7. Nipah and Hendra Viruses Encephalitis

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7.1. Introduction

Nipah and Hendra viruses are two new zoonotic viruses that have emerged during the past decade (Murray et al., 1995b; Chua et al., 2000a). Both are members of the Paramyxoviridae family, and they share similar antigenic, serologic, as well as ultrastructural characteristics. Molecular studies have also shown that they are closely related in terms of their nucleotide and amino acid sequences (Harcourt et al., 2000). Because of their homology and the fact that these two viruses differ much more from other members of the Paramyxoviridae family, a new genus called *Henipavirus* (*He*ndra + *Nipa*h) was created for these two viruses (Wang et al., 2000, 2001; Bossart et al., 2002).

Hendra virus first appeared in 1994 when it caused a disease affecting horses, which subsequently involved three human cases in Queensland, Australia. Between 1998 and 1999, Nipah virus resulted in widespread infection of pigs and humans in Malaysia and, to a lesser extent, Singapore (Murray et al., 1995a; Selvey et al., 1995; Chua et al., 1999). Between 2001 and 2004, several further outbreaks of Nipah virus have also occurred in Bangladesh (Hsu et al., 2004; ICCDR, B, 2004; Quddus et al., 2004; WHO, 2004).

7.2. Hendra Virus Infection

In August–September 1994, an outbreak of an acute respiratory illness among horses occurred in Australia, in which 13 of 20 affected horses died (Murray et al., 1995a, 1995b). The infection affected two humans, a horse trainer and a stablehand, who suffered from acute respiratory disease, from which the horse trainer died despite hospitalization (Selvey et al., 1995). The stablehand recovered subsequently. A new virus was isolated from tissues of infected horses and was named Hendra

virus after the Brisbane, Queensland, suburb where the original outbreak occurred. A second human death occurred in October 1995, when a farmer from Mackay, about 1000 km north of Brisbane, died from a neurological illness (O'Sullivan et al., 1997). He had had close contact with two ill horses about a year earlier and had become ill with mild meningoencephalitis but recovered. After being asymptomatic for more than a year, he developed seizures, coma, and died. He tested seropositive for Hendra virus, and retrospective tests on tissues from the Mackay horses revealed the presence of Hendra virus. In January 1999, an isolated equine death occurred in Cairns, north Queensland, with no evidence of further infection (Barclay and Paton, 2000).

7.2.1. Epidemiology

The outbreak of Hendra virus infection primarily occurred among horses. Human infection occurred in individuals who had had close personal contact with infected horses, for example, nursed ill horses (Murray et al., 1995a, 1995b; Selvey et al., 1995; O'Sullivan et al., 1997). Noninfected individuals associated with the outbreak premises were serologically negative, and no human-to-human transmission has been reported (Selvey et al., 1995). Hendra virus was isolated from the oral cavity and urine of infected horses as well as in the urine of experimentally infected cats and guinea pigs (Williamson et al., 1998). Horses could be infected experimentally through infected urine contaminating their feed.

The natural reservoir for the Hendra virus is likely to be fruit bats of the *Pteropus* species, also known as flying foxes, although the bats remain asymptomatic. Neutralizing antibodies for Hendra virus have been demonstrated in all four species of *Pteropus* in Australia, and the virus has been isolated from fetal tissue and uterine fluid of these bats (Young et al., 1996; Halpin et al., 2000). However, there was no serological evidence of infection in persons with close and prolonged contact with bats (Selvey et al., 1996). The mechanism by which the virus could have spread to horses is unknown, although it has been noted that the outbreaks occurred during the breeding season of the fruit bats. The isolation of the virus from fetal tissues and fluid suggests that contact with aborted fetuses and products of the birthing process could be a possible mechanism of transmission (Halpin et al., 2000).

7.2.2. Clinical Features

Infected horses developed an acute febrile respiratory illness. Human infection resulted in an acute influenza-like illness with marked respiratory symptoms, fever, and myalgia in the Hendra cases (Selvey

et al., 1995). One of the two patients (the horse trainer) died despite intensive care treatment after a week of illness, whereas the other patient (the stablehand) recovered over a 6-week period.

On the other hand, the patient from Mackay presented primarily with neurological symptoms with an unusual temporal pattern (O'Sullivan et al., 1997). A history of exposure was followed by a mild meningoencephalitic illness from which he recovered. He then remained asymptomatic for about 13 months before developing a severe encephalitic illness and died. The patient presented with irritability followed by focal seizures. He then developed a right hemiplegia and became comatose. He died after 25 days without recovering consciousness.

7.2.3. Diagnosis

Serological diagnosis of Hendra virus infection has been carried out by demonstrating antibodies using enzyme-linked immunosorbent assay (ELISA) or serum neutralization tests. Viral isolation was initially carried out from postmortem tissues, but as the virus has been classified as a biohazard level 4 pathogen, this cannot be readily done except in appropriate facilities.

MRI brain imaging of the patient with neurological illness revealed distinctive focal high signal cortical changes on T2-weighted sequences, and the electroencephalogram (EEG) showed persistent periodic epileptiform discharges (O'Sullivan et al., 1997). His cerebrospinal fluid examination showed increased leukocytes with a predominance of lymphocytes, raised protein, but normal glucose levels.

7.2.4. Pathology

Postmortem studies showed severe interstitial pneumonia in the first patient with respiratory illness (Selvey, et al., 1995). On the other hand, in the second fatal human case, the main abnormalities were in the brain, and findings were focal necrosis in the cortex, basal ganglia, brain stem, and cerebellum. Multinucleated endothelial cells were seen in several organs (O'Sullivan et al., 1997). Immunohistochemistry indicated the presence of viral antigens in the neuronal cytoplasm as well as the neuropil (felt work of interwoven cytoplasmic process of nerve cells in gray matter).

7.3. Nipah Virus Infection

Between September 1998 and June 1999, there was an outbreak of acute viral encephalitis in several pig-farming villages in Peninsular

Malaysia. (CDC, 1999a, 1999b; Chua et al., 1999; Goh et al., 2000). The outbreak started near Ipoh, in the northern state of Perak, but subsequently spread to involve villages 300 km to the south in Negeri Sembilan in the Sikamat and Bukit Pelanduk districts, including Sungai Nipah village, where patients, whose specimens yielded the first viral isolates, lived (Chua et al., 2000a). Overall, more than 265 patients were affected in Malaysia with more than 105 deaths (Parashar et al., 2000). Several abattoir workers in Singapore, which imports pigs from Malaysia, were also affected (Chew et al., 2000; Lee et al., 1999; Paton et al., 1999). The outbreak ended with the mass culling of pigs in the affected areas.

More recently, several outbreaks of acute Nipah encephalitis occurred in Bangladesh: in the Meherpur and the Naogaon districts in 2001 and 2003 (Hsu et al., 2004) and in the Rajbari, Faridpur, Golpagonj, Manikgonj, Joypurat, and Naogoan districts in January 2004 and the Faridpur district in April 2004 (ICCDR, B, 2004; Quddus et al., 2004; WHO, 2004). Unlike the earlier Malaysian outbreak of Nipah virus infection, no significant diseases among animals were noted, and exactly how the infection was transmitted to humans is uncertain.

7.3.1. Epidemiology

In the Nipah outbreak in Malaysia, direct contact with infected pigs, their secretions, or fresh pig products was responsible for viral transmission to humans. Patients were originally thought to have Japanese encephalitis (JE) because it occurred in pig-farming villages, but distinguishing features included infection predominantly in adults rather than children, clustering of cases in members of the same home with an attack rate much higher than JE, a high proportion of patients in direct contact with pigs as opposed to unaffected individuals living in the same neighbourhood, a history of illness in the pigs belonging to the affected farmers, and the fact that many patients had prior immunization against JE (Chua et al., 1999; Goh et al., 2000). These observations were confirmed by case-control studies showing that patients had activities with more direct contact with pigs. Parashar et al. (2000) found that Nipah-infected patients were more likely than community farm controls (persons from farms without reported Nipah infection) to have ill/dying pigs (59% vs. 24%) and were more likely than case farm controls (persons from infected farms without illness) to have had activities requiring direct contact with pigs (86% vs. 50%). Other affected occupations included abattoir workers, pork sellers, as well as army personnel brought in to cull infected pigs (Tan et al., 1999; Chew et al., 2000; Premalatha et al., 2000; Ali et al., 2001). Exposure to ill animals other than pigs (e.g., dogs) were also reported in a few patients

suggesting possible viral transmission from other animals (Tan et al., 1999; Parashar et al., 2000).

The risk of nosocomial transmission of Nipah virus during the Malaysian outbreak was thought to be low. In a study on 338 health care workers exposed to infected patients during the outbreak, three subjects were positive for IgG antibodies on enzyme-linked immunosorbent assay (ELISA) but as their serum anti-Nipah IgM and neutralizing antibodies were negative, they were felt to be false positives (Mounts et al., 2001). However, in an MRI study of asymptomatic seropositive cases, MRI findings in the brain typical of Nipah infection was demonstrated in a nurse who had cared for Nipah-infected patients. She had no exposure to infected animals and is likely a case of human-to-human transmission, probably from exposure to infected patients' secretions (Tan et al., 2000; Tan and Tan, 2001). This is not surprising as the Nipah virus could be isolated from patients' respiratory secretions and urine (Chua et al., 2001a).

On the other hand, in the outbreaks in Bangladesh, no clusters of ill animals were observed (Hsu et al., 2004; WHO, 2004). A variety of animals were tested for antibodies to Nipah antigens but were negative except for bats (*P. giganteus*) (Hsu et al., 2004). Nipah-infected patients were more likely than nonpatients to have close contact with other Nipah-infected patients. The likelihood therefore was that in Bangladesh, the transmission occurred due to close contact with other infected persons.

The primary reservoir for Nipah virus is likely to be the fruit bat, of the species *Pteropus.* In a study of 324 bats from 14 species, serum neutralizing antibodies to Nipah virus were demonstrated in five species, including *P. vampyrus* and *P. hypomelanus,* but attempts to isolate the virus or amplify viral sequences from these bats were negative (Yob et al., 2001). However, the Nipah virus was isolated from the urine of *P. hypomelanus* roosting in fruit trees in Tioman, an island off the east coast of Peninsular Malaysia (Chua et al., 2002). In the search for the natural host for Nipah virus, another new paramyxovirus was discovered in urine of *P. hypomelanus* as well: Tioman virus (Chua et al., 2001b). The latter virus is as yet not known to cause any human or animal disease. In a serological survey of the inhabitants of Tioman Island, no individual was found to be seropositive for Nipah or Tioman antibodies, suggesting that direct viral transmission from bats to humans does not occur (Chong et al., 2003).

If the fruit bat is the reservoir host, half-eaten fruits could have been ingested by pigs, in which the virus was then amplified and transmitted to human beings. In fact, the virus has been isolated from such fruits (Chua et al., 2002). In the case of the Bangladesh outbreaks, the exact mechanism of the spread of virus to humans is unknown, although direct contact with bat secretions is a possibility (Hsu et al., 2004; WHO et al., 2004).

Subsequent person-to-person spread may have occurred as evidenced by clustering of cases in certain households (ICCDR, B, 2004).

7.3.2. Clinical Features

Nipah virus primarily caused an encephalitic illness both in the Malaysia/Singapore and the Bangladesh outbreaks. The incubation period ranged from a few days to 2 weeks (Chong et al., 2000; Goh et al., 2000). Most persons who were exposed to the virus became symptomatic, with the ratio of symptomatic versus asymptomatic (subclinical) infection assessed to be 3:1 (Tan et al., 1999).

The main clinical features were fever, headache, dizziness, vomiting, seizures, and reduced level of consciousness (Chong et al., 2000; Goh et al., 2000). In fact, more than 50% of patients with acute Nipah encephalitis had a reduced level of consciousness during the acute infection. This together with the presence of abnormal pupillary reflexes, absent doll's-eye reflex, a profound tachycardia and hypertension (suggesting involvement of the medullary vasomotor center) in many seriously ill patients suggested involvement of the brain stem (Goh et al., 2000). Persistent segmental myoclonus characterized by focal rhythmic jerking of muscles, occurring in about one-third to one-half of patients, was a distinctive feature, and the involvement especially of the diaphragm as well as the head and neck muscles suggested brain stem and/or upper cervical involvement. Another distinctive feature was hypotonia and areflexia, seen in about one-half of the patients. This was believed to be due to spinal root involvement secondary to meningeal inflammation (Chew et al., 2000). Other clinical features included seizures, nystagmus, cerebellar ataxia, tremor, dysarthria, and dysphasia.

Pneumonia was reported in 3 of the 11 patients from Singapore with abnormal chest radiograph findings (Paton et al., 1999). However, in Malaysian patients, respiratory tract symptoms (such as cough) were minor and occurred in about 14% in the series from the University of Malaya Medical Centre, Kuala Lumpur (Goh et al., 2000). A small number of patients presented with systemic symptoms, but no evidence for encephalitis both clinically and on cerebrospinal fluid examination, and were considered to have nonencephalitic infection (Goh et al., 2000). Mortality during the acute infection was high and estimated overall to be about 40% (Parashar et al., 2000). Mortality was associated with severe brain stem involvement, the presence of the Nipah virus in the cerebrospinal fluid suggesting high virus replication in the central nervous system and concomitant diabetes mellitus, probably due to immunoparesis (Chua et al., 2000b; Chong et al., 2001b; Goh et al., 2000).

Clinical descriptions of cases from the Bangladesh outbreaks similarly describe an acute febrile illness associated with headache and diminishing consciousness in the majority of patients and seizures (Hsu et al., 2004; ICCDR, B, 2004; Quddus et al., 2004; WHO, 2004). Respiratory symptoms (e.g., cough and dyspnea) also occurred, and chest radiographs in some patients demonstrated bilateral infiltrates consistent with acute respiratory distress syndrome (ARDS) (Hsu et al., 2004; ICCDR, B, 2004; WHO, 2004).

7.3.3. Investigations

In the majority of patients, cerebrospinal fluid (CSF) examination showed features typical of viral encephalitis with raised mononuclear leukocytes, elevated protein, and normal glucose levels. Blood investigation revealed nonspecific thrombocytopenia and leukopenia with raised alanine and aspartate transaminases in up to two-thirds of patients (Chong et al., 2000; Goh et al., 2000).

Diagnosis is confirmed by the detection of IgM and IgG antibodies against Nipah viral antigens in serum and CSF, using an IgM-capture enzyme-linked immunosorbent assay (ELISA) test and an indirect IgG ELISA test. The ELISA tests for Nipah antibodies had a high specificity and are therefore useful screening tests (Daniels et al., 2001). In the Malaysian outbreak, the rate of positive IgM was 60% to 70% by the 4th day and 100% by the 12th day of illness. IgG antibody was 100% positive by the 25th day of illness (Ramasundrum et al., 2000). Prior to the complete characterization of the virus, Nipah virus was suspected to be "Hendra-like" because cells infected with Nipah virus reacted strongly to anti-Hendra antibodies. Nipah and Hendra viruses shared enough epitopes for Hendra viral antigens to be used in the prototype serologic test sensitive enough to detect Nipah virus antigens (CDC, 1999a).

Viral isolation is not routinely carried out as Nipah virus is classified as a biosafety level 4 agent and requires specialized facilities. However, the virus was isolated from CSF, urine, and respiratory secretions of patients during the Malaysian outbreak (Chua et al., 2000b, 2001a). Direct visualization by electron microscopy in negatively stained CSF specimens was found to be useful but unable to differentiate between Nipah and Hendra viruses (Chow et al., 2000). In cell cultures, it was possible to differentiate between the various paramyxoviruses using features such as the types and distribution of nucleocapsid aggregates and viral envelope differences. The presence of single-fringe viral envelope and peripherally distributed, type 1 nucleocapsid is characteristic of Nipah virus (Hyatt et al., 2001).

Brain MRI is a useful diagnostic investigation. Ahmad Sarji et al. (2000) carried out MRI brain scans in 23 patients with Nipah encephalitis, 14 during the acute illness (during the first week) and a further 10 during the later phase of illness (about 54 days after onset) and demonstrated small and discrete high-signal-intensity lesions, measuring 2 to 7 mm in diameter, in the subcortical and deep white matter of the brain. These were best seen in the T2-weighted fluid attenuated inversion recovery (FLAIR) sequences (Fig. 7.1). These lesions were multiple and disseminated without specific predilection to any site in the brain and probably corresponded with the necrotic plaques seen in postmortem tissues (see below). No correlation appeared to exist between MRI findings and clinical focal neurological signs. MRI investigations of asymptomatic seropositive subjects revealed similar changes in about 16%, suggesting that these lesions can occur without any clinical symptoms or signs (Tan et al., 2000).

Electroencephalography (EEG) showed diffuse slow waves with the degree of slowing correlating with the severity of illness. In deeply comatosed patients, bitemporal, independent periodic complexes were seen and were associated with 100% mortality (Chew et al., 1999).

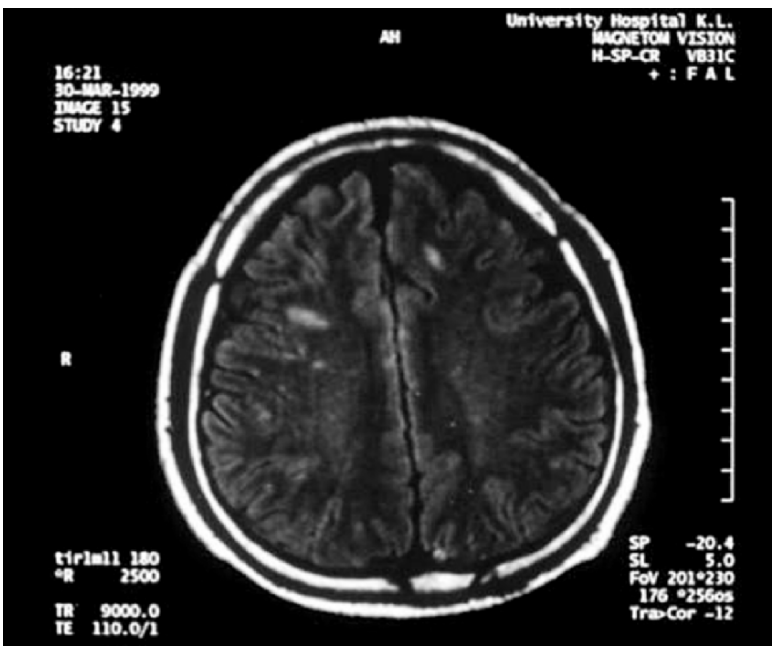

Figure 7.1. T2-weighted fluid attenuated inversion recovery (FLAIR) MRI showing multiple discrete subcortical lesions in a patient with acute Nipah virus encephalitis.

7.3.4. Treatment and Outcome

Treatment is mainly supportive with mechanical ventilatory support for seriously ill patients. Seizures were treated with anticonvulsants. Ribavirin, a broad-spectrum antiviral agent, was tried on an empirical basis in patients in the Malaysian outbreak. Ribavirin was administered orally initially and then intravenously, when available, to those who were unable to take it orally. In a comparison of 140 treated patients with 54 untreated patients serving as historical controls (i.e., those who developed the illness before ribavirin was made available), it was found that fewer treated patients died (32% vs. 54%, $p = 0.011$), suggesting that ribavirin reduced the mortality of patients (Chong et al., 2001a). However, it was possible that treated patients who were seen later in the outbreak were also given better general medical care than untreated patients seen earlier, as physicians became more experienced in the outbreak.

7.3.5. Relapsed and Late-Onset Encephalitis

Several patients who had recovered from the original encephalitis developed second and occasionally third episodes of neurological dysfunction. These patients constitute about 7.5% of the survivors and redeveloped symptoms about 8.4 months after exposure (Tan et al., 2002). In addition, about 3.4% who were either asymptomatic or had mild nonencephalitic illness initially developed a late-onset neurological illness (late-onset encephalitis). Common clinical features in relapsed or late-onset disease were fever, headache, seizures, and focal neurological deficits. Mortality was 18%. MRI brain scan findings in these patients were markedly different from that of acute encephalitis and showed large, patchy, confluent lesions involving the cortex (Ahmad Sarji et al., 2000; Tan et al., 2002) (Fig. 7.2). These clinical and radiological features were strikingly similar to the fatal case of Hendra viral encephalitis (O'Sullivan et al., 1997).

7.3.6. Pathology

Several autopsy examinations were carried out during the Malaysian outbreak. Wong et al. (2002a) reported on the pathological findings. Macroscopically, features were nonspecific, although occasionally, small lesions suggestive of necrosis could be observed. One of the earliest histopathological findings was the formation of multinucleated syncytium in the vascular endothelium. More commonly, vasculitis with endothelial ulceration and varying degrees of inflammation and fibrinoid necrosis and associated with thrombosis were seen (Fig. 7.3).

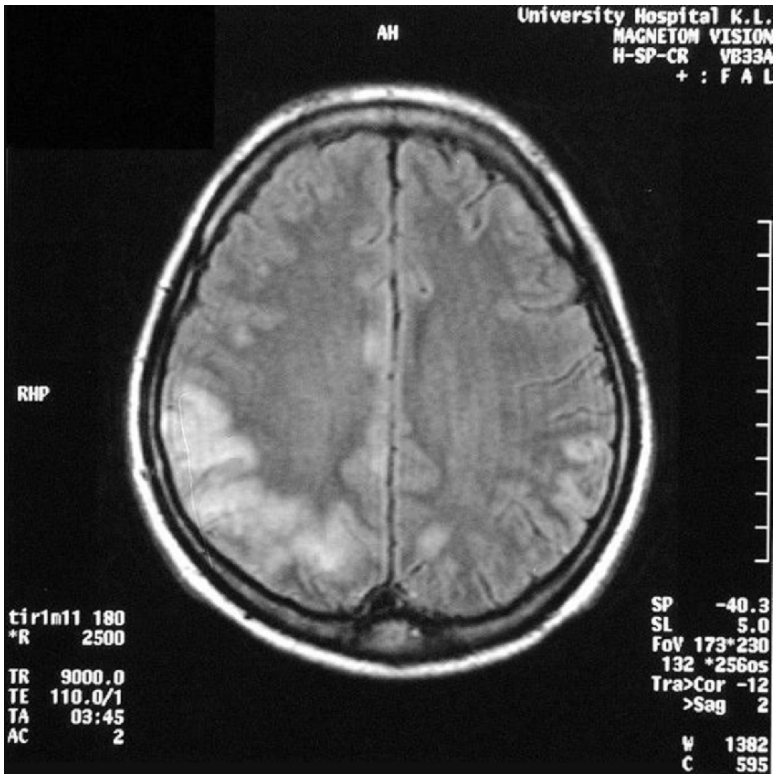

Figure 7.2. T2-weighted FLAIR MRI sequence in a patient with relapsed Nipah encephalitis showing large, patchy, confluent lesions involving the cortex.

Immunohistochemistry demonstrated the presence of viral antigen within the vascular endothelium. These vascular lesions were most widespread in the brain affecting the small but sparing the larger blood vessels. Surrounding these vessels were necrotic plaques believed to be a combination of microinfarction (from thrombosis) and direct viral infection of surrounding extravascular parenchyma. Some surviving neurons demonstrate cytoplasmic or nuclear inclusions, which were viral inclusions on immunohistochemistry. Electron microscopy showed that these inclusions were viral nucleocapsids (Hyatt et al., 2001).

Other organs were also involved, and after the central nervous system, the most severely affected organ was the lung in which there was focal fibrinoid necrosis of the lung parenchyma associated with vasculitis. In addition, there was inflammation associated with multinucleated giant cells in the alveolar spaces. Again, the presence of viral antigens was confirmed on immunohistochemistry in these giant cells, blood vessels, and, in one rare case, in the bronchial epithelium (Wong et al., 2002a).

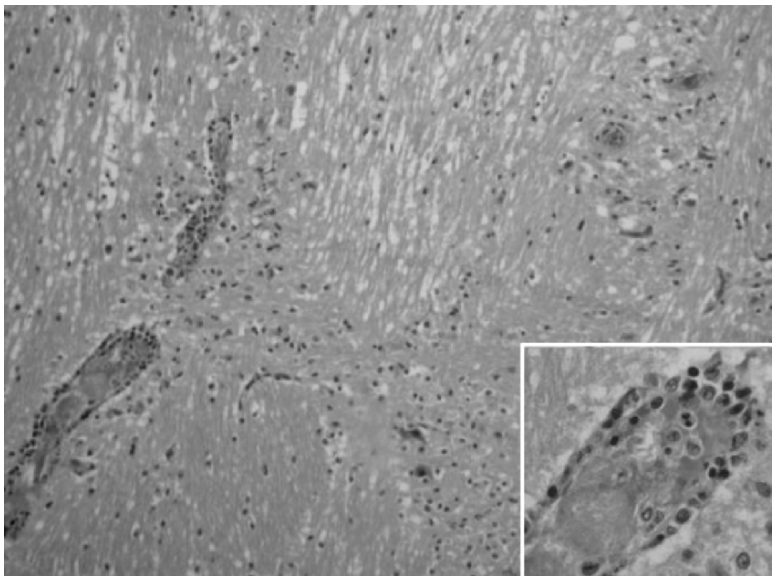

Figure 7.3. Vasculitis-induced thrombosis in two small blood vessels in the pons causing adjacent microinfarction (necrotic plaque) in the upper half of the figure (H&E; magnification, ×10 objective). The inset shows a close-up of the vasculitic vessel with thrombus within its lumen (magnification, ×40 objective).

Pathology of relapse encephalitis was different (Wong et al., 2002a). In the one autopsy case studied, vasculitis, perivascular cuffing, and necrotic plaques were not seen and instead viral inclusions were much more extensive and prominent, occupying the entire neuronal cytoplasm as well as being abundant in the neuropil. This suggested that relapse was due to viral reinfection rather than postinfectious demyelination (Wong et al., 2002a, 2002b).

7.3.7. Pathogenesis

After viral exposure, probably via the respiratory or alimentary tracts, and virus replication at site or sites presently unknown, viremia occurs with systemic spread of the virus, as evidenced by widespread vasculitis (Wong et al., 2002a, 2002b). The endothelium, being susceptible to viral infection, could be a secondary site for viral replication. In the brain, vasculitis would have resulted in a breach in the blood–brain barrier, allowing the spread of the virus into brain parenchyma. The predominatly central nervous system symptoms would have resulted from a combination of cerebral ischemia/microinfarction (from vasculitis and thrombosis) as well as from direct neuronal invasion.

7.4. Conclusion

Hendra and Nipah viruses are but two of the several recently discovered viruses isolated from bats. Others include other paramyxoviruses, viz. Menangle and Tioman viruses, and new members of the genus *Rubulavirus* (Chant et al., 1998; Philbey et al., 1998; Chua et al., 2001b). The former caused stillbirths among piglets and an influenza-like illness and rash in two human workers, whereas the latter has not yet been shown to cause human disease.

The implication of these new viral infections is that the virus can jump the species barrier, infect a naive animal host, and cross over to humans; occasionally at great cost, as can be seen in the Nipah and to a lesser extent the Hendra virus outbreaks. How the virus spreads from bats to the secondary animal hosts is not yet known for certain, and there is also the question of safety of human populations living near bats. Although the surveys in Australia and Malaysia showed no evidence of direct viral transmission from bats to humans (Selvey et al., 1996; Chong et al., 2003), the appearance of Nipah outbreaks in Bangladesh (Hsu et al., 2004; ICCDR, B, 2004; Quddus et al., 2004; WHO, 2004), with no apparent intermediate animal host and possible human-to-human transmission, makes it an all the more important question to answer. Factors that may influence viral transmission (both epidemiological and clinical) also remain to be elucidated.

Development of animal models for these infections will help further the understanding of their pathology and pathogenesis (Wong et al., 2003) as well as the development and testing of vaccines and antiviral therapy.

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