of HIV antigens, such as tat and gp120, from infected macrophages may also have a role in inducing cell damage [18]. Inflammatory mediators, such as leukotriene-B<sub>4</sub> and prostaglandin-D<sub>2</sub>, are also elevated in the CSF of patients with neurological complications of HIV [19]. Hence, although cytokines may prove to be important in the induction of neuropathology, they are not the only molecules that can mediate this effect. On the other hand, cytokines may also be involved in determining the behaviour of the virus itself. Swingler, Easton and Morris [20] found that in an *in vitro* system certain cytokines could augment the expression of a reporter gene driven by the long-terminal repeat region of HIV.

Antibody also plays a role in determining the outcome of CNS viral infection, through a variety of mechanisms, including antibody-dependent cell-mediated cytotoxicity (ADCC), complement-mediated lysis and viral

neutralization. Early humoral immunity, presumably involving immunoglobulin (Ig)-M antibodies, has been found to be protective in a murine model of retrovirus encephalitis [21]. In retrovirus infection of mice, anti-viral antibody is able to prevent the spread of virus to the CNS from its peripheral site of infection, and also within the CNS [22]. Antibody can also inhibit the cell-to-cell spread of rabies virus by neutralization. In addition, there is evidence that it may exert an effect once virus has been taken up by a cell, resulting in decreased transcription of viral mRNA and decreased replication. This implies that a novel antiviral mechanism is active, probably involving the endocytosis of antibody [23•]. Another novel mechanism involving antibody-mediated control of virus replication in nerve cells involves Sindbis virus. Levine *et al.* [24] studied the antibody-mediated clearance of Sindbis virus from the brain of mice with severe combined immune deficiency (SCID). This was achieved without neuronal destruction and did not involve ADCC or complement-mediated lysis. It was, however, associated with decreased viral RNA in cells — as determined by *in situ* hybridization — suggesting interruption at the transcriptional level. Furthermore, the likelihood of recrudescence of disease from the persistent virus varies according to the antibody used to effect the control of the initial infection [25]. The influence of the quality of the initial antibody response — generated in an infection — on the likelihood of disease arising from persistent virus is a point worth bearing in mind in the design of vaccines.

It is also interesting to note that, as well as determining the effect of the virus on the CNS, the presence or absence of a good antibody response may modulate the immunopathology that results from a different arm of the effector response. Wright and Buchmeier [26] found that administration of anti-viral antibody to adult mice infected intracranially with LCMV, was able to suppress viral replication in the CNS, and protect the mice from the usually fatal T cell-mediated immunopathology.

### Immunosuppression and viral infection

The nervous system is an immunoprivileged site, even so, immunosuppression may alter the balance between virus and host, resulting in alteration of viral behaviour within the CNS, and the potential for disease production. Immunosuppression may allow a usually innocuous virus to cause progressive cell destruction, as seen in progressive multifocal leucoencephalopathy in which JC virus, a polyoma virus, causes destruction of oligodendrocytes [27]. The interaction of viral factors with the effects of immunosuppression in the control of viral behaviour is demonstrated in experiments with the in1814 mutant of HSV type 1. (The in1814 mutant has an insertion in the tegument protein, Vmw65 —  $\alpha$  TIF, transinducing factor — abolishing its activity [28]. When HSV infects a cell, Vmw65 is thought to interact with a cellular protein, Oct 1, to form a complex capable of transactivating herpes immediate-early genes [29, 30].) Wild-type HSV establishes a latent infection in Balb/c mice but is lethal

to SCID mice. In immune competent mice, in1814 establishes latency, but does not replicate. In SCID mice, however, it is able to establish a slowly progressive productive infection in the CNS [31]. This system illustrates features required if a virus is to produce a persistent infection, namely a low destructive capacity combined with the avoidance of the immune response, the latter due either to virus specific mechanisms, or to immunosuppression of the host.

Viruses may themselves have mechanisms that down-regulate the immune response. *In vitro* studies of HSV-infected fibroblasts suggest that this virus can inhibit the activity of natural killer cells [32] and cytotoxic T cells [33]. Host-produced cytokines may also have a role in reducing the immune response. Transforming growth factor (TGF)- $\beta$  may be produced within the brain and have an effect in suppressing immune responses [34]. Infection of rats with Borna virus, a negative strand RNA virus [35], can cause a T cell-mediated encephalitis. The administration of TGF- $\beta$  reduces the immune response in this disease and ameliorates the development of encephalitis [36].

### Conclusions

This review has attempted to concentrate on the interaction between virus, cell and immune factors in governing the outcome of infection within the CNS, and in the production of neurological disease. The search for viruses involved in human neurological disease continues with, for example, Coronavirus RNA and protein being found in active plaques, perivascular cells and lipid-filled macrophages of some multiple sclerosis patients [37] and Epstein Barr virus being detected by polymerase chain reaction (PCR) in brain biopsies submitted for a number of neurological diseases [38]. It is clear that some of the virus and immune-mediated pathologies that are now being described in animal models may be subtle and raise the possibilities of viruses being involved in novel ways in derangements of nervous system function in human disease.

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