The Inflammatory Response to Viral Infection of the Central Nervous System

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1. Introduction

Encephalitis, inflammation of the brain, is a rare condition in humans characterised by a triad of clinical features that include fever, headache and an altered level of consciousness that can persist for over 24 hours. There are many causes for the condition ranging from infection with an array of pathogens to autoimmune reactions. A major cause, and the one which this review will focus on, are viral infections (Whitley and Gnann, 2002). A diverse range of viruses are capable of invading the Central Nervous System (CNS) and infecting a variety of cell types within it (Tyler, 2009a; Tyler, 2009b). Examples from a range of viral species will be used to illustrate initial infection of the CNS, early innate immune responses, viral evasion of these early responses and the development of the adaptive immune response. The majority of viral CNS infections are transient but some can lead to long-term disability or death.

Structurally, the CNS consists of the brain and spinal cord that are both encased within bone. Three membranes surround the brain, which are collectively called the meninges. These are the thick dura mater, the web-like arachnoid mater and the delicate pia mater. This structure can itself become inflamed giving rise to meningitis. Within this, the brain is divided at a gross level into the forebrain containing the frontal, parietal, occipital and temporal lobes, and the hindbrain containing specialist structures including the hippocampus, pituitary and cerebellum. Below this is the brainstem that connects the brain to the spinal cord that extends through the body of all vertebrates. Neuronal processes leave the spinal cord to innervate the organs and structures of the body through sensory and motor neurons, forming the peripheral nervous system. The cellular content of the CNS is varied. The key cell-type is the neuron although this only constitutes around 5% of the cellular total and can vary between regions of the brain. Neurons typically have a large cell body containing the nucleus, from which extensions, termed axons extend. At the termini of axons, small processes or dendrites make contact with other neurons creating an extensive network of connections enabling the brain to act as an information-processing centre for the organism. In addition to neurons are cells collectively termed glia. Glial cells support neurons through a range of functional phenotypes. Astrocytes assist in sustaining neuronal metabolism and neurotransmission. Oligodendrocytes have elongated processes that

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surround axons and produce myelin, which effectively insulates axonal processes and enables efficient electrical transmission along axons. Finally, microglia provide an immune function within the CNS, acting as the resident macrophage population removing dead cells, and are particularly important in the development of the foetal brain.

Infection of the leptomeninges (arachnoid mater and pia mater) generally does not result in serious disease. However, infection of glial cells and neurons can result in acute disease through direct cellular destruction and/or the initiation of immune pathology. The loss of non-renewable, specialized cells responsible for cognition, mobility and almost all organ function can have profound impact on the host. The immediate consequence of infection is inflammation, manifest as viral encephalitis, the outcome of which can vary from a transient episode to long term neurological deficit or in the death of the host. The pathology of disease is characterised by a cellular thickening of blood vessels or perivascular cuffing and a proliferation of astrocytes, or gliosis. Changes are also observed in the cerebrospinal fluid (CSF) including the presence of predominantly mononuclear cells (pleocytosis), increased levels of protein and antibodies. Infecting viruses can be detected in the CSF using specific molecular tests such as the polymerase chain reaction (PCR).

When considering any host-pathogen relationships within the CNS, the issue of immune-privilege must be acknowledged. The CNS is considered an immuno-privileged site, a term first coined by Peter Medawar resulting from the observation that allografts placed in certain locations, such as the eye or brain were not rejected by the immune system with the rapidity observed in other organs (Brent, 1990). The cause of this privilege has been attributed almost exclusively to the barrier separating the periphery from the CNS, which is collectively termed the blood-brain barrier (BBB). At a basic level this is formed by the endothelium found on the luminal side of blood vasculature within the brain (Bailey et al., 2006). Tight junctions between endothelial cells prevent entry of cells, large molecules and proteins. Furthermore, under normal conditions these endothelial cells express no or very low levels of adhesion molecules preventing attachment of immune cells and blocking a potential route of entry for cells involved in immune surveillance. Surrounding the endothelial cells, on the CNS side, is a thick basement membrane that is itself surrounded by processes from astrocytes termed astrocytic end feet. The BBB is present over 99% of the brain vasculature. The exceptions are those structures that are involved in hormone secretion such as the pituitary and hypothalamus where free movement of proteins is required between neural tissue and the blood. In addition there are no lymphatic vessels within the parenchyma of the brain that would provide a conduit for antigen presenting dendritic cells to move directly to lymphoid tissue. However, it would be wrong to assume that the absence of comparable cells and structures found in the periphery represents an absence of these functions. Numerous studies are now identifying the multiple mechanisms of immune surveillance that support the CNS, initiating both protective and damaging immune responses (Hickey, 2001). Likewise there are now defined routes by which immune cells can enter the CNS, particularly in response to infection (Ransohoff et al., 2003). The appropriate and timely orchestration of these events is critical in responding to viral infection particularly in the early chemokine and cytokine signals that trigger the innate immune response. Much research has been conducted in recent years on these early responses with some mediators being observed repeatedly, such as the chemokine CXCL10, in response to viral infection. This in turn has dramatic consequences for the vasculature without apparently changing the efficacy of the BBB. Adjunct to this is the activation of the adaptive response to viral infection within the CNS. In this area less is known of the
mechanism that leads to antigen presentation from this site. Key questions that are raised by this include the mechanism of antigen transfer from the CNS to sites of antigen presentation, the timescale of these events and the fate of effector cells that eventually migrate to the site of infection. The coordination of immune activation and appropriate response are poorly understood and can result in a continuum of outcomes that vary from complete resolution of the infection, latent infection by the virus, severe encephalitis and cell-mediated destruction of host cells.

This review will follow the theoretical events that occur following neuroinvasion in humans by viruses that result in encephalitis. This will include:

- A brief overview of viruses that cause encephalitis. This focuses mainly on those that infect humans although includes a number that have been used extensively in murine experimental models.
- An examination of the early innate immune response generated within the CNS to viral infection. This will also consider the cell-types that these signals target that prepare the tissue for the immune response. Also of importance will be the mechanisms that viruses use to subvert the immune response at this early stage.
- Consideration of antigen presentation from CNS, which has been traditionally been considered an immuno-privileged site. In addition, the process of antibody-secreting cell development within lymphoid tissue and then migration to the CNS will be reviewed.
- The review will conclude with the final steps of leukocyte recruitment across the BBB and discuss the variable outcomes to infection with different viruses. This appears to be determined as much by the response of the host as the virulence of the virus.

2. Viruses that cause encephalitis

Many viruses have been identified as the causative agent of encephalitis. Table 1 gives a summary of many of the virus species that are associated with human and/or animal encephalitis. It is noteworthy that most have ribonucleic acid (RNA) genomes. Many are found worldwide although some, particularly those that are associated with an arthropod vector, have a more limited distribution. Most are established causative agents of disease.

2.1 Herpesviruses that cause encephalitis

Herpes viruses are one of the few groups that preferentially infect neurons or show neurotropism. Herpes simplex virus type 1 infects trigeminal ganglia and establishes a latent infection. Periodically, the virus reactivates and returns to the site which the ganglia innervates, causing a vesicular rash. Virus is released into the exudate and can be transmitted onwards through contact. This cycle occurs repeatedly through the life of the infected individual but occasionally this can develop into encephalitis in both immunocompetent and particularly immunocompromised individuals (Kleinschmidt-DeMaster and Gilden, 2001). A recent investigation into the causes of encephalitis in England found that 42% could be attributed to infectious disease with 19% being caused by herpes simplex virus and 5% being caused by varicella zoster virus (Granerod et al., 2010). A further 30% were caused by immune-mediated encephalitis. However, the largest group (42%) were of unknown origin, some of which could have had a viral aetiology but not diagnosed. Similar findings were made from a retrospective study of encephalitis in
Australia with Herpes simplex viruses being the most common virus associated with human encephalitis (Huppatz et al., 2010), although in this study, flaviviruses such as Murray Valley encephalitis virus and Kunjin virus were also reported.

2.2 Arthropod-borne viruses that cause encephalitis
Prominent amongst the viruses that cause encephalitis are the flaviviruses (Gould and Solomon, 2008). These are transmitted by an arthropod vector (mosquitoes and ticks) and thus disease characteristically occurs in human populations in synchrony with seasonal peaks in vector abundance. In the Northern Hemisphere this is typically late summer for mosquito-borne infections. Infection with arthropod-borne viruses usually results in replication in lymphoid tissue close to the bite site that results in a transient viraemia and febrile illness. The viraemia following infection with some viruses, either through its transient nature or low titres, does not result in onward transmission to the vector such as the case of West Nile virus and humans are a dead end host. However, in other cases, human infection is critical to maintaining the infection such as in outbreaks of chikungunya virus (see below). Encephalitis usually occurs subsequent to the resolution of viraemia. The flavivirus genus includes viruses such as mosquito-borne West Nile virus, which following its introduction into the United States in 1999 resulted in a sharp increase in human cases of encephalitis as it rapidly spread west (Granwehr et al., 2004). In experimental studies, West Nile virus is highly neurovirulent in mice, spreading rapidly through the brain. Figure 1 illustrates the rapid spread of West Nile virus following intranasal infection. The morphology of infected cells indicates that they are predominantly neuronal. Another prominent member of this genus is Japanese encephalitis virus, present throughout Asia. Mortality rates can reach 30% with a further 50% having severe neurological sequelae (Tyler, 2009a). In addition to mosquito-borne viruses, the flaviviruses also include tick-borne viruses of which the most prominent is tick-borne encephalitis virus (TBEV) found in an uninterrupted belt from Western Europe to the far east of Asia (Mansfield et al., 2009). The virus is transmitted by Ixodes species of ticks and is currently increasing in many countries, likely through changes in human behaviour such as leisure pursuits that bring humans into areas inhabited by ticks. TBEV causes asymptomatic infection in wildlife but can result in severe encephalitis in humans. The exception to this is a variant of TBEV named louping ill virus found almost exclusively in the United Kingdom that has been rarely associated with human illness but causes fatal encephalitic disease in sheep and grouse (Sheahan et al., 2002).

Another group of mosquito-borne viruses capable of causing encephalitis is the genus Alphavirus (Zacks and Paessler, 2009). Prominent among these are the equine encephalitides of North, Central and South America. Although less rarely associated with human encephalitis, infection with Eastern equine encephalitis virus can be devastating with mortality rates ranging between 50 and 75%. As the names of these viruses indicates, they also cause severe encephalitis in horses with mortality rates reaching up to 90%. This group also includes emerging viruses such as chikungunya virus, that has caused explosive outbreaks of febrile disease in countries around the Indian Ocean that have sporadically been transported back to Europe by infected individuals. In one year this resulted in autochthonous transmission of chikungunya virus in Italy potentially vectored by the invasive mosquito species Aedes albopictus (Seyler et al., 2008).
2.3 Lyssaviruses that cause encephalitis

One group of viruses of special note are the lyssaviruses of which every member is believed to be neurotropic and capable of causing encephalitis. This group is dominated by the rabies virus, a virus that infects all mammals and is transmitted by animal bites that with rare exceptions cause fatal encephalitis in all those where infection reaches the brain (Rupprecht et al., 2002). Estimates are vague on the actual number of annual deaths due to rabies, particularly in regions of the world with poorly developed public health infrastructures but figures upwards of 50,000 are regularly quoted. Transmission of rabies virus occurs when virus is deposited within skin or muscle following a bite and if not treated either by wound cleansing or vaccination, infects neurons close to the wound. The virus then ascends rapidly to the CNS by retrograde axonal transport leading to replication within the spinal cord and brain (Schnell et al., 2010). Vaccination post-bite is highly effective and has virtually eliminated rabies as a human disease in the Americas and Europe. The main human burden of this disease falls in Asia and Africa where the cost of treatment or ignorance prevents its use. The lyssavirus genus also contains viruses that are associated with certain bat species and that on occasion have resulted in human fatalities following contact with an infected bat. These viruses appear to have a limited geographical distribution (Fooks et al., 2003; Johnson et al., 2010).
2.4 Emerging viruses that cause encephalitis

Others species are considered emerging viruses (Tyler, 2009a). Human immunodeficiency virus is a prime example of a virus emerging as a recognised disease in the 1970s. The virus emerged in the human population following a jump from primates into man in Africa at a much earlier point in the century (Zhu et al., 1998; Tebit and Arts, 2011). It has spread rapidly and continues to be a global public health problem. In addition to progressive immunodeficiency, up to one third of individuals infected with HIV-1 develop encephalitis (Lipton and Gendelman, 1995).

Another virus that has emerged, particularly in Italy as one of the major causes of encephalitis, is Toscana virus and the closely related virus Naples sandfly fever (Tyler, 2009a). The virus is transmitted by sandflies (Phlebotomus perniciosus) during the summer months causing mild febrile illness that can develop into severe encephalitis (Dionisio et al., 2001). Further examples include the recently described Hendra and Nipah viruses (Tyler, 2009b). Hendra virus was first isolated in 1994 as a result of disease affected horses in Brisbane, Australia, that in turn lead to disease amongst veterinarians who treated the animals (Tulsi et al., 2011). Subsequent investigation demonstrated that the virus had made the jump from its natural host, pteropid fruit bats, into an amplifying host, the horse, from which it then made the jump to humans. A similar sequence of events occurred with the emergence Nipah virus that caused an outbreak of acute encephalitis among Malaysian pig handlers in 1998, with farmed pigs acting as the amplifying host. Immunohistochemical analysis of autopsy specimens observed extensive parenchymal necrosis with widespread Nipah virus antigen staining associated with the smooth muscle of blood vessels (Wong et al., 2002). Again, fruit bats were identified as the original host of the virus and the Malaysian outbreak was controlled by mass slaughter of pigs. Repeated sporadic outbreaks of Nipah virus infections continue to occur in Asia.

2.5 Rare viral encephalopathies

Although more commonly associated with haemorrhagic fever, Lassa fever virus can cause encephalopathy during the course of infection (Cummins et al., 1992). A related virus, lymphocytic choriomeningitis virus, is used as a model for encephalitis in mice. Measles virus is a common childhood infection which usually resolves rapidly with no neural involvement. However a small number of cases develop subacute sclerosing panencephalitis (SSPE), a fatal condition in which the virus persists in the brain, spreading through the CNS causing progressive demyelination and destruction of neurons (Allen et al., 1996).

3. Innate response to viral infection of the Central Nervous System

Entry of virus into the CNS can be haematogenous, resulting from changes in the vasculature that enable virus to cross the blood-brain-barrier either directly or within infected cells crossing the endothelium. Alternatively entry can be neuronal of which rabies virus and herpes viruses are the best characterised. By the haematogenous route there is likely to have been prior virus replication in the periphery often resulting in a detectable viraemia and an opportunity for systemic immune activation and an adaptive response demonstrable by the presence of anti-virus antibodies in serum. There is usually a febrile phase that often resolves and the appearance of virus-neutralising antibodies in the blood that rapidly controls viraemia. Entry by the neuronal route may occur with minimal virus replication in the periphery and therefore the first contact between the immune response
<table>
<thead>
<tr>
<th>Virus Family</th>
<th>Genome</th>
<th>Virus Species</th>
<th>Geographical Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenoviridae</td>
<td>Double-stranded DNA</td>
<td>Adenovirus</td>
<td>Worldwide</td>
</tr>
<tr>
<td></td>
<td>Single-stranded RNA</td>
<td></td>
<td></td>
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<tr>
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<td>(negative sense, segmented)</td>
<td>Lassa fever virus</td>
<td>West Africa</td>
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<td></td>
<td></td>
<td>Lymphocytic choriomeningitis virus</td>
<td>Worldwide</td>
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<tr>
<td>Bunyaviridae</td>
<td>Single-stranded RNA</td>
<td>California encephalitis virus</td>
<td>North America</td>
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<td></td>
<td>(negative sense, segmented)</td>
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<td></td>
<td></td>
<td>Rift Valley fever virus</td>
<td>Worldwide</td>
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<td></td>
<td></td>
<td>Toscana virus</td>
<td>Africa</td>
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<td></td>
<td></td>
<td>La Crosse virus</td>
<td>Europe</td>
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<td></td>
<td></td>
<td></td>
<td>North America</td>
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<tr>
<td>Flaviviridae</td>
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<td>Japanese encephalitis virus</td>
<td>Asia</td>
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<td></td>
<td>(negative sense, non-</td>
<td>Kyasanur Forest virus</td>
<td>India</td>
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<td></td>
<td>segmented)</td>
<td>Louping ill virus</td>
<td>United Kingdom</td>
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<td>Murray Valley encephalitis virus</td>
<td>Australia</td>
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<td></td>
<td></td>
<td>St. Louis encephalitis virus</td>
<td>North and South America</td>
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<td></td>
<td></td>
<td>Tick-borne encephalitis virus</td>
<td>Europe and Asia</td>
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<td></td>
<td></td>
<td>West Nile virus</td>
<td>Worldwide</td>
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<tr>
<td>Herpesviridae</td>
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<td>Human herpes virus 1</td>
<td>Worldwide</td>
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<tr>
<td></td>
<td></td>
<td>(Herpes simplex virus)</td>
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<td>Human herpes virus 3</td>
<td>Worldwide</td>
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<td></td>
<td></td>
<td>(Varicella zoster virus)</td>
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<td></td>
<td>Human herpes virus 6</td>
<td>Worldwide</td>
</tr>
<tr>
<td>Orthomyxoviridae</td>
<td>Single stranded RNA</td>
<td>Influenza A virus</td>
<td>Worldwide</td>
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<tr>
<td></td>
<td>(negative sense, segmented)</td>
<td></td>
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<tr>
<td>Paramyxoviridae</td>
<td>Single stranded RNA,</td>
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<td></td>
<td></td>
<td>Measles virus</td>
<td>Worldwide</td>
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<td></td>
<td></td>
<td>Nipah virus</td>
<td>Asia</td>
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<td></td>
<td></td>
<td>Hendra virus</td>
<td>Australia</td>
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<tr>
<td>Picornaviridae</td>
<td>Single stranded RNA</td>
<td>Poliovirus</td>
<td>Africa</td>
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<tr>
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<td>(positive sense, non-</td>
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Innate immune responses are activated by recognition of pathogen-associated molecular patterns or PAMPs. For viruses, this includes surface glycoproteins and structures associated with single- or double-stranded RNA. Recognition is mediated by a number of protein families, the most prominent being the toll-like receptors (TLRs), NOD-like receptors and RIG-I helicases (Creagh and O’Neill, 2006; Rehwinkel and Reis e Sousa, 2010). These receptors are expressed in many compartments of the cell and trigger the production of type I interferons, a key element in controlling virus replication and spread (Randall and Goodbourn, 2008). Type 2 interferons are produced exclusively by lymphoid cells, which are absent early in infection but contribute to later control of pathogens. Interferons in turn activate the up-regulation of a large number of proteins that act to control infection at the cellular level and attract immune effector cells. Three mechanisms of direct inhibition of virus replication have been identified. Activation of protein kinase R (PKR) in response to
double stranded RNA (e.g. virus replication intermediates) which inhibits eukaryotic translational factor 2 that in turn restricts synthesis of viral proteins. Activation of 2′5′ oligoadenylate synthetase (OAS), which activates RNase L and in turn degrades viral RNA. Finally, the Mx family of proteins are activated that target nucleocapsids (viral structures that contain the genome bound to viral proteins including the nucleoprotein, a protein encoded by many viruses). Whilst the CNS has unique immunological status there is increasing evidence that there is a vigorous innate immune response to viral infection of cells within it (Savarin and Bergman, 2008). For example, TLRs are selectively up-regulated in the brain in response to infection with different viruses (McKimmie et al., 2005). Neurons can produce a range of innate immune-associated proteins including type 1 interferons in response to infection with rabies virus (Prehaud et al., 2005), Theilers virus and La Crosse virus (Delhaye et al., 2006). Microglial cells are capable of producing a wide range of inflammatory mediators in response to activation (Rock et al., 2004) or direct infection (Nakamichi et al., 2005).

Unsurprisingly, viruses have evolved a range of mechanisms to subvert the innate immune response, particularly through inhibition of the interferon system (Randall and Goodbourn, 2008). These often involve direct interaction of virally expressed proteins with signaling intermediates that would normally trigger the expression of interferon or interferon-induced proteins. Many such viral proteins have multiple roles, both in viral replication and subversion of the immune response. The influenza virus NS1 protein inhibits export of host mRNA from the nucleus during infection (Satterley et al., 2007) whereas the La Crosse virus nonstructural protein NSs targets inhibition of type 1 interferon production (Blaqori et al., 2007). The rabies virus phosphoprotein blocks interferon signaling through interaction with interferon regulatory factor 3 (Brzozka et al., 2005) and STAT proteins (Chelibi-Alix et al., 2006; Brzozka et al., 2006; Vidy et al., 2007).

Whilst innate immune inhibition occurs within the cell, neuroinvasion by viruses rapidly induces immune responses across the CNS (See Figure 2). A key family of proteins produced in response to infection are the chemokines whose primary role is to attract immune effector cells to sites of infection. The chemokine family consists of a growing number of small proteins (<100 amino acids) that are produced at sites of pathology. They contain four conserved cysteine residues that form structurally critical disulphide bonds. The position of the first two cysteines, either adjacent (CC) or separated by a single amino acid (CXC) dictates the current naming of chemokines (Baggiolini, 1998). These in turn bind to specific receptors expressed on target cells. A summary of a number of well-characterized chemokines is shown in Table 2.

<table>
<thead>
<tr>
<th>Current name</th>
<th>Former name</th>
<th>Receptors</th>
<th>Receptor expressed on</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL2</td>
<td>MCP1</td>
<td>CCR2</td>
<td>Monocytes, activated T cells</td>
</tr>
<tr>
<td>CCL3</td>
<td>MIP-1α</td>
<td>CCR1,4,5</td>
<td>Activated T cells</td>
</tr>
<tr>
<td>CCL4</td>
<td>MIP-1β</td>
<td>CCR5</td>
<td>Monocytes, activated T cells</td>
</tr>
<tr>
<td>CCL5</td>
<td>RANTES</td>
<td>CCR1,3,4,5</td>
<td>Monocytes, activated T cells</td>
</tr>
<tr>
<td>CXCL10</td>
<td>IP-10</td>
<td>CXCR3</td>
<td>Activated T cells</td>
</tr>
</tbody>
</table>

Table 2. Summary of a small number of human chemokines and their receptors
Studies of in vivo models generally show transcriptional up-regulation of a wide range of chemokines in the CNS following viral infection. Numerous studies using murine models of infection have consistently shown up-regulation of chemokines including those on rabies virus (Wang et al., 2005) and this is associated with infiltration of mononuclear cells, particularly T cells (Johnson et al., 2008). Likewise, West Nile virus infection up-regulates a wide range of pro-inflammatory genes within the murine brain (Venter et al., 2005), prominent among these is CXCL10. Figure 2 shows the transcriptional up-regulation of a small panel of chemokines from time-points that correspond with those in Figure 1. Most show a detectable up-regulation by day 4 with CXCL10 showing the greatest increase both temporally and in magnitude. Functionally, expression of CXCL10 leads to recruitment of CD8+ T-cells and control of infections such as West Nile virus and lymphocytic choriomeningitis virus (Klein et al., 2005; Christensen et al., 2006). Conversely, the majority of T cells entering the CNS express the CXCL10 receptor CXCR3 (Stiles et al., 2006) and expression of this receptor is critical for targeting CD8+ T cells to specific regions of the CNS (Zhang et al., 2008).

Fig. 2. Expression of interferon β and selected chemokine transcripts following intranasal infection with West Nile virus. Groups of three mice were infected with West Nile virus by the intranasal route and sampled at the time points indicated. Total RNA was extracted from whole brain and the level of transcript was measured by quantitative real-time reverse transcription polymerase chain reaction as previously described (Johnson et al., 2008).

Many cytokines are toxic to neurons and oligodendrocytes including IL1β, TNF-α and interferon-γ. Uncontrolled cytokine expression would inevitably lead to tissue damage, a situation that occurs in auto-immune mediated encephalopathies such as multiple sclerosis and Alzheimer’s disease. It is therefore not surprising to find mechanisms for controlling inflammation within the CNS. Innate immune responses are suppressed by TGF-β, produced by resting and active astrocytes (Constam et al., 1992). During inflammation, the family of suppressors of cytokine signaling (SOCS) proteins are induced by cytokine expression and block signal transduction activated by these same cytokines providing...
feedback inhibition of inflammation (Yoshimura et al., 2007). There is also evidence that these proteins are selectively expressed in the brain to control inflammation in response to viral infection (Mansfield et al., 2010). A delicate balance is required to ensure that inflammation, a natural response to infection and vital to initiate the process of infection resolution does not result in excessive cellular destruction. It is also clear that innate immune responses are not alone capable of controlling and eliminating the cause of infection and that an adaptive response is required.

4. Antigen presentation, lymphatic drainage and the development of the adaptive immune response

The adaptive immune response to CNS infection is presented with a unique challenge compared to other sites within the body. There appears to be limited antigen presentation capacity within the CNS and no lymphatic drainage vessels. There is no lymphoid tissue within the CNS in which antigen responses can develop and activated immune cells that develop externally to the CNS need to cross the BBB in order to reach locations where they are required. Direct viral infection can lead to disruption of the BBB such as the infection of endothelial cells by Semliki Forest virus in a murine model of disease (Soilu-Hanninen et al., 1994). However, during the early stages of most infections the BBB remains intact, indeed it is possible that failure to open the BBB may be a contributory factor in the failure to control rabies virus infection (Roy et al., 2007). The ability to detect, present and rapidly respond to the presence of virus are critical determinants in controlling infection.

Throughout the body, the principal cell-type that detects non-host antigens are plasmacytoid dendritic cells that present antigen on the cell surface to other cell-types of the adaptive immune system. They are also the main type I interferon producing cells. However, cells with dendritic cell morphology and phenotype are absent from the CNS (Hart and Fabre, 1981). A possible alternative within the CNS is the microglia cell component, which in some areas of the CNS form over 15% of the cellular composition (Rock et al., 2004). In their resting form, microglia express very low levels of MHC class II molecules, but this along with a range of co-stimulatory molecules are up-regulated when microglia enter an activated state enabling antigen presentation (Constam et al., 1992; Fischer and Reichmann, 2001). In addition, there appears to be a discrete population of dendritic cells associated with the vascular endothelium within the brain that could provide antigen-presenting function (Gieber et al., 2005) and could act to draw immune effectors into the CNS.

A key challenge to understanding the development of the adaptive immune response is to find appropriate means of introducing antigen or pathogen into the CNS without disruption of the BBB. It has long been recognised that direct injection of large volumes (upto 30μl) causes a dramatic increase of intra-cranial pressure leading to the expulsion of most of the inoculum into peripheral locations (Cairns, 1950; Mims 1960). One means of overcoming this has been the inoculation of laboratory animals with stereotactically guided small volumes of virus (< 2 μl). Stevenson and co-workers used this approach to study influenza virus infection in mice. Inoculation of virus into the CSF or intranasally resulted in a rapid induction of IgM (2-4 days) and IgG (3-5 days), and proliferative responses from cells prepared from cervical lymph nodes were observed 10 days after inoculation. However, direct inoculation of virus into the brain parenchyma failed to induce either antibody or cellular responses despite demonstration of virus replication within the brain (Stevenson et
al., 1997). This scenario reflects the antibody responses observed in rabies patients. Infection of peripheral nerves occurs in the skin or muscle, subsequent retrograde axonal transport enables the virus to ascend directly into the CNS accompanied by a rapid increase in replication. It is possible in most infections with rabies virus that no replication occurs within the periphery and thus rabies virus bypasses normal immune surveillance and response. Hence few patients have detectable levels of antibody on admission with disease (Noah et al., 1998; Hunter et al., 2010). These observations suggest that under some circumstances, the adaptive immune response to antigen or whole virus is delayed.

A further obstacle to development of the immune response is the absence of a discrete lymphatic vasculature. This has gradually undergone revision with the recognition that this must occur. There is a net influx of fluid into the CNS that requires some form of drainage (Cserr and Knopf, 1992). Up to 50% of protein introduced into the brain of laboratory animals could be detected in the cervical lymphatics and that this can be extremely rapid with tracer being detected in deep cervical lymph nodes within six hours (Yamada et al., 1991). It is now recognised that there are discrete drainage routes from the CNS and these include passage through the arachnoid villi into the blood supplying the dural sinus. There is also drainage to the lymph system along spaces adjacent to the cranial and spinal nerves, and through the Virchow-Robin perivascular spaces surrounding brain blood vessels (Knopf et al., 1995; Kida et al., 1995). Antibody secreting cells responding to CNS antigen can be detected in cervical lymph nodes and removal of these lymph nodes in experimental animals following introduction of antigen ablates the antibody response (Knopf et al., 1995). However, it is not clear that the response to virally infected cells and thus cellular associated virus will have the same dynamics and kinetics as soluble antigen. Recent studies have shown that activated CD4+ cells can traffic from the brain parenchyma to the nasal mucosa and cervical lymph nodes, using a similar route to that of soluble antigen (Goldmann et al., 2006). This opens the possibility that classical antigen presentation is possible from within the CNS leading to development of virus specific immune cells within lymphoid tissue that are external in the presence of an intact BBB. Although this fails to explain the late emergence of antibodies to rabies virus in patients that present with disease (Noah et al., 1998).

5. Leukocyte recruitment and immune control of infection

The mechanism that directs activated immune cells from lymphoid tissue to particular organs has not been elucidated. However, some form of targeting is likely to occur in order to achieve successful resolution of infection and it is likely that chemokines play a key role in this. Activated T cells can enter the CNS in an antigen-independent manner and rates of entry do increase when inflammatory responses are triggered in the periphery. However retention and anti-viral function are dependent on MHC restricted antigen recognition. Cells can enter the CNS through a number of routes (Ransohoff et al., 2003). The first is to cross the choroid plexus stromal venules, the region of the brain where CSF is produced, and can lead to an influx of cells within the CSF. Secondly, lymphocytes can enter through the vasculature supplying the pia membrane. Finally, lymphocytes can migrate into the parenchyma across vascular endothelium throughout the brain. This leads to the common histological feature observed during encephalitis of the perivascular cuff (Figure 3). Lymphocyte entry through the vasculature is divided into a series of events controlled by the expression of a range of adhesion molecules expressed on the lymphocyte and
endothelium (Ransohoff et al., 2003). The first stage is rolling or tethering as the cell becomes attached to the blood vessel. Critical amongst these are the intercellular adhesion molecules (ICAMs) that are stimulated within vascular endothelium by a range of cytokines directly or through positive feedback once adhesion has occurred between the lymphocyte and the endothelium (Dietrich, 2002). Finally the lymphocyte undergoes diapedesis, where processes extend between the endothelial cells, driven in part by chemokine-induced chemotaxis.

Fig. 3. Example of a perivascular cuff forming around a parenchymal blood vessel observed within the brain following infection with a lyssavirus. The fixed section of murine brain has been paraffin mounted and stained with haematoxylin and eosin. There is distinct accumulation of mononuclear cells around the vessel. Further details can be found in Hicks et al. (Hicks et al., 2009).

The current understanding of effector cell infiltration of the CNS suggests that the earliest responders to viral infection are T cells, whereas B cell development takes longer and these enter the CNS later in the course of infection (Savarin and Bergmann, 2008). T cells control virally infected cells whereas antibody is critical in controlling virus spread. However, in some instances there may also be antibody-mediated control of infected host cells through antibody-dependent cellular cytotoxicity. This mechanism may be more prominent in humans than in mice. B cells originate in the bone marrow and after development in this site, migrate as immature cells to secondary lymphoid tissues to complete the maturation process. B cells that encounter protein antigens through their B cell receptor can interact with primed T cells in a cognate-dependent manner (MacLennan et al., 2003). Productive contacts with T cells result in the B cell differentiating into antibody-secreting plasma cells in extrafollicular sites such as the splenic red pulp or medulla, in lymph nodes or entering the germinal centre reaction (MacLennan et al., 2003; Cozine et al., 2005). There are differences between these two
Extrafollicular responses develop to T-dependent and T-independent antigens and produce the first wave of IgM and IgG, the latter of which is generally of modest affinity. High affinity persisting, effective life-long antibody responses and memory B cells derive from germinal centres. In germinal centres B cells undergo somatic hypermutation with subsequent competition for antigen, and require further survival signals from T cells. Plasma cells that derive from this response then usually migrate to sites such as the bone marrow and secrete antibody. Thus, these effector cells can contribute to protection from sites distal from the sites of infection. Nevertheless, cells generated through the response may also be able to migrate to sites of inflammation, possibly through mechanisms such as their expression of CXCR3 resulting in migration to sites with elevated levels of CXCL10.

What is significant in the context of the current discussion is that the type of B cell response induced and the kinetics in which it develops can vary markedly between different pathogens. Fundamental work on the development of antibodies has focused on the response to bacterial infection. For instance, in responses to alum-precipitated proteins extrafollicular and germinal centre responses develop in parallel, with both detectable within the first 5 days of immunization in mice. In contrast, extrafollicular responses to Salmonella infection are detectable within 3 days of systemic infection yet germinal centre responses do not develop until the infection has all but resolved (Cunningham et al., 2007). Furthermore, after infection with mycobacteria antibody responses may not be detectable until weeks after infection. This means that there are likely to be host-dependent and pathogen-dependent factors that shape the type and timing of the antibody response that is induced during infection. For rabies infection of humans this is likely to be important and possibly more influenced by pathogen-intrinsic factors, since post-exposure antibody is clearly able to control the infection if present early enough.

The majority of cells undergoing migration across the CNS vasculature appear to be CD3+ T cells (Man et al., 2007). This is illustrated in Figure 4 where the average counts of T or B cells have been compared across three regions of the brain of a mouse infected with rabies virus. In each region over 90% of cells counted are T cells. Under normal conditions, low numbers of B cells can be detected in the CNS with an activated phenotype (Anthony et al., 2003). These presumably provide a low level of immune surveillance. The development of inflammation within the CNS causes this proportion to increase. The explanation for this may be in the longer development and maturation of B cells compared to other cell types prior to migration to the CNS.

The evolution of intrathecal production of antibody has been demonstrated through introduction of antigen through a permanent cannula (Knopf et al., 1998). This study demonstrated trafficking of B cells into the CNS and detection of antigen-specific intrathecal antibody 14 days after injection. It was also observed that there was localisation of mononuclear cells at the site of antigen introduction suggesting a homing process. Similar kinetics have been demonstrated for antibody secreting cell entry into the CNS following viral infection with mouse hepatitis virus (Tschen et al., 2002). Four pathways have been proposed to account for the presence of antibody secreting cells within the CNS (Meinl et al., 2006). These include migration of plasmablasts to the CNS, migration of memory B cells that subsequently differentiate into antibody-secreting cells, development of antibody secreting cells within lymphoid follicles in the meninges and antigen independent differentiation of B cells within the CNS itself. Recruitment and survival within the CNS is controlled by chemokines particularly CXCL12 / CXCL13 (Krumholz et al., 2006), and B cell promoting factors such as BAFF produced locally by astrocytes (Avery et al., 2003; Schneider, 2005).
The presence of antigen secreting cells can have both positive and negative effects within the CNS (Meinl et al., 2006). B cell secretion of cytokines such as TGF-β can down-regulate inflammation whereas expression of IL-6 and IL-12 can increase tissue destruction. Similarly, secretion of antibodies that bind to autoantigens can lead to tissue damage as observed in multiple sclerosis lesions where B cells are prominent. However, secretion of antibodies against viruses will have a direct beneficial impact by controlling virus spread. One critical observation of B cells within the CNS is that they have undergone hypersomatic mutation, confirming that they have at some stage been present within a lymphoid germinal centre (Baranzini et al., 1999). This in turn suggests that the development of mature antibody secreting B cells requires a distinct period of time before it eventually reaches the CNS and that if excessive cell death has occurred during this period permanent damage may result.

![Fig. 4. Illustration of the proportion of T and B cells present with different regions of the brain following infection with rabies virus. Sections of murine brain were prepared and stained with anti-CD3 for T cells and anti-CD45R/B220 for B cells.](image)

### 6. Conclusions

This review has attempted to follow the course of events that lead to the development of viral encephalitis from the viruses that cause it, through the early and late events of the host response. The proposed mechanisms that viruses use to cross the blood-brain barrier are limited and evidence for each is incomplete. The first, and one used by the most devastating viruses, is through direct infection of neurons within the periphery and effectively bypassing the BBB to infect neurons within the CNS. Examples include rabies virus and herpes viruses. Pulzova and co-workers suggested three further methods by which pathogens can cross the endothelial barrier (Pulzova et al., 2009). The first involves direct infection of endothelial cells although this appears to be rare for viruses that invade the CNS. Secondly crossing between cells, again this is likely to be rare in the absence of other factors that might lead to a breakdown of the BBB although it has been proposed for West Nile virus (Verma et al., 2009). Finally, entry may result following transit within an infected cell, the so-called Trojan horse mechanism suggested for infection with HIV. Once within the CNS, viruses can spread rapidly. The consequences of uncontrolled viral-induced damage and subsequent un-regulated inflammation within the CNS can have severe consequences to the host. The brain is enclosed within the skull and the tough dural membranes so that there can be no increase in brain-case volume. This could occur in
response to increases in extracellular fluids or cellular content during inflammation with consequences for arterial influx and the possibility of ischaemia (Ransohoff et al., 2003). Furthermore, destruction of non-replaceable neurons can leave the patient with permanent disability, commonly observed following infections with Japanese encephalitis virus and West Nile virus, or death as happens following infection with rabies virus. Indeed, T cell infiltration has been associated with paralysis in experimental models of rabies virus infection (Sugamata et al., 1992).

Infection and cellular destruction triggers the innate immune response that attracts immune-effector cells with the aim of controlling virus replication. Viruses, in turn, possess mechanisms to inhibit the innate immune response, or in the case of herpesviruses are able to remain latent within infected neurons. This delays the host response providing a window for early rounds of virus replication. A further delay occurs through limited antigen presentation within the CNS. Early responders tend to be T cells and often appear to dominate the response when viewed at a single time-point (see Figure 4), whereas B-cells tend to enter later during the course of infection (Savarin and Bergmann, 2008). CD8 cells in particular have been shown in experimental models to be critical for clearance of some viral infections (Borrow et al., 1992) and control of others such as Herpes simplex infection (Lang and Nikolich-Zuglich, 2005). By contrast, antibody production within the CNS is considered critical to the control of rabies virus (Hooper et al., 2009).

The difficulty in identifying the aetiological agent of encephalitis presents a real challenge to treatment. Herpes simplex viruses are the most common viral cause of encephalitis and are readily treatable with nucleoside analogues such as acyclovir. However, resistance has been observed leading to the development of alternatives such as helicase inhibitors and TLR agonists (Grauer et al., 2008; Wilson et al., 2009). Nitric oxide inhibitors have been suggested as a possible therapy in HIV to inhibit BBB disruption during HIV encephalopathy (Giovannoni et al., 1998). Recently, the use of therapeutic coma has been promoted as a method for treating rabies virus infection, particularly following the survival of a teenager using this treatment (Willoughby et al., 2005). However, numerous attempts to replicate this have not met with success (Maier et al., 2010; Hunter et al., 2010). Effective prevention for rabies, as with many other virus infections is best achieved by prior vaccination. Future alternatives to treatment of viral encephalitis may focus on innate immune agonists that will assist in the early anti-viral response.

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8. References


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Many infectious agents, such as viruses, bacteria, and parasites, can cause inflammation of the central nervous system (CNS). Encephalitis is an inflammation of the brain parenchyma, which may result in a more advanced and serious disease meningoencephalitis. To establish accurate diagnosis and develop effective vaccines and drugs to overcome this disease, it is important to understand and elucidate the mechanism of its pathogenesis. This book, which is divided into four sections, provides comprehensive commentaries on encephalitis. The first section (6 chapters) covers diagnosis and clinical symptoms of encephalitis with some neurological disorders. The second section (5 chapters) reviews some virus infections with the outlines of inflammatory and chemokine responses. The third section (7 chapters) deals with the non-viral causative agents of encephalitis. The last section (4 chapters) discusses the experimental model of encephalitis. The different chapters of this book provide valuable and important information not only to the researchers, but also to the physician and health care workers.

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