INFECTIONS OF THE CNS

Japanese encephalitis — the prospects for new treatments

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Abstract | Japanese encephalitis is a mosquito-borne disease that occurs in Asia and is caused by Japanese encephalitis virus (JEV), a member of the genus *Flavivirus*. Although many flaviviruses can cause encephalitis, JEV causes particularly severe neurological manifestations. The virus causes loss of more disability-adjusted life years than any other arthropod-borne virus owing to the frequent neurological sequelae of the condition. Despite substantial advances in our understanding of Japanese encephalitis from in vitro studies and animal models, studies of pathogenesis and treatment in humans are lagging behind. Few mechanistic studies have been conducted in humans, and only four clinical trials of therapies for Japanese encephalitis have taken place in the past 10 years despite an estimated incidence of 69,000 cases per year. Previous trials for Japanese encephalitis might have been too small to detect important benefits of potential treatments. Many potential treatment targets exist for Japanese encephalitis, and pathogenesis and virological studies have uncovered mechanisms by which these drugs could work. In this Review, we summarize the epidemiology, clinical features, prevention and treatment of Japanese encephalitis and focus on potential new therapeutic strategies, based on repurposing existing compounds that are already suitable for human use and could be trialled without delay. We use our newly improved understanding of Japanese encephalitis pathogenesis to posit potential treatments and outline some of the many challenges that remain in tackling the disease in humans.

Japanese encephalitis is the most commonly diagnosed epidemic encephalitis in the world and is found throughout South and Southeast Asia, encompassing an area delimited by Pakistan to the west, the Philippines and Japan to the east and the Australian Torres Strait Islands to the south. The most comprehensive estimate of incidence within the past decade suggests that 69,000 cases of Japanese encephalitis occur every year[1]. However, other estimates vary widely — from 50,000 to 175,000 cases per year — and, in reality, this number can fluctuate with cycles of transmission and is likely to be an underestimate. Japanese encephalitis is thought to cause the loss of 709,000 disability-adjusted life years annually[2], making it the most important arboviral disease of man by this measure, ranking above dengue in the 2004 WHO global burden of disease report[3], the last time that Japanese encephalitis was featured as an individual diagnosis in the report.

Japanese encephalitis is caused by Japanese encephalitis virus (JEV), a member of the genus *Flavivirus*, family Flaviviridae, which includes dengue, West Nile and Zika viruses[4]. Although several members of the genus *Flavivirus* cause encephalitis, JEV is remarkable in the high likelihood and severity of neurological disease that it elicits; by contrast, systemic features such as haemorrhage and jaundice are infrequently described for JEV. Over the past decade, many advances have been made in our understanding of the biology of the virus, yet in the same period only four treatment trials have been conducted into therapies for Japanese encephalitis. To date, no trials on Japanese encephalitis have translated into improvements in treatment; however, in total, only 381 patients with proven Japanese encephalitis have been randomly assigned in treatment trials. Nevertheless, in vitro and animal model studies have revealed many drugs that have activity against JEV, which could be suitable for use in humans. Studies of pathogenesis and virology have uncovered the mechanisms by which these drugs might work and identified potential new strategies, often based on repurposing existing compounds, for Japanese encephalitis treatment. Here, we review basic biological and clinical aspects of the disease and discuss new advances in our understanding of Japanese encephalitis that have highlighted compounds with anti-JEV activity in vitro and in animal models and could be used in humans. In addition, we discuss some tentative sample size calculations for Japanese encephalitis treatment trials using a clinical scoring system specifically

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Key points

- Japanese encephalitis is a severe disease caused by Japanese encephalitis virus, genus *Flavivirus*, family Flaviviridae, which is endemic to most of rural Asia and for which no specific treatment exists.
- Japanese encephalitis causes loss of more disability-adjusted life years than any other arthropod-borne virus owing to the frequent neurological sequelae of the condition.
- Pathogenesis studies indicate that inhibition of viral replication, viral spread and the host response are needed in combination for optimal therapy.
- Animal models and in vitro experiments highlight a number of compounds that are potentially suitable for treatment of Japanese encephalitis in humans that could be tested without delay.
- The minimum clinically significant treatment effect has probably been underestimated, and previous clinical trials of Japanese encephalitis have been too small; larger, pragmatic trials are needed.

developed for measurement of outcome from encephalitis in field settings such as those where Japanese encephalitis is endemic. In this way, we aim to stimulate new activity in the field to improve treatments for individuals with Japanese encephalitis. Although vaccines for Japanese encephalitis are available, the disease will never be eradicated owing to its zoonotic cycle. Moreover, its propensity to cause unpredictable outbreaks in unexpected places make vaccination planning challenging, underscoring the need to develop treatments for this devastating condition.

Virology and epidemiology

JEV is a single-stranded, positive-sense RNA virus[5] (FIG. 1) — features that it shares in common with other flaviviruses. JEV circulates among wild wading birds in Asia and is transmitted by mosquitoes of the genus *Culex*. Seminal epidemiological studies in Japan in the mid-20th century established pigs as amplifying hosts for JEV[6,7]. However, the dominance of pigs as amplifying hosts has been challenged, as countries such as Bangladesh that have predominantly Muslim populations and very little pig farming still have an appreciable burden of Japanese encephalitis in humans[8]. In 2017, a complete JEV genome was identified by unbiased RNA sequencing in a patient coinfected and clinically diagnosed with yellow fever in Cunene province, Angola[9], raising the possibility that the geographic range of JEV might be greater than previously thought. Non-vector transmission of JEV between pigs via respiratory secretions has also been described under experimental conditions[10], although the relevance of this process to the natural cycle of the virus is unclear.

In endemic areas, Japanese encephalitis affects children mostly; however, cases are observed in adults when JEV is introduced into areas previously considered nonendemic[11,12] or when non-immune adults are introduced into Japanese-encephalitis-endemic areas[13,14]. Japanese encephalitis is mostly a rural disease[12], although urban and peri-urban transmission is also reported[15,16]. Historically, two patterns of JEV transmission were recognized: seasonal epidemic transmission that peaks in late summer in temperate regions (23–43° N) and year-round low-level endemic transmission in tropical regions (1–13° N)[17]. In reality, outbreaks do occur in tropical regions and between 13° and 23° N[18], but cases occur at all times of year and are often underrecognized[19]. The incidence of Japanese encephalitis varies from 0.003 per 100,000 people in areas with established high-quality vaccination programmes, such as Japan and Korea, to 3.7 per 100,000 people in areas such as Cambodia, Indonesia and Malaysia[1], but these numbers are probably underestimates.

Unlike in pigs, no onward transmission occurs from humans because viraemia is insufficient to be infectious to the mosquito vector, making humans a dead-end host for JEV. The vast majority of JEV infection in humans is asymptomatic or causes an illness so mild as to not present to health facilities. An estimated 0.1–1% of JEV infections culminate in encephalitis[20,21], although, given the estimated annual number of cases (~69,000 (REF. 1)) and the size of the population at risk (2–3 billion people), the actual risk could be lower. After exposure, neutralizing antibody develops[22] and disease is not seen in adult populations in endemic areas, implying that immunity is lifelong. Episodic re-exposure of individuals to JEV probably also aids maintenance of lifelong immunity. Consequently, childhood residence in areas in which Japanese encephalitis is endemic results in near universal exposure and almost all adults in these regions are JEV seropositive[23]. Immunity is determined by subclinical exposure, not by age, although cases can also be observed in old age as immunity wanes[24].

Clinical features

JEV infection begins as an undifferentiated febrile illness. In some cases, febrile illness might be the only manifestation of infection[25], with no subsequent encephalitis, although it is unknown what proportion of JEV infection in humans causes self-limiting illness without involvement of the CNS. Nonspecific features such as coryza, diarrhoea or rigours can occur 3–4 days before the onset of acute encephalitis syndrome, which presents as clouding of consciousness, headache, vomiting and often seizures. Focal neurological signs are variable and can reflect the anatomical sites of damage. Parkinsonian features, including mask-like blank staring faces and tremors, reflect involvement of the basal ganglia[26]; other movement disorders include lip-smacking, bruxism, choreoathetosis and hemiballismus. Poliomyelitis-like flaccid paralysis indicates damage of anterior horn cells in the spinal cord[27], and cranial nerve signs include facial palsies, ptosis and abnormalities of eye movements. Obvious generalized tonic–clonic seizures can occur, and subtle motor seizures can also be observed in some instances, especially in advanced disease in children[28]. Features outside the brain have also been described, including pulmonary oedema (which can be neurogenic as a result of brainstem involvement)[13], hepatomegaly, splenomegaly, modestly raised liver enzymes and thrombocytopenia[29]. When Japanese encephalitis occurs in adults, similar signs are seen[30].

As well as the poliomyelitis-like flaccid paralysis due to anterior horn cell damage, JEV has occasionally been associated with Guillain–Barré syndrome (GBS), which provides an interesting parallel with Zika-virus-related

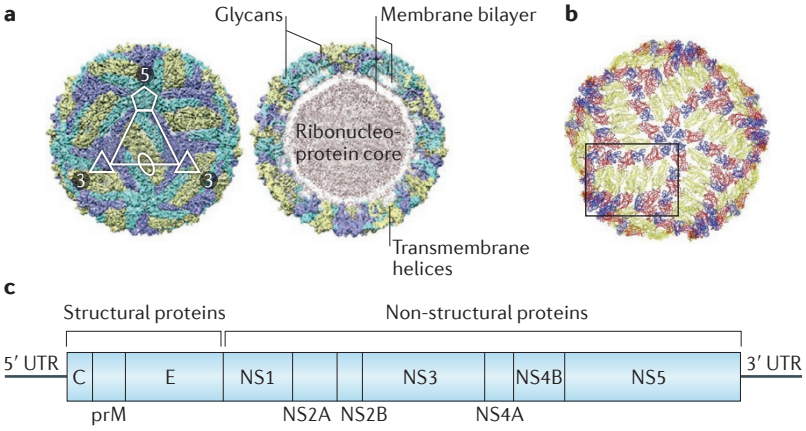


Figure 1 | **JEV structure. a** | The structure of the Japanese encephalitis virus (JEV) virion solved by cryo-electron microscopy (from Wang et al.[176], with permission). The JEV virion is ~50 nm in diameter and contains a central nucleocapsid core of viral RNA and core (capsid) protein. In the mature virion, this core is surrounded by a lipid bilayer envelope in which the viral membrane and envelope proteins are embedded[4]. **b** | The structure of JEV solved by X-ray crystallography of the envelope protein. As with other flaviviruses, the outer surface of the mature JEV virion is smooth and consists of 180 copies of envelope and membrane protein, with the envelope proteins arranged as 90 head-to-tail homodimers in an icosahedron, symmetry T = 3 (REFS 176,177). **c** | JEV genome organization. The JEV genome is 11 kb of single-stranded positive-sense RNA, comprising a single 10.7 kb open reading frame (ORF) flanked by 5′ and 3′ untranslated regions (UTRs), which function in replication. At the 5′ end of the ORF are the core (C; encoding the capsid), pre-membrane (prM) and envelope (E) proteins, which together make up the viral particle and are referred to as structural proteins. The remaining nonstructural (NS) proteins are expressed during replication. Part **a** is reproduced from REF. 176, Macmillan Publishers Limited. Part **b** is adapted from Luca, V. C., AbiMansour, J., Nelson, C. A. & Fremont, D. H. Crystal structure of the Japanese encephalitis virus envelope protein. *J. Virol.* **86**, 2337–2346 (2012), with permission from the American Society for Microbiology https://doi.org/10.1128/JVI.06072-11 (REF. 177).

disease. Patients with JEV-associated GBS have shown electrophysiological evidence of demyelination and, occasionally, axonal damage, and inflammatory infiltrates of lymphocytes and monocytes have also been observed in a fatal case[31]. A good response to steroids and intravenous immunoglobulin was described in one individual[32], but the use of plasmapheresis has not been reported. In poliomyelitis-like acute flaccid paralysis, which is seen for JEV[27] and West Nile virus[33], anterior horn cell involvement can be seen histologically at post-mortem[34]. GBS has also been reported in other flavivirus infections, such as dengue[35] and West Nile virus[36]. In the single case of GBS attributed to West Nile virus, neuropathy was predominantly demyelinating, but an axonal component was also present; therefore, anterior horn cell infection is difficult to completely rule out in this instance[36]. Immune-mediated transverse myelitis[37], acute disseminated encephalomyelitis[38] and *N*-methyl-D-aspartate (NMDA) receptor encephalitis[39] have also been reported following JEV infection. Although JEV can impair neurogenesis in experimental systems[40], the few reports that exist of human infection in pregnancy describe either abortion (with virus isolated from fetal CNS in one individual) or normal delivery[41,42]. Infection of pregnant sows also causes abortion and can be a major threat to pig farming[43]. JEV has not been associated with microcephaly, as has Zika virus. This difference might partly be explained by the epidemiology: most JEV infections are in children, so women in endemic areas are immune before they reach child-bearing age.

JEV is one of many causes of an acute encephalitis syndrome in which patients present with impaired consciousness; as such, diagnosis of Japanese encephalitis requires laboratory confirmation. Abnormalities of routine peripheral blood tests of patients with Japanese encephalitis are nonspecific; neutrophilia is common, and hyponatraemia can be present. Analysis of cerebrospinal fluid (CSF) typically shows a lymphocytic pleocytosis, but CSF can be acellular in some cases[28,44,45]. Diagnosis of Japanese encephalitis is confirmed by demonstration of JEV-specific immunoglobulin M (IgM) in CSF by enzyme-linked immunosorbent assay (ELISA), which is present in nearly all patients by day 7 of illness[46]. Repeat lumbar puncture can be necessary if early testing is negative and the diagnosis needs to be confirmed; in practice, during outbreaks, this confirmation is rarely needed if many patients have already tested positive. If CSF is unavailable, serum testing should be interpreted with caution, because it could just indicate coincidental systemic infection in a patient who has another cause of acute neurological disease. In areas coendemic for other flaviviruses, such as dengue virus, the test might yield false positives, and more specific neutralization assays for both JEV and cocirculating viruses could be necessary. Viral nucleic acids and infectious virus can also be found in CSF but much less frequently than JEV-specific IgM[47,48]. Unlike many other members of the *Flavivirus* genus, JEV is very infrequently cultured or detected by reverse transcription PCR (RT-PCR) from blood during acute illness, although it has been cultured from clot-derived white blood cells after coculture with healthy donor peripheral blood mononuclear cells (PBMCs)[49]. JEV also cannot be detected in urine by RT-PCR[50]. Many imaging findings are described in patients with Japanese encephalitis[51], but diagnostic utility has been assessed only for hypodense lesions in the thalami on CT (FIG. 2). Although this finding was 100% specific, the sensitivity was only 23% in a Japanese-encephalitis-endemic area, and thalamic hypodensities are also found in most other flavivirus encephalitides — as such, these features must be interpreted in the geographical context[52].

The reported outcomes of Japanese encephalitis vary widely, with mortality ranging from around 5–50%[14,19,24,28,29,42,44,53–85]; the neurological sequelae observed are dependent in part on the duration of follow-up[66]. The average mortality in studies published over the past 30 years is 18% (95% CI 14–21%; FIG. 3a). Over the same period, the proportion of individuals who survive but do not experience a full neurological recovery is 44% (95% CI 35–53%; FIG. 3b), meaning that just over half of patients who develop clinical Japanese encephalitis do not recover completely. Longer follow-up studies indicate that many children who had apparently made a full recovery had not returned to the same educational level[66].

Latency. Although Japanese encephalitis is typically an acute infection, with an initial clinical course lasting 2–3 weeks[12], some evidence shows that the virus

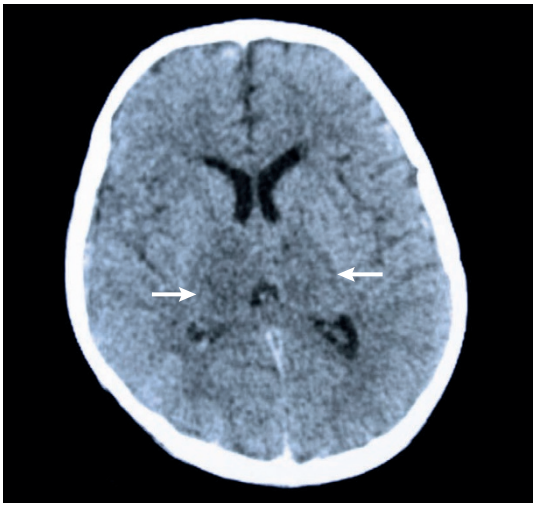


Figure 2 | **Features of Japanese encephalitis.** CT scan in an individual with Japanese encephalitis. Bilateral thalamic hypodensity in a 7 year-old child in Ho Chi Minh City, Vietnam, is illustrated (white arrows). In this setting, this finding has a sensitivity of 22% and specificity of 100% for a diagnosis of Japanese encephalitis[52]. However, this feature is also seen in other flaviviral encephalitides; therefore, interpretation needs to take into account the local epidemiology. Photo T. Solomon; figure adapted with permission from REF. 52, Springer.

can persist in the nervous system[86] and PBMCs[87] for 4–8 months after illness onset. This phenomenon seems to be rare in individuals with Japanese encephalitis, with few published reports in humans. There is some evidence of latent JEV infection in mice[88], and West Nile virus RNA has been identified in the urine of individuals infected with this pathogen many years after initial illness[89]; therefore, the phenomenon might not be confined to JEV. JEV-infected microglial and neuronal mouse cell lines can produce infectious virus for many weeks[90,91], and human monocytes have also been shown to produce JEV for weeks[92], providing a possible cellular basis for persistent infection. However, the consequences of this finding in living patients remains unknown, and persistent infection does not seem to be a common issue in clinical practice.

Genotype and geographic spread

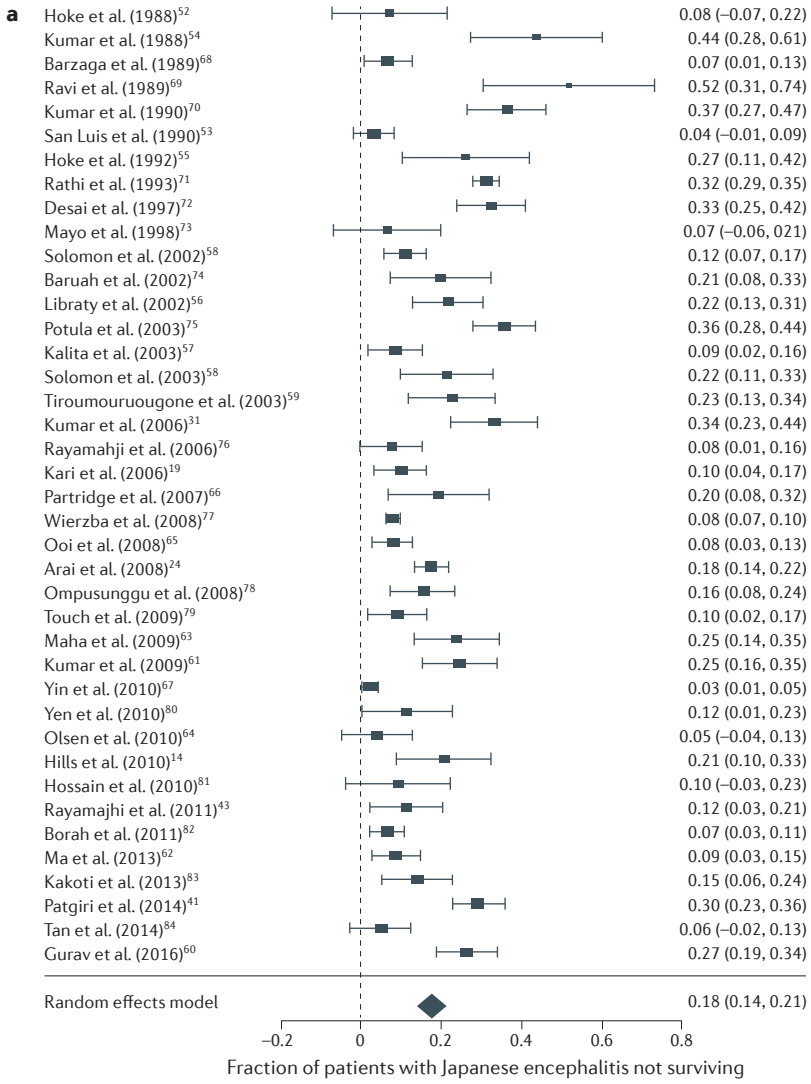
JEV can be divided into five genotypes, all of which can be traced back to a common ancestor that probably emerged in the region of modern-day Indonesia and Malaysia in Southeast Asia[93,94] (FIG. 4a). Early isolates of JEV from the 1930s to the 1950s were all genotype III. However, genotype I JEV is gradually replacing genotype III[95] (FIG. 4b), although most human cases are still genotype III[96] (FIG. 4c). JEV genotype I is better adapted than genotype III to the mosquito vector, with a shorter extrinsic incubation time[97]; however, this adaptation comes at the cost of a narrower host range[96]. Climate might also have an effect on genotype distribution, as genotypes Ia, II and IV tend to be found in tropical Asia and genotypes Ib and III in temperate Asia[94], although genotype III has also been isolated in many tropical areas.

JEV continues to spread and reach new populations. For example, a genotype II strain of JEV reached the Australian Torres Strait Islands in 1995 (REFS 11,98). Rare Japanese encephalitis cases continued to occur in this area over the next few years, including one on the Australian mainland[99], where JEV was also isolated from mosquitoes[100]. Since this time, Japanese encephalitis cases in this region have abated, and although there have been subsequent intermittent isolations of JEV, no human cases have been reported in Australia or the outer islands since 1998 (REF. 101). Why JEV has not become more firmly established in Northern Australia and the outer islands is not clear, as the ecological conditions are similar to many regions where the virus is endemic. The presence of mosquitoes that have a tendency to feed on vertebrates that do not become highly viraemic following JEV infection is one potential explanation[101].

Many regions of the world also have conditions that seem to be appropriate for JEV yet no circulation of the virus. The related flaviviruses St Louis encephalitis virus and West Nile virus both circulate in the USA, which has given rise to concern around the potential for introduction of JEV in this region[102]. Parts of southern Europe also have suitable conditions for JEV, and nucleic acid sequences that seem to correspond to parts of the JEV genome have been detected in birds[103] and mosquitoes[104] in Italy. However, the sequences identified were very short (only 167 and 215 base pairs), full-length viral genome or infectious virus has not been isolated and no evidence of human infection has been reported[105]. West Nile and Usutu viruses circulate in the same region; therefore, ongoing flavivirus surveillance could be expected to detect JEV if it does emerge. JEV has always been thought to be exclusively transmitted by mosquitoes, which affects its potential geographic range. However, the experimental demonstration of vector-free transmission via respiratory secretions in pigs[10] indicates that a JEV reservoir could be maintained in temperate locations with short mosquito seasons. How JEV is maintained in more northern, temperate endemic areas is not known; JEV might persist over winter in mosquitoes, or in lizards, frogs or snakes, or could be reintroduced in migrating birds (although studies of genotype distribution argue against reintroduction)[101]. The ecology and geographic expansion of JEV is reviewed in more detail elseswhere[101].

Japanese encephalitis vaccination

Several different vaccines have been used to control Japanese encephalitis since the 1950s (TABLE 1; reviewed in REF. 106). Early isolates of JEV, which were the foundation of vaccine development, were all genotype III. Randomized controlled trials have shown the efficacy of vaccination against Japanese encephalitis and showed that neutralizing antibody correlated with protection[53]. Early Japanese encephalitis vaccines were mostly derived from mouse brain, but this manufacturing method has now been replaced by cell culture methods owing to safety concerns and relatively poor immunogenicity of the former method in humans[106]. The Japanese encephalitis vaccines that are most commonly used today are based on



the attenuated strain SA14-14-2 that was derived from the JEV SA14 strain (which itself has fairly low pathogenicity[107]) grown from a pool of *Culex pipiens* larvae from Xi'an in 1954. The live attenuated Japanese encephalitis vaccine SA14-14-2 generates neutralizing antibody against wild genotype III strains (Beijing1 and SA14) in humans[108–111] but has not been tested against genotype I strains. Inactivated, adjuvanted SA14-14-2 (marketed in Europe and North America as IXIARO and in Australasia as JESPECT) induces neutralizing antibody to JEV genotypes I–IV and gives similar seroconversion rates for genotype I and III viruses[112,113], although titres against genotype I tend to be lower than those for genotype III. In the past few years, a new vaccine has been developed on the basis of an Indian genotype III strain, Kolar821564XY (TABLE 1). This Japanese encephalitis vaccine, JENVAC, also induces neutralizing antibody against genotypes I–IV[114]. Whether protection induced by a genotype III vaccine will be as durable for genotype I remains to be seen, as genotype I disease has been reported to occur after incomplete genotype III vaccination[115]. Moreover, JEV genotype V is also emerging[116], although cases in humans are rare. Current vaccines might not be as protective against genotype V as other genotypes, although this notion is based on a small sample thus far[117].

Vaccine use has resulted in a decrease in Japanese encephalitis incidence in many Asian countries[118,119], but estimates suggest that 80% of cases still occur in areas with Japanese encephalitis vaccination programmes[1]. Why so many cases occur despite vaccination programmes is not clear; possible explanations are a lack of vaccine coverage, problems in the cold chain, a lack of vaccine effectiveness or waning immunity in areas where JEV circulation is intermittent. In 2006, India — which, along with China, accounts for much of Japanese encephalitis disease burden — introduced the live Japanese encephalitis vaccine SA14-14-2, and a small case–control study in Lucknow (Uttar Pradesh, North India) showed a 6-month vaccine efficacy of 94.5%[120]. However, post-marketing studies suggest a lower efficacy[121], and although the number of cases reported to the National Vector Borne Disease Control Program (NVBDCP) of India decreased after vaccine introduction, they have since begun to rise again. Several sources have documented especially low seroconversion rates to Japanese encephalitis vaccine SA14-14-2 in India, from 58% to 74%[114,121,122]. However, clear examples can be found of circumstances in which serum concentrations of neutralizing antibody to contemporary strains have waned to undetectable levels in most of the general population, yet a sustained reduction has been observed in the incidence of Japanese encephalitis[123]. This finding underscores the fact that measurement of neutralizing antibody is a surrogate for protection, which also

Figure 3 | **Clinical outcome of Japanese encephalitis.** Forest plots showing mortality (part **a**) and proportion of patients with neurological sequelae (part **b**) from studies of individuals with Japanese encephalitis published in the past 30 years. Data points represent point estimates of percentage mortality; size of point represents study sample size. Bars represent 95% CIs.

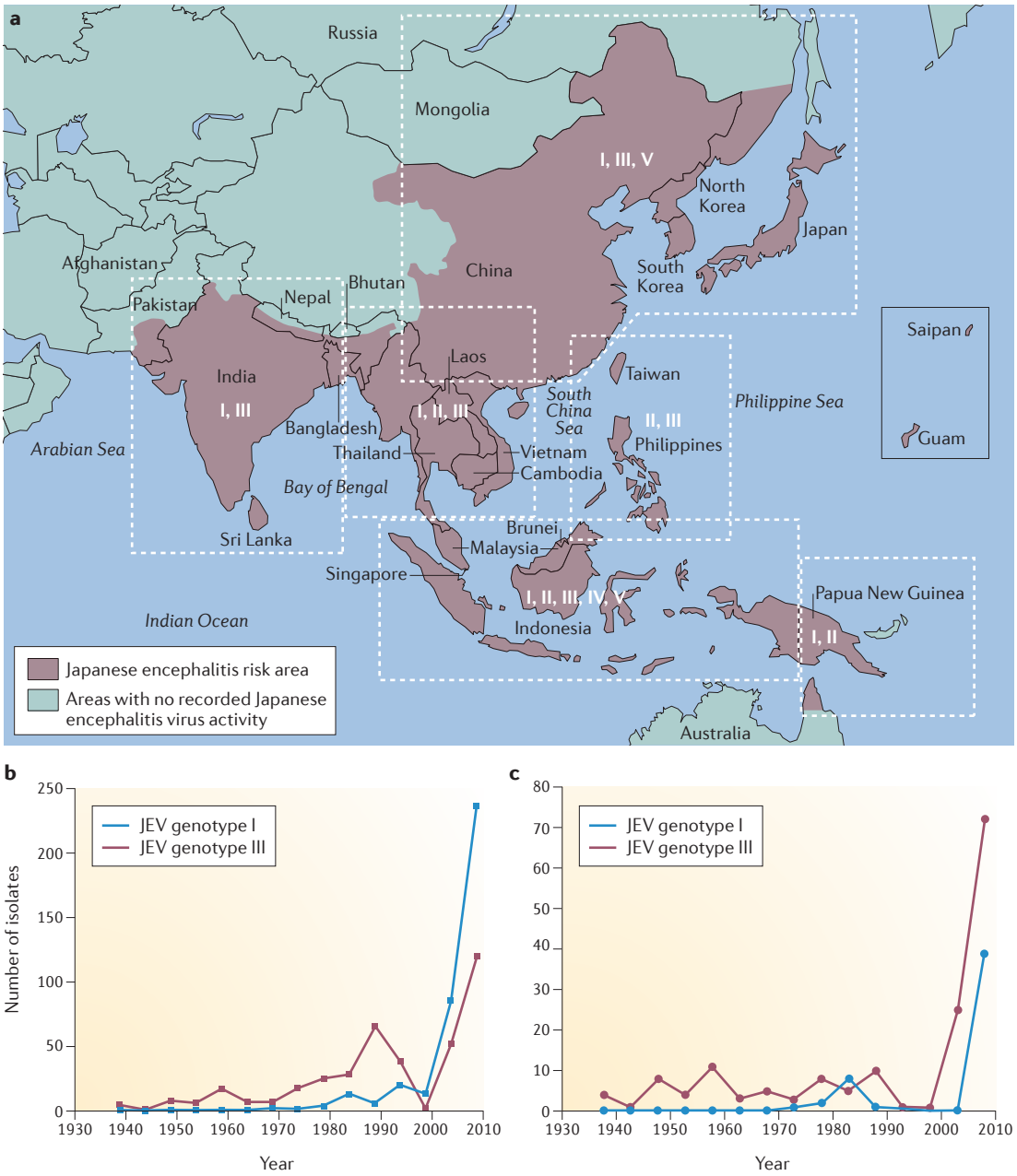


Figure 4 | **Origin and genotype spread of JEV in Southeast Asia. a** | Japanese encephalitis virus (JEV) probably originated in the region of Indonesia and Malaysia before spreading across Asia. **b** | Relative proportion of JEV genotypes I and III isolated from any source (humans, other mammals, birds and mosquitoes) according to decade of collection. **c** | Number of JEV isolates from humans over time. Blue line represents JEV genotype I; red line represents JEV genotype III. Part **a** is adapted from REF. 178, Centers for Disease Control and Prevention, USA, and from Solomon, T. et al. Origin and evolution of Japanese encephalitis virus in southeast Asia. *J. Virol.* **77**, 3091–3098 (2003), with permission from the American Society for Microbiology https://doi.org/10.1128/JVI.77.5.3091-3098.2003 (REF. 93). Parts **b** and **c** are adapted from Han, N. et al. Comparison of genotypes I and III in Japanese encephalitis virus reveals distinct differences in their genetic and host diversity. *J. Virol.* **88**, 11469–11479 (2014), with permission from the American Society for Microbiology https://doi.org/10.1128/JVI.02050-14 (REF. 96).

involves cellular immunity. Consistent with these findings is the observation that priming of memory B cells in mice is protective in the absence of serum neutralizing antibody[124]. What accounts for the low seroconversion rate of Japanese encephalitis vaccine SA14-14-2 in India and whether this finding has clinical importance remain unknown. Interference by dengue virus in India has been hypothesized to interfere with SA14-14-2 immunogenicity[122].

Whatever the reasons for the continued existence of Japanese encephalitis cases in endemic areas and the possible contributions of failures of the vaccine

programmes or vaccines themselves, Japanese encephalitis remains a major problem in Asia. Although efforts to prevent Japanese encephalitis undoubtedly need to be strengthened, the continued occurrence of clinical disease despite vaccination campaigns highlights the need to develop new treatments and to improve the care of the patients who contract the disease.

Pathogenesis and therapeutic targets

Pathogenesis. Despite substantial advances in the past few years, much remains to be learned about the pathogenesis of Japanese encephalitis (FIG. 5a). Studies in mouse models have characterized the initial pathogenesis of JEV infection: after peripheral inoculation with JEV, a round of replication occurs in the local lymph nodes[125], and virus can be found peripherally in monocytes and some T cells[126]. The initial replication is then followed by viraemia and the spread of infection to the CNS (FIG. 5b). JEV replicates well in monocytes[92,127] and dendritic cell lineages[127–130] from humans and mice, although replication in human neuronal cell lines is much more efficient[92]. JEV probably infects cells of the macrophage and dendritic cell lineage in the skin — in an analogous fashion to dengue virus[131] — with the infected cells then carried to local lymph nodes, from where viraemia and then CNS infection can occur, if replication is sufficient[125]. The incubation period of JEV in mice is around 5 days after subcutaneous injection, whereas the incubation period after intranasal inoculation of JEV in macaques is 7–10 days[132]. In humans, the incubation period is often described as 5–15 days; however, published data describing the incubation period in humans are scant and they suggest a median of around 8.4 days, similar to the macaque model[133].

Spread of JEV to the CNS can be prevented by neutralizing antibody, which alone is sufficient to prevent encephalitis[134] (FIG. 5a). The importance of antibody in protection from JEV infection is highlighted by the observation that mice lacking B cells are extremely sensitive to JEV infection[135], and priming[136] or adoptive transfer[124] of JEV immune memory B cells is protective even in the absence of pre-challenge neutralizing antibody.

Entry into the CNS. The mechanism by which JEV enters into the brain remains elusive. The observations of peripheral replication and viraemia in animal models, along with the distribution of lesions in the brain[125,137], strongly suggest that JEV enters the CNS from the blood. JEV antigen is detected in the vascular endothelium[137–139], but whether this observation reflects actual replication is unknown: a 'Trojan horse' mechanism of CNS entry via an infected leukocyte cannot be discounted completely[49,87,126].

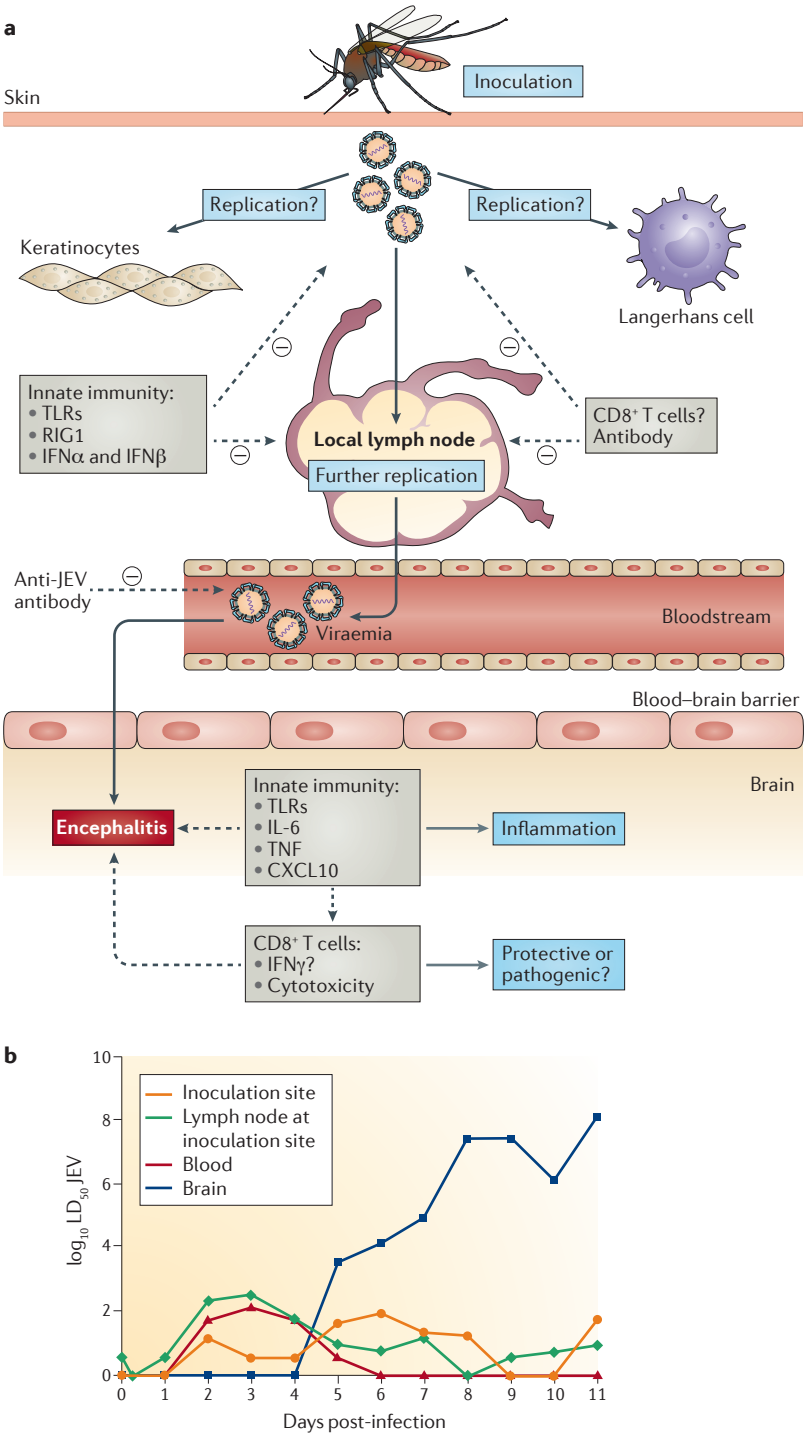
The effect of JEV infection on the blood–brain barrier (BBB) has been studied mostly in mouse models of Japanese encephalitis. JEV is not a natural pathogen of mice, which are generally resistant to the disease unless JEV is inoculated when the mice are very young or via the intracerebral route. Although there is substantial variation in the characteristics of mouse models

Table 1 | **Vaccines against Japanese encephalitis**

Description	Type	Virus strain	Common name	Country of origin, manufacturer and/or developer
Early vaccines, no longer in use				
Mouse brain	Inactivated	Nakayama	BIKEN	Japan, BIKEN
Mouse brain	Inactivated	Nakayama	Green Cross	Korea, Green Cross
Mouse brain	Inactivated	Beijing-1	NA	Japan
Primary hamster kidney	Inactivated	P3	NA	China
Currently available vaccines				
Vero cell	Inactivated	P3	NA	China
Primary hamster kidney	Live attenuated	SA14-14-2	NA	China, Chengdu Biological Products
Vero cell	Inactivated	Beijing-1	JEBIKV	Japan, BIKEN
Vero cell	Inactivated	Beijing-1	ENCEVAC	Japan, Kaketsuken
Vero cell	Inactivated	SA14-14-2	1C51, IXIARO	Intercell, Valneva
Vero cell	Inactivated	Kolar-821564XY	JENVAC	India, Bharat Biotech
Yellow fever 17D recombinant vectored	Live attenuated	SA14-14-2 (envelope)	Imojev, Chimerivax JE	Acambis, Sanofi Pasteur

The early, mouse-brain-derived vaccines against Japanese encephalitis are no longer in use owing to concerns regarding adverse events, including acute disseminated encephalomyelitis (ADEM), although the incidence of ADEM after vaccination was never accurately estimated. The virus strains used in vaccine development are all genotype III. Of the currently used inactivated vaccines for Japanese encephalitis, most are made for domestic markets and not sold internationally. The exception is IXIARO (known as JESPECT in Australia and New Zealand), which is licensed in Europe, North America and many other countries. IXIARO is the principal vaccine used for prevention of Japanese encephalitis in travellers. In areas endemic for Japanese encephalitis, the most commonly used vaccine now is the Chinese live attenuated vaccine, SA14-14-2, which has been studied extensively in China and other countries and which was WHO prequalified in 2013. IXIARO is made from inactivated Japanese encephalitis virus (JEV) SA14-14-2, which was chosen for ease of production as it does not require containment level 3 facilities. Imojev, the first recombinant vaccine licensed, is based on the yellow fever 17D vaccine with the premembrane and envelope genes replaced by those of JEV SA14-14-2. The same technology was used to develop the first licensed dengue vaccine, Dengvaxia. NA, not applicable.

between laboratories in this regard, BBB breakdown can be readily demonstrated in models where JEV is pathogenic and produces encephalitis in the infected animals[140]. The mechanism underlying this breakdown is uncertain, with some evidence that it might be induced by soluble mediators[141]. However, BBB breakdown seems to be a consequence rather than the cause of JEV infection of the brain[142] and, although it contributes to pathology, is not absolutely required for virus entry into the CNS.



CNS pathology and inflammation. Although JEV can replicate in many cell types in vitro, inside the CNS its principal target cells are neurons[137–139,143]. In the macaque model of Japanese encephalitis, JEV antigen is also found in microglia and occasionally in blood-derived macrophages[143]. Marked inflammation is present in the brain of patients with Japanese encephalitis — a phenomenon that might be amenable to treatment. Inflammatory infiltrates in the brains of humans infected with JEV have not been very well characterized but are dominated by T cells and, to a lesser extent, by monocytes and macrophages[137].

Reports of the composition of T cells in inflammatory infiltrates in the brains of people with Japanese encephalitis have been inconsistent. $CD4^+$ T cells were the most predominant T cells observed at post-mortem in seven children who died from Japanese encephalitis[138], but $CD8^+$ T cells were most prevalent in two adults who died from the disease[144]. $CD4^+$ T cells were also found to be enriched in the CSF of 15 children infected with JEV[145], although the small number of cases makes it difficult to draw a firm conclusion that the T cell infiltrates in adults and children are different. Histologically, fatal cases of Japanese encephalitis in humans are characterized by perivascular cuffing and vascular leakage, sometimes with small haemorrhages, and small areas of well-demarcated ('punched-out') focal necrosis[137,138,146]. Viral antigen is found in inflamed areas of brain but is also seen in noninflamed areas of brain[132,146]. Given the facts that JEV spreads to the brain via the blood and viraemia is transient and hard to demonstrate in humans, these noninflamed brain regions are likely to represent areas that are affected by JEV later in the disease process by

Figure 5 | **Overview of the pathogenesis of Japanese encephalitis. a** | After inoculation into the skin, virus is thought to replicate in Langerhans dendritic cells (as observed for dengue virus[131]) and/or in keratinocytes (as observed for West Nile virus). Japanese encephalitis virus (JEV) is then carried to the local lymph node, where further replication takes place[125]. Viraemia occurs, and the virus crosses the blood–brain barrier and enters the CNS. Major outstanding questions (indicated by question marks) include which are the true target cells for JEV in the skin, is there any role for the $CD8^+$ T cell or antibody response in the early stages before viraemia, is there any mechanism other than viraemia to gain entry to the CNS, how does JEV cross the blood–brain barrier and which immune components are protective or pathogenic once inside the brain? Solid lines indicate viral spread, dotted lines indicate potential effects of the immune system. Circled minus symbols indicate potential immune effects that restrict viral replication and/or spread. The protective effect of the immune response inside the brain is less clear; the response could be just as pathogenic as it is protective. **b** | Viral load of JEV after peripheral subcutaneous inoculation with $10^{1.5}$ median lethal dose (LD_{50}) of JEV Beijing strain into mice. The data show that viral replication is controlled at the site of inoculation and in the blood, but once virus enters the CNS, unrestricted multiplication occurs[125]. CXCL10, CXC-chemokine receptor 10; RIG1, retinoic acid-inducible gene 1 protein (also known as DDX58); TLR, Toll-like receptor. Part **a** is adapted with permission from REF. 179, Oxford University Press.

contiguous or axonal spread of the virus[139], which suggests that a therapy that blocks viral spread will limit damage to the CNS. Alternatively, this pattern might represent viral protein spread by axonal transport, with no active viral replication in the noninflamed areas. Ultimately, neurons probably die through a combination of direct viral-mediated damage, immune-mediated damage (see later in the article) and apoptosis, which together give rise to the clinical symptoms of the condition. Features of raised intracranial pressure are reported in both clinical and pathological studies of Japanese encephalitis[28,138,139], indicating that measures to reduce this pressure might also be beneficial.

Several lines of evidence support a pathological role for CNS inflammation in patients with Japanese encephalitis. In animal models of Japanese encephalitis, the BBB breaks down, which results in a large influx of monocytes and lymphocytes into the brain[147–149]. Consistent with pathological findings in fatal cases of Japanese encephalitis in humans and in animal models of the disease, high levels of inflammatory mediators can be detected in the CSF of patients infected with JEV, and increasing concentrations of tumour necrosis factor (TNF) and IL-6 correlate with poor outcome in these individuals[150,151]. Blockade of TNF with etanercept reduces inflammation and improves survival in JEV-infected mice[152], and manipulation of pro-inflammatory responses by other means — such as genetic ablation of Toll-like receptor 4 (TLR4) — protects mice against the lethal effects of Japanese encephalitis[147]. Generally, mice that survive acute JEV infection in these models show strong type I interferon responses, which result in a greater restriction of viral replication, less uncontrolled inflammation in the CNS and enhanced resistance to JEV. However, treatment with IFNα did not affect outcome from Japanese encephalitis when it was tested in a clinical trial[59].

Adaptive immunity and immunopathogenesis. Although the role of antibodies in protection against Japanese encephalitis is well described, the role of T cells, both in protection and pathology, is less clear. Evidence for a protective role by T cells in mouse models of Japanese encephalitis is contradictory, with some investigators finding no role for T cells[136], some finding a partially protective role[135,153] and still others finding complete protection[154,155]. In one mouse model of the disease, depletion of $CD8^+$ T cells resulted in a 100-fold increase in virus in the CNS, whereas mice that did not undergo T cell depletion had high levels of $IFN\gamma^+CD8^+$ T cells in the brain[135]. These pivotal experiments could explain some of the observed differences between mouse models, but — more importantly — they suggest that either antiviral or anti-inflammatory therapies alone are not maximally effective and that studies of combination therapies aimed at suppressing both virus replication and pathologic host responses together should be investigated. Some evidence also supports this paradoxical role of host immunity in humans: memory T cell responses in individuals who were asymptomatic after JEV exposure were found to be predominantly $CD8^+$ and were highly cross-reactive with dengue virus, whereas in individuals who had recovered from Japanese encephalitis T cell responses were mostly $CD4^+$ and not cross-reactive[156]. Among individuals who had recovered from Japanese encephalitis, the quality of the T cell response correlated with the clinical outcome, and responses dominated by $CD4^+$ T cells that produce TNF alone, and not IFNγ or IL-2, were associated with incomplete recovery (FIG. 6). This observation and the observation that most of the T cells found in the brain and CSF in patients with Japanese encephalitis are $CD4^+$ (REFS 138,145) support the notion that $CD4^+$ T cells are pathogenic in this disease. Taken together, these results suggest that immunity is effective if JEV can be contained in the periphery, but once inside the CNS, prevention of virus-induced damage comes at the cost of substantial immune-mediated neuronal injury.

Management

Given the potential severity of Japanese encephalitis, and its ability to emerge in previously nonendemic areas, the lack of work done to test treatments for this disease is surprising. To date, no specific treatment for Japanese encephalitis has been shown to work. However, supportive management is beneficial in patients with Japanese encephalitis, and several complications of the disease that increase risk of death are treatable. For example, the presence of seizures, which are associated with raised intracranial pressure[28], indicates a poor prognosis, and prognosis is worse still in the presence of intractable seizures or status epilepticus[28,66]. Seizure activity, which can be subtle, should be actively sought out and aggressively treated, and some observational evidence supports the use of routine sedation to improve outcome[60]. Although a clinical trial of high-dose (0.6 mg/kg) dexamethasone given to all patients with suspected Japanese encephalitis did not show a benefit[56], agents such as corticosteroids or mannitol are nevertheless commonly administered to patients with Japanese encephalitis who have intractable seizures or other signs of raised intracranial pressure. Although the trial of dexamethasone in patients with Japanese encephalitis showed this treatment to be safe, several prominent examples of commonly used treatments for other disorders have been shown to be harmful when studied systematically, such as bolus fluids in children with shock in a malaria-endemic area[157]. This finding further underlines the need for more definitive evidence to validate the use of common supportive therapies in Japanese encephalitis.

Other complications, particularly those associated with immobility such as pressure sores and contractures, should be prevented by good nursing care. However, this care often falls to the family in some areas endemic for Japanese encephalitis. Attention should be paid to maintain fluid balance and avoid both underhydration and overhydration in patients with the disease[44,60]. In unconscious patients, clinicians should be vigilant for aspiration pneumonia, which should be treated upon early clinical suspicion. In practice, many patients who present with fever and altered consciousness are treated with broad-spectrum antibiotics, as uncertainty often exists regarding the presence of bacterial infection such as meningitis or brain abscess. However, routine antibiotics are

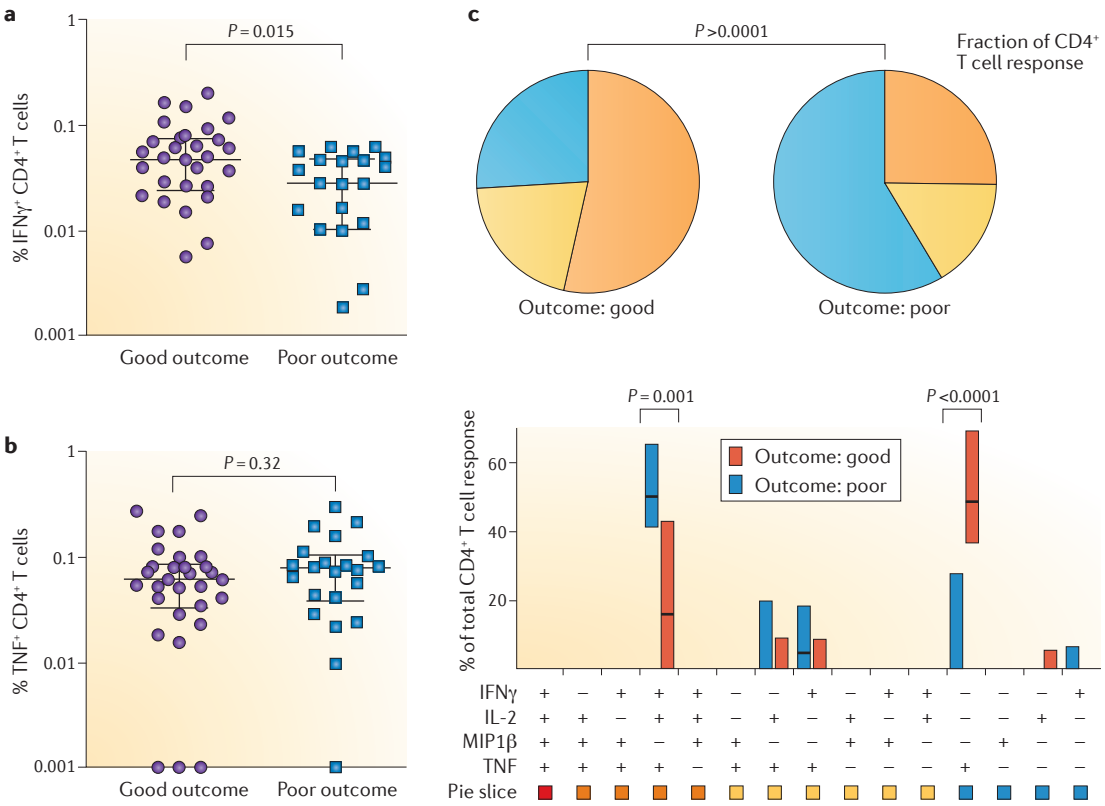


Figure 6 | **CD4⁺ T cell responses in patients recovered from Japanese encephalitis. a** | Overall, IFNγ responses are modestly smaller in patients with incomplete recovery from Japanese encephalitis and residual neurological disability (poor outcome) than in those who recover completely (good outcome)[156]. **b** | Tumour necrosis factor (TNF) responses are not significantly different between individuals who have a poor outcome and those who have a good outcome. **c** | The balance of cytokines produced by CD4⁺ T cells is different between patients who have a poor or a good outcome, with responses in the poor outcome group dominated by TNF single-producing cells, whereas responses in the good outcome group are biased towards CD4⁺ T cells that make IFNγ, IL-2 and TNF (so-called polyfunctional T cells). Coloured boxes and pie slice fractions represent the number of the measured cytokines (IFNγ, IL-2, TNF or MIP1β) made by a cell: red indicates all four cytokines, orange indicates three cytokines, yellow indicates two cytokines and blue indicates one cytokine. MIP1β, macrophage inflammatory protein 1 (also known as CCL4). Figure adapted from Turtle, L. et al. Human T cell responses to Japanese encephalitis virus in health and disease. *J. Exp. Med.* **213**, 1331–1352 (2016), with permission from Rockefeller University Press https://doi.org/10.1084/jem.20151517 (REF 156).

not necessary in Japanese encephalitis; therefore, if the diagnosis of Japanese encephalitis is secure or strongly suspected, and CSF analysis or imaging does not suggest bacterial infection, then antibiotics need not be used.

Clinical trials in Japanese encephalitis. To date, few randomized clinical trials have tested treatments for Japanese encephalitis (TABLE 2). Replication of JEV is inhibited in vitro by type I interferon and ribavirin, but neither agent, when tested alone, altered the outcome in patients with Japanese encephalitis[59,62]. High-dose dexamethasone did not affect the outcome from Japanese encephalitis (see previous discussion)[56], but this trial was small and underpowered. A preliminary study of intravenous immunoglobulin given for virus-neutralizing and anti-inflammatory effects showed that this approach was safe and feasible, but the study was also not powered to detect an improvement in outcome[45]. However, the study showed enhancement of the antibody response to JEV from intravenous immunoglobulin treatment in some individuals, a phenomenon that is well described in animal models of Japanese encephalitis[158]. Lastly, two studies of minocycline — one in acute encephalitis syndrome of any cause (hypothesized to improve BBB integrity) and one in Japanese encephalitis — recruited only 29 and 44 patients with Japanese encephalitis, respectively, and therefore, again, were underpowered[159,160].

Existing compounds as therapies

None of the trials on Japanese encephalitis to date has demonstrated a benefit; however, many approaches remain untested. Numerous compounds are available that have anti-JEV activity (reviewed elsewhere[161]), including several that have already been used in humans for other indications[162] (TABLE 3). All of the compounds in TABLE 3 that have direct evidence of anti-JEV activity — except for arctigenin[163] and clinidipine[164] — have been used in clinical trials in children or are in routine use in children.

Most of the therapies for Japanese encephalitis that were tested previously in mouse models were administered at the time of or shortly after JEV infection[165,166]. The exceptions are an anti-JEV monoclonal antibody, which was partially protective when given on day 5 after infection, at which time JEV was detectable in the brain[134]; minocycline, which was effective after symptom onset[167]; and etanercept (a recombinant fusion protein of TNF receptor to IgG1), which was administered at days 3 and 5 after infection[152] (TABLE 3). Fenofibrate was effective only if administered several days before JEV infection[168] and so is unsuitable for clinical testing, although useful mechanistic information about the disease might be learned from this agent. Pentoxifylline has been studied as an adjunctive treatment for both malaria and dengue fever because of its anti-TNF activity[169,170], but it also inhibits JEV replication in vitro and has protective effects in mouse models of Japanese encephalitis[171]. The pathological role of TNF in Japanese encephalitis is further supported by the protective effect of direct TNF blockade with etanercept as well as observations that link high levels of TNF with poor outcome in humans with the disease[150,156]. Although etanercept improved survival of JEV-infected mice and reduced neuroinflammation, JEV replication was marginally increased[152].

As described previously in the article, some mouse studies demonstrate that animals with intact immune systems die with uncontrolled inflammation after JEV infection, whereas those with impaired immunity (for example, depletion of $CD8^+$ T cells) die with less inflammation but a much higher viral burden[135]. This finding implies that targeting viral replication and inflammation concurrently could have a synergistic effect, which might be achieved using the available agents in TABLE 3 or using combinations of agents that have already been tested, such as steroids, ribavirin and interferon; however, this strategy has not yet been attempted in humans.

Lastly, a theoretic basis supports a benefit for some other drugs in Japanese encephalitis; thus, these agents merit testing, at least in an animal model of the disease (TABLE 3). Knockout of *Tlr4* protects mice against the lethal effects of Japanese encephalitis; therefore, this receptor represents a potential therapeutic target[147]. TLR4 is antagonized by eritoran, which has been used in phase III trials for sepsis and has also been shown to protect mice in a model of influenza[172]. Recruitment of inflammatory monocytes into the brain can be prevented by blockade of the interaction between integrin $\alpha_4\beta_1$ and vascular cell adhesion protein 1, which represents the mechanism of action of the multiple sclerosis treatment natalizumab. Blockade of this pathway also partly protects mice from lethal West Nile infection[173]. Inhibition of c-Jun N-terminal kinase 1 (JNK1; also known as MAPK8) reduced neuroinflammation, viral load and mortality in a mouse model of Japanese encephalitis[174]; consequently, JNK1 is a potential drug target in humans, but different compounds would be required from those used in mice. High IL-6 levels in CSF correlate with

Table 2 | **Randomized clinical trials of treatments for Japanese encephalitis**

Study	Drug schedule tested	No. of patients randomly assigned to treatment	No. of patients with confirmed Japanese encephalitis	Primary end point	Outcome	Intervention of benefit?
Hoke et al. (1992)[56]	Dexamethasone 0.6 mg/kg followed by 0.2 mg/kg every 6 h for 5 days	65	55	Death at 25 days	Dexamethasone: 6 of 24 died; placebo: 8 of 30 died	No
Solomon et al. (2003)[59]	IFNα2a 106 U/m^2 body surface area for 7 days	112	87	Death or severe sequelae at discharge and 3 months	Interferon: 16 of 57 poor outcome[a]; placebo 13 of 50 poor outcome	No
Kumar et al. (2009)[62]	Ribavirin 10 mg/kg daily for 7 days	153	153	Early death (in hospital)	Ribavirin: 19 of 70 died; placebo: 21 of 83 died	No
Rayamajhi et al. (2015)[45]	IVIG (ImmunoRel) 400 mg/kg daily for 5 days	22	13	Feasibility — not powered for a clinical end point	IVIG: 5 of 9 recovered completely and 1 died; placebo: 2 of 9 recovered completely and 2 died	No
Kumar et al. (2016)[159]	Minocycline 5 mg/kg daily followed by 2.5 mg/kg daily (age <12 years) or 200 mg followed by 100 mg every 12 h for 7 days	281	29	Death at 3 months	Minocycline: 2 of 13 died; placebo 5 of 14 died (JEV-positive only)	No
Singh et al. (2016)[160]	Minocycline 5–6 mg/kg daily in two divided doses for 10 days	44	44	Not stated	Minocycline: 2 of 22 died; placebo 5 of 22 died	No
Total number of patients	–	677	381	–	–	–

All studies were double blind and placebo controlled. Five studies tested treatments for Japanese encephalitis, and one study[159] tested minocycline for acute encephalitis syndrome and included patients with Japanese encephalitis. Minocycline has also been studied in patients with Japanese encephalitis alone, although this study was underpowered[160]. None of these studies showed a detectable benefit on any clinical outcome measure. IVIG, intravenous immunoglobulin; JEV, Japanese encephalitis virus. [a]Poor outcome is death or severe neurological sequelae.

mortality in humans[151]; therefore, the anti-IL-6 antibody tocilizumab might also have therapeutic potential in patients with Japanese encephalitis.

Considerations for new treatment trials

Most treatment trials in patients with Japanese encephalitis have been designed to show an absolute reduction in mortality or improvement in outcome of around 20–25%, which translates into a relative reduction in mortality (or poor outcome) of greater than 50% in most cases — a large effect. The sample sizes of humans with Japanese encephalitis have been modest, ranging from 44 to 153 patients per study, with an overall total of only 381 patients with confirmed Japanese encephalitis ever to be recruited into randomized treatment trials (TABLE 3).

An alternative approach that could enable the detection of a smaller effect size than in existing trials would be to use a linear outcome measure, such as an outcome score, instead of a categorical good-versus-bad outcome. Although many scoring systems exist to measure neurological disability and outcome after various types of neurological injury, few have been developed specifically for encephalitis, and fewer still have been validated in the setting where Japanese encephalitis occurs. The Liverpool Outcome Score (LOS) is a simple numerical scoring system that has been developed and applied to patients with Japanese encephalitis as well as other forms of encephalitis across different age groups and cultures[63,64,156,175] (TABLE 4). This score classifies outcomes as complete recovery (V), recovery with sequelae but with the (age-appropriate) ability to live independently (IV), recovery with disability precluding independent living (III), fully dependent for daily activities (II) and death (I). The score is easy to use and is suitable for use in the field, an important consideration for Japanese encephalitis trials. An increase of 1 point on this scale represents a clinically meaningful improvement.

Four studies have been conducted using the LOS[63,64,156,175]; we estimate that 38% of patients in these studies have a poor outcome, defined as death or neurological sequelae sufficiently bad to preclude independent living (assuming an 18% mortality (FIG. 3) in those studies that recruited only follow-up cases). An average improvement of 1 point on the LOS scale would result in a reduction in the proportion of patients with poor outcome to 27%. For a trial to have 80% power to show this difference at a 5% significance level would require 569 patients to be recruited, assuming 10% loss to follow-up. Treating the LOS as a continuous variable could allow the sample size to be reduced to 356, but this number is probably an underestimate owing to the ordinal categorical nature of the LOS (that is, a patient cannot have an outcome score of 3.5). The real figure will lie somewhere in the range of 356–569, which would still require approximately the same number of patients, or more, with confirmed Japanese encephalitis in a single treatment trial than have ever been randomized to date.

The choice of follow-up time point also requires careful consideration. An end point reached during the inpatient stay is likely to have less loss to follow-up than a later end point as trial participants are relatively confined in a fixed location. Conversely, a longer follow-up of 3–6 months gives a more accurate picture of the real clinical outcome[66], but patient follow-up in this scenario presents a challenge, particularly in the rural settings where Japanese encephalitis occurs. Assessment of combinations of therapies, including supportive measures, perhaps using factorial design, will make trial design more complex still. Consequently, it is easy to see how

Table 3 | **Compounds potentially suitable for Japanese Encephalitis trials**

Compound	Evidence and comments	Refs
Compounds protective against Japanese encephalitis in animal models		
Anti-JEV monoclonal antibody (503)	82% of mice protected with 200 µg per mouse antibody given 5 days post-infection, when virus is detected in the brain	134
Rosmarinic acid	80% of mice protected by 25 mg/kg from day 1 post-infection	166
Minocycline	70% of mice protected at 90 mg/kg daily from 6 days post-infection; inhibited JEV replication in vivo	167
Arctigenin	100% of mice protected by 20 mg/kg daily from day 1 post-infection. Only used at phase I in pancreatic cancer; no data in children	163
Pentoxifylline	50% of mice protected at 100 mg/kg daily given immediately after JEV infection and 100% protection at >200 mg/kg daily; inhibited JEV replication in vitro	171
Fenofibrate	80% of mice protected by 100 mg/kg daily from day 4 pre-infection to day 9 post-infection	168
Nitazoxanide	70% of mice protected at 75 mg/kg daily from day 1 post-infection and 90% protection at 100 mg/kg daily; inhibited replication of JEV in vitro	165
Etanercept	80% survival with 100 µg etanercept (30% without) on days 3 and 5 post-infection, reduced CNS inflammation and reduced production of inflammatory mediators in vitro	152
Compounds with anti-JEV activity in vitro		
Ivermectin	Inhibited JEV, yellow fever, dengue and West Nile virus replication in vitro	162
Niclosamide	Inhibited JEV replication in vitro; also inhibits Zika virus replication in human neural progenitor cells	164
Cilnidipine	Inhibited JEV replication in vitro; no data in children	164
Compounds with theoretical activity or indirect evidence		
Eritoran	TLR4 antagonist: *Tlr4* knockout reduces Japanese encephalitis lethality in mice and eritoran protects mice against lethal influenza	147
Tocilizumab	Anti-IL-6 antibody: IL-6 levels in CSF correlate with mortality in Japanese encephalitis in humans	151
Natalizumab	Anti-VLA4 antibody: protective in a mouse model of West Nile virus	173
PGL5001	JNK inhibitor: JNK inhibition by a different compound protective in a mouse model of JEV	174

Compounds with evidence of anti-JEV activity in mouse models are shown in the top panel, and those with anti-JEV activity (inhibition of replication) in cell culture are shown in the middle panel. All these compounds except arctigenin and cilnidipine have been used in children. Pentoxifylline and nitazoxanide also inhibit JEV replication in vitro. Ivermectin inhibits flavivirus NS3 helicase activity[162] and inhibited JEV production in vitro with EC_{50} 0.3 µM. Niclosamide and cilnidipine inhibited JEV-induced cytopathic effect and replication[164], EC_{50} 5.8 and 6.52 µM, respectively. The bottom panel shows compounds with theoretical benefit in Japanese encephalitis. Eritoran (synthetic TLR4 antagonist), tocilizumab (anti-IL-6 antibody), natalizumab (anti-VLA4 antibody) and PGL5001 (JNK inhibitor) all have indirect evidence of benefit; their anti-JEV potential is hypothetical and requires testing in animal models. CSF, cerebrospinal fluid; EC_{50}, effective concentration for half-maximum response; JEV, Japanese encephalitis virus; JNK, c-Jun N-terminal kinase; NS3, nonstructural protein 3; TLR4, Toll-like receptor 4; VLA4, very late antigen 4.

Table 4 | **The Liverpool Outcome Score for assessing outcome from encephalitis**

Feature	Stage			
	V	IV	III	II
History from child, parents and/or carers				
Speech	The same as other children of this age	–	Reduced	Not speaking or communicating
Feeding	The same as other children	–	Occasionally needs help	Always needs more help
Can the child be left alone?	Too young or yes	–	Yes, briefly in familiar environment	No
Behaviour	Normal	Gets angry easily; other behavioural problems	NA	Severely abnormal
Recognition (other than the main carer)	Too young or yes	–	Some	None
School and/or work; home activity if too young	Back to normal at school and/or work, or if too young, able to do the same tasks at home	Not doing as well; not able to do the same tasks as before	Dropped a school grade, no longer attending school and/or work or not able to do anything at home	–
Seizures in the last 2 months	No seizures and not on antiepileptic drugs	Seizure-free on antiepileptic drugs	Has had seizures	Seizures most days
Ability to dress	The same as other children of the same age	–	Occasionally needs extra help	Always needs more help than other children of the same age
Urinary and faecal continence	The same as other children of the same age	–	–	Needs more help or has incontinence of bowel or bladder
Hearing	Normal	Reduced in one or both ears	Cannot hear at all	–
Observation of the child				
Sitting	Too young or can sit independently	–	Needs help	Not at all
Able to move from sitting to standing	Too young or can walk independently	–	Needs help	Not at all
Observe the child walking for 5 m	Too young or normal	–	Abnormal but independent with crutches or stick	Not able to walk
Put both hands on head; ask child to copy	Too young or normal with both hands	Abnormal with one or both hands	Unable to put one or both hands on head	–
Pick up small object	Too young or normal pincer grasp both hands	–	Unable with one hand or abnormal with one hand or both hands	Unable with both hands

For each domain, the child is scored II–V according to residual neurological or behavioural deficits as detailed in the table[175]. The final outcome score is the lowest score of any domain. For example, if an 8-year-old child is normal in every respect other than not being able to be left alone except briefly in a familiar environment, then the outcome score is III. A fatal case has a score of I. –, values that are not used for the domain in question.

the existing studies on treatment of Japanese encephalitis have not been sufficient to answer key questions and improve patient outcomes. Future trials, therefore, will need to be designed carefully and have a large patient sample size if we are to successfully assess promising new treatments for Japanese encephalitis.

Conclusion

Japanese encephalitis remains a devastating disease with no specific treatment other than best supportive care. Despite the availability of vaccines, 69,000 cases of the disease are estimated to occur every year in Asia, and this figure is probably an underestimate. However, developments in our understanding of Japanese encephalitis within the past few years point to several potential avenues of treatment. Several compounds that are already approved for human use could be tested without delay. Such trials should be larger than previous studies and will almost certainly need to be multicentre trials. Recruitment of individuals with Japanese encephalitis can be challenging as many other conditions mimic the disease and outbreaks are unpredictable and can occur in remote and resource-limited settings. For a successful trial, sufficient infrastructure for good-quality diagnostics is needed to ensure confirmation of every case. Given the apparently paradoxical role of the immune system in Japanese encephalitis, with evidence of both protective and pathological elements, strong consideration should be given to trials of combination therapy that test treatment regimens comprising both anti-inflammatory and antiviral drugs. Some potential treatments, such as a JEV-specific monoclonal antibody, might provide a clinical proof of principle, if successful, to develop treatments for other arboviral encephalitides and could speed up the development of treatments for other emerging flaviviruses in the future.

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