Transactions of the Royal Society of Tropical Medicine and Hygiene (2006) **100**, 1135—1145

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevierhealth.com/journals/trst

A preliminary neuropathological study of Japanese encephalitis in humans and a mouse model

Allison C. German[a,b], Khin Saw Aye Myint[c], Nguyen Thi Hoang Mai[d], Ian Pomeroy[e], Nguyen Hoan Phu[d], John Tzartos[e], Peter Winter[a,b], Jennifer Collett[a,b], Jeremy Farrar[f], Alan Barrett[g], Anja Kipar[h], Margaret M. Esiri[e], Tom Solomon[a,b,*]

[a] *Division of Medical Microbiology, University of Liverpool, Liverpool, UK*
[b] *Division of Neurological Sciences, University of Liverpool, Liverpool, UK*
[c] *Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand*
[d] *Centre for Tropical Diseases, Cho Quan Hospital, Ho Chi Minh City, Viet Nam*
[e] *Department of Neuropathology, Radcliffe Infirmary, Oxford, UK*
[f] *University of Oxford—Wellcome Trust Clinical Research Unit, Ho Chi Minh City, Viet Nam*
[g] *Department of Pathology, Center for Biodefense and Emerging Infectious Diseases, and Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston, TX, USA*
[h] *Department of Veterinary Pathology, University of Liverpool, Liverpool, UK*

Received 28 November 2005; received in revised form 20 February 2006; accepted 20 February 2006
Available online 30 June 2006

KEYWORDS
Arbovirus;
Japanese encephalitis;
Zoonosis;
Blood brain barrier

Summary Japanese encephalitis virus is a mosquito-borne flavivirus that causes approximately 10 000 deaths annually in Asia. After a brief viraemia, the virus enters the central nervous system, but the means of crossing the blood—brain barrier is uncertain. We used routine histological staining, immunohistology and electron microscopy to examine brain material from four fatal human cases, and made comparisons with material from a mouse model. In human material there was oedema, perivascular inflammation, haemorrhage, microglial nodules and acellular necrotic foci, as has been described previously. In addition, there was new evidence suggestive of viral replication in the vascular endothelium, with endothelial cell damage; this included occasional viral antigen staining, uneven binding of the vascular endothelial cells to *Ulex europaeus* agglutinin I and ultrastructural changes. Viral antigen was also found in neurons. There was an active astrocytic response, as shown by glial fibrillary acidic protein staining, and activation of microglial cells was demonstrated by an increase in major

* Corresponding author. Viral Brain Infections Group, Divisions of Medical Microbiology and Neurological Sciences, 8th Floor Duncan Building, Liverpool L69 3GA, UK. Tel.: +44 151 706 4381; fax: +44 151 706 5805.
E-mail address: tsolomon@liv.ac.uk (T. Solomon).

0035-9203/$ — see front matter
doi:10.1016/j.trstmh.2006.02.008

histocompatibility complex class II expression. Similar inflammatory infiltrates and a microglial reaction were observed in mouse brain tissue. In addition, β-amyloid precursor protein staining indicated impaired axonal transport. Whether these findings are caused by viral replication in the vascular endothelium or the immune response merits further investigation.

1. Introduction

Japanese encephalitis (JE) is an acute encephalitis caused by infection with Japanese encephalitis virus (JEV), a member of the genus *Flavivirus*, family Flaviviridae, closely related to West Nile virus (Solomon, 2004). JEV is found in Asia and, like West Nile virus, is spreading, with recent outbreaks in Nepal and Australia (Mackenzie et al., 2004; Solomon, 2004; Weaver and Barrett, 2004). Numerically, JE is a more important disease than West Nile Virus, with an estimated 30 000—50 000 encephalitis cases and 10 000—15 000 deaths annually, mostly among children in Asia (Tsai, 2000). JEV is zoonotic and is transmitted naturally among birds, especially egrets, herons and other water birds, by mosquitoes, principally *Culex* species (Scherer and Buescher, 1959). Pigs are also important amplifying hosts (Scherer and Buescher, 1959). Humans become infected with the virus following the bite of an infected mosquito; most human infections are asymptomatic or cause a non-specific febrile illness, which is thought to correspond to a self-limiting viraemia. However, in a proportion of those infected the virus crosses the blood—brain barrier to cause central nervous system (CNS) disease. Researchers are uncertain about the mechanism by which the virus crosses the blood—brain barrier; most data suggest the vascular endothelium is a more likely route than the olfactory mucosa, but whether the virus is passively transported or actively replicates in the endothelium is unclear (Solomon and Vaughn, 2002).

The few pathological studies of JE in humans describe characteristic 'punched-out' necrotic foci, often associated with blood vessels (Desai et al., 1995; Johnson et al., 1985; Miyake, 1964), but their nature is unknown, and there have been few immunohistological and/or ultrastructural investigations. The mouse is often cited as a useful model for JE, because of the similar clinical features to humans, but there has been little work comparing the pathological changes in the mouse model with those in human disease (Hase et al., 1990a; Miyake, 1964). We therefore conducted an immunohistological and ultrastructural study of JE in humans, looking in particular at the effect on the vascular endothelium. We also looked for similar changes in limited material that was available from a previous study of JEV in the mouse model (Cao et al., 1995).

2. Materials and methods

2.1. Patients and samples

2.1.1. Humans

Specimens were collected from four fatal JE cases admitted during prospective clinical studies of JE between 1995 and 1997, at the paediatric and adult intensive care units at the Centre for Tropical Diseases, Ho Chi Minh City, Viet Nam (Solomon et al., 2002, 2003). JEV infection was confirmed on admission by measurement of IgM antibodies in the serum and cerebrospinal fluid (CSF) using a rapid IgM dot enzyme immunoassay (Solomon et al., 1998). Serum and CSF samples were subsequently analysed for IgM and IgG anti-JEV antibodies using a double sandwich capture ELISA (Innis et al., 1989), and viral culture attempted, as described previously (Solomon et al., 2002). Where permission was granted, a post-mortem needle biopsy of the brain through the infra-occipital route, and/or an autopsy, was performed as soon after death as possible. Tissue samples were fixed in 10% non-buffered formalin for a minimum of 4 weeks for histopathological examination and 2% glutaraldehyde for electron microscopic examination.

2.1.2. Mouse

During previous studies (Cao et al., 1995; Nitayaphan et al., 1990), 3—4-week-old female outbred (NIH Swiss strain) white mice (Harlan, USA), weighing 20—25 g, were inoculated intracerebrally with 20 μl of either an attenuated derivative of the JEV Nakayama strain or JEV strain SA-14. The original Nakayama strain was attenuated by six passages through HeLa cells, characterized by alterations in the E protein. This strain (Nakayama-O/HeLa p6) failed to induce overt encephalitis in mice when inoculated intracerebrally (Cao et al., 1995), whereas the SA-14 strain had a lethal neurovirulent phenotype in mice when inoculated by the intracerebral route (Hase et al., 1993). Three mice (one infected with Nakayama-O/HeLa p6 and two infected with SA-14) were euthanized by cervical spine dislocation 5 d post-infection, and the brains were fixed in 10% non-buffered formalin for histopathological examination. Two mice inoculated intracerebrally with an equivalent volume of phosphate-buffered saline were used as controls.

2.2. Histology and immunohistology

Tissues were routinely embedded in paraffin wax and 5 μm sections cut and stained with haematoxylin and eosin (HE) and Toluidine Blue for histological examination. For immunohistological examination, 5 μm sections were mounted on poly-lysin coated slides. Briefly, sections were deparaffinized and then rehydrated through a series of graded alcohols. Optimal conditions for antigen retrieval, blocking and antibody dilutions were independently determined by comparative titration experiments. Endogenous peroxidase was blocked through incubation with hydrogen peroxide and antigen retrieval pre-treatment performed as necessary, using formic acid and/or heated citrate buffer (pH 6.0). Sections were incubated with normal serum to avoid non-specific binding of antibodies and then incubated for 15—18 h at 4 °C with the primary antibodies. For human tissues,