

Inflammatory Diseases of the CNS I: Encephalitis

Neuropathology Articles in ACNR

A series of neuropathology articles will be published in the next few issues of ACNR with the aim of fostering interdisciplinary team discussion. The number of team meetings is increasing, bringing together all the disciplines involved in the diagnosis, management and treatment of patients with neurological disease. An essential part of this process is education. The more we understand the basic principles in each other's discipline, the better the communication and care of patients.

In the first three articles we shall deal with Inflammatory Diseases of the CNS, not as extensive reviews but as short accounts of the basic pathological principles used by neuropathologists to establish diagnoses and to plan research.

Inflammatory Diseases of the CNS I: Encephalitis by Dr Ingrid Mazanti and Professor Roy Weller, Southampton

Inflammatory Diseases of the CNS II: Meningitis and Cerebral Abscess by Dr Susan Robinson and Dr William Stewart, Glasgow

Inflammatory Diseases of the CNS III: Sarcoidosis and Non-Metastatic Diseases associated with Neoplasia by

Professor Francesco Scaravilli, London

Professor Roy O. Weller, Series Editor

Introduction

Intracranial infections involving the bones of the skull or spine (osteomyelitis), the extradural compartments, the pachymeninges (dura), subdural space, leptomeninges and subarachnoid space are mainly caused by bacteria and occasionally by fungi and viruses (leptomeningitis). The organisms that infect the CNS itself (the brain and the spinal cord) are mainly viruses and bacteria, but fungi and protozoa, such as *Toxoplasma* and amoebae, do occasionally invade the CNS.

Encephalitis can be defined as diffuse inflammation of brain tissue, or spinal cord (encephalomyelitis or just myelitis). Two major forms of encephalitis occur: those due to virus infections and those due to autoimmune inflammation - Acute Disseminated Encephalomyelitis (ADEM).

Viruses responsible for encephalitis

The table lists the main viruses involved in encephalitis and encephalomyelitis. Many are enteroviruses; encephalitis caused by respiratory viruses appears to be less common¹. In many parts of the world encephalitides are seasonal due to the spread of viruses by insects (arboviruses)². New virus infections of the CNS, such as West Nile Fever³ and Nipah⁴, continue to evolve.

Immunosuppression is an important predisposing factor for infections of the central nervous system by viruses such as the wart virus (papova virus). Viruses mainly gain access to the body via the respiratory, gastrointestinal or genitourinary mucosae and through insect bites. From the site of inoculation, viruses spread to lymph nodes and then enter the brain via the blood. Certain viruses, e.g. rabies, enter the CNS by retrograde axoplasmic transport along nerves from peripheral wounds. Other viruses, such as human immunodeficiency virus (HIV), are thought to enter the CNS within infected mononuclear cells/macrophages. DNA viruses (eg herpes simplex) and RNA viruses (eg measles) enter neurons and glia by attaching to receptors on the cell surfaces and then replicate within the cell. New virus particles may bud from the surfaces of cells or are released through lysis of the infected cells. In autoimmune encephalomyelitis (ADEM) there is no causative virus in the CNS, although the inflammatory changes of perivenous inflammation and perivenous demyelination may be widespread and are thought to result from an autoimmune reaction, frequently in response to a previous systemic virus infection.

Pathology

The pathology of the various viral infections listed in the



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Table: Viruses causing encephalitis in various regions of the CNS (modified from (1))

Viruses infecting the central nervous system and its coverings	
Meningitis	Virus affecting mainly the white matter (leukoencephalitis or leukoencephalopathy)
Echovirus Coxsackieviruses Other enteroviruses Herpes simplex virus 2 Mumps Human immunodeficiency virus Lymphocytic choriomeningitis virus	Papovavirus (JC virus) Human immunodeficiency virus
Virus affecting mainly grey matter (polioencephalitis/polioencephalomyelitis)	Virus affecting mainly both grey and white matter (panencephalitis/panmyelitis)
Poliovirus Coxsackievirus A4, A7 or B3 Echovirus 2 or 9 Enteroviruses 70 and 71 Rabies Arboviruses (especially Japanese encephalitis virus and Tick-borne encephalitis viruses)	Herpes simplex virus 1, Herpes simplex virus 2 Varicella-zoster virus Cytomegalovirus Human immunodeficiency virus Measles Arboviruses (insect borne) Early herpes simplex virus infection Atypical herpes simplex encephalitis

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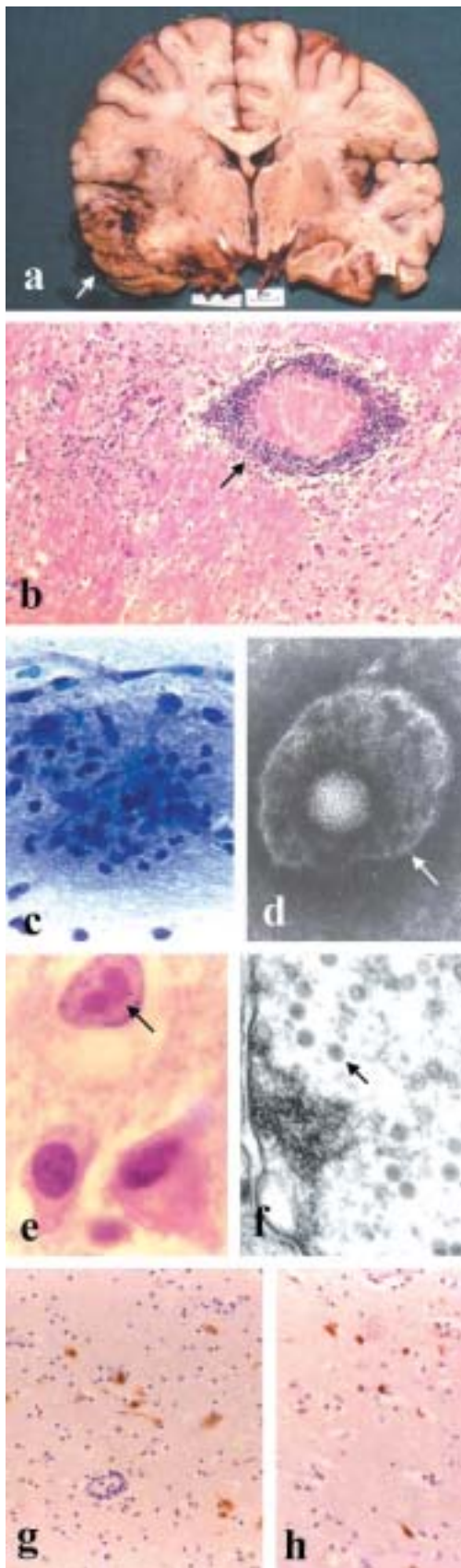


table varies, depending upon the causative virus, the location in the brain or cord affected and upon the acute or chronic nature of the disease. For example, poliomyelitis classically infects and destroys spinal and bulbar motor neurons, whereas herpes simplex infection (HSV-1) is localised to the temporal lobes. Nevertheless, the major pathological reactions within the CNS are very similar throughout a whole range of virus infections. As an example of an acute encephalitis, the pathology of herpes simplex encephalitis is described in some detail below.

Herpes simplex (HSV-1) encephalitis is probably the most common acute necrotising encephalitis in the UK. Patients present with headache, fever, seizures and decreasing levels of consciousness. CT and MRI reveal swelling and necrosis of the temporal lobes that is often bilateral. In patients who survive for many years, there is cystic change and atrophy of the temporal lobes. HSV is present in cold sores on the lips and in the trigeminal ganglia; but whether the close anatomical relationship between the trigeminal ganglia and the temporal lobes plays a role in the spread of infection to the brain remains unresolved.

Macroscopically, the brain at autopsy in a patient dying with acute herpes simplex encephalitis shows haemorrhage, swelling and necrosis of the temporal lobes (figure 1a), often with atrophy and cystic change.

The diagnostic features of encephalitis are seen microscopically. They consist of death of neurons and other cells, inflammation and the presence of the infecting virus. Dying neurons are recognised by their shrunken (pyknotic) nuclei, and pale, pink cytoplasm in haematoxylin and eosin (H&E) stained sections. The major inflammatory cells are lymphocytes; they enter the brain via post-capillary venules, accumulate around veins and spread diffusely into the infected brain tissue (Figure 1b). Microglial cells become activated, proliferate and may form small collections (microglial stars) around dead virus-infected neurons that they ingest (neuronophagia) (figure 1c). A small number of neutrophil polymorphonuclear leukocytes may be present in the early stages of the disease whereas reactive astrocytosis and macrophages are seen in the more advanced stages of the encephalitis. Identification of HSV-1 in the tissue allows a definitive diagnosis to be made. This may entail the culture of virus or the detection of antibodies to HSV in the CSF; more commonly now, viral DNA is detected by PCR.

Viruses are detected in biopsy or autopsy brain tissue

Figure 1: Herpes simplex encephalitis:

(a) Coronal section of the cerebral hemispheres showing haemorrhagic necrosis in both temporal lobes; the left temporal lobe (arrow) is more affected than the right. (b) Histology of a haematoxylin and eosin (H&E) stained section showing lymphocytes around a vein (arrow) and spreading into the surrounding brain tissue. (c) A collection of microglial cells (microglial star) with rod-shaped nuclei, around an infected neuron (smear preparation stained with toluidine blue) (d) A herpes virus particle isolated from brain, showing the central icosahedral nucleocapsid and the outer membrane (arrow) derived from the cell membrane. (Negatively stained PTA Transmission Electron Micrograph (TEM) x 114,000) (e) High magnification photomicrograph of an intranuclear viral inclusion (arrow); the nucleus bottom left shows diffuse hyperchromasia also indicating the presence of virus (H&E). (f) Herpes virus particles (arrow) in a nucleus. (TEM x 30,000) (g) HSV protein within brain cells is stained brown by immunocytochemistry. (h) HSV DNA in brain cells is stained brown by in situ hybridisation. (Reproduced by kind permission of Prof JAR Nicoll (Figs a, b, e, g, h.) Dr PJ Gallagher (Figure f) and Ms Sue Cox (Figure d). The plate of figures was kindly prepared by Anton Page and Roger Alston)

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in a variety of ways. Virus particles can be isolated by homogenising fresh tissue in glutaraldehyde, centrifuging and negatively staining the virus in the supernatant with phosphotungstate (figure 1d); this was a major diagnostic technique before the introduction of immunocytochemistry and PCR, and remains a key investigation for the characterisation of novel virus infections. In H&E stained sections of brain tissue, viral inclusions may be seen in the nuclei of infected cells, or the virus may be spread more diffusely through the nucleus (figure 1e). Viral particles in the nuclei lack the outer membrane of the intact virus (compare electron micrographs figure 1d and f). Viral proteins within cells are detected by immunocytochemistry (figure 1g), provided the antibodies are available, and viral DNA by *in situ* hybridisation (figure 1h).

The pathological features illustrated in figures 1 b & c are characteristic of the body's defences against virus infections. Infected cells produce interferons (IFN). IFN- α and IFN- β induce other cells to resist infection by the virus; IFN- γ acts as a potent pro-inflammatory factor that activates NK cells, monocytes and macrophages. NK lymphocytes are rapid response cells that kill cells infected by virus although the mechanism of such killing is unclear. Specific antibodies to viral glycoproteins are produced by B lymphocytes/plasma cells and together with complement, they neutralise free virus especially in the blood. T lymphocytes play a key role in encephalitis as MHC Class I-restricted CD8⁺ cytotoxic T lymphocytes destroy cells infected with virus. Immunosuppression interferes with these defence mechanisms; as a consequence, viruses that do not normally infect the CNS, such as cytomegalovirus

(CMV) and papova virus, may proliferate in the CNS causing CMV-encephalitis and progressive multifocal leukoencephalopathy respectively.

Differential diagnoses

One major differential diagnosis at biopsy or autopsy is between virus infection and autoimmune encephalomyelitis (ADEM)³. The regional specificity of many virus infections within the CNS may suggest a diagnosis of viral encephalitis as may the intense lymphocyte infiltration and presence of viral protein or nucleic acid by histology, immunocytochemistry and *in situ* hybridisation. PCR or antibody studies of CSF may identify a virus. However, in more than 50% of cases no virus is identified by these techniques even though it is suspected². ADEM is usually preceded by a systemic viral illness some 2-3 weeks previously, such as measles in the developing world or a wide variety of pulmonary or gastrointestinal infections in Europe and USA. Although often centred on the cerebral white matter, ADEM may affect many areas of the CNS including the spinal cord. Distinction from a virus infection may be difficult and may depend upon the history and the lack of evidence of a virus infection in the CNS. Bacterial and fungal infections mainly result in neutrophil polymorphonuclear leukocyte infiltration and focal necrosis associated with the presence of bacteria or fungi. Differentiating these diseases from the lymphocyte-dominated inflammation of viral encephalitis and ADEM is, therefore, relatively straight-forward. The other major differential diagnosis is multiple sclerosis (MS). MS appears to be an autoimmune disorder and is characterised by demyelination, perivenous lymphocytes, microglial activation, monocyte/macrophage invasion reactive astrogliosis, and varying degrees of axonal degeneration. However, MS plaques are usually focal and well-demarcated areas that lack overt evidence of infecting virus. Confusion occurs most often between MS and ADEM, although mostly the MS plaques are well circumscribed whereas the areas of demyelination seen in ADEM are frequently confined to narrow areas around veins².

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