Chapter 10

Japanese Encephalitis Vaccine

Tom Solomon

For most people, the first time they have ever heard of Japanese encephalitis (JE) is when visiting a travel clinic in preparation for a trip to Asia. They are then faced with the seemingly impossible decision of whether to spend money on a vaccine that has a rather bad reputation or to risk developing the disease, which would be much worse.[1] Many of the physicians that have to advise them feel ill equipped to do so. From a Western perspective, JE is considered a rare and exotic disease, but for those living and working in southern and eastern Asia, JE is a daily reality. Although confined to this region, the arthropod-borne JE virus (JEV) is numerically one of the most important causes of viral encephalitis worldwide, with an estimated 50,000 cases and 15,000 deaths annually.[2,3] In addition to the large number of deaths, approximately half the survivors of JE have severe neuropsychiatric sequelae, imposing a large socioeconomic burden on the community. Yet, ironically, this is a disease for which several safe and effective vaccines exist, and newer ones are in development. A safe and effective formalin-inactivated vaccine has been available for nearly 40 years, but production difficulties and its cost have restricted its usage in Asia to wealthier countries, whereas its use by Western travelers is limited by concerns over side effects. In the absence of robust epidemiologic data, vaccination practices have at times fluctuated according to highly publicized cases of JE in travelers or of adverse events related to vaccination. A newer live attenuated vaccine, developed by the Chinese, is cheaper to produce, but its uptake outside China has been limited by regulatory issues and concerns over its production. In recent years, the application of molecular biologic approaches have helped elucidate the structure of flaviviruses, giving insights into viral pathogenesis and have allowed development of newer recombinant vaccines.

Sadly, some remarkable achievements in vaccinology have not always been matched by equal vigor in public health policy and implementation, and the disease continues to grow in importance globally. This chapter reviews the epidemiology, clinical features, and pathogenesis of JE and then focuses on the formalin-inactivated mouse brain–derived (BIKEN) vaccine, which is available to travelers, before briefly considering some of the other vaccines used in Asia as well as newer vaccines in development.

HISTORIC PERSPECTIVE

Epidemics of encephalitis were described in Japan from the 1870s onward, with major epidemics approximately every 10 years. In September 1923, Japan suffered a large earthquake, and in the following summer, which was particularly dry, one of the largest encephalitis outbreaks occurred, with 6,551 cases in 6 months and a 55% case fatality rate.[4–7] The term type B encephalitis was originally used to distinguish these summer epidemics from von Economo's encephalitis lethargica ("sleeping sickness,"

known as type A), but the B has since been dropped. In 1933, a filterable agent was transmitted from the brain of a fatal case and caused encephalitis in monkeys. The prototype Nakayama strain of JEV was isolated from the brain of a fatal case in 1935, and the disease has been recognized across Asia since then (Figure 10-1).[8] The virus was later shown to be a member of the genus *Flavivirus* (family Flaviviridae), named after the prototype yellow fever virus (L *flavus* yellow). Although of no taxonomic significance, the ecologic term arbovirus ("arthropod-borne virus") is used to describe the fact that JEV is one of more than 500 viruses transmitted between vertebrate hosts by arthropods (insects, ticks, sand flies, and biting midges).[9]

INFECTIOUS AGENT

Flaviviruses

The *Flavivirus* genus is one of three genera in the family Flaviviridae. The other two are the genus *Hepacivirus*, which includes hepatitis C virus, and the genus *Pestivirus*, which includes bovine viral diarrhea viruses. The *Flavivirus* genus contains approximately 70 members and includes many important

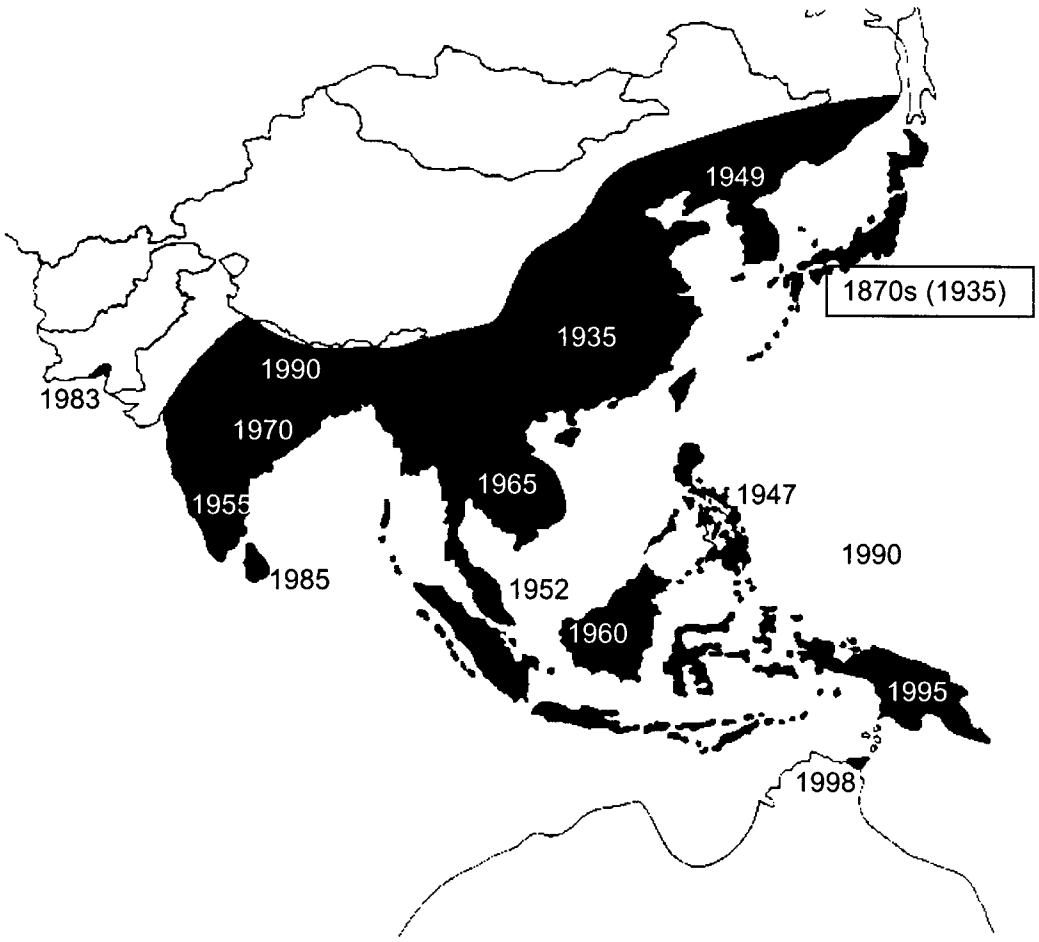

Figure 10-1. Current distribution of Japanese encephalitis. The approximate dates of the first major outbreaks or first virus isolations since epidemics of encephalitis were first described in Japan in the 1870s are shown. Adapted from Solomon T.[8]

Table 10-1. **Medically Important Flaviviruses**

Virus	*Main Clinical Syndromes**	*Main Vectors*	*Natural Hosts*	*Main Geographic Area*
Mosquito-borne viruses				
Japanese encephalitis	CNS	*Culex tritaeniorhyncus* and others	Waterfowl (egrets, herons), chickens, pigs	Asian subcontinent, Southeast Asia, Pacific Rim
West Nile	FAR, CNS	*Culex pipiens* and others	Passeriform birds (jays, blackbirds, sparrows, crows)	Africa, Middle East, southern Europe, North America
St. Louis encephalitis	CNS	*Culex pipiens, C. tarsalis, C. nigripalpus*	Passeriform birds (pigeons, sparrows)	North and South America
Murray Valley encephalitis	CNS	*Culex annulirostris*	Waterfowl, rabbits, marsupials	New Guinea, Australia
Dengue (serotypes 1–4)	FAR, HF	*Aedes aegypti, A. albopictus*	Humans, (macaque monkeys in Africa)	Most countries in the tropics
Yellow fever	Hepatitis, HF	*Aedes* and other species	Primates (monkeys, chimpanzees, baboons), humans	South America, Africa
Tick-borne viruses				
Tick-borne encephalitis	CNS	*Ixodes* spp†	Forest rodents (mice, hedgehogs)	Commonwealth of Soviet States
Omsk hemorrhagic fever	HF	*Dermacentor* spp	Rodents (muskrats, voles)	Siberia
Kyasanur forest disease virus	HF	*Haemaphysalis* spp	Rodents, birds, bats, monkeys	Karnataka State, India

Adapted from Solomon T and Mallewa MJ.[11]

*CNS = central nervous system infection; FAR = fever arthralgia rash syndrome; HF = hemorrhagic fever.

†Tick-borne encephalitis virus is also transmitted via infected milk.

causes of human disease (Table 10-1).[10,11] Flaviviruses are thought to have evolved from a common ancestor as recently as 10,000 years ago and are rapidly evolving to fill new ecologic niches.[12,13] Within the genus, the JE serogroup contains JEV, West Nile virus, St. Louis encephalitis virus, and Murray Valley encephalitis virus.[14,15] Other flaviviruses that cause hemorrhagic fever include yellow fever virus (see Chapter 5, "Yellow Fever Vaccine") and dengue viruses (see Chapter 13, "Dengue Fever Vaccine"). Dengue viruses are endemic in much of Asia and are serologically cross-reactive with JEV, which has implications for diagnosis and pathogenesis. Additional flaviviruses circulating in Asia include Tembusu in Thailand and Langat in Malaysia, but these do not appear to be important causes of disease.

Structure and Replication Strategy

Flaviviruses consist of a single strand of positive-sense ribonucleic acid (RNA) wrapped in a nucleocapsid and surrounded by a glycoprotein-containing envelope. The RNA comprises a short 5′ untranslated region (UTR), a longer 3′ UTR, and between them a single open reading frame.[16] This codes for a single polyprotein, which is co- and post-translationally cleaved by viral and host proteases into three structural proteins (core or C; premembrane or PrM; and envelope or E) and seven nonstructural (NS)

proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5). The C protein is highly basic and combines with the RNA to form the nucleocapsid. The PrM is closely associated with the E protein, forming a heterodimer, and is thought to act as a "chaperone" to it, impairing its function until after virion release. Immediately prior to virion release, the PrM protein is cleaved by a furin-like protease to its mature M protein form. This allows the formation of E protein homodimers, which are thus activated.[17] The E protein is the largest structural protein, consisting of nearly 500 amino acids with up two potential gylcosylation sites. It is the major target for the humoral immune response and is thought to be important in viral entry into host cells. The flavivirus receptor has yet to be identified, but a highly sulphated heparan sulphate molecule might contribute to receptor binding.[18,19] Studies with monoclonal antibodies and, more recently, x-ray crystallography have determined the composition of the E protein's three domains.[20,21] Domain III is the putative receptor-binding domain (by which virions attach to the yet-to-be-identified host cell receptor), domain II is the dimerization domain, and domain I has a central beta barrel and is the hinge domain that links the other two. Following viral attachment to the cell surface, flaviviruses enter cells by endocytosis. Subsequent fusion of the virus's lipid membrane with the endosome membrane allows viral RNA to penetrate into the cytoplasm of the infected cell.[16] Recent cryoelectron microscopy studies have shown an arrangement of 90 E protein dimers lying flat on the surface of the virion that rearrange to form E homodimers as the pH drops, exposing an internal fusion peptide and a patch of viral membrane for fusion. Interestingly, recent studies have shown that the E1 protein of alphaviruses has a striking similarity to the flavivirus E protein in terms of structure and function.[22] Together, they have been labeled class II fusion peptides.[23]

Prototypes and Genotypes of Japanese Encephalitis Virus

Soon after the identification of the prototype Nakayama JEV strain, other strains were isolated that have proved to be important in the history of vaccine development. Beijing-1 was isolated in the People's Republic of China (referred to hereafter as China) in 1949 and P3 soon after in 1950. (At this time, the capital of China was known as Peking—hence the letter P.) In 1982, the Chinese isolated strain SA14, from which the live attenuated vaccine strain (SA14-14-2) would ultimately be derived. JaOARS982 was isolated by the Japanese in 1982 and became important as the first fully sequenced strain.

Comparisons of virus strains by serologic cross-reactivity, and later by the use of monoclonal antibodies, suggested that viruses could be grouped according to antigenic differences.[24,25] Based on limited nucleotide sequencing of the *PrM* and *E* genes, four genotypes of virus were identified (differentiated by at least 12% divergence), which roughly correlated with the antigenic groupings, though a single isolate might represent a putative fifth genotype.[25–28] The different genotypes are not evenly distributed across Asia. Because genotypes I and III were found mainly in northern areas where JE occurs in large summer epidemics and II and IV were found in southern endemic areas, it was postulated that the different genotypes were responsible for the different epidemiologic patterns.[26,27,29] However, recent, more detailed studies have shown that all genotypes of JEV are found in the Indonesia-Malaysia region, but in other parts of Asia only the less divergent, more recently evolved genotypes (I, II, and III) are found.[30] These findings suggest that the virus arose from its flavivirus ancestor in the Indonesia-Malaysia region and evolved here into the different genotypes, only the more recent of which have spread to new geographic areas.[8,31] It has been suggested that differences between the genotypes could have implications for vaccine development, but there are few data to support this (see below).

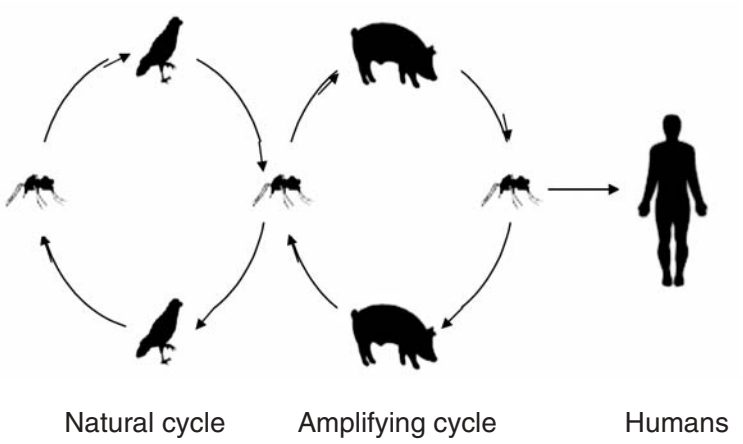

Figure 10-2. The transmission cycle of Japanese encephalitis virus. The virus is transmitted naturally between aquatic birds by *Culex* mosquitoes; during the rainy season, when there is an increase in the number of mosquitoes, the virus "overflows" into pigs and other domestic animals and then into humans, who do not transmit the virus further (dead-end hosts).

EPIDEMIOLOGY

Enzootic Cycle

JEV is transmitted naturally in an enzootic cycle between birds, pigs, and other vertebrate hosts by mosquitoes, especially *Culex tritaeniorhyncus, Culex vishnui, Culex pseudovishnui, Culex gelidus,* and other species that breed in pools of stagnant water (such as rice paddies) (Figure 10-2). Although many animals can be infected with JEV, only those with high viremias are important for the natural cycle. Birds are thought to be important in maintaining and amplifying JEV in the environment, and migrating birds, particularly the black-crowned night heron (*Nycticorax nycticorax*) and the Asiatic cattle egret (*Bubulcus ibis coromandus*), are thought to be important in the virus's dispersal to new geographic areas.[32,33] In addition, windblown mosquitoes might have a role.[34] The means by which JEV overwinters (ie, survives the winter months when there is little mosquito activity) is not certain. The virus can be transmitted vertically from an infected female into her eggs, and overwintering in the eggs of *Aedes* mosquitoes might be one mechanism.[35] In many parts of Asia, pigs are kept close to the home, and they thus serve as important bridging hosts, bringing the virus close to humans. In Indonesia, the lower prevalence of the antibody to JEV in Borneo than in neighboring Bali has been attributed to the near-absence of swine in the predominantly Muslim culture.[36] Whereas the virus does not normally cause encephalitis in birds or pigs, it can cause pregnant sows to abort, and it also causes encephalitis in horses.

Geographic Distribution

In the past 50 years, the geographic area affected by JEV has expanded (see Figure 10-1; Table 10-2).[37,38] Differences in diagnostic capabilities and in reporting of encephalitis make it impossible to plot this expansion precisely. However, the timing of the first reported cases or new epidemics in each area gives an impression of the relentless spread of JE. In China, outbreaks of summer encephalitis occurred from 1935, and the virus was first isolated there in 1940; there are currently 10,000 to 20,000 cases per year, though in the early 1970s there were more than 80,000 cases annually.[39] In the far eastern states of the former Soviet Union, JE first occurred in 1938. In 1949, large epidemics of JE were reported from

Table 10-2. **Risk of Japanese Encephalitis by Country**

Country	*Affected Areas*	*Transmission Season*	*Comments*
Australia	Islands of Torres Strait	Probably year-round transmission risk	Localized outbreak in Torres Strait in 1995 and sporadic cases in 1998 in Torres Strait and on mainland Australia at Cape York Peninsula
Bangladesh	Little data but probably widespread	Possibly July to December as in northern India	Outbreak reported from Tangail District, Dhaka Division; sporadic cases in Rajshahi Division
Bhutan	No data	No data	No comments
Brunei	Presumed to be sporadic-endemic as in Malaysia	Presumed year-round transmission	No comments
Burma (Myanmar)	Presumed to be endemic-hyperendemic countrywide	Presumed to be May to October	Repeated outbreaks in Shan State in Chiang Mai valley
Cambodia	Presumed to be endemic-hyperendemic countrywide	Presumed to be May to October	Cases reported from refugee camps on Thai border and from Phnom Penh
India	Reported cases from all states and union territories except Arunachal Pradesh, Dadra, Daman, Diu, Gujarat, Himachal, Jammu, Kashmir, Lakshadweep, Meghalaya, Nagar Haveli, Orissa, Punjab, Rajasthan, and Sikkim	South India, May to October in Goa, October to January in Tamil Nadu, and August to December in Karnataka, second peak April to June in Mandhya Pradesh District; Andhra Pradesh, September to December; northern India, July to December	Outbreaks in Andhra Pradesh, Assam, western Bengal, Bihar, Goa, Karnataka, Manipur, Tamil Nadu, and Uttar Pradesh; urban cases reported (eg, in Luchnow)
Indonesia	Bali, Irian Jaya (Papua), Kalimantan, Lombok, Moluccas, Nusa Tenggara, and Sulawesi	Probably year-round risk; varies by island; peak risks associated with rainfall, rice cultivation, and presence of pigs; peak periods of risk November to March, also June and July in some years	Human cases recognized on Bali, Java, and possibly in Lombok
Japan	Rare-sporadic cases on all islands except Hokkaido	June to September, except April to December on Ryukyu Islands (Okinawa)	Vaccine not routinely recommended for travel to Tokyo and other major cities; enzootic transmission without human cases observed on Hokkaido
Korea	North Korea, no data; South Korea, sporadic-endemic with occasional outbreaks	July to October	Last major outbreaks in 1982 and 1983; sporadic cases reported in 1994 and 1998
Laos	Presumed to be endemic-hyperendemic countrywide	Presumed to be May to October	No comments
Malaysia	Sporadic-endemic in all states of Peninsula, Sarawak, and probably Sabah	Year-round transmission; October to February in Sarawak	Most cases from Johor, Penang, Perak, Sarawak, and Selangor

Continued on next page

Table 10-2. **Risk of Japanese Encephalitis by Country** ***(Continued)***

Country	*Affected Areas*	*Transmission Season*	*Comments*
Nepal	Hyperendemic in southern lowlands (Terai)	July to December	Vaccine not recommended for travelers visiting only high-altitude areas
Pakistan	Might be transmitted in central deltas	Presumed to be June to January	Cases reported near Karachi; endemic areas overlap those for West Nile virus; lower Indus Valley might be an endemic area
Papua New Guinea	Normanby Islands and Western Province	Probably year-round risk	Localized sporadic cases
People's Republic of China	Cases in all provinces except Xizang (Tibet), Xinjiang, Qinghai; endemic–periodically epidemic in temperate areas; Hong Kong, rare cases in new territories; Taiwan, endemic, sporadic cases islandwide; hyperendemic in southern China	Northern China, May to September; southern China, April to October (southern Fujian, Guangdong, Guangxi, Guizhou, Hunan, Jiangxi, Sichuan, and Yunnan provinces); Hong Kong, April to October; Taiwan, April to October with a June peak	Vaccine not routinely recommended for travelers to urban areas only; Taiwan, cases reported in and around Taipei and the Kaohsiung-Pingtung river basins
Philippines	Presumed to be endemic on all islands	Uncertain; speculations based on locations and agroecosystems; West Luzon, Mindoro, Negros, and Palawan, April to November; elsewhere year-round, with greatest risk April to January	Outbreaks described in Luzon, Manila, and Nueva Ecija
Russia	Far-eastern maritime areas south of Khabarousk	Peak period July to September	First human cases in 30 years recently reported
Singapore	Rare cases	Year-round transmission, with April peak	Vaccine not routinely recommended
Sri Lanka	Endemic in all but mountainous areas; periodically epidemic in northern and central provinces	October to January; secondary peak of enzootic transmission May to June	Recent outbreaks in central (Anuradhapura) and northwestern provinces
Thailand	Hyperendemic in north; sporadic-endemic in south	May to October	Annual outbreaks in Chiang Mai Valley; sporadic cases in Bangkok suburbs
Vietnam	Endemic-hyperendemic in all provinces	May to October in the North, year-round in the South	Highest rates in and near Hanoi
Western Pacific	Two epidemics reported in Guam and Saipan since 1947	Uncertain; possibly September to January	Enzootic cycle might not be sustainable; epidemics might follow introductions of virus

Adapted from the Centers for Disease Control and Prevention[37,38] and updated following the Global Alliance for Vaccines and Immunizations, Southeast Asia and Western Pacific Regional Working Group's Japanese Encephalitis Meeting: Setting the Global Agenda on Public Health Solutions and National Needs, Bangkok, Thailand, 2002.

South Korea for the first time. Epidemics in northern Vietnam followed in 1965 (currently 1,000 to 3,000 cases nationally per year) and in Chiang Mai, northern Thailand, in 1969 (currently 1,500 to 2,500 cases nationally each year). JE was recognized in southern India from 1955 but was confined to the south until the 1970s. Since then, large outbreaks (2,000 to 7,000 cases per year) have been reported from eastern and northeastern states. The fact that adults and children were equally affected in these Indian states supports the idea that the virus was introduced here for the first time. The late 1970s also saw the first cases in Burma (Myanmar) and Bangladesh and large epidemics (up to 500 cases per year) in Nepal. In 1985, Sri Lanka experienced its first epidemic, with 410 cases and 75 deaths. JEV continues to spread west, with cases occurring in Pakistan and new epidemics in the Kathmandu valley of Nepal.[40–42]

Charting the progression of JE southeast across Asia and the Pacific Rim is difficult because sporadic cases in endemic areas do not command the same attention as the massive epidemics associated with temperate climates. JE has appeared sporadically on the Western Pacific islands, with outbreaks in Guam in 1947 and Saipan (Northern Mariana Islands) in 1990.[43,44] In Malaysia, the disease is endemic, with the virus first isolated in the 1950s and approximately 100 cases annually.[45] Further east, JE occurs sporadically in the Philippines and New Guinea. The first cases occurred in the Australian Torres Strait islands in 1995, and JE was reported for the first time on the Australian mainland, north of Cairns, in 1998.[34,46]

The reasons for the spread of JE are incompletely understood but probably include changing agricultural practices, such as increasing irrigation (which allows mosquito breeding) and animal husbandry (which provides host animals). A single rice paddy can produce more than 30,000 adult mosquitoes in a day.[47] The risk of acquiring JE after a single mosquito bite is low. Even where transmission is intense, the infection rate of mosquitoes rarely exceeds 3%.[48] However, by one estimate, the minimum probability of an infectious mosquito bite in Tamil Nadu, India, was 0.47 to 0.77 per year.[49,50]

In developed countries, such as Japan, Taiwan, and South Korea, the number of cases of JE has fallen, probably because of a combination of mass vaccination of children, spraying of pesticides, changing pig-rearing practices, separation of housing from farming, better housing with air conditioning, and less availability of mosquito breeding pools.[51] The impact of factors other than vaccination is best demonstrated in Singapore. This country was previously endemic for JE but now has no disease, even though there is no vaccination program.[48] In some developed Asian countries, although JE is now rare in children, it is still seen in adults, particularly the elderly.[39]

Epidemic versus Endemic Disease

Broadly speaking, two epidemiologic patterns of JE are recognized.[39] In northern areas (northern Vietnam, northern Thailand, Korea, Japan, Taiwan, China, Nepal, and northern India), large epidemics occur during the summer months, whereas in southern areas (southern Vietnam, southern Thailand, Indonesia, Malaysia, Philippines, Sri Lanka), JE tends to be endemic; cases occur sporadically throughout the year, with a peak after the start of the rainy season.[39]

A variety of explanations for this different pattern have been offered. The observation that JEV genotypes I and III circulate in northern "epidemic" regions and II and IV in southern "endemic" regions led to the proposal that different genotypes might explain the differing clinical epidemiology.[26,27] However, the recent arrival of a "northern genotype I" isolate in Australia, the observation that genotype III is associated with epidemic disease in northern Vietnam and endemic disease in southern Vietnam, and the identification of a putative fifth genotype suggested that this paradigm was not right.[28,52,53] The distri-

bution of genotypes is now thought to relate to the virus's origin in the Indonesia-Malaysia region and that the spread of the more recently evolved genotypes is from there also.[8,31]

An alternative hypothesis is that the clinical epidemiology relates to climate. Comparisons of climatic data from northern and southern Vietnam suggested that temperature might be a key determinant of disease pattern.[53] Whereas rainfall patterns are almost identical in northern and southern Vietnam, the temperature is very different, and the number of encephalitis cases appears to follow temperature closely. In the south, the temperature remains high throughout the year, and the number of cases each month is unchanged. In the north, a rise in JE cases during the summer months corresponds with a rise in temperature. The prolonged mosquito larval development time and longer extrinsic incubation period of JEV at cooler temperatures, which thus reduce the rate of virus transmission, could be one explanation for these observations.

Epidemiology of Human Disease

Humans become infected with JEV coincidentally when living or traveling in close proximity to the virus's enzootic cycle. Although most cases occur in rural areas, JEV is also found on the edge of some Asian cities, including Ho Chi Minh City in Vietnam and Bangkok in Thailand, and outbreaks have been reported from Lucknow, India.[48,54] Epidemiologic studies have shown that following the monsoon rains, mosquitoes breed prolifically, and as their numbers grow, so does their carriage of JEV and the infection rate of pigs.[55,56] Human infection soon follows. In sentinel studies, previously unexposed pigs placed in endemic areas were infected with the virus within weeks.[57]

Although the virus has occasionally been isolated from human peripheral blood, viremias are usually brief and titers low; thus, man is considered a dead-end host from which transmission does not normally occur (see Figure 10-2).[58] Cross-sectional serologic surveys have shown that in rural Asia, the majority of the population is infected with JEV during childhood or early adulthood.[59] Approximately 10% of the susceptible population is infected each year.[38] However, most infections of humans are asymptomatic or result in a nonspecific, flu-like illness; estimates of the ratio of symptomatic to asymptomatic infection vary between 1 in 25 and 1 in 1,000.[60,61]

When epidemics first occur in new locations, for example, in Sri Lanka, India, and Nepal, adults as well as children are affected.[62] The susceptibility of immunologically naive adults was also demonstrated by the incidence of JE among American troops during conflicts in Japan, Korea, and Vietnam.[63–67] The risk of developing symptoms seems to be higher in these troops than in the local population (possibly because for the local population, previous exposure to other flaviviruses reduces the severity of infection with JEV).

Incidence

Although JE is a disease that is reportable to the World Health Organization (WHO), official figures vastly underestimate the true incidence. For example, in the Philippines and Indonesia, few cases are officially reported, yet hospital-based studies show up to 50% of encephalitis patients have JE. One reason for underreporting has been the difficulty in establishing the diagnosis, but with the availability of new diagnostic tests based on immunoglobulin M (IgM) capture enzyme-linked immunosorbant assays (ELISAs), this should become easier.[68–71] JE is primarily a disease of children and young adults. In most affected areas, the incidence is 1 to 10 per 10,000. In northern Thailand, the incidence has been estimated to be up to 40 per 100,000 for ages 5 to 25 years, declining to almost zero for those over 35 years.[59,72] The

incidence is lower in infants and young children (< 3 years old) than in older children, possibly reflecting behavioral factors, for example, playing outside, particularly after dusk.[39]

Although a figure of 35,000 to 50,000 reported cases is often quoted, estimates of the disease burden based on incidence rates suggest that the number of cases is much higher. Data from Taiwan and Thailand looking at the incidence among nonvaccinated children during placebo-control JE vaccine trials suggest an incidence of 1.8 to 2.5 per 10,000, whereas unvaccinated children in trials in China had an incidence of 5.7 to 64 per 10,000.[48,59,73] Using 1994 population estimates that 700 million Asian children (< 15 years) live in rural areas at risk of JEV and assuming a representative incidence rate of 2.5 per 10,000 (and no vaccination), the annual incidence of JE was estimated to be 175,000 cases, with 43,750 deaths and 78,750 survivors having severe disabilities.[73] Allowing for vaccine coverage, the expected number of cases was more than 125,000.[73]

Epidemiology among Travelers and Expatriates

Until the 1980s, vaccination against JE was rarely considered for travelers and expatriates. Then, in 1982, an American student who was spending a year at Peking University developed JE and died. His father, a Washington lawyer, was outraged that the vaccine was not even an option in the United States and used his good connections with government officials to ensure that the vaccine soon became available through the United States Centers for Disease Control and Prevention (CDC) as an investigational new drug.[74] The vaccine subsequently received full licensure after efficacy trials conducted by the United States Army in Thailand (see "Efficacy").

No systematically collected data exist on the incidence of JE among travelers or expatriates (including military personnel and other foreign residents), though cases are reported in the literature and have been surveyed informally.[48] A review of 24 cases reported to the CDC between 1978 and 1992 showed that 11 had occurred in expatriates, 8 of whom were military personnel or their dependents and 1 or 2 of whom were thought to be tourists.[38] Outcome information was available for 15, of whom 6 died: 5 were disabled, and 4 recovered. Taking Department of Transport figures indicating that 2 to 3 million US citizens travel by air to Asia each year, and allowing for the fact that most travelers have brief itineraries that do not include staying in areas with an exposure risk and that some will have been immunized, an annual incidence was estimated at roughly one per million. Based on these data, immunization against JE was recommended by the United States Advisory Committee on Immunization Practices (ACIP) for those staying in Asia for a month or more, or a shorter time if likely to be at a greater risk of exposure (see following discussion).[38] However, with so many unknown variables, it is difficult to know how reliable this estimate of risk really is. An alternative approach is to extrapolate from the incidence rates in the local population. Assuming an annual incidence of 10 per 10,000 and recognizing that most cases occur in a 5-month period, the risk of developing JE during a 1-month visit in the transmission season was estimated at 1 per 5,000 or 1 per 20,000 per week.[38] These rates are similar to the attack rates for nonimmunized Western military personnel exposed during field operations in Asia between 1945 and 1991 (0.05 to 2.1 per 10,000 per week).[60,75]

The cases of JE that have occurred in tourists have shown limitations in our understanding of the risk factors. Three foreigners staying in hotels in Bali, Indonesia, for 2 weeks or less developed JE. In the 1980s, an Australian child developed the disease after a 2-week holiday but recovered.[76] A female Swedish tourist developed JE in March 1994, and a fatal case occurred in January 1995 in a Danish man.[77,78] Two of these cases occurred after the ACIP recommendations had been published and in line with those rec-

ommendations, the tourists had not been vaccinated because they were on short trips and were not visiting a known epidemic area. It has been suggested that Bali might reflect a unique situation because of the close proximity of tourist hotels and beaches to areas with intense enzootic viral transmission. Although it might be argued that there will inevitably be some unlucky individuals who will develop disease despite following the recommendations for vaccination, it could alternatively be argued that these cases in Bali indicate the failings of the vaccination policy. The recently published guidelines are less specific about the duration of a trip. Further cases among travelers were reported in 1996, but none appear to have been reported since then, possibly because the publicity caused by these cases resulted in more travelers being vaccinated.[48]

Vaccination policies in countries other than the United States have tended to follow this reactive rather than proactive approach. For example, the Israeli public health authorities now recommend JE vaccine for all Israeli travelers going to Thailand following a single case that occurred in a nonvaccinated traveler.[1] In the Kathmandu valley of Nepal, JE cases have been seen among Nepalis since 1995, and serosurveys of pigs have shown widespread virus activity.[41,79] Long-term expatriates are now being vaccinated, but, unfortunately, it seems that vaccination for travelers will not be recommended until the first case in a traveler is seen.[1]

CLINICAL FEATURES

Patients with JE typically present after a few days of nonspecific febrile illness, which can include coryza, diarrhea, and rigors. This is followed by headache, vomiting, and a reduced level of consciousness, often heralded by a convulsion. In some patients, particularly older children and adults, abnormal behavior might be the only presenting feature, resulting in an initial diagnosis of mental illness: during the Korean conflict, American servicemen with JE were initially diagnosed as having "war neurosis."[65] A proportion of patients make a rapid, spontaneous recovery (so-called abortive encephalitis). Others present with aseptic meningitis and have no encephalopathic features.[80]

Seizures

Seizures occur frequently in JE and have been reported in up to 85% of children and 10% of adults.[64,81,82] In some children, a single seizure is followed by a rapid recovery of consciousness, resulting in a clinical diagnosis of febrile seizure. Generalized tonic-clonic seizures occur more often than focal motor seizures. Multiple or prolonged seizures and status epilepticus are associated with hypoxic brain metabolism, raised intracranial pressure, clinical signs consistent with brainstem herniation, and a poor prognosis.[80] In a proportion of children, subtle motor seizures occur, causing twitching of a digit, an eye, or the mouth; eye deviation; nystagmus; excess salivation; or irregular respiration. Without electroencephalographic monitoring, these subtle motor seizures might be difficult to document.[80]

Parkinsonism and Other Movement Disorders

The classic description of JE includes a Parkinson's disease–like dull, flat, "mask-like" facies with wide, unblinking eyes, tremor, generalized hypertonia, and cogwheel rigidity. These features were reported in 70 to 80% of American service personnel and 20 to 40% of Asian children.[81,83] Opisthotonos (Figure 10-3) and rigidity spasms, particularly on stimulation, occur in about 15% of patients and are associated with a

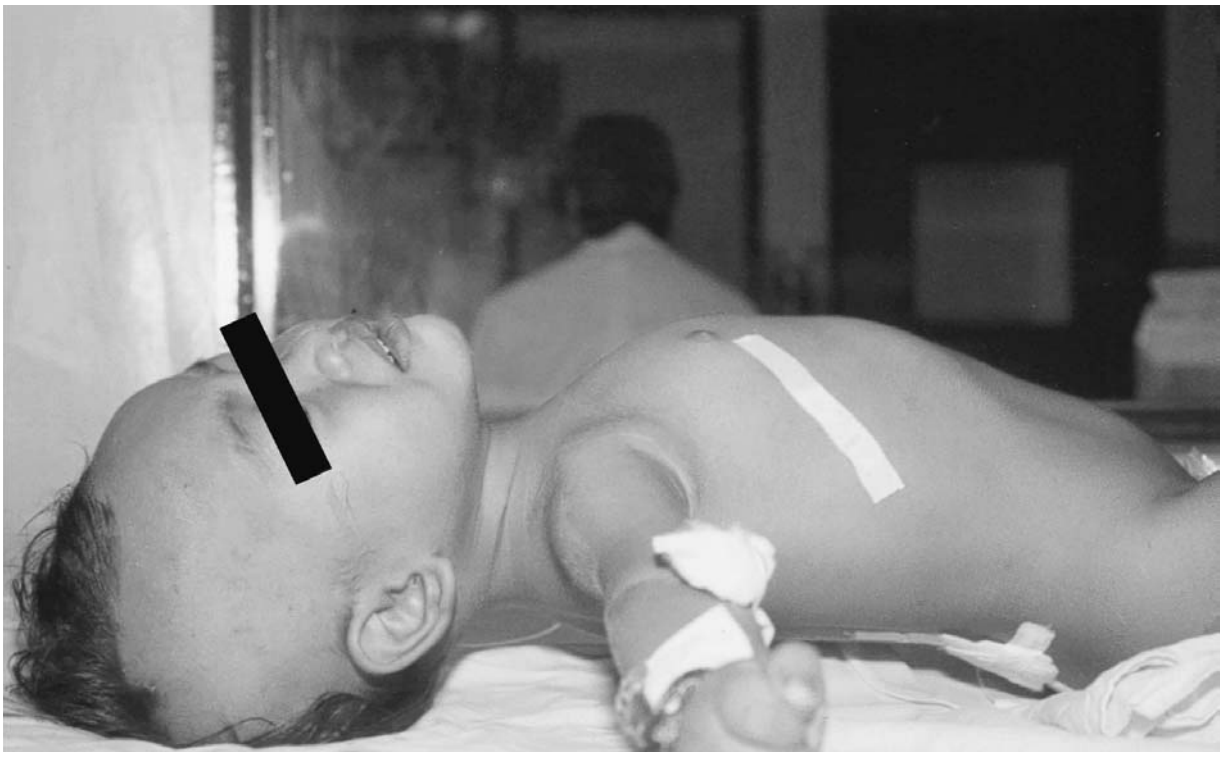

Figure 10-3. Opisthotonos and other movement disorders are common in Japanese encephalitis. Photograph by T. Solomon reproduced with permission from Solomon T.[84]

poor prognosis.[81,83,84] Other extrapyramidal features include head nodding and pill-rolling movements, opsoclonus-myoclonus, choreoathetosis, bizarre facial grimacing, and lip smacking.[81,83,85] Radiologic studies support earlier pathologic studies implicating the basal ganglia and thalamus in the parkinsonian syndromes seen in JE.[85–87] Upper motor neuron facial nerve palsies occur in approximately 10% of children and can be subtle or intermittent.

Acute Flaccid Paralysis

A subgroup of patients infected with JEV present with a poliomyelitis-like acute flaccid paralysis.[88] Following a short febrile illness, there is a rapid onset of flaccid paralysis in one or more limbs, despite a normal level of consciousness. Weakness occurs more frequently in the legs than in the arms and is usually asymmetric. Of these patients, 30% subsequently developed encephalitis, with a reduced level of consciousness and upper motor neuron signs, but, in the majority, acute flaccid paralysis is the only feature. At follow-up (1 to 2 years later), there is persistent weakness and marked wasting in the affected limbs (Figure 10-4).[88] Nerve conduction studies demonstrated markedly reduced compound muscle action potentials, and electromyography showed a chronic partial denervation pattern, suggesting anterior horn cell damage.[88] Flaccid paralysis also occurs in comatose patients with JE, being reported in 5 to 20%.[64,89] Electrophysiologic studies have confirmed anterior horn cell damage, and magnetic resonance imaging (MRI) of the spinal cord has shown abnormal signal intensity on T2-weighted images, which is probably the radiologic correlate of the inflammation in the anterior horn of the spinal cord that is seen at autopsy.[7,86]

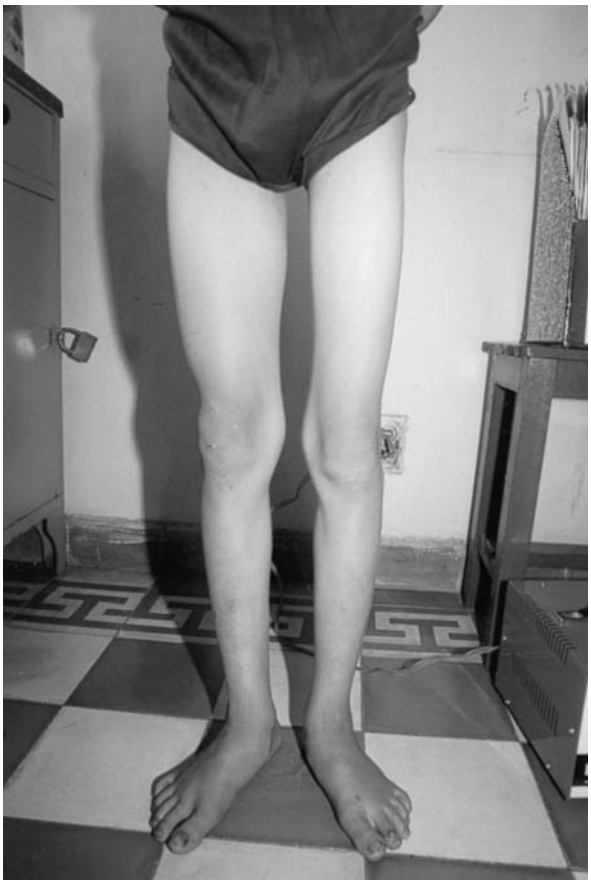

Figure 10-4. Poliomyelitis-like acute flaccid paralysis. This child has marked weakness and wasting a year after the initial presentation. Photograph by T. Solomon reproduced with permission from Solomon T et al.[88]

Investigations

A peripheral neutrophil leukocytosis is seen in most patients, and hyponatremia can occur as a consequence of the syndrome of inappropriate antidiuretic hormone secretion (SIADH). The cerebrospinal fluid (CSF) opening pressure is elevated in approximately 50% of patients. Typically, there is a moderate CSF pleocytosis of 10 to 100 cells per mm^3, with predominant lymphocytes, mildly elevated protein (50 to 200 mg %), and a normal glucose ratio. However, polymorphonuclear cells may predominate early in the disease or there might be no CSF pleocytosis.[81]

In approximately 50% of patients with JE, computed tomography (CT) scans show bilateral, nonenhancing, low-density areas in one or more of the thalamus, basal ganglia, midbrain, pons, and medulla.[90,91] MRI is more sensitive, typically demonstrating more extensive lesions, including damage in the cerebral hemispheres, cerebellum, and anterior spinal cord of patients with flaccid paralysis.[86,92] Thalamic lesions of mixed intensity on T1 and T2 are often seen and are suggestive of hemorrhage.[85,86] They may be useful in distinguishing JE from herpes simplex encephalitis, where the changes are characteristically frontoparietal.[93] Single photon emission CT (SPECT) studies carried out acutely can show hyperperfusion in the thalamus and putamen.[94] Follow-up studies have shown hypoperfusion in the same areas as well as in the frontal lobes.[85]

A variety of electroencephalographic abnormalities have been reported in JE, including theta and delta coma, burst suppression, periodic lateralized epileptiform discharges and other epileptiform activity and, occasionally, alpha coma.[80,91,93,95]

Diagnosis

The differential diagnosis of JE is broad and includes other viral encephalitides (arboviruses, herpesviruses, enteroviruses, postinfectious and postvaccination encephalomyelitis), other central nervous system (CNS) infections (bacterial and fungal meninigitis, tuberculosis, cerebral malaria, leptospirosis, tetanus, abscesses), other infectious diseases with CNS manifestations (typhoid encephalopathy, febrile convulsions), and noninfectious diseases (tumors, cerebrovascular accidents, Reye's syndrome, toxic and alcoholic encephalopathies, epilepsy).[3] Distinguishing encephalitis from partially pretreated bacterial meningitis and cerebral malaria might be particularly difficult.

Attempts at isolating JEV from clinical specimens are usually unsuccessful, probably because of low viral titers and the rapid production of neutralizing antibodies. Isolates can sometimes be obtained from brain tissue (either at autopsy or from a postmortem needle biopsy) or from CSF, in which case it is associated with a failure of antibody production and a high mortality rate.[96] Immunohistochemical staining of CSF cells or autopsy tissue with anti-JEV polyclonal antibodies can be positive.[97,98] However, in most cases, JE is diagnosed serologically. The hemagglutination inhibition test was used for many years, but it had various practical limitations and, because it required paired sera, could not give an early diagnosis.[99] In the 1980s, IgM and IgG capture ELISAs were developed and have become the accepted standard for diagnosis of JE.[69,100] The presence of anti-JEV IgM in the CSF has a sensitivity and specificity of greater than 95% for CNS infection with JEV.[101] ELISAs are now commercially available and have also been modified to a kit form that requires no specialist equipment, which might be useful for diagnosing JE in small rural hospitals.[70,71] JEV ribonucleic acid (RNA) has been detected in human CSF samples using reverse transcriptase polymerase chain reaction.[40,102] However, its reliability as a routine diagnostic test has not been shown.

MANAGEMENT AND ANTIVIRAL TREATMENT

Treatment for JE is supportive and involves controlling convulsions and raised intracranial pressure when they occur. For many years, corticosteroids were given, but a double-blind randomized placebo-controlled trial of dexamethasone failed to show any benefit.[103] Aspiration pneumonia is a common occurrence in patients with a reduced gag reflex. Careful nursing care and physiotherapy are needed to reduce the risk of bedsores, malnutrition, and contractures. There is no established antiviral treatment for JE or any other flavivirus infection. A variety of compounds have shown antiviral activity in vitro and/or in animal models of infection.[104] In animal studies, passive immunization with polyclonal or mixed monoclonal antibodies given peripherally and intrathecally was effective.[105] This has also been attempted in a small number of patients, but experience from similar treatment of tick-borne encephalitis suggests it is unlikely to be useful unless given before encephalitis has developed and might even worsen outcome.[106,107]

Recently, salicylates and nonsteroidal anti-inflammatory drugs were shown to suppress the in vitro replication of JEV and prevent apoptosis of infected cells.[108,109] This did not appear to be via suppression of nuclear factor κB activation but might be via mitogen-activated kinase.[109] Interferon-α, a glycoprotein cytokine that is produced naturally in response to viral infections, including JE, had been the most promising antiviral candidate.[110] In tissue culture, recombinant interferon is effective against JEV and other arboviruses, including West Nile virus.[111,112] In the 1980s, it was given in open trials to a small number of Thai JE patients with encouraging results[113] However, a recently completed double-

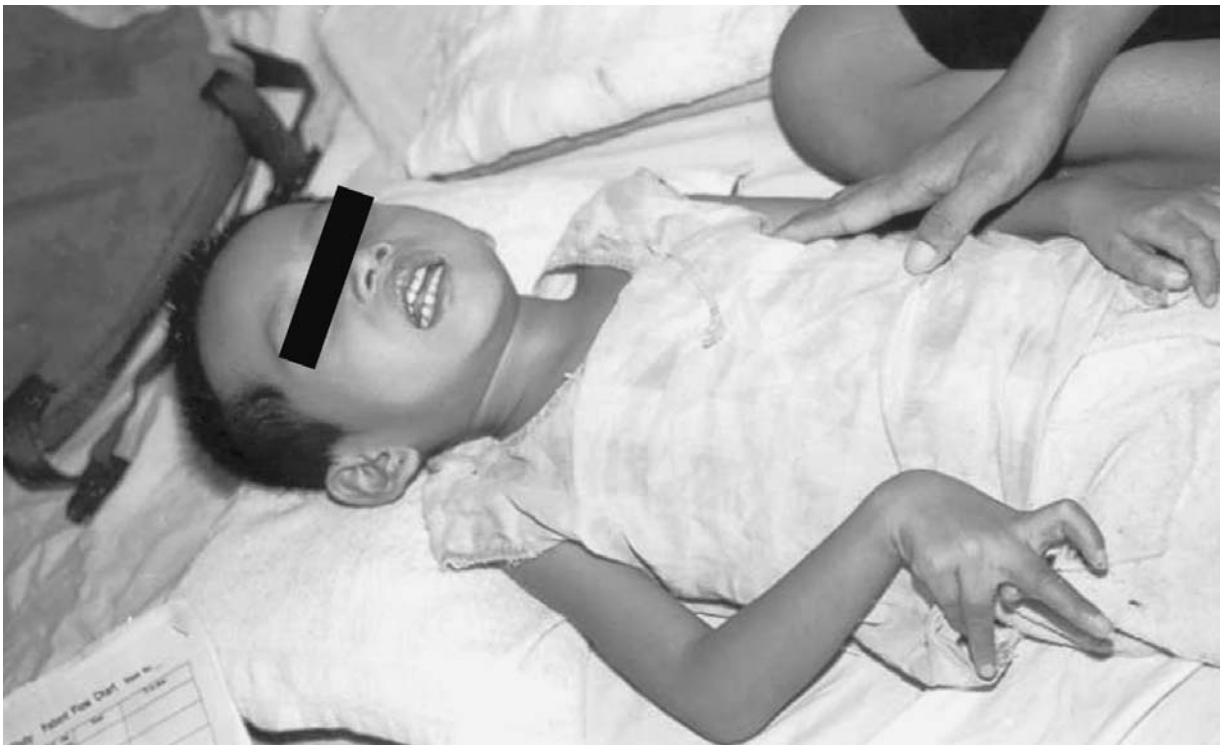

Figure 10-5. Sequelae of Japanese encephalitis: flexion deformities are apparent in this child 2 months after the initial illness. Photograph by T. Solomon reproduced with permission from Solomon T.[84]

blind placebo-controlled trial in Vietnamese children with JE (the first randomized controlled antiviral trial for any flavivirus) showed that it made no impact on the overall outcome.[114]

Outcome

Approximately 30% of hospitalized patients with JE die, and around half of the survivors have severe neurologic sequelae. However, in areas with better hospital facilities, there is a reduction in mortality but a concomitant increase in the number of patients with severe sequelae. Poor prognostic indicators include a depressed level of consciousness, abnormal breathing and decerebrate posturing, multiple seizures, raised intracranial pressure, isolation of virus from the CSF, low levels of JE virus–specific IgM and IgG in CSF and serum, and immune complexes in the CSF.[80,81,115,116] Other indicators that have been found in some, but not all, populations studied include higher admission temperature, absent abdominal reflexes, hyponatremia, low serum iron, and elevated CSF white cell counts and protein.[80,81,117–119] Approximately 30% of survivors have frank motor deficits. These result in a mixture of upper and lower motor neuron weakness and cerebellar and extrapyramidal deficits.[66,120] Hyperextension of the legs, with "equine feet," and fixed-flexion deformities of the arms are common (Figure 10-5). Twenty percent of patients have severe cognitive and language impairment (most with motor impairment too), and 20% have convulsions.[121,122] A higher rate of sequelae is reported for children than for adults.[123] In addition, the more detailed studies have shown that approximately half of those who were classed in the "good recovery" group have subtle sequelae, such as learning difficulties, behavioral problems, and subtle neurologic signs.[121]

Pathogenesis

Only about 1 in 25 to 1 in 1,000 humans infected with JEV develop clinical features of infection.[60,61] These can range from a mild, flulike illness to a fatal meningoencephalomyelitis. The factors determining which of all the humans infected with JEV will develop disease are unknown but could include viral factors, such as route of entry, titer of the inoculum, strain virulence, and host factors such as age, genetic make-up, general health, and preexisting immunity.

Humans become infected from the bite of an infected mosquito. Following inoculation, the virus is thought to replicate in the skin before being transported to local lymph nodes. Langerhans' dendritic cells migrating from the skin to the lymph nodes have recently been implicated in this transport in experimental intradermal infection of BALB/c mice with West Nile virus and in volunteers receiving candidate live attenuated dengue virus vaccines.[124,125]

The means by which JEV crosses the blood-brain barrier is unknown. In experimental studies with a hamster model of the related flavivirus, St. Louis encephalitis virus, the olfactory route was shown to be important.[126] Intranasal spraying is also an effective means of experimentally inoculating monkeys.[127] However, immunohistochemical staining of human JE autopsy material has shown diffuse infection throughout the brain, indicating a hematogenous route of entry.[98] Although experimental evidence suggests that replication within endothelial cells might be an important means of crossing the blood-brain barrier in some flaviviruses, for JEV, passive transfer across the endothelial cells appears a more likely mechanism.[128,129] Other factors that compromise the integrity of the blood-brain barrier have also been implicated as risk factors for neuroinvasion. In several studies, a disproportionate number of fatal cases had neurocysticercosis at autopsy, and it has been suggested that head trauma (eg, due to a road traffic accident) during the incubation period could facilitate viral entry into the central nervous system.[130–132]

Virulence Determinants

In animal models, JEV strains differ in both their neuroinvasiveness (following peripheral inoculation) and neurovirulence (following intracranial inoculation). This might be a consequence of the high viremia achieved by some strains. In mice, JEV strains with higher neurovirulence produce higher viremias than those with lower neurovirulence.[133,134] An analysis of the nucleotide and amino acid sequence showed that changes in the structural, nonstructural, and noncoding regions were associated with neurovirulent strains. The E protein has been shown to have a major role in determination of the virulence phenotype, and single amino acid substitutions are sufficient to cause loss of neurovirulence or neuroinvasiveness.[135,136] Two mechanisms mediated by the E protein might be involved: attachment of the virus to the receptor and fusion of viral and host cell membranes. The putative receptor-binding site of flaviviruses lies in an exposed hydrophilic region of domain III of the envelope protein, which in some mosquito-borne flaviviruses includes the integrin-binding motif arginine-glysine-aspartate (RGD). Substitutions around position E-306 on the exposed lateral surface of domain III, at or close to this RGD motif, are associated with loss of neuroinvasiveness.[133,137,138] Another group of flavivirus variants with altered virulence has amino acid changes in the putative hinge region. For example, in several studies of JEV and Murray Valley encephalitis virus, neutralization escape variants with low neuroinvasiveness for mice have shown changes around positions 52 and 270 to 277 of the E protein, both of which lie in this hinge region.[139–141] A substation at E279 in a chimeric yellow fever–Japanese encephalitis virus (see below) was recently shown to affect neurovirulence for mice and monkeys.[142]

Histopathology

At autopsy, CNS findings in JE reflect the inflammatory response to widespread neuronal infection with the virus.[7,143,144] The leptomeninges are normal or hazy. The brain parenchyma is congested with focal petechiae or hemorrhage in the gray matter. When survival is prolonged beyond 7 days, blotchy, necrolytic zones are seen. The white matter usually appears normal. In some patients, the gray matter of the spinal cord is confluently discolored, resembling that of poliomyelitis.[145] The thalamus, basal ganglia, and midbrain are heavily affected, providing anatomic correlates for the tremor and dystonias that characterize JE. At the histologic level, invasion of neurons by JEV is followed by perivascular cuffing, infiltration of inflammatory cells (T cells and macrophages) into the parenchyma, and phagocytosis of infected cells.[7,143] T cells in the brains of fatal cases stained with monoclonal antibodies are CD8+ and CD8− (presumed to be CD4+) and are localized at the perivascular cuff. Both cell types are found in the CSF in acute infection, though the predominant cell type is CD4+.[143] In patients that die rapidly, there might be no histologic signs of inflammation, but immunohistochemical studies reveal viral antigen in morphologically normal neurons.[143,146] This might explain the normal CSF findings in a proportion of patients with Japanese encephalitis.

Immune Response

Interferon and interferon inducers are active against JEV in mice and monkeys, and endogenous interferon-α has been detected in the plasma and CSF of humans with JE.[101,147,148] In addition, both humoral and cellular immune responses occur following infection with JEV. The humoral immune response in JE has been well characterized. In primary infection (ie, when JEV is the first flavivirus with which an individual has been infected), a rapid and potent IgM response occurs in serum and CSF within days of infection. By day 7, most patients have elevated titers.[101] Attempts to isolate virus are usually negative in such patients. However, the failure to mount an IgM response is associated with positive virus isolation and a fatal outcome.[96] Antibodies to JEV may protect the host by restricting viral replication during the viremic phase, before the virus crosses the blood-brain barrier.[149] Evidence from other flaviviruses suggests it may also limit damage during established encephalitis by neutralizing extracellular virus and facilitating lysis of infected cells by antibody-dependent cellular cytotoxicity.[150]

In surviving patients, class switching occurs, and within 30 days, most have IgG in the serum and CSF. Asymptomatic infection with JEV is also associated with elevated IgM in the serum but not in the CSF. In patients with secondary infection (ie, those who have previously been infected with a different flavivirus such as dengue infection or yellow fever vaccination), there is an anamnestic response to flavivirus group common antigens.[100] This secondary pattern of antibody activation is characterized by an early rise in IgG with a subsequent slow rise in IgM.

In animal models of JE, the cellular immune response appears to contribute to the prevention of disease during acute infection by restricting virus replication before the nervous system is invaded. Athymic nude mice have increased susceptibility to experimental infection with JEV and transfer of spleen cells from mice immunized with live attenuated virus conveys immunity to infection.[151,152] Spider monkeys, which are normally unaffected by intracerebrally inoculated JEV, develop rapidly progressive encephalitis when T-cell function has been impaired by cyclophosphamide.[153]

In humans infected with St. Louis encephalitis virus, impairment of T-cell function by human immunodeficiency virus (HIV) appears to increase the risk of developing encephalitis.[154] By analogy with other

human viral infections, including influenza, HIV, Epstein-Barr virus, and dengue, cytotoxic T lymphocytes might be important in the control and, possibly, the clearance of JEV.[155,156] Preliminary experimental evidence is in agreement with this: T-lymphocyte responses were characterized in 7 convalescent JE patients and 10 vaccine recipients of the formalin-inactivated vaccine, JEV-specific T-cell proliferation (including CD4+ and CD8+ T-lymphocyte responses) was demonstrated in both groups.[157] JEV-specific and flavivirus–cross-reactive CD4+ T lymphocytes that recognize E protein in an HLA-restricted manner were recently demonstrated in two vaccine recipients.[158]

Effect of Heterologous Antiflavivirus Antibodies

Many JE-endemic areas are also endemic for dengue and other flaviviruses, such as Tembusu and Langat viruses. In dengue infection, the presence of heterologous antibodies to other dengue virus serotypes appears to be associated with more severe disease (due to a postulated Fc-γ receptor–mediated antibody-dependent enhancement of virus entry into macrophages) (see Chapter 13, "Dengue Fever Vaccine").[159–161] However, in JE the evidence suggests that rather than making the disease more severe, the presence of prior dengue antibodies might afford some protection against severe disease.[72,118,162,163] Thus, patients with a secondary flavivirus infection are less likely to die or have severe sequelae than are those with primary infection.[118] Younger children with JE tend to have a worse outcome, which might be a reflection of their having had less exposure to other flaviviruses.[123] Preexisting dengue virus antibodies have also been postulated as one reason why the apparent-to-inapparent infection ratio is much lower in indigenous populations (1 in 300) than it was in nonindigenous American service personnel (1 in 25).[39,60] In a similar way, during the 1962 Florida epidemic of St. Louis encephalitis virus, the age-adjusted clinical attack rates were much lower in those with prior dengue immunity than in those without.[164]

NONVACCINE PREVENTIVE MEASURES

Broadly speaking, measures to control JE include those that interfere with the virus's enzootic cycle and those that prevent disease in humans. Measures to control breeding of *Culex* mosquitoes, such as the application of larvicides to rice fields and insecticide spraying, have largely proved ineffectual. Alternative, more ecologically friendly methods that might be useful include the application of the natural insecticide neem (which is also a fertilizer for the rice) to rice fields, placing larvivorous fish in rice paddies, and using intermittent irrigation of rice paddies, which disrupts the mosquitoes' breeding but does not impair rice yields (indeed, it might provide better yields for the water consumed).[165,166] *Culex* mosquitoes breed preferentially on cattle, yet cattle are dead-end hosts for JEV. Thus, using cattle to divert mosquitoes away from swine and humans (zooprophylaxis) might have a role.[167,168]

Inactivated and live attenuated vaccines (described below) have been used to protect swine against JEV; however, widespread vaccination is not feasible in most settings. Residents and travelers to endemic areas should take personal protection to reduce the number of *Culex* bites. These include minimizing outdoor exposure at dusk and dawn, wearing clothing that leaves a minimum of exposed skin, using insect repellents containing at least 30% DEET (N,N-diethyl-3-methlybenzamide), and sleeping under bed nets. Although these measures might be possible for the short-term visitor, they are not practical for many residents of endemic areas.

Table 10-3. **Summary of Vaccines against Japanese Encephalitis**

Description	*Virus Strain*	*Common Name*	*Manufacture/Developer*	*Notes*
INACTIVATED VACCINES				
Mouse brain	Nakayama	BIKEN	BIKEN, Japan	Manufactured for international distribution
	Nakayama	Green Cross	Green Cross, S. Korea	Some available internationally
	Beijing-1	—	Japan	Manufactured for the domestic market
Primary hamster kidney	P3	—	China	Previously China's principal vaccine
Vero cell	P3	—	China	Recently licensed in China
	Beijing-1	—	Japan	In development
	SA14-14-2	—	US Army	In development
	P3	—	Aventis Pasteur	Abandoned after it caused febrile reactions in clinical trials
LIVE ATTENUATED VACCINES				
Primary hamster kidney	SA14-14-2	—	China	Widely used in China, also in trials in Nepal and South Korea
	SA14-5-3	—	China	Abandoned after clinical trials because poorly immmunogenic
RECOMBINANT VACCINES				
Canarypox vectored	—	NYVAC-JE	—	Abandoned because poorly immunogenic
Vaccinia vectored	—	ALVAC-JE	—	Abandoned because poorly immunogenic in vaccinia-immune individuals
17D yellow fever vectored	SA14-14-2	ChimeriVax-JE	Acambis	In development
DNA vaccine	Various	—	Japan	In development

VACCINES FOR JAPANESE ENCEPHALITIS

The only JE vaccine currently available for travelers is a formalin-inactivated mouse brain–derived vaccine. This comes from the Research Foundation for Microbial Disease of Osaka University (BIKEN), Japan and is distributed by Aventis Pasteur (Table 10-3).[169] A vaccine is also sometimes internationally available from Green Cross in Korea. In addition, formalin-inactivated mouse brain–derived vaccines are produced for local use in Taiwan, Thailand, and Vietnam. Two other vaccines are used widely in China: an inactivated vaccine grown in primary hamster kidney (PHK) cells and a live attenuated vaccine, known as SA14-14-2, which is having a great impact on JE control in China. In addition, a formalin-inactivated Vero cell–derived vaccine has recently been licensed in China, and other tissue culture–derived vaccines and genetically engineered recombinant vaccines are in development.

Inactivated Mouse Brain–Derived JE Vaccine

Work on inactivated mouse brain–derived vaccines began soon after JEV was first isolated in the 1930s in Japan. An immunogenic, efficacious, and relatively safe inactivated vaccine has been available for at least 30 years and has been used widely in wealthier Asian countries. In poorer Asian countries, its use has been limited by cost, difficulty of production, and issues over availability, whereas its use in travelers has been dominated by issues related to vaccine safety.

Vaccine Production

Following the isolation of JEV in the 1930s, the Japanese and Russians produced crude vaccines by growing the virus in mouse brain and then inactivating it in formalin.[170,171] During World War II, a similar vaccine was developed for the US Army by Albert Sabin (later of poliomyelitis fame) and colleagues. It was shown to be immunogenic and was given to 60,000 American soldiers during an encephalitis outbreak in Okinawa in 1945.[63] For several years postwar, the US armed forces used a chick embryo–derived inactivated vaccine but later abandoned it because the available data suggested it was not immunogenic or efficacious. Since the 1950s, the mouse brain vaccine has been refined by research institutes in Japan, and by 1966 it had been introduced for routine use in children. The current vaccine undergoes centrifugation, ultrafiltration, protamine sulphate precipitation, and then formalin inactivation, followed by further clarification, ultrafiltration and concentration, ammonium sulphate precipitation, ultracentrifugation on a sucrose density gradient, and then dialysis and concentration.[48] National standards in Japan specify minimal immunogenicity and potency in mice (compared with a vaccine standard), maximal protein content, and undetectable myelin basic protein (assay limit of detection 2 ng/mL). The vaccine is stabilized with gelatin and sodium glutamate and preserved with thimerosal. In Japan, the vaccine is distributed in liquid form, but for international distribution it is lyophilized (freeze dried). The production procedures of formalin-inactivated mouse brain vaccine in other Asian countries are similar to that in Japan.

Stability and Storage

The liquid and freeze-dried inactivated vaccines should be stored at 2°C to 8°C but should not be frozen. Lyophilized vaccine is stable at 4°C for at least a year. It retains more than 90% of its potency after 28 weeks at 22°C and 95% of its potency after 4 weeks at 37°C. Once it has been reconstituted, inactivated vaccine is stable for at least 2 weeks at 22°C, but at 37°C, the potency declines to 85%.[172]

Virus Strains Used

Most formalin-inactivated vaccines (including the BIKEN and Green Cross vaccines) are based on the original Nakayama strain of JEV isolated in 1935 and maintained by continuous mouse brain passage since then. However, since 1989 the vaccine produced for the domestic market in Japan has been prepared using the Beijing-1 strain, isolated in China in 1948 (see Table 10-3). The Beijing-1 strain has a higher potency, and in challenge experiments in mice, it elicited broader cross-reacting antibodies against various wild-type strains of JEV.[173] However, during a phase III clinical trial comparing monovalent Nakayama vaccine to bivalent Nakayama/Beijing-1 vaccine, the efficacy of the two vaccines was the same (see "Efficacy").[59] Recently, the question of whether these vaccines (which are based on genotype III

strains of virus) are efficacious against other genotypes of JEV has been raised. A WHO-supported study is addressing this question, but no clinical or epidemiologic data suggest that it is a problem.

Dosage and Route

A variety of dosage regimens are used depending on the setting. The differing immunization schedules have been derived from immunogenicity studies, though, as explained later in this chapter, these have not always been conducted to a single standard. The volume of Nakayama-based vaccine administered per dose is 1 mL subcutaneously (0.5 mL for children age 1 to 2 years; 1.0 mL for children 3 years and older). Because the Beijing-1 vaccine is more potent, it is given at half this volume.

Immunogenicity Studies

Assays of Humoral Immunity. To determine the neutralizing antibody titer that is protective against JEV, mice were passively immunized with anti-JEV antibody and then challenged with live virus. The dose of challenge virus used in these experiments was 105 median lethal dose (MLD) of JEV (ie, 105 times the lethal dose50—the dose that kills 50% of the unimmunized mice), which is thought to be a typical dose transmitted by an infectious mosquito bite. In such challenge experiments, mice that had neutralizing antibody titers greater than 1 in 10 were found to be protected, whereas mice with lower titers were not.174,175 Neutralizing antibody titers greater than 1 in 10 are also therefore taken to indicate postvaccination seroconversion and protection in humans, though no direct data support this.48

To measure neutralizing antibody titers, the plaque reduction neutralization test is most often used. The essence of the test is that when JEV is grown on a monolayer of Vero cells, or other appropriate cell substrate, it causes plaques to form. The number of plaques formed is reduced if the virus has been mixed with serum containing neutralizing antibody, and this reduction in plaques gives a measure of the antibody titer. No international standard for the exact procedure or choice of end points has been established. Thus, the challenge virus strains, cell systems, addition of exogenous complement (which facilitates binding of antibody to virus), and end points (ranging from 50% to 90% reduction in plaques) vary between laboratories. A comparison of results from three laboratories showed good correlation, and the issue is currently being examined further with the support of the WHO initiative for vaccine research.[176]

Immune Responses to Inactivated Vaccine. Studies of the immune response in vaccine recipients revealed important differences between residents of endemic areas and travelers, which have led to different vaccination schedules being recommended. When Asian children were vaccinated with a primary regimen of two doses of Nakayama or Beijing-1 strain–derived vaccines, 94 to 100% had neutralizing antibody to the homologous strain, though seroconversion rates against heterologous antigenic groups were lower.[177] Nearly 100% seroconversion was achieved following a 1-year booster dose. In contrast, seroconversion rates in travelers and military personnel from the United Kingdom and United States following a two-dose primary vaccination regimen were lower (33 to 80%).[178–181] By 6 to 12 months after vaccination, only 10% of vaccinees still had antibody titers greater than 1 in 8. A three-dose primary schedule was more effective, giving seroconversion in more than 90% of recipients and higher geometric mean titers.[180,181] The difference in vaccine immunogenicity between the two populations is presumed to be due to a degree of natural immunity among the Asian children because of exposure to JEV and other

flaviviruses, particularly dengue; however, the differences in the age of the populations studied (most of the travelers and military personnel are adults) might also be important.

Cellular Immunity

Although immunogenicity studies of vaccine efficacy have focused on humoral immunity, cellular immune responses produced by inactivated vaccines might also be important. CD4+ and CD8+ T-cell memory has been demonstrated by lymphoproliferative responses to JE antigens in virus-like particles containing only structural proteins.[157] In addition, as described above, inactivated JE vaccine induces both JE-specific and flavivirus cross-reactive human leukocyte antigen (HLA)-restricted CD4+ cytotoxic T cells directed against the E protein of the virus. Thus, T-cell memory could be protective even in vaccinees who appear to be seronegative.[157,158]

Primary Vaccination Schedules

In endemic parts of Asia, a two-dose primary immunization schedule is used. Children typically receive their first dose at any age between 12 and 36 months; the second dose is given 7 to 30 days later (Table 10-4).[169] A booster dose is given at 1 year and then additional boosters are given every 1 to 3 years. In Japan and South Korea, where the incidence of JE has declined, the first dose is given at 18 months to 3 years. In Thailand, vaccination is initiated at 18 months, whereas in Sarawak, Malaysia, it is started at 9 months.

For travelers (and military personnel), a three-dose primary schedule is recommended because, as explained previously, a two-dose regimen fails to produce neutralizing antibody in approximately 20% of subjects (Table 10-5). ACIP recommends three doses on days 0, 7, and 30. If there is insufficient time available before departure, an accelerated regimen (days 0, 7, and 14) is recommended.[38] Both regimens produce nearly 100% seroconversion, but the accelerated regimen produces lower antibody titers when measured subsequently. Although not ideal, even two doses given 7 days apart produce antibody in 80% of recipients and so might be better than nothing. An interval of at least 10 days is recommended between the last dose of vaccine and the commencement of a trip because of the low risk of adverse events requiring medical attention (see "Adverse Events").[38,169]

Booster Doses

Precise recommendations are not possible because only limited data are available. Studies of US Army vaccine recipients showed that antibody titers were maintained for up to 3 years in nearly 95% of recipients, but field studies suggested greater variability.[180] Boosters at 2 to 3 years are currently recommended. In different parts of Asia, the practice has varied over time: annual boosters during childhood were given in Korea until the 1980s; in Japan boosters are given approximately every 5 years; in poorer countries that are able to afford only primary vaccination in a limited number of children, no boosters are given at all. Natural boosting by exposure to JEV, dengue, or other flaviviruses might be important in parts of Asia.

Immunosuppressed Recipients, Pregnancy, and Lactation

Because it is not a live vaccine, the BIKEN and other inactivated JE vaccines are safe in immunosuppressed individuals. In one comparison of infants with vertically acquired HIV and HIV-negative infants of HIV-infected women, there was a trend toward lower JEV seroconversion rates after vaccination in the HIV-positive children.[182] No specific information is available on vaccination in pregnancy or in lactation.

Table 10-4. **Vaccination Policy in Countries at Risk of Japanese Encephalitis**

Country	*Policy*	*Vaccine Used*	*Schedule*	*Manufacture and Distribution*
Australia	High-risk areas (Torres Strait islands)	Inactivated Nakayama mouse brain	3 doses given to adults and children in affected areas	5,000 doses, imported from Japan
China	All children	Inactivated P3 in PHK cells	2 doses, 1 wk apart at age 0–6 mos; boosters at 2, 3, and 7 yrs	100 million doses manufactured in total, being phased out
		Vero cell–derived inactivated P3	Used as booster for those that received PHK-derived P3 for primary immunization	200,000 doses in 2001
		Live attenuated SA14-14-2	0–2 yrs, boosters at age 2 and age 7 yrs	50 million doses annually
India	In response to epidemics	Inactivated Nakayama mouse brain	Children, 2 doses	Imported from Japan and Vietnam
Japan	All children	Inactivated Beijing-1 mouse brain	2 doses up to 4 wks apart at age 3 yrs; boosters at 1 yr, 9–12 yrs, and 14–15 yrs	11 million doses, manufactured locally
Malaysia	Implemented in stages, beginning with Sarawak	Inactivated Nakayama mouse brain	2 doses 1 mo apart at age 9 mos; booster at 18 mos	Imported from Japan
Nepal	Children in high-risk areas	Inactivated Nakayama mouse brain; live attenuated SA14-14-2 also used in trials	2 doses of inactivated; live attenuated used as a single dose	Inactivated imported from Japan; live attenuated imported from China
North Korea	All children	Inactivated Nakayama mouse brain	—	3.5 million doses manufactured locally
South Korea	All children	Inactivated Nakayama mouse brain; live attenuated SA14-14-2 has also been used in trials	2 doses, 1 to 4 wks apart at age 3 yrs; boosters at 1 yr and every 2 yrs up to age 14	6 million doses of inactivated manufactured locally; live attenuated imported from China
Taiwan	All children	Inactivated Nakayama mouse brain	2 doses 2 wks apart at age 15–27 mos; boosters at 1 yr and age 6 yrs	1.5 million doses manufactured locally
Thailand	Targeted to children in high-risk areas; incorporated into EPI* in northern provinces	Inactivated Nakayama mouse brain	2 doses up to 4 wks apart at age 18–24 mos; booster at 1 yr and age 7 yrs	1–2 million doses manufactured in country
Vietnam	Targeted to children in high-risk areas; being incorporated into EPI	Inactivated Nakayama mouse brain	2 doses 1 wk apart in children aged 1–5 yrs; booster 1 yr later	7 million doses manufactured in Hanoi in 2002 (previously 2 million annually)

Adapted from Monath[169] and updated following the Global Alliance for Vaccines and Immunizations, Southeast Asia and Western Pacific Regional Working Group's Japanese Encephalitis Meeting: Setting the Global Agenda on Public Health Solutions and National Needs, Bangkok, Thailand 2002.

The following countries have no vaccination policy: Bangladesh, Brunei, Cambodia, Indonesia, Laos, Myanmar (Burma), and Philippines .

EPI = Expanded Program on Immunization; PHK = primary hamster kidneys.

Table 10-5. **Summary of Information for Travelers**

General measures
In the evenings, travelers should wear clothing with long sleeves and trousers and use insect repellent containing DEET to minimize the risk of mosquito bites. They should stay in air-conditioned or screened rooms, or use bed nets, aerosol insecticides, and mosquito coils.
Indications for vaccination
BIKEN formalin-inactivated vaccine is recommended for travelers spending prolonged periods in rural areas where JE is endemic or epidemic (see Table 10-2), or those on shorter trips if likely to include extensive outdoor evening and nighttime exposure to biting mosquitoes in rural areas. Previously, "prolonged" was specified as a month or more, but because in some parts of Asia the epidemiology is not well described and JE has occurred in short-term travelers, recent guidelines have been less specific, and some authors have recommended more liberal use of vaccine.*
Primary dosage schedules
• Preferred regimen: three 1.0 mL doses of BIKEN formalin-inactivated vaccine given subcutaneously on days 0, 7, and 30 • Accelerated 2 week regimen if traveling soon: three 1.0 mL doses of formalin-inactivated vaccine given on days 0, 7, and 14 (this regimen gives near 100% protection but lower antibody titers) • One-week regimen: two 1.0 mL doses of formalin-inactivated vaccine given on days 0 and 7 (gives seroconversion in 80% of recipients, so is probably better than nothing)
For any regimen, the last dose should be given at least 10 days before travel to allow time for the immune response to develop and access to medical care in the event of a delayed adverse reaction. For infants and children < 3 years, a 0.5 mL dose should be given using the same routes and the same schedules. The vaccine is not recommended in pregnancy unless the risk of acquiring JE outweighs the theoretic risks of the vaccine
Boosters
Recommended at 2 years, then every 2 to 3 years if continuing risk of exposure to JEV (though conclusive data are lacking)
Adverse events
Approximately 20% of vaccinees have local cutaneous or mild systemic reactions (fever, headache, myalgias). Approximately 0.6% of vaccinees have had more serious allergic reactions (urticaria, angioedema, respiratory distress, anaphylaxis) that respond to adrenaline, antihistamines, or steroids. Vaccinees should be observed for 30 minutes after vaccination. Those with a history of urticaria or allergic reactions are at greater risk of adverse events. Severe neurologic adverse events are very rare (approximately 1 per million doses).

Adapted from Shlim DR and Solomon T[1] and the US Centers for Disease Control.[37,38]
DEET = N,N-diethyl-3-methylbenzamide; JEV = Japanese encephalitis vaccine.

Because of the theoretic risk to the fetus, vaccine is not normally recommended in pregnant women unless there is thought to be a strong risk of infection.

Efficacy

Two randomized double-blind placebo-controlled trials have assessed the efficacy of formalin-inactivated JE vaccines. In 1965, nearly 134,000 individuals in Taiwan were given either a single dose (22,000) or a double dose (112,000) of the Nakayama vaccine (a less purified form than today's vaccine), and nearly 132,000 children were given tetanus toxoid as placebo. The JE attack rates per 100,000 recipients were 18.2 in the placebo group, 9.0 in the single-dose group, and 3.6 in the double-dose group. Thus, a single dose of vaccine yielded 50% (95% CI: 26% to 88%) efficacy, and two doses gave 80% (95% CI: 71% to 93%) efficacy. The BIKEN JE vaccine was assessed in northern Thailand in the 1980s.[59] Children aged 1 year or older, in three groups of approximately 22,000, received two doses (1 week apart) of the BIKEN Nakayama vaccine, two doses of bivalent Nakayama/Beijing-1 vaccine, or tetanus toxoid as placebo. After a 2-year observation period, the JE attack rate in the placebo group was 51 per 100,000, whereas

in both vaccine groups it was 5 per 100,000, giving a protective efficacy of 91% (95% CI: 70% to 90%).[59] These results were accepted by the United States Food and Drug Administration as evidence for efficacy, leading to licensure of the BIKEN vaccine.[38] Interestingly, during the follow-up period, the risks of dengue and dengue hemorrhagic fever were slightly lower in the vaccinated groups, which does not support the theoretic possibility that vaccination against JE might increase the risk of severe dengue disease (as discussed in "Effects of Heterologous Antiflavivirus Antibodies").

Adverse Events

Local and Nonspecific Adverse Events. In studies in the United States, Thailand, and the United Kingdom, approximately 20% of individuals reported localized tenderness, redness, or swelling, and 5 to 10% reported mild systemic side effects (headache, low-grade fever, myalgias, malaise, and gastrointestinal symptoms).[59,176,178,181,183,184] The incidence of these adverse events decreased with each dose in the three-dose primary vaccine series.

Neurologic Adverse Events. Because of the vaccine's neural-tissue substrate (mouse brain) there has always been a concern that an immune response raised against mouse neural tissue could attack the human nervous system causing autoimmune-type conditions such as acute disseminated encephalomyelitis (ADEM), Guillain-Barré syndrome, or related neuritis, polyneuritis, and demyelinating diseases. As discussed previously, the current purification procedures ensure that the amount of myelin basic protein in the vaccine is below the limit of detection (< 2 ng/mL), though there are no reported data on measurements of other neural proteins known to be associated with ADEM, such as proteolipid protein and myelin-oligodendrocyte glycoprotein. Experimental infection of guinea pigs and cynomolgus monkeys with 50 times the normal dose of vaccine did not result in clinical or histopathologic evidence of encephalomyelitis. Furthermore, as outlined later in this chapter, data from clinical studies suggest that the risk of serious neurologic adverse events is about one in a million, which is comparable to that for other vaccines, such as measles.

Most attempts at early monitoring of adverse events were simply documented cases, with no attempt at examining a control group. Eight neurologic reactions (mostly neuritis) were reported among 53,000 American soldiers vaccinated in 1945 with a crude, inactivated vaccine on Okinawa island, Japan, but similar cases occurred among nonvaccinees.[63] In Japan, a countrywide survey between 1957 and 1966 found 26 temporally-related events (meningitis, convulsions, demyelinating disease, polyneuritis), but the rates of vaccination and comparison with controls were not available.[171] Surveillance data from the manufacturers in Japan from 1965 through to 1989 suggested neurologic adverse events rates in children of 1 to 2.2 per million doses.[171,185,186] In the 1990s, following two ADEM cases temporally related to vaccination, a retrospective survey of 162 Japanese medical institutions identified 7 further cases over 22 years and estimated the incidence to be less than one per million vaccinees, though the denominator of vaccine recipients was poorly defined.[186,187] Similarly, ADEM following JE vaccination in a Danish traveler prompted a review of the national database for further cases.[185] This identified 2 additional cases in adults and gave an estimated risk of 1 in 50,000 to 1 in 75,000 vaccinees, far greater than in any other report.[188] In total, 16 ADEM cases were reported between 1992 and 1996 from Japan, South Korea, and Denmark (identified by passive reporting and retrospective case finding). Guillain-Barré, optic neuritis, and Bell's palsy have also on occasion been reported following JE vaccination, but the causal relationship is uncertain.

Hypersensitivity Reactions. As the formalin-inactivated JE vaccine became available to travelers from Europe, North America, and Australia, hypersensitivity reactions not previously reported were described.

These consisted of urticaria, angioedema, and bronchospasm. The incidence is reported to be 2 to 6 per 1,000 vaccinees in travelers and military personnel, whereas a study in Korean children gave a risk factor of 0.3 per 1,000 for similar reactions.[183,189–192] A review of vaccine administration data from Okinawa in 1945 suggests that allergic side effects were also seen there.[63] In a prospective study of nearly 15,000 US marines, the median time interval between immunization and onset of symptoms was 16 to 24 hours after the first dose and 96 hours after the second dose, though it could occur up to 14 days later.[189,190] Reactions could occur after the second or third dose even if the first dose had been given uneventfully. Most cases respond to outpatient treatment with antihistamines or corticosteroids, but hospitalization and intravenous steroids have been required. Three deaths due to anaphylaxis, or possibly a cardiovascular collapse syndrome with a different pathogenesis, have been attributed to the vaccine.[169,193,194] Other allergic phenomena reported include transient generalized pruritus and, rarely, erythema multiforme, erythema nodosum, and serum sickness–like disease with joint manifestations. Because of the risk of adverse reactions, vaccinees should be observed for 30 minutes after vaccination, and immunization should be completed at least 10 days before departure.[38]

The cause of these allergic reactions is not known. Numerous lots from different manufacturers have been implicated. Case-control studies have indicated an increased risk in those with a history of allergic disorders, such as urticaria and rhinitis, or of asthma; female sex; and young (adult) age.[189,191] Alcohol consumption in the prior 48 hours might also be implicated.[195] IgE antibodies to gelatin (used as a vaccine stabilizer) were demonstrated in three Japanese children with systemic allergic reactions, whereas IgG against gelatin appeared to be more important in those with later cutaneous reactions.[196,197]

The risk of adverse events was assessed by examining the postmarketing surveillance data from Japan and the United States. The rate of total adverse events per 100,000 doses was 2.8 in Japan and 15.0 in the United States. In Japan, 17 neurologic disorders were reported from April 1996 to October 1998, for a rate of 0.2 per 100,000 doses. In the United States, no serious neurologic adverse events temporally associated with JE vaccine were reported from January 1993 to June 1999. Rates for systemic hypersensitivity reactions were 0.8 and 6.3 per 100,000 doses in Japan and the United States, respectively. Data passively collected by the United States' Vaccine Adverse Events Reporting System (VAERS) indicate that characteristic hypersensitivity reactions with a delayed onset continue to occur among JE vaccine recipients.[198]

Inactivated Cell Culture–Derived Vaccines

Because of the limitations of the formalin-inactivated vaccines grown in mouse brain (cost, complexity of production, and concerns over adverse reactions) and the desire to improve immunogenicity, attention has focused on inactivated vaccines grown in cell culture. A variety of JEV strains have been used in a range of different tissues.

Studies in China showed primary hamster kidney cells (PHK cells, primary cell cultures derived from the kidneys of Syrian golden hamsters) gave the highest yield of JEV.

A formalin-inactivated vaccine produced from growing the P3 strain of JEV in PHK cells has been used in China since the 1960s and for many years was the country's principal JE vaccine.[48] The vaccine, which is not purified, is stabilized with 0.1% human serum albumin and presented as a liquid formulation. The primary course consists of two 0.5 mL subcutaneous doses given 1 week apart to children age 6 to 12 months, then boosters at 1 year, school entry, and age 10 years. After primary immunization, 60 to 70% of children have seroconverted, and booster doses elicit good recall immunity.[169] In five randomized field trials in

China involving a total of 480,000 children, the vaccine's efficacy ranged from 76 to 95%.[48] However, the need for repeated booster doses and its relatively low efficacy have meant that the vaccine is gradually being replaced with the live attenuated vaccine (see below).

Vaccines grown in Vero cells have also been developed. Vero is a continuous cell line derived from African green monkeys and is a conventional substrate for vaccine production with the advantage of quality, absence of animal proteins and allergens, and lower cost. One such vaccine, a formalin-inactivated P3 virus grown in Vero cells, has recently been licensed in China, and 200,000 doses were produced in 2001.[199] A similar vaccine developed by Aventis Pasteur reached clinical trials but was discontinued because of nonspecific febrile reactions, the cause of which was not known.[169] An inactivated Vero cell Beijing-1 strain vaccine is being developed by two Japanese companies, and a Vero cell–derived inactivated vaccine based on the live attenuated SA14-14-2 strain (see below) is being developed by the Walter Reed Army Institute of Research (WRAIR) in the United States.[200] Because this uses an attenuated rather than a virulent virus, production is easier, requiring only biosafety level 2 rather than level 3 facilities. In clinical trials, immune responses were disappointing after primary immunization (40 to 70%) but better after a booster dose.[169]

Live Attenuated SA14-14-2 Vaccine

The accomplishment by Dr. Yu Yong Xin and colleagues in developing a live attenuated vaccine against JEV has been likened to Max Theiler's Nobel prize–winning derivation of the 17D yellow fever vaccine strain.[73,201] Live attenuated SA14-14-2 vaccine was licensed in China in 1988, and more than 200 million doses have been delivered since then with an excellent record of safety and efficacy.

Vaccine Development

To produce a live attenuated JEV strain, wild-type strains were passaged empirically in a range of cell culture systems, including PHK, chick embryo (CE), and mouse embryo skin cells. A lack of virulence in mice, hamsters, or pigs suggested the possibility of safe use in humans. JEV strain SA14 was isolated from *Culex pipiens* larvae collected in Xian, China, in 1954.[201] It was passaged 11 times in weanling mice, then 100 times in PHK cells, at which stage it was no longer neurovirulent in monkeys but was not stable. To produce a stable, avirulent virus, it was then inoculated intraperitoneally into mice, harvested from the spleen, plaque-purified further in CE cells, and passaged subcutaneously in mice and orally in hamsters before further purification in PHK cells.[48] The resultant strain, SA14-5-3, did not revert to virulence after intracerebral passage in suckling mice and was still immunogenic. It was safe in humans but in large field trials in southern China had poor immunogenicity in flavivirus-naive subjects. To increase immunogenicity, the virus was therefore passaged subcutaneously in suckling mice five times and twice plaque-purified on PHK cells to produce strain 14-14-2, which was equally attenuated. SA14-14-2 is more immunogenic for mice than inactivated PHK vaccines and was protective against challenge experiments with JEV strains from Thailand, Indonesia, and Vietnam, which represented diverse genotypes.[202] The vaccine is produced from seed virus by infecting PHK cells and is manufactured as a freeze-dried product stabilized with gelatin and sorbitol. After reconstitution with normal saline, the vaccine must be used within 4 hours.

Biologic Characteristics. The virus neurovirulence in animals has been studied extensively. Compared with the parental SA14 strain, SA14-14-2 is attenuated in immunocompetent mice, hamsters, and nude and cytoxan-treated mice and in monkeys inoculated by the intrathalamic and intraspinal routes.[48] The

virus replicates in C6/36 mosquito cells and also in *Culex tritaeniorhyncus* and other mosquitoes.[203] As for any live virus, one safety concern is the possibility of continuous circulation and reversion to a virulent form. There is a theoretic possibility that by feeding on a viremic, recently vaccinated human, a mosquito could become infected with SA14-14-2, and replicating in the mosquito, the strain could revert to virulence. However, the evidence suggests that this is unlikely to occur. Human viremias following vaccination are likely to be below the oral infection threshold of mosquitoes, and a related vaccine strain (SA-14-2-8) did not infect *Culex tritaeniorhyncus* orally.[169,204]

Genetic Basis of Attenuation. Although there are many silent mutations and amino acid changes between SA14-14-2 and the parental strain SA14, comparison with two other attenuated strains from earlier in the vaccine's development (SA14-2-8 and SA14-5-3) suggests that eight common amino acid changes from SA14 are important in attenuation; these include six changes in the virus's E protein (E107, E138, E176, E279, E315, E439) and single changes in nonstructural proteins NS2B, NS3, and NS4. Plotting the E-protein mutations onto the three-dimensional model of the flavivirus envelope protein showed they occurred in domains I, II, and III.[21,169] To examine which combination of E-protein changes might be important in attenuation, the mutations were reverted to wild-type sequence singly or in combinations using an infectious chimeric clone that incorporates the structural genes of SA14-14-2 into the yellow fever 17D backbone (see "Chimeric Yellow Fever–JE Vaccine").[205] Virulence was assessed by intracerebral inoculation of mice. These studies showed that attenuation depended on at least three or four mutations, making it extremely unlikely that the virus would revert to a virulent form.[169]

Immunogenicity, Efficacy, and Adverse Events

SA14-14-2 vaccine has undergone many clinical trials in China and, more recently, South Korea and Nepal. After a single dose of SA14-14-2, 85 to 100% of children had seroconverted, but two doses given 1 to 3 months apart gave 99 to 100% seroconversion with higher geometric mean titers.[48,201,206,207] This regimen allows full protection of infants born before or during the summer transmission season and is compatible with incorporation into the Expanded Program on Immunization (EPI) schedule at 9 and 12 months of age. Currently, a third dose at school entry is administered in China, but it is not certain that this is necessary.

The vaccine's efficacy was demonstrated in five open-label field studies in China between 1988 and 1993 that involved nearly 600,000 children.[48] Comparisons of the incidence of JE in vaccinated and unvaccinated children showed a protective efficacy of approximately 98%. These findings were confirmed in a more rigorous, relatively simple and inexpensive postlicensure case-control study in which the prevalence of immunization was compared between 56 JE cases and 1,299 age- and village-matched controls.[208] The effectiveness of one dose was 80% (95% CI: 44% to 93%) and of two doses 1 year apart, 97.5% (CI: 86% to 99.6%). The efficacy of single-dose vaccine given just before the JE season was assessed in a similar case-control study in Nepal in 1999, when approximately 160,000 children were vaccinated.[209] None of 20 JE cases had received vaccine, compared with 326 of 557 age- and sex-matched village controls, giving a protective efficacy of 99.3% (CI: 94.9% to 100%). Interestingly, the data suggested protection occurred a median 2 weeks after vaccination. The mechanism of this early protection is unknown, and whether this single-dose vaccination with SA14-14-2 gives longer term protection is under investigation.

In the field studies involving more than 600,000 children, the vaccine had a very low incidence of side effects, which included fever, rash, nausea, and dizziness. Fever occurred in less than 5 per 10,000 recipients.[48]

Vaccine safety was also assessed in a postlicensure, randomized, placebo-controlled trial in 26,000 children.[210] One month after vaccination, the two groups had similar rates of hospitalization and illness. There were no cases of postvaccine anaphylaxis or neurologic disease.

The Vaccine's Future

SA14-14-2 vaccine has not had regulatory approval outside of China because of concerns about the PHK substrate, which is not an accepted cell line for vaccine production; uncertainty about the quality control tests for adventitious agents; and other issues related to good manufacturing practice (GMP). However, the WHO has recently developed guidelines to facilitate the international acceptance of the vaccine.[73] The key issues identified are testing of the hamster colonies (which should preferably be closed, "specified pathogen–free" colonies) and testing of the vaccine seeds to prove freedom from adventitious agents. In addition, evidence of continued attenuation for batches in animal tests and evidence of phenotypic stability will be important. Concerns have also been expressed about documentation of the raw materials, including bovine serum and hamster cells, used in the production of the original seed virus. These might be harder to address retrospectively, but given the vaccine's efficacy and apparent safety and the more experience with the vaccine grows, the less important these are likely to be.

COST-EFFECTIVENESS OF JE VACCINATION IN ASIA

Cost effectiveness analyses of JE vaccination have been conducted in Thailand and China.[211,212] In Thailand, it was estimated that incorporating the inactivated vaccine into routine immunization at 18 months (at a cost of $2.28 (US) per person) would prevent 124 cases (per 100,000 people), with a cost-effectiveness of $15,715, and would save $72,922 (in treatment costs, disability care, and loss of future earnings) for each prevented JE case. JE vaccination was thought to be worth implementing unless the incidence was below 3 per 100,000 population.[211] In Shanghai, China, a cost-effectiveness analysis estimated that immunization with inactivated P3 vaccine would prevent 420 JE cases and 105 deaths, saving 6,456 disability-adjusted life years (DALYs) per 100,000 people. The live attenuated SA14-14-2 vaccine would prevent a similar number of cases and deaths. Both vaccines resulted in costs savings compared with no vaccination, but the live vaccine would result in a greater cost saving ($512,456 per 100,000 people versus $348,246) because it is cheaper to produce.[212]

VACCINES IN DEVELOPMENT

Newer JE vaccines in development include genetically engineered recombinant vaccines, in which the JEV structural genes are delivered by established vaccine strains and DNA vaccines.

Vaccinia-Vectored Vaccines

Replication-deficient canarypox (ALVAC) and highly attenuated vaccinia viruses (NYVAC) have been used as vectors for delivering *PrM-E* or *PrM-E-NS1* genes.[213,214] The recombinant vaccines induced protective immunity in mice and monkeys in challenge experiments and proceeded to clinical studies. Because of the possibility that prior vaccination against smallpox would limit the response to these pox-vectored vaccines, the recombinant vaccines were tested in vaccinia-immune and vaccinia-naive individuals.[215]

ALVAC-JE was poorly immunogenic in all subjects. In contrast, NYVAC-JE elicited neutralizing antibody and T-cell responses in vaccinia-naive recipients but not in those that had previously been vaccinated against smallpox. Because the main commercial interest was for immunization of adult travelers, approximately half of whom were likely to be vaccinia immune, development of the vaccine has not continued.[169]

Chimeric Yellow Fever–JE Vaccine

In an alternative approach, the *PrM-E* genes of attenuated JEV strain SA14-14-2 were inserted into an infectious clone of the 17D yellow fever vaccine strain.[216] The chimeric virus (ChimeriVax-JE; Acambis, Cambridge, UK) replicated efficiently in vitro and was shown to be immunogenic, efficacious, and safe in mice and nonhuman primates, being even more attenuated than the original 17D yellow fever strain.[217,218] Attenuation of the chimeric virus was shown to depend on clusters of at least three of the six amino acid changes in the E protein.[205] The chimeric virus was incapable of infecting mosquitoes by oral feeding and had reduced replication after indirect intrathoracic inoculation, allaying fears of secondary transmission after vaccination and suggesting that it is unlikely to be transmitted by mosquitoes' biting recently vaccinated individuals.[203] The vaccine has been given to 12 human volunteers in a phase I trial and was shown to be safe and immunogenic.[219] Interestingly, in both humans and monkeys, prior yellow fever immunity did not reduce the response to the chimeric vaccine.[217] A similar approach using the same 17D yellow fever virus backbone is being used to develop chimeric vaccines against West Nile virus and dengue (see Chapter 13, "Dengue Fever Vaccine").[220]

DNA Vaccine

Plasmid DNA vaccines containing JEV *PrM-E* genes under the control of a cytomegalovirus promoter have produced promising results in mice and swine. In mice, intramuscular or intradermal inoculation with two doses of plasmid DNA produced neutralizing antibodies, T-cell memory, and CD8+ cytotoxic T-cell responses against the E protein, and protected against lethal JE challenge.[221] In swine, two intramuscular doses produced high antibody titers and high anamnestic responses to challenge with live attenuated virus.[222] Another vaccine in development includes the secretory signal sequence derived from tissue plasminogen activator fused to either the full-length or partial JEV envelope protein gene. Cells transfected with the latter construct secreted E protein and produced better protection against intracerebral challenge in mice.[223]

CONCLUDING COMMENTS

Since outbreaks of encephalitis were first recognized in Japan in the 1870s, the story of JE has been one of remarkable achievements in virology and vaccine development against a disease that continues to spread and for which there is no antiviral treatment. In some parts of Asia, these achievements have translated into public health policy and have had a large impact on disease control, whereas in developing countries, JE is still a major cause of morbidity and death. For travelers, uncertainties still remain about the risk-benefit ratio of the inactivated JE vaccine. The development of newer, safe vaccines might make these decisions easier for travelers, but there is a need to ensure that such vaccines reach those most in need of them.

ACKNOWLEDGMENTS

I thank David Vaughn, Scott Halstead, Julie Jacobson, and Jane Cardosa for helpful discussions and all the participants of the Global Alliance for Vaccines and Immunizations, Southeast Asia and Western Pacific Regional Working Group's Japanese Encephalitis Meeting: Setting the Global Agenda on Public Health Solutions and National Needs, Bangkok, Thailand 2002, for regional updates on Japanese encephalitis disease burden and vaccination policy. Some of the work described was funded by the Wellcome Trust of Great Britain.

REFERENCES

1. Shlim DR, Solomon T. Japanese encephalitis vaccine for travelers. Exploring the limits of risk. Clin Infect Dis 2002;35:183–8.
2. Tsai TF. Factors in the changing epidemiology of Japanese encephalitis and West Nile fever. In: Saluzzo JF, Dodet B, editors. Factors in the emergence of arbovirus diseases. Paris: Elsevier; 1997. p. 179–89.
3. Solomon T. Viral encephalitis in Southeast Asia. Neurol Infect Epidemiol 1997;2:191–9.
4. Barker F, Bird GA. The Japanese earthquake, notes from Koby on the medical history. BMJ 1924:469.
5. Anonymous. Infectious diseases abroad: outbreak in Japan of a disease resembling encephalitis lethargica. Lancet 1924:1251–2.
6. Kaneko R, Aoki Y. Uber die Encephalitis epidemica in Japan. Engebrisse der Inneren Medizin Kinderheilkunde 1928;34:342–456.
7. Miyake M. The pathology of Japanese encephalitis. Bull World Health Organ 1964;30:153–60.
8. Solomon T. Recent advances in Japanese encephalitis. J Neurovirol 2003;9:274–83.
9. Solomon T, Whitley RJ. Arthropod-borne viral encephalitides. In: Scheld M, Whitley RJ, Marra C, editors. Infections of the central nervous system. Philadelphia, PA: Lippincott Williams and Wilkins; 2004. p.
10. Solomon T. Viral haemorrhagic fevers. In: Cook G, Zumlar A, editors. Manson's tropical diseases. London: WB Saunders; 2002. p. 773–93.
11. Solomon T, Mallewa MJ. Dengue and other emerging flaviviruses. J Infect 2001;42:104–15.
12. Zanotto PM, Gould EA, Gao GF, et al. Population dynamics of flaviviruses revealed by molecular phylogenies. Proc Natl Acad Sci U S A 1996;93:548–53.
13. Gould EA, Zanotto PM, Holmes EC. The genetic evolution of flaviviruses. In: Saluzzo JF, Dodet B, editors. Factors in the emergence of arbovirus diseases. Paris: Elsevier; 1997. p. 51–63.
14. Tsai TF, Popovici F, Cernescu C, et al. West Nile encephalitis epidemic in southeastern Romania. Lancet 1998;352:767–71.
15. Petersen LR, Marfin AA. West Nile virus. A primer for the clinician. Ann Intern Med 2002;137:173–9.
16. Chambers TJ, Hahn CS, Galler R, Rice CM. Flavivirus genome organisation, expression and replication. Annu Rev Microbiol 1990;44:649–88.
17. Stadler K, Allison SL, Schalich J, Heinz FX. Proteolytic activation of tick-borne encephalitis virus by furin. J Virol 1997;71:8475–81.
18. Chen Y, Maguire T, Hileman RE, et al. Dengue virus infectivity depends on envelope protein binding to target cell heparan sulphate. Nat Med 1997;3:866–71.
19. Su CM, Liao CL, Lee YL, Lin YL. Highly sulfated forms of heparin sulfate are involved in Japanese encephalitis virus infection. Virology 2001;286:206–15.
20. Roehrig JT, Hunt AR, Johnson AJ, Hawkes RA. Synthetic peptides derived from the deduced amino acid sequence of the E-glycoprotein of Murray Valley encephalitis virus elicit antiviral antibody. Virology 1989;171:49–60.
21. Rey FA, Heinz FX, Mandl C, et al. The envelope glycoprotein from tick-borne encephalitis virus at 2Å resolution. Nature 1995;375:291–8.
22. Lescar J, Roussel A, Wien MW, et al. The fusion glycoprotein shell of Semliki Forest virus. An icosahedral assembly primed for fusogenic activation at endosomal pH. Cell 2001;105:137–48.
23. Heinz FX, Allison SL. The machinery for flavivirus fusion with host cell membranes. Curr Opin Microbiol 2001;4:450–5.
24. Kobayashi Y, Hasegawa H, Oyama T, et al. Antigenic analysis of Japanese encephalitis virus by using monoclonal antibodies. Infect Immun 1984;44:117–23.
25. Hasegawa H, Yoshida M, Fujita S, Kobayashi Y. Comparison of structural proteins among antigenically different Japanese encephalitis virus strains. Vaccine 1994;12:841–4.

26. Chen WR, Tesh RB, Rico-Hesse R. Genetic variation of Japanese encephalitis virus in nature. J Gen Virol 1990;71:2915–22.
27. Chen WR, Rico-Hesse R, Tesh RB. A new genotype of Japanese virus from Indonesia. Am J Trop Med Hyg 1992;47:61–9.
28. Uchil PD, Satchidanandam V. Phylogenetic analysis of Japanese encephalitis virus. Envelope gene based analysis reveals a fifth genotype, geographic clustering, and multiple introductions of the virus into the Indian subcontinent. Am J Trop Med Hyg 2001;65:242–51.
29. Williams DT, Wang LF, Daniels PW, Mackenzie JS. Molecular characterization of the first Australian isolate of Japanese encephalitis virus, the FU strain. J Gen Virol 2000;81:2471–80.
30. Solomon T, Ni H, Beasley DW, et al. Origin and evolution of Japanese encephalitis virus in Southeast Asia. J Virol 2003;77:3091–8.
31. Solomon T, Ni H, Beasley DW, et al. The origin and evolution of Japanese encephalitis virus. New evidence from the fourth genotype, ICID 2002. 2002.
32. Johnsen DO, Edelman R, Grossman RA, et al. Study of Japanese encephalitis virus in Chiangmai Valley, Thailand. V. Animal infections. Am J Epidemiol 1974;100:57–68.
33. Rodrigues FM, Guttikar SN, Pinto BD. Prevalence of antibodies to Japanese encephalitis and West Nile viruses among wild birds in the Krishna-Godavari Delta, Andhra Pradesh, India. Trans R Soc Trop Med Hyg 1981;75:258–62.
34. Hanna J, Ritchie S, Phillips DA, et al. An outbreak of Japanese encephalitis in the Torres Strait, Australia, 1995. Med J Aust 1996;165:256–60.
35. Rosen L. The natural history of Japanese encephalitis virus. Annu Rev Microbiol 1980;40:395–414.
36. Wuryadi S, Suroso T. Japanese encephalitis in Indonesia. Southeast Asian J Trop Med Public Health 1989;20:575–80.
37. Centers for Disease Control and Prevention. Encephalitis, Japanese. In: CDC, editor. Health information for international travel 2003-2004. Atlanta (GA): CDC; 2003.
38. Centers for Disease Control and Prevention. Inactivated Japanese encephalitis virus vaccine. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Morb Mortal Wkly Rep 1993;42:1–14.
39. Vaughn DW, Hoke CH. The epidemiology of Japanese encephalitis. Prospects for prevention. Epidemiol Rev 1992;14:197–221.
40. Igarashi A, Tanaka M, Morita K, et al. Detection of West Nile and Japanese encephalitis viral genome sequences in cerebrospinal fluid from acute encephalitis cases in Karachi, Pakistan. Microbiol Immunol 1994;38:827–30.
41. Zimmerman MD, Scott RM, Vaughn DW, et al. Short report. An outbreak of Japanese encephalitis in Kathmandu, Nepal. Am J Trop Med Hyg 1997;57:283–4.
42. Akiba T, Osaka K, Tang S, et al. Analysis of Japanese encephalitis epidemic in western Nepal in 1997. Epidemiol Infect 2001;126:81–8.
43. Hammon WM, Tiggert WD, Sather GE. Epidemiologic studies of concurrent "virgin" epidemics of Japanese B encephalitis and mumps on Guam, 1947–1948, with subsequent observations including dengue through 1957. Am J Trop Med Hyg 1958;67:441–67.
44. Paul WS, Moore PS, Karabatsos N, et al. Outbreak of Japanese encephalitis on the island of Saipan, 1990. J Infect Dis 1993;167:1053–8.
45. Paterson PY, Ley HL Jr, Wisseman CL Jr, et al. Japanese encephalitis in Malaya. Am J Hyg 1952;56:320–33.
46. Anonymous. Japanese encephalitis on the Australian mainland. Commun Dis Intell 1998;22:80.
47. Olson JG, Atmosoedjono S, Lee VH, Ksiazek TG. Correlation between population indices of *Culex tritaeniorhynchus* and *Cx. gelidus* (*Diptera: Culicidae*) and rainfall in Kapuk, Indonesia. J Med Entomol 1983;20:108–9.
48. Tsai TF, Chang J, Yu XX. Japanese encephalitis vaccines. In: Plotkin SA, Orenstein WA, editors. Vaccines. Philadelphia: WB Saunders; 1999. p. 672–710.
49. Gajanana A, Rajendran R, Samuel PP, et al. Japanese encephalitis in south Arcot district, Tamil Nadu, India. A three-year longitudinal study of vector abundance and infection frequency. J Med Entomol 1997;34:651–9.
50. Gajanana A, Thenmozhi V, Samuel PP, Reuben R. A community-based study of subclinical flavivirus infections in children in an area of Tamil Nadu, India, where Japanese encephalitis is endemic. Bull World Health Organ 1995;73:237–44.
51. Innis BL. Japanese encephalitis. In: Porterfield JS, editor. Exotic viral infections. London: Chapman and Hall; 1995. p. 147–74.
52. Pyke AT, Williams DT, Nisbet DJ, et al. The appearance of a second genotype of Japanese encephalitis virus in the Australasian region. Am J Trop Med Hyg 2001;65:747–53.
53. Solomon T, Dung NM, Kneen R, et al. Japanese encephalitis. J Neurol Neurosurg Psychiatry 2000;68:405–15.
54. Gingrich J, Nisalak A, Latendresse JR, et al. Japanese encephalitis virus in Bangkok. Factors influencing vector infections in three suburban communities. J Med Entomol 1992;29:426–44.
55. Buescher EL, Schere WF. Ecological studies of Japanese encephaltis in Japan. IX. Epidemiological correlations and conclusions. Am J Trop Med Hyg 1959;8:719–22.

56. Peiris JSM, Amerasinghe FP, Amerasinghe PH, et al. Japanese encephalitis in Sri Lanka—the study of an epidemic. Vector incrimination, porcine infection, and human disease. Trans R Soc Trop Med Hyg 1992;86:307–13.
57. Burke DS, Ussery M, Elwell MR, et al. Isolation of Japanese encephalitis virus strains from sentinel pigs in northern Thailand, 1982. Trans R Soc Trop Med Hyg 1985;79:420–1.
58. Chan YC, Loh TF. Isolation of Japanese encephalitis virus from the blood of a child in Singapore. Am J Trop Med Hyg 1966;15:567–72.
59. Hoke CH, Nisalak A, Sangawhipa N, et al. Protection against Japanese encephalitis by inactivated vaccines. N Engl J Med 1988;319:608–14.
60. Halstead SB, Grosz CR. Subclinical Japanese encephalitis. I. Infection of Americans with limited residence in Korea. Am J Hyg 1962;75:190–201.
61. Huang CH. Studies of Japanese encephalitis in China. Adv Virus Res 1982;27:71–101.
62. Umenai T, Krzysko R, Bektimorov TA, Assaad FA. Japanese encephalitis. Current worldwide status. Bull World Health Organ 1985;63:625–31.
63. Sabin AB. Epidemic encephalitis in military personnel. Isolation of Japanese B virus on Okiowa in 1945, serologic diagnosis, clinical manifestations, epidemiological aspects, and use of mouse brain vaccine. JAMA 1947;13:281–93.
64. Dickerson RB, Newton JR, Hansen JE. Diagnosis and immediate prognosis of Japanese B encephalitis. Am J Med 1952;12:277–88.
65. Lincoln AF, Silvertson SE. Acute phase of Japanese B encephalitis. Two hundred and one cases in American soldiers, Korea 1950. JAMA 1952;150:268–73.
66. Richter RW, Shimojyo S. Neurologic sequelae of Japanese B encephalitis. Neurology 1961;11:553–9.
67. Ketel WB, Ognibene AJ. Japanese B encephalitis in Vietnam. Am J Med Sci 1971;261:271–9.
68. Burke DS, Nisalak A. Detection of Japanese encephalitis virus immunoglobulin M antibodies in serum by antibody capture radioimmunoassay. J Clin Microbiol 1982;15:353–61.
69. Bundo K, Igarashi A. Antibody-capture ELISA for detection of immunoglobulin M antibodies in sera from Japanese encephalitis and dengue hemorrhagic fever patients. J Virol Methods 1985;11:15–22.
70. Solomon T, Thao LTT, Dung NM, et al. Rapid diagnosis of Japanese encephalitis by using an IgM dot enzyme immunoassay. J Clin Microbiol 1998;36:2030–4.
71. Cuzzubbo AJ, Endy TP, Vaughn DW, et al. Evaluation of a new commercially available immunoglobulin M capture enzyme-linked immunosorbant assay for diagnosis of Japanese encephalitis infections. J Clin Microbiol 1999;37:3738–41.
72. Grossman RA, Edelman R, Chiewanich P, et al. Study of Japanese encephalitis virus in Chiangmai valley, Thailand. II. Human clinical infections. Am J Epidemiol 1973;98:121–32.
73. Tsai TF. New initiatives for the control of Japanese encephalitis by vaccination. Minutes of a WHO/CVI meeting, Bangkok, Thailand, 13–15 October 1998. Vaccine 2000;18 Suppl 2:1–25.
74. Trillin C. American chronicles. The New Yorker 1985:61-94.
75. Benenson MW, Top FH, Gresso W, et al. The virulence to man of Japanese encephalitis virus in Thailand. Am J Trop Med Hyg 1975;24:974–80.
76. MacDonald WBG, Tink AR, Ouvrier RA, et al. Japanese encephalitis after a two-week holiday in Bali. Med J Aust 1989;150:558–66.
77. Wittesjö B, Eitrem R, Niklasson B, et al. Japanese encephalitis after a 10-day holiday in Bali. Lancet 1995;345:856.
78. Buhl MR, Black FT, Andersen PL, Laursen A. Fatal Japanese encephalitis in a Danish tourist visiting Bali for 12 days. Scand J Infect Dis 1996;28:189.
79. Basnyat B, Zimmerman MD, Shrestha Y, et al. Persistent Japanese encephalitis in Kathmandu. The need for immunization. J Travel Med 2001;8:270–1.
80. Solomon T, Dung NM, Kneen R, et al. Seizures and raised intracranial pressure in Vietnamese patients with Japanese encephalitis. Brain 2002;125:1084–93.
81. Kumar R, Mathur A, Kumar A, et al. Clinical features and prognostic indicators of Japanese encephalitis in children in Lucknow (India). Indian J Med Res 1990;91:321–7.
82. Poneprasert B. Japanese encephalitis in children in northern Thailand. Southeast Asian J Trop Med Public Health 1989;20:599–603.
83. Solomon T, Thao LTT, Dung NM, et al. Clinical features of Japanese encephalitis. Prognostic and pathophysiological significance in 50 patients. Paper presented at the Seventh International Congress for Infectious Diseases, Hong Kong, 1996.
84. Solomon T. Japanese encephalitis. In: Gilman S, Goldstein GW, Waxman SG, editors. Neurobase. Vol. 4. San Diego: Medlink Publishing; 2000.
85. Misra UK, Kalita J. Movement disorders in Japanese encephalitis. J Neurol 1997;244:299–303.

86. Kumar S, Misra UK, Kalita J, et al. MRI in Japanese encephalitis. Am J Med Sci 1997;39:180–4.
87. Murgod UA, Muthane UB, Ravi V, et al. Persistent movement disorders following Japanese encephalitis. Neurology 2001;57:2313–5.
88. Solomon T, Kneen R, Dung NM, et al. Poliomyelitis-like illness due to Japanese encephalitis virus. Lancet 1998;351:1094–7.
89. Kumar R, Agarwal SP, Waklu I, Misra PK. Japanese encephalitis—an encephalomyelitis. Indian Pediatr 1991;23:1525–33.
90. Shoji H, Murakamo T, Murai I, et al. A follow-up study by CT and MRI in 3 cases of Japanese encephalitis. Neuroradiology 1990;32:215–9.
91. Misra UK, Kalita J, Jain SK, Mathur A. Radiological and neurophysiological changes in Japanese encephalitis. J Neurol Neurosurg Psychiatry 1994;57:1484–7.
92. Huang CR, Chang WN, Lui CC, et al. Neuroimages of Japanese encephalitis. Report of 3 patients. Chin Med J (Engl) 1997;60:105–8.
93. Misra UK, Kalita J. A comparative study of Japanese and herpes simplex encephalitis. Electromyogr Clin Neurophysiol 1998;38:41–6.
94. Kimura K, Dosaka A, Hashimoto Y, et al. Single-photon emission CT findings in acute Japanese encephalitis. Am J Neuroradiol 1997;18:465–9.
95. Gourie-Devi M, Deshpande DH. Japanese encephalitis. In: Prasad LS, Kulczycki LL, editors. Paediatric problems. New Delhi: S Chand; 1982. p. 340–56.
96. Leake CJ, Burke DS, Nisalak A, Hoke CH. Isolation of Japanese encephalitis virus from clinical specimens using a continuous mosquito cell line. Am J Trop Med Hyg 1986;35:1045–50.
97. Mathur A, Kumar R, Sharma S, et al. Rapid diagnosis of Japanese encephalitis by immunofluorescent examination of cerebrospinal fluid. Indian J Med Res 1990;91:1–4.
98. Desai A, Shankar SK, Ravi V, Chandramuki A. Japanese encephalitis virus antigen in the brain and its topographical distribution. Acta Neuropathol (Berl) 1995;89:368–73.
99. Clark CH, Casals J. Techniques for hemagglutination inhibition with arthropod viruses. Am J Trop Med Hyg 1958;7:561–73.
100. Innis BL, Nisalak A, Nimmannitya S, et al. An enzyme-linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. Am J Trop Med Hyg 1989;40:418–27.
101. Burke DS, Nisalak A, Ussery MA, et al. Kinetics of IgM and IgG responses to Japanese encephalitis virus in human serum and cerebrospinal fluid. J Infect Dis 1985;151:1093–9.
102. Meiyu F, Huosheng C, Cuihua C, et al. Detection of flavivirus by reverse transcriptase-polymerase chain reaction with the universal primer set. Microbiol Immunol 1997;41:209–13.
103. Hoke CH, Vaughn DW, Nisalak A, et al. Effect of high dose dexamethasone on the outcome of acute encephalitis due to Japanese encephalitis virus. J Infect Dis 1992;165:631–7.
104. Leyssen P, De Clercq E, Neyts J. Perspectives for the treatment of infections with Flaviviridae. Clin Microbiol Rev 2000;13:67–82.
105. Zhang M, Wang M, Jiang S, Ma W. Passive protection of mice, goats, and monkeys against Japanese encephalitis with monoclonal antibodies. J Med Virol 1989;29:133–8.
106. Ma WY, Jiang SZ, Zhang MJ, et al. Preliminary observations on treatment of patients with Japanese B encephalitis with monoclonal antibody. J Med Coll PLA 1992;7:299–302.
107. Waldvogel K, Bossart W, Huisman T, et al. Severe tick-borne encephalitis following passive immunization. Eur J Pediatr 1996;155:775–9.
108. Chen CJ, Raung SL, Kuo MD, Wang YM. Suppression of Japanese encephalitis virus infection by non-steroidal anti-inflammatory drugs. J Gen Virol 2002;83:1897–905.
109. Liao CL, Lin YL, Wu BC, et al. Salicylates inhibit flavivirus replication independently of blocking nuclear factor kappa B activation. J Virol 2001;75:7828–39.
110. Burke DS, Morill JC. Levels of interferon in the plasma and cerebrospinal fluid of patients with acute Japanese encephalitis. J Infect Dis 1987;155:797–9.
111. Harinasuta C, Wasi C, Vithanomsat S. The effect of interferon on Japanese encephalitis virus *in vitro.* 1984;15:564–8.
112. Anderson JF, Rahal JJ. Efficacy of interferon alpha-2b and ribavirin against West Nile virus *in vitro.* Emerg Infect Dis 2002;8:107–8.
113. Harinasuta C, Nimmanitya S, Titsyakorn U. The effect of interferon alpha on two cases of Japanese encephalitis in Thailand. Southeast Asian J Trop Med Public Health 1985;16:332–6.
114. Solomon T, Dung NM, Wills B, et al. Interferon alfa-2a in Japanese encephalitis. A randomised double-blind placebo-controlled trial. Lancet 2003;361:821–6.
115. Burke DS, Lorsomrudee W, Leake CJ, et al. Fatal outcome in Japanese encephalitis. Am J Trop Med Hyg 1985;34:1203–10.

116. Desai A, Ravi V, Guru SC, et al. Detection of autoantibodies to neural antigens in the CSF of Japanese encephalitis patients and correlation of findings with the outcome. J Neurol Sci 1994;122:109–16.
117. Dapeng L, Jindou S, Huijun Y, Renguo Y, Ze W. Prognostic factors of early sequelae and fatal outcome of Japanese encephalitis. Southeast Asian J Trop Med Public Health 1995;26:694–8.
118. Libraty DH, Nisalak A, Endy TP, et al. Clinical and immunological risk factors for severe disease in Japanese encephalitis. Trans R Soc Trop Med Hyg 2002;96:173–8.
119. Misra UK, Kalita J, Srivastava M. Prognosis of Japanese encephalitis. A multivariate analysis. J Neurol Sci 1998;161:143–7.
120. Simpson TW, Meiklehohn G. Sequelae of Japanese B encephalitis. Am J Trop Med Hyg 1947;27:727–31.
121. Kumar R, Mathur A, Singh KB, et al. Clinical sequelae of Japanese encephalitis in children. Indian J Med Res 1993;97:9–13.
122. Huy BV, Tu HC, Luan TV, Lindqvist R. Early mental and neurological sequelae after Japanese B encephalitis. Southeast Asian J Trop Med Public Health 1994;25:549–53.
123. Schneider RJ, Firestone MH, Edelman R, et al. Clinical sequelae after Japanese encephalitis. A one year follow up study in Thailand. Southeast Asian J Trop Med Public Health 1974;5:560–8.
124. Johnston LJ, Halliday GM, King NJ. Langerhans cells migrate to local lymph nodes following cutaneous infection with an arbovirus. J Invest Dermatol 2000;114:560–8.
125. Wu SJ, Grouard-Vogel G, Sun W, et al. Human skin Langerhans cells are targets of dengue virus infection. Nat Med 2000;6:816–20.
126. Monath TP, Cropp CP, Harrison AK. Mode of entry of a neurotropic virus into the central nervous system. Reinvestigation of an old controversy. Lab Invest 1983;48:399–410.
127. Myint KSA, Raengsakulrach B, Young GD, et al. Immunocytochemical detection of Japanese encephalitis (JE) virus antigen in the CNS of rhesus macaques inoculated intranasally with JE virus. Am J Trop Med Hyg 1994;51 Suppl:274.
128. Dropulie B, Masters CL. Entry of neurotropic arboviruses into the central nervous system. An in vitro study using mouse brain endothelium. J Infect Dis 1990;161:685–91.
129. Liou ML, Hsu CY. Japanese encephalitis virus is transported across the cerebral blood vessels by endocytosis in mouse brain. Cell Tissue Res 1998;293:389–94.
130. Liu YF, Teng CL, Liu K. Cerebral cysticercosis as a factor aggravating Japanese B encephalitis. Chin Med J (Engl) 1957;75:1010.
131. Shankar SK, Rao TV, Mruthyunjayana BP, et al. Autopsy study of brains during an epidemic of Japanese encephalitis in Karnataka. Indian J Med Res 1983;78:431–41.
132. Shiraki H. Encephalitis due to arboviruses. Japanese encephalitis. In: Celers RDaJH, editor. Clinical virology. The evaluation and management of human viral infections. Philadelphia: WB Saunders; 1970. p. 155–75.
133. Ni H, Barrett ADT. Molecular differences between wild-type Japanese encephalitis virus strains of high and low mouse neuroinvasiveness. J Gen Virol 1996;77:1449–55.
134. Huang CH, Wong C. Relation of the peripheral multiplication of Japanese B encephalitis virus to the pathogenesis of the infection in mice. Acta Virol 1963;7:322–30.
135. Ni H, Burns NJ, Chang GJ, et al. Comparison of nucleotide and deduced amino acid sequence of the 5′ non-coding region and structural protein genes of the wild-type Japanese encephalitis virus strain SA14 and its attenuated vaccine derivatives. J Gen Virol 1994;75:1505–10.
136. Ni H, Chang GJ, Xie H, et al. Molecular basis of attenuation of neurovirulence of wild-type Japanese encephalitis virus strain SA14. J Gen Virol 1995;76:409–13.
137. Holzmann H, Heinz FX, Mandl C, et al. A single amino acid substitution in envelope protein of tick borne encephalitis virus leads to attenuation in the mouse model. J Gen Virol 1990;64:5156–9.
138. Lee E, Lobigs M. Substitutions at the putative receptor-binding site of an encephalitic flavivirus alter virulence and host cell tropism and reveal a role for glycosaminoglycans in entry. J Virol 2000;74:8867–75.
139. Hasegawa H, Yoshida M, Shiosaka T, et al. Mutations in the envelope protein of Japanese encephalitis virus affect entry into cultured cells and virulence in mice. Virology 1992;191:158–65.
140. Cecilia D, Gould EA. Nucleotide changes responsible for loss of neuroinvasiveness in Japanese encephalitis virus neutralisation-resistant mice. Virology 1991;181:70–7.
141. McMinn PC, Dalgarno L, Weir RC. A comparison of the spread of Murray Valley encephalitis viruses of high or low neuroinvasiveness in the tissues of Swiss mice after peripheral inoculation. Virology 1996;220:414–23.
142. Monath TP, Arroyo J, Levenbook I, et al. Single mutation in the flavivirus envelope protein hinge region increases neurovirulence for mice and monkeys but decreases viscerotropism for monkeys. Relevance to development and safety testing of live, attenuated vaccines. J Virol 2002;76:1932–43.
143. Johnson RT, Burke DS, Elwell M, et al. Japanese encephalitis. Immunocytochemical studies of viral antigen and inflammatory cells in fatal cases. Ann Neurol 1985;18:567–73.

144. Zimmerman HM. The pathology of Japanese B encephalitis. Am J Pathol 1946;22:965–91.

145. Haymaker W, Sabin AB. Topographic distribution of lesions in central nervous system in Japanese B encephalitis. Nature of the lesions with report of a case on Okinawa. Arch Neurol Psychiatry 1947;57:673–92.

146. Li ZS, Hong SF, Gong NL. Immunohistochemical study of Japanese B encephalitis. Chin Med J (Engl) 1988;101:768–71.

147. Liu JL. Protective effect of interferon alpha on mice experimentally infected with Japanese encephalitis virus. Chin J Microbiol 1972;5:1–9.

148. Ghosh SN, Goverdhan MK, Sathe PS, et al. Protective effect of 6-MFA, a fungal interferon inducer against Japanese encephalitis virus in bonnet macaques. Indian J Med Res 1990;91:408–13.

149. Hammon WM, Sather GE. Passive immunity for arbovirus infection. I. Artificially induced prophylaxis in man and mouse for Japanese (B) encephalitis. Am J Trop Med Hyg 1973;22:524–34.

150. Carmenaga DL, Nathonson N, Cole GA. Cyclophosphamide-potentiated West Nile encephalitis. Relative influence of cellular and humoral factors. J Infect Dis 1974;130:634–41.

151. Yu WX, Wang JF, Zheng GM, Li HM. Response of normal and athymic mice to infection by virulent and attenuated Japanese encephalitis virus. Chin J Virol 1985;1:203–9.

152. Jia LL, Zheng A, Yu YX. Study on the immune mechanism of JE attenuated live vaccine (SA_{14}-14-2 strain) in immune inhibited mice. Chin J Immunol Microbiol 1992;12:364.

153. Nathanson N, Cole GA. Fatal Japanese encephalitis virus infection in immunosuppressed spider monkeys. Clin Exp Immunol 1970;6:161–6.

154. Okhuysen PC, Crane JK, Pappas J. St. Louis encephalitis in patients with human immunodeficiency virus infection. Clin Infect Dis 1993;17:140–1.

155. Bukowski JF, Kurane I, Lai CJ, et al. Dengue virus-specific cross-reactive CD8+ human cytotoxic T lymphocytes. J Virol 1989;63:5086–91.

156. McMichael AJ. Cytotoxic T lymphocyte specific for influenza virus. Curr Top Microbiol Immunol 1994;189:75–91.

157. Konishi E, Mason PW, Innis BI, Ennis FA. Japanese encephalitis virus-specific proliferative responses of human peripheral blood T lymphocytes. Am J Trop Med Hyg 1995;53:278–83.

158. Aihara H, Takasaki T, Matsutani T, et al. Establishment and characterization of Japanese encephalitis virus-specific, human CD4+ T-cell clones. Flavivirus cross-reactivity, protein recognition, and cytotoxic activity. J Virol 1998;72:8032–6.

159. Halstead SB. Pathogenesis of dengue. Challenges to molecular biology. Science 1988;239:476–81.

160. Halstead SB, O'Rourke EJ. Antibody-enhanced dengue virus infection in primate leukocytes. Nature 1977;265:739–41.

161. Gollins SW, Porterfield JS. Flavivirus infection enhancement in macrophages. An electron microscopic study of viral cellular entry. J Gen Virol 1985;66:1969–82.

162. Edelman R, Schneider R, Chieowanich P, et al. The effect of dengue virus infection on the clinical sequelae of Japanese encephalitis. A one year follow-up study in Thailand. Southeast Asian J Trop Med Public Health 1975;6:308–15.

163. Grossman RA, Edelman R, Willhight M, et al. Study of Japanese encephalitis virus in Chiangmai Valley, Thailand. 3. Human seroepidemiology and inapparent infections. Am J Epidemiol 1973;98:133–49.

164. Bond JO. St. Louis encephalitis and dengue fever in the Caribbean area. Evidence of possible cross protection. Bull World Health Organ 1969;:160–3.

165. Rao DR, Reuben R, Nagasampagi BA. Development of combined use of neem *(Azadirachta indica)* and water management for the control of culicine mosquitoes in rice fields. Med Vet Entomol 1995;9:25–33.

166. Rao DR, Reuben R, Venugopal MS, et al. Evaluation of neem, *Azadirachta indica*, with and without water management, for the control of culicine mosquito larvae in rice-fields. Med Vet Entomol 1992;6:318–24.

167. Lacey LA, Lacey CM. The medical importance of riceland mosquitoes and their control using alternatives to chemical insecticides. J Am Mosq Control Assoc Suppl 1990;2:1–93.

168. Reuben R, Thenmozhi V, Samuel PP, et al. Mosquito blood feeding patterns as a factor in the epidemiology of Japanese encephalitis in southern India. Am J Trop Med Hyg 1992;46:654–63.

169. Monath TP. Japanese encephalitis vaccines. Current vaccines and future prospects. Curr Top Microbiol Immunol 2002;267:105–38.

170. Smorodintsev AA, Shubladse AK, Neustroer VD. Etiology of autumn encephalitis in the far east of the USSR. Arch Ges Virus Forsch 1940;1:549–59.

171. Tsai TF, Yu YX. Japanese encephalitis vaccines. In: Plotkin SA, Mortimer EAJ, editors. Vaccines. Philadelphia: WB Saunders; 1994. p. 671–713.

172. Gowal D, Singh G, Bhau LN, Saxena SN. Thermostability of Japanese encephalitis vaccine produced in India. Biologicals 1991;19:37–40.

173. Kitano T, Yabe S, Kobayashi M, et al. Immunogenicity of JE Nakayama and Beijing-1 vaccines. JE and HFRS Bull 1986;1:37–41.
174. Lubiniecki AS, Cypess RH, Hammon WM. Passive immunity for arbovirus infection. II. Quantitative aspects of naturally and artificially acquired protection in mice for Japanese (B) encephalitis virus. Am J Trop Med Hyg 1973;22:535–42.
175. Oya A. Japanese encephalitis vaccine. Acta Paediatr Jpn 1988;30:175–84.
176. Defraites RF, Gambel JM, Hoke CH Jr, et al. Japanese encephalitis vaccine (inactivated, BIKEN) in US soldiers. Immunogenicity and safety of vaccine administered in two dosing regimens. Am J Trop Med Hyg 1999;61:288–93.
177. Nimmannitya S, Hutamai S, Kalayanarooj S, Rojanasuphot S. A field study on Nakayama and Beijing strains of Japanese encephalitis vaccines. Southeast Asian J Trop Med Public Health 1995;26:689–93.
178. Poland JD, Cropp CB, Craven RB, Monath TP. Evaluation of the potency and safety of inactivated Japanese encephalitis vaccine in US inhabitants. J Infect Dis 1990;161:878–82.
179. Henderson A. Immunisation against Japanese encephalitis in Nepal. Experience of 1152 subjects. J R Army Med Corps 1984;130:188–91.
180. Gambel JM, DeFraites R, Hoke C Jr, et al. Japanese encephalitis vaccine. Persistence of antibody up to 3 years after a three-dose primary series. J Infect Dis 1995;171:1074.
181. Sanchez JL, Hoke CH, McCowan J, et al. Further experience with Japanese encephalitis vaccine. Lancet 1990;335:972–3.
182. Rojanasuphot S, Shaffer N, Chotpitayasunondh T, et al. Response to JE vaccine among HIV-infected children, Bangkok, Thailand. Southeast Asian J Trop Med Public Health 1998;29:443–50.
183. Ruff TA, Eisen D, Fuller A, Kass R. Adverse reactions to Japanese encephalitis vaccine. Lancet 1991;338:881–2.
184. Andersen MM, Ronne T. Side-effects with Japanese encephalitis vaccine. Lancet 1991;337:1044.
185. Plesner AM, Arlien-Soborg P, Herning M. Neurological complications and Japanese encephalitis vaccination. Lancet 1996;348:202–3.
186. Ohtaki E, Matsuishi T, Hirano Y, Maekawa K. Acute disseminated encephalomyelitis after treatment with Japanese B encephalitis vaccine (Nakayama-Yoken and Beijing strains). J Neurol Neurosurg Psychiatry 1995;59:316–7.
187. Ohtaki E, Murakami Y, Komori H, et al. Acute disseminated encephalomyelitis after Japanese B encephalitis vaccination. Pediatr Neurol 1992;8:137–9.
188. Plesner AM, Arlien-Soborg P, Herning M. Neurological complications to vaccination against Japanese encephalitis. Eur J Neurol 1998;5:479–85.
189. Berg SW, Mitchell BS, Hanson RK, et al. Systemic reactions in US Marine Corps personnel who received Japanese encephalitis vaccine. Clin Infect Dis 1997;24:265–6.
190. Plesner AM, Ronne T. Allergic mucocutaneous reactions to Japanese encephalitis vaccine. Vaccine 1997;15:1239–43.
191. Plesner A, Ronne T, Wachmann H. Case-control study of allergic reactions to Japanese encephalitis vaccine. Vaccine 2000;18:1830–6.
192. Sohn YM. Japanese encephalitis immunization in South Korea. Past, present, and future. Emerg Infect Dis 2000;6:17–24.
193. Sakaguchi M, Nakashima K, Takahashi H, et al. Anaphylaxis to Japanese encephalitis vaccine. Allergy 2001;56:804–5.
194. Sakaguchi M, Inouye S. Two patterns of systemic immediate-type reactions to Japanese encephalitis vaccines. Vaccine 1998;16:68–9.
195. Robinson P, Ruff T, Kass R. Australian case-control study of adverse reactions to Japanese encephalitis vaccine. J Travel Med 1995;2:159–64.
196. Sakaguchi M, Yoshida M, Kuroda W, et al. Systemic immediate-type reactions to gelatin included in Japanese encephalitis vaccines. Vaccine 1997;15:121–2.
197. Sakaguchi M, Miyazawa H, Inouye S. Specific IgE and IgG to gelatin in children with systemic cutaneous reactions to Japanese encephalitis vaccines. Allergy 2001;56:536–9.
198. Takahashi H, Pool V, Tsai TF, Chen RT. Adverse events after Japanese encephalitis vaccination. Review of post-marketing surveillance data from Japan and the United States. The VAERS Working Group. Vaccine 2000;18:2963–9.
199. Ding Z, Shi H, Pang C. [Production of purified Japanese encephalitis vaccine from Vero cells with roller bottles]. Zhonghua Yi Xue Za Zhi 1998;78:261–2.
200. Sugawara K, Nishiyama K, Ishikawa Y, et al. Development of Vero cell-derived inactivated Japanese encephalitis vaccine. Biologicals 2002;30:303–14.
201. Xin YY, Ming ZG, Peng GY, et al. Safety of a live-attenuated Japanese encephalitis virus vaccine (SA14-14-2) for children. Am J Trop Med Hyg 1988;39:214–7.
202. Xin YY, Zhang GM, Zheng Z. Studies of the immunogenicity of live and killed Japanese encephalitis (JE) vaccines to challenge with different Japanese encephalitis virus strains. Chin J Virol 1989;5:106–10.

203. Bhatt TR, Crabtree MB, Guirakhoo F, et al. Growth characteristics of the chimeric Japanese encephalitis virus vaccine candidate, ChimeriVax-JE (YF/JE SA14-14-2), in *Culex tritaeniorhynchus*, *Aedes albopictus*, and *Aedes aegypti* mosquitoes. Am J Trop Med Hyg 2000;62:480–4.

204. Chen BQ, Beaty BJ. Japanese encephalitis vaccine (2-8 strain) and parent (SA 14 strain) viruses in *Culex tritaeniorhynchus* mosquitoes. Am J Trop Med Hyg 1982;31:403–7.

205. Arroyo J, Guirakhoo F, Fenner S, et al. Molecular basis for attenuation of neurovirulence of a yellow fever virus/Japanese encephalitis virus chimera vaccine (ChimeriVax-JE). J Virol 2001;75:934–42.

206. Tsai TF, Yong-Xin Y, Putvatan R, et al. Immunogenicity of live attenuated SA14-14-2 Japanese encephalitis vaccine—a comparison of 1- and 3-month immunization schedules. J Infect Dis 1998;177:221–3.

207. Sohn YM, Park MS, Rho HO, et al. Primary and booster immune responses to SA14-14-2 Japanese encephalitis vaccine in Korean infants. Vaccine 1999;17:2259–64.

208. Hennessy S, Zhengle L, Tsai TF, et al. Effectiveness of live-attenuated Japanese encephalitis vaccine (SA14-14-2). A case control study. Lancet 1996;347:1583–6.

209. Bista MB, Banerjee MK, Shin SH, et al. Efficacy of single-dose SA 14-14-2 vaccine against Japanese encephalitis. A case control study. Lancet 2001;358:791–5.

210. Liu ZL, Hennessy S, Strom BL, et al. Short-term safety of live attenuated Japanese encephalitis vaccine (SA14-14-2). Results of a randomized trial with 26,239 subjects. J Infect Dis 1997;176:1366–9.

211. Siraprapasiri T, Sawaddiwudhipong W, Rojanasuphot S. Cost benefit analysis of Japanese encephalitis vaccination program in Thailand. Southeast Asian J Trop Med Public Health 1997;28:143–8.

212. Ding D, Kilgore PE, Clemens JD, et al. Cost-effectiveness of routine immunization to control Japanese encephalitis in Shanghai, China. Bull World Health Organ 2003;81:334–42.

213. Konishi E, Pincus S, Paoletti E, et al. A highly attenuated host range-restricted vaccinia virus strain, NYVAC, encoding the *prM*, *E* and *NS1* genes of Japanese encephalitis virus prevents JEV viremia in swine. Virology 1992;190:454–8.

214. Mason PW, Pincus S, Fournier MJ, et al. Japanese encephalitis virus-vaccinia recombinants produce particulate forms of the structural proteins and induce high levels of protection against lethal JEV infection. Virology 1991;180:294–305.

215. Kanesa-thasan N, Smucny JJ, Hoke CH, et al. Safety and immunogenicity of NYVAC-JEV and ALVAC-JEV attenuated recombinant Japanese encephalitis virus—poxvirus vaccines in vaccinia-nonimmune and vaccinia-immune humans. Vaccine 2000;19:483–91.

216. Chambers TJ, Nestorowicz A, Mason PW, Rice CM. Yellow fever/Japanese encephalitis chimeric viruses. Construction and biological properties. J Virol 1999;73:3095–101.

217. Guirakhoo F, Zhang ZX, Chambers TJ, et al. Immunogenicity, genetic stability, and protective efficacy of a recombinant, chimeric yellow fever-Japanese encephalitis virus (ChimeriVax-JE) as a live, attenuated vaccine candidate against Japanese encephalitis. Virology 1999;257:363–72.

218. Monath TP, Levenbook I, Soike K, et al. Chimeric yellow fever virus 17D-Japanese encephalitis virus vaccine. Dose-response effectiveness and extended safety testing in rhesus monkeys. J Virol 2000;74:1742–51.

219. Monath TP, McCarthy K, Bedford P, et al. Clinical proof of principle for ChimeriVax. Recombinant live, attenuated vaccines against flavivirus infections. Vaccine 2002;20:1004–18.

220. Guirakhoo F, Pugachev K, Arroyo J, et al. Viremia and immunogenicity in nonhuman primates of a tetravalent yellow fever-dengue chimeric vaccine. Genetic reconstructions, dose adjustment, and antibody responses against wild-type dengue virus isolates. Virology 2002;298:146–59.

221. Konishi E, Yamaoka M, Khin Sane W, et al. Induction of protective immunity against Japanese encephalitis in mice by immunization with a plasmid encoding Japanese encephalitis virus premembrane and envelope genes. J Virol 1998;72:4925–30.

222. Konishi E, Yamaoka M, Kurane I, Mason PW. Japanese encephalitis DNA vaccine candidates expressing premembrane and envelope genes induce virus-specific memory B cells and long-lasting antibodies in swine. Virology 2000;268:49–55.

223. Ashok MS, Rangarajan PN. Protective efficacy of a plasmid DNA encoding Japanese encephalitis virus envelope protein fused to tissue plasminogen activator signal sequences. Studies in a murine intracerebral virus challenge model. Vaccine 2002;20:1563–70.