Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

ADVANCES IN VIRUS RESEARCH, VOL 60

PATHOGENESIS OF FLAVIVIRUS ENCEPHALITIS

Thomas J. Chambers* and Michael S. Diamond†

*Department of Molecular Microbiology and Immunology
St. Louis University Health Sciences Center, School of Medicine
St. Louis, Missouri 63104
†Department of Molecular Microbiology, Medicine, Pathology, and Immunology
Washington University School of Medicine
St. Louis, Missouri 63110

I. Introduction

Within the flavivirus family, viruses that cause natural infections of the central nervous system (CNS) principally include members of the Japanese encephalitis virus (JEV) serogroup and the tick-borne encephalitis virus (TBEV) serocomplex. Neuroinvasion follows infection of host organisms in the periphery by bites of chronically infected mosquito and tick vectors. Syndromes that result from CNS infection in humans range from mild aseptic meningitis to acute encephalitis of variable morbidity and mortality and are often complicated by neurologic sequelae among survivors. The pathogenesis of these diseases involves complex interactions of viruses, which differ in neurovirulence potential, and a number of host factors, which govern susceptibility to infection and the capacity to mount effective antiviral immune responses both in the periphery and within the CNS. Animal models

0065-3527/03 $35.00

have been instrumental for providing insight into how virus-specific and host factors influence the course of disease. Rodent models have been used in classic experiments on pathogenesis and continue to be relied upon for studies of viral neurovirulence determinants and immune system requirements for a successful antiviral response, particularly because of readily available knockout strains. Nonhuman primates have also been useful for some studies of peripheral immune responses to encephalitic viruses and for quantitating the disease burden caused by replication of virus in the CNS (monkey neurovirulence testing) and have also been applied as models for immunization and challenge. This review summarizes progress in the field of flavivirus neuropathogenesis since previous reviews on this topic (Monath, 1986; Monath and Heinz, 1996). Mosquito-borne and tick-borne viruses are considered together, although it is important to note that there are differences in the pathogenesis of these two groups of viruses.

II. Host Factors

It is generally acknowledged that the ratio of apparent to inapparent infections with flaviviruses is quite low (on the order of 1:100 to 1:300), implying that a number of host factors are involved in protection against CNS disease. The most well known of these are age, genetic factors, and preexisting flavivirus immunity. The effect of age has been recognized in both clinical and experimental studies of flavivirus encephalitis (Eldadah *et al*., 1967a; Grossberg and Scherer, 1966; Luby *et al*., 1967; O'Leary *et al*., 1942; Powell and Kappus, 1978; Weiner *et al*., 1970), in some cases accompanied by effects of gender (Andersen and Hanson, 1974). In terms of human infections, clinical disease with JEV is primarily a pediatric entity, suggesting that certain features of the developing nervous system predispose to pathogenesis of encephalitis. Experimental evidence in support of this is based on the known propensity of arboviruses to infect and spread more rapidly through nervous tissue of young rodents; however, resistance to fatal infection occurs abruptly soon after the weanling stage. This resistance occurs in conjunction with neural ontogeny and is associated with restricted replication of virus in neurons as a function of their degree of differentiation, with low levels of virus being observed in mature neurons in some models (Hase *et al*., 1993; Ogata *et al*., 1991). However, this phenomenon is very dependent on the intrinsic level of viral neurovirulence, being most apparent with strains of lower

virulence (Eldadah *et al*., 1967a; Ogata *et al*., 1991; Oliver *et al*., 1997), and is also affected by the dose and route of inoculation (Eldadah *et al*., 1967a; Fitzgeorge and Bradish, 1980). Changes in the expression of cellular receptors and/or intracellular factors required for efficient replication have been invoked as explanations for the age-related resistance. In support of these hypotheses, it is known that the replication block can be overcome by the neuroadaptation of flaviviruses, which results in genetic changes in the envelope region, as well as in nonstructural regions of the genome (Chambers and Nickells, 2001; McMinn, 1997; Schlesinger *et al*., 1996).

Studies with the mouse model of Sindbis virus encephalitis suggest that the differential expression of neural genes in young versus older mice is the determinant of age-related resistance. Candidate genes involved in this process include apoptotic regulators, interferon-responsive genes, and other classes that are regulated developmentally (Labrada *et al*., 2002; see Section IX). These factors are presumed to operate by activating innate antiviral effector systems in neurons and promoting survival of these cells after viral infection.

Despite these experimental data, age-dependent resistance to encephalitic viruses in areas endemic for human disease is influenced by the effects of immunization and recurrent inapparent infections with homologous and heterologous viruses, such that the cumulative immune responses in adults may confer protection or ameliorate the severity of disease (Kurane, 2002; Solomon and Vaughn, 2002). However, susceptibility of young children (below the age of 9 months) to yellow fever virus (YFV) 17D vaccine-associated encephalitis is well documented (Freestone, 1994), and resistance to this adverse event in older individuals is clearly not based on immunity from recurrent subclinical infections. This supports the phenomenon of age-related susceptibility to encephalitis. A predisposition of elderly individuals to develop encephalitis from West Nile Virus (WNV) and St. Louis encephalitis (SLE) virus has also been observed. In this case, lack of previous cross-reactive immunity may be an important factor, but comorbid illnesses may contribute to the risk of complications, and the decline in immune function with advanced age is also likely to play a role (Grubeck-Loebenstein and Wick, 2002). In support of this, immunocompromised individuals encountering flavivirus infections appear to be less able to mount effective immune responses, as in reports of HIV-infected subjects with complicated CNS disease (Neogi *et al*., 1998; Okhuysen *et al*., 1993; Szilak and Minamoto, 2000; Wasay *et al*., 2000) and in transplant recipients sustaining WNV infection (Iwamoto *et al*., 2003). YFV 17D vaccine appears to be tolerated in

HIV infection, provided immunosupression is not severe, although this isuue deserves further investigation (Kengsakul *et al.*, 2002; Receveur *et al.*, 2000).

Coexistent infection with other infectious agents has been suggested as a modifying factor for flavivirus encephalitis. The association between cysticercosis and JEV infection was investigated in a controlled study that did not provide evidence for predisposition to viral encephalitis (Azad *et al.*, 2003). Association of TBEV encephalitis with borrelia infection has been described (Korenberg *et al.*, 2001), but probably reflects coexistence of these pathogens in the tick vector. Autopsy studies have suggested that herpes simplex infection may predispose to JEV encephalitis by altering the integrity of the blood–brain barrier (Hayashi and Arita, 1977). Experimentally, infection of mice with *Toxocara canis* or *Trichinella spiralis* predisposes to JEV encephalitis as a result of T-cell immunosuppression (Cypess *et al.*, 1973; Gupta and Pavri, 1987; Lubiniecki *et al.*, 1974; Pavri *et al.*, 1975), suggesting the possibility of increased disease severity in endemic areas where parasitic pathogens and encephalitic viruses coexist.

The susceptibility of mice to flavivirus encephalitis is controlled genetically and is associated with host factors that map to chromosome 5 at the oligoadenylate synthetase (OAS) gene cluster (Mashimo *et al.*, 2002; Perelygin *et al.*, 2002; Sangster *et al.*, 1994). Although OAS is involved in the activation of RNase L, the mechanism of resistance associated with the gene is not known and could conceivably relate to other potential functions of OAS proteins in cellular responses to viral injury (Samuel, 2002). The importance of the OAS gene in human susceptibility to these viruses requires investigation, as differences exist between murine and human gene clusters. Evidence shows that 2′, 5′-OAS is activated in response to peripheral flavivirus infection in humans (Bonnevie-Nielsen *et al.*, 1989, 1995), but studies to correlate this with the effectiveness of the antiviral responses are not yet available.

The effect of different class I HLA-A and B alleles on the immune response to flavivirus infection has been investigated for dengue viruses, where associations have been found with increased and decreased susceptibility to dengue hemorrhagic fever (Loke *et al.*, 2001; Stephens *et al.*, 2002), suggesting a relationship between the extent of T-cell activation and severe disease. It is unclear whether similar results will be found for encephalitic viruses, as these associations occurred in the context of secondary infections and presumably affect the strength of the memory rather than the primary T-cell response.

Interactions between flavivirus untranslated regions (UTRs) and intracellular proteins indicate another potential level of host-mediated control over virus infection (Brinton, 2000). Multiple proteins have been reported to bind to the 3′ UTR of the positive strand of various flaviviruses, including EF-1α and Mov34, which bind to the positive strand 3′ UTR of WNV and JEV, respectively (Blackwell and Brinton, 1995, 1997; Ta and Vrati, 2000), and as many as eight cellular proteins in the case of dengue (De Nova-Ocampo *et al*., 2002). Several proteins have also been reported to bind to the 3′ UTR of the negative strand of dengue virus (Yocupicio-Monroy *et al*., 2003) and four proteins in the case of West Nile virus (Li *et al*., 2002). These proteins have been suggested to participate in flavivirus replication by influencing viral transcription and/or translation in conjunction with host intracellular membranes. There is some evidence that interactions of the viral RNA with such proteins are in fact important for virus replication (Li *et al*., 2002). It is not known whether compartmentalization of their interactions in different cell types is a determinant of tissue tropism in infected hosts.

III. Arthropod Factors Affecting Pathogenesis

It has become evident from studies on arthropod vectors that certain components of their saliva influence pathogenesis in vertebrate hosts. For mosquitoes, flaviviruses are deposited principally in the extravascular tissue during probing, as virus that is injected intravascularly is reingested rapidly during the blood meal (Burke and Monath, 2001; Turell and Spielman, 1992; Turell *et al*., 1995). Instead of a rapid dissemination of virus via the bloodstream, flaviviruses may then undergo some replication locally in subcutaneous tissues accompanied by spread to regional lymph nodes through lymphatic channels. Dendritic cells in the skin are likely to serve as a vehicle for the transport of virus to lymphoid tissues.

Components of the insect saliva modulate the earliest steps in flavivirus infection by altering the local host immune response. Feeding by *Aedes aegypti* or *Culex pipiens* mosquitoes or administration of sialokinin-I, a mosquito salivary protein, downregulates interferon-γ (IFN-γ) production and upregulates the T_H2 cytokines interleukin (IL)-4 and IL-10 (Zeidner *et al*., 1999). Salivary gland extracts from *Dermacentor* and *Ixodes* ticks decrease natural killer activity (Kubes *et al*., 1994, 2002), suppress the antiviral actions of interferons in cell culture (Hajnicka *et al*., 2000), and enhance the transmission of TBEV in rodents (Labuda *et al*., 1993). Overall, these findings suggest that insect

factors facilitate flavivirus transmission by interfering with aspects of both innate and adaptive responses. Whether these effects are required to establish systemic infection and neuroinvasion is not known.

IV. Extraneural Infection

Flavivirus neuropathogenesis involves both *neuroinvasiveness* (capacity to enter the CNS) and *neurovirulence* (replication within the CNS) (Monath, 1986), both of which can be manipulated experimentally. In rodent models, neurovirulence is an inherent property of most of these viruses, and the quantity of virus needed to cause infection in the CNS is usually quite small. In classic studies of arbovirus pathogenesis (Albrecht, 1968; Huang and Wong, 1963), distinctions were made among neuropathogenic phenotypes on the basis of replication efficiency and pathogenic potential in the peripheral tissues versus the CNS, with various phenotypes being distinguished (high peripheral susceptibility, low neurotropism; low neuroinvasiveness, high neurotropism; and high neuroinvasiveness, high neurotropism). These phenotypes were related to different clinical outcomes, which ranged from inapparent infection to acute encephalitis of varying severity, and are influenced in the rodent model by host age and species (Kozuch *et al*., 1981). These concepts have held up over time and have been supplemented by additional data concerning viral determinants of virulence and host innate and adaptive immune mechansisms. A main principle that applies is the relationship between peripheral virus burden and the propensity to cause neuroinvasion. Viruses with a low capacity to replicate in the periphery generally can be classified as low in neuroinvasive potential, regardless of their intrinsic level of neurovirulence. A relationship between systemic virus burden and viremia is also apparent (Albrecht, 1968), with the potential of the virus to generate viremia being a correlate of neuroinvasion as it applies to most naturally acquired encephalitic infections. Aerosol-acquired infections are probably an exception, but some of these may also cause systemic infection with viremia after gaining access to the lower respiratory tract. Mucosal infection of the alimentary tract has also been implicated both experimentally and in naturally acquired cases of TBEV encephalitis (Gresikova *et al*., 1975; Odeola and Oduye, 1977). Furthermore, data from a number of studies indicate that such factors as the time of onset, magnitude, and duration of the viremia, as well as the integrity of the host innate immune system, also influence the risk of entry into the CNS, prior to the onset of the virus-specific

immune response (see Section VIII). Type I interferons and macrophages in particular have been identified as important factors in limiting infection and clearing systemic virus. Thus this process is largely a balance between the replicative efficiency of the virus and the effectiveness of early host defenses in clearing viremia.

V. Cellular Receptors for Flaviviruses

The cellular receptors that mediate attachment and entry of flaviviruses have been only partially characterized, and to date there has been no definitive identification of the molecules required for these processes in either peripheral or CNS tissues. Although several groups have used biochemical approaches to identify candidate protein receptors (Kopecky *et al*., 1999; Martinez-Barragan and del Angel, 2001; Ramos-Castaneda *et al*., 1997) on mammalian cells, their physiologic relevance remains unclear, as there is heterogeneity in the proteins that bind flaviviruses in different cell types. Most recently, CD209 or DC-SIGN (the dendritic cell-specific intercellular adhesion molecule 3–grabbing nonintegrin) has been proposed as a cell surface ligand for dengue virus (Navarro-Sanchez *et al*., 2003; Tassaneetritep *et al*., 2003). Additional studies are required to evaluate the *in vivo* significance of DC-SIGN as an attachment or entry ligand and whether this is a common determinant of tropism for other flaviviruses. One possibility to reconcile these observations is that multiple independent cellular receptor molecules are utilized either during the spread of flaviviruses within the host or during entry into the CNS. A number of early studies have described binding of flaviviruses to mouse brain substances (reviewed in Albrecht, 1968), suggesting that this tissue is enriched for a unique receptor activity, which may enhance tropism for this organ, particularly in developing brain (Kimura-Kuroda *et al*., 1992). In this regard, it has been shown that mouse and monkey brain membrane-receptor preparations preferentially bind neurovirulent strains of flaviviruses, but not attenuated variants (Ni and Barrett, 1998; Ni *et al*., 2000). Receptor variability *in vivo* may be a general mechanism for promoting wide tissue tropisms of arthropod-borne viruses, which require cycling in both arthropod and vertebrate hosts. Some data suggest that different cell surface proteins may be utilized for the entry of insect versus vertebrate cells (Martinez-Barragan and del Angel, 2001; Munoz *et al*., 1998). Tissue tropism in mosquitos has also been observed to correlate with expression of a specific receptor molecule (Yazi-Mendoza *et al*., 2002).

Heparan sulfate has been proposed as a flavivirus receptor based on studies showing the dependence of dengue virus infectivity on binding of the E protein to heparan sulfate on target cells (Chen *et al.*, 1997; Hilgard and Stockert, 2000). Subsequent reports have demonstrated that infectivity of TBEV, yellow fever virus (YFV), JEV, and Murray Valley encephalitis (MVE) viruses is affected by cell surface interactions with glycosaminoglycans that are proposed to mediate initial low-affinity binding to the cell surface (Germi *et al.*, 2002; Lee and Lobigs, 2000; Mandl *et al.*, 2001; Su *et al.*, 2001), but the role of heparin as an authentic receptor for virulent flaviviruses remains uncertain. For instance, the serial passage of JEV and MVE viruses in cell culture results in selection for viruses that exhibit increased binding to heparin but decreased virulence *in vivo* (Lee and Lobigs, 2002). Similar observations have been made with alphaviruses (Bernard *et al.*, 2000; Klimstra *et al.*, 1998), where heparin binding was associated with cell culture adaptation of primary virus isolates and attenuation of viral virulence. Thus enhanced binding to glycosaminoglycans is a marker for attenuation of JEV and MVE viruses in the mouse model and correlates with rapid clearance of the glycosaminoglycan-binding variants from the circulation compared to more pathogenic strains (Lee and Lobigs, 2002). The mechanism responsible for this process has not been defined. The relationship of this observation to classic studies on neuroinvasion is also unclear, as virulent strains were originally characterized by their ability to undergo rapid uptake from the circulation, presumably as a result of highly efficient binding and entry to target cells (Albrecht, 1968). Wild-type and glycosaminoglycan-binding variants may differ, however, with respect to their entry into cells that are permissive for replication or are involved in virus clearance (Lee and Lobigs, 2002).

Antibody-dependent enhancement (ADE) of infection has been reported for some encephalitic flaviviruses. ADE is believed to occur through FcγR I (CD64) and FcγR II (CD32), although a second type of ADE that requires complement has also been described (Cardosa *et al.*, 1983, 1986). In tissue culture, ADE occurs with several flaviviruses in cells of myeloid lineage (Brandriss and Schlesinger, 1984; Brandt *et al.*, 1982; Cardosa *et al.*, 1983, 1986; Diamond *et al.*, 2000b; Halstead and O'Rourke, 1977; Halstead *et al.*, 1980, 1984; Schlesinger and Brandriss, 1983). Most of these data are relevant to the pathogenesis of dengue infection, and the significance for encephalitic viruses is less certain. However, there is some experimental evidence that ADE may be involved (Hawkes, 1964), and this notion is consistent with the concepts described for dengue viruses based on the wide antibody

cross-reactivity among flaviviruses with respect to the E protein. Neutralizing homologous or cross-reactive antiflavivirus antibodies can enhance neurovirulence and mortality associated with YFV and JEV infection (Gould and Buckley, 1989; Gould *et al*., 1987; Lobigs *et al*., 2003b). However, in some cases, the pathogenesis was associated with complement-mediated cytolysis and not with enhancement of infection *in vivo* (Gould *et al*., 1987). The strongest data in support of ADE in dengue infections are epidemiological in nature. In this regard, there is not abundant evidence to support a phenomenon of enhancement of JEV or other encephalitic viruses by preexisting cross-reactive antibodies in natural infections. There is evidence of cross-protection in experimental models, particularly among JEV serogroup members, and for amelioration of JEV encephalitis by prior immunity to related viruses, including dengue (Kurane, 2002; Solomon and Vaughn, 2002). Experimentally, the protective effect is presumably antibody mediated and is most apparent following infection with live virus or transfer of serum from animals infected with virus (Broom *et al*., 2000; Tesh *et al*., 2002). Antibody reponses elicited by inactivated virus do not exhibit much cross-protection in animals and may lead to enhanced infections (Broom *et al*., 2000; Lobigs *et al*., 2003b). Consistent with these observations, recipients of inactivated JEV vaccine or live-attenuated dengue vaccine did not generate neutralizing activity in sera against WNV (Kanesa-Thasan *et al*., 2002). Severe forms of TBEV encephalitis have been observed after passive immunization with hyperimmune globulin (Waldvogel *et al*., 1996), but it is not clear if this represents an enhancement phenomenon as opposed to either failure of antibody to penetrate the CNS or suppression of peripheral or CNS immune responses by high-titer immunoglobulin. The entire issue is somewhat limited by the fact that the encephalitic viruses may not necessarily target cells with abundance of Fc receptors, such as monocyte–macrophages, which are generally considered more important for the pathogenesis of dengue viruses.

VI. Cellular Tropism of Encephalitic Flaviviruses

In cell culture, flaviviruses readily infect a variety of cell types, including epithelial, endothelial, and fibroblasts (Avirutnan *et al*., 1998; Bielefeldt-Ohmann, 1998; Diamond *et al*., 2000b; Kurane *et al*., 1992), but the relationship of these findings to *in vivo* replication is uncertain. After peripheral inoculation, flaviviruses probably do not replicate extensively in the skin, but are spread from local lymph

nodes by immature dendritic or Langerhans cells, which are permissive for infection (Byrne *et al.*, 2001; Johnston *et al.*, 1996, 2000; Libraty *et al.*, 2001; McMinn *et al.*, 1996; Wu *et al.*, 2000). Within 1 day of infection, epidermal Langerhans cells that express viral antigens migrate from the skin to the draining lymph node (Byrne *et al.*, 2001; Wu *et al.*, 2000) while expressing maturation markers such as B7-1, B7-2, class II MHC molecules, CD11b, and CD83 (Ho *et al.*, 2001). These cells produce tumor necrosis factor (TNF)-α and IFN-α (Ho *et al.*, 2001; Libraty *et al.*, 2001) and become more resistant to flavivirus infection (Wu *et al.*, 2000). Thus infected dendritic cells probably serve to promote antigen presentation in the lymph node and also participate in the spread of infection to lymphoid compartments. The consequences of DC infection, whether apoptosis (as for alphaviruses) or persistent infection, as in the case of Kunjin virus replicons (Varnavski and Khromykh, 1999), and their effects on subsequent shaping of the immune response remain important areas for further investigation. For instance, data from other viral models indicate that there is a quantitative requirement for activated dendritic cells in order to induce T-cell responses (Ludewig *et al.*, 1998). Survival versus death of these cells as a result of virus infection may have an important impact on this requirement. After replication in lymphoid tissue, encephalitic viruses are believed to exit via efferent lymphatics (Malkova and Frankova, 1959) and gain access to the circulation, whereby systemic infection is established.

Although a tropism of encephalitic viruses for lymphoid tissues has been observed, the identities of the cell types in other compartments that support replication to the levels needed to generate a viremia sufficient to cause neuroinvasion have not been determined definitively. Replication in various peripheral tissues occurs, but vascular endothelial cells have not necessarily been implicated as important sites of replication (Albrecht, 1968 and references therein). However, it should be noted that dengue, JEV, and probably other flaviviruses can enter and, in some cases, establish infection in endothelial cells (Dropulic and Masters, 1990; Liou and Hsu, 1998) and modulate their activation state and cytokine production (Anderson *et al.*, 1997; Avirutnan *et al.*, 1998; Bosch *et al.*, 2002; Huang *et al.*, 2000). *In vitro* studies with endothelial cells are complicated by the fact that variable responses can be observed depending on the cell types and assay systems; however, given the potential role of these cells in immune activation, further studies on the effects of flavivirus infection on cytokine and chemokine production by these cells are needed.

In the CNS, neurons are the primary targets for encephalitic flaviviruses (Eldadah *et al*., 1967b; Hase *et al*., 1987; Iwasaki *et al*., 1986; Kimura-Kuroda *et al*., 1992; Wang *et al*., 1997, 1998; Weiner *et al*., 1970; Xiao *et al*., 2001). Although viral replication and antigen production have also been observed in cultured oligodendrocytes (Jordan *et al*., 2000) and astrocytes (Chen *et al*., 2000; Liu *et al*., 1988; Suri and Banerjee, 1995), the significance of these reports is difficult to judge, as there is scant evidence for infection of glial cells *in vivo*. Neurotropism and neurovirulence are governed to a large extent by determinants in the viral E protein, as indicated by an abundance of genetic data indicating that mutations in this protein modulate these phenotypes (McMinn, 1997; Ni and Barrett, 1998; Ni *et al*., 2000), presumably through their effects on receptor targeting and postreceptor events involved in virus entry. In the absence of an experimental system to manipulate the virus receptor on neurons, one cannot conclude whether high neurotropism of these viruses is dependent solely on the binding of the E protein. Comparison of sequence data from virulent and attenuated strains of encephalitic viruses also suggests that the nonstructural region and 3′ UTRs contain determinants that influence pathogenesis. It is important in these types of studies to differentiate effects of genetic mutations on overall replication fitness of the virus versus specific effects in terms of interactions of viral proteins and RNA structures with host factors that affect pathogenesis uniquely. Thus the molecular basis for neurotropism is not understood adequately, and further studies using genetic clones of well-characterized viruses in animal models should help address this issue. Furthermore, the use of primate models to investigate the issue is needed greatly, as the observations in rodent models may not have direct correlates to human infections.

VII. Immune Responses to Flaviviruses and Their Role in Pathogenesis

Susceptibility to flavivirus encephalitis implies a failure at some stage of the immune response that theoretically may be defined in either qualitative or quantitative terms. There is substantial clinical and experimental evidence for a correlation between protection against encephalitic disease and the presence of virus-specific antibodies, but the molecular and cellular basis for the development of this response has not been defined thoroughly. In studies that have shown protection by antibodies, the roles of other immune system components in

the process have not often been assessed. Furthermore, there is increasing evidence that flaviviruses have evolved mechanisms to manipulate the effector functions of both innate and adaptive immune responses. The magnitude and importance of these responses probably vary from one experimental model to another and account for differences observed in studies that have examined the immune system in the context of a either a primary or a memory response.

A. Innate Immunity

1. Interferons

In vitro and *in vivo* studies have demonstrated that interferon-dependent responses are relevant to protection against flavivirus infections. Dendritic cells in the skin may be the first cells to produce type I interferons (IFN-α or -β) in response to flavivirus infection and initiate this antiviral response (Libraty *et al*., 2001). These interferons inhibit flavivirus infection by preventing translation and replication of infectious viral RNA at least partially through an RNase L-, Mx1-, and Protein Kinase R (PKR)-independent mechanism (Anderson and Rahal, 2002; Diamond and Harris, 2001; Diamond *et al*., 2000a). These studies have been supported by experiments in immunodeficient and therapeutic mouse models of disease. Pretreatment of mice with IFN-α or its inducers prevents or ameliorates flavivirus infections (Brooks and Phillpotts, 1999; Charlier *et al*., 2002; Harrington *et al*., 1977; Leyssen *et al*., 2003b; Lucas *et al*., 2003), and mice that are deficient in type I IFNs or their receptor have increased susceptibility to flaviviruses (Johnson and Roehrig, 1999; Lobigs *et al*., 2003a). The role of type II IFN (IFN-γ) in protection versus immunopathogenesis of flavivirus encephalitis is less clear, as this cytokine has a multitude of effects on the host response to these viruses. In part, this includes induction of proinflammatory and antiviral molecules, including nitric oxide (Lin *et al*., 1997), and enhancement of the phagocytic activity of monocytes/macrophages through increased Fc receptor expression (Rothman and Ennis, 1999). Data from various models support the importance of IFN-γ production in the context of a T_H1 virus-specific immune response for the control of infection with encephalitic viruses (Johnson and Roehrig, 1999; Liu and Chambers, 2001; Lobigs *et al*., 2003a). Distinctions should be made, however, concerning the effects of IFN-γ in the periphery and in the context of the CNS immune response (see Section X).

Flaviviruses appear to be capable of attenuating some of the IFN-dependent antiviral effector mechanisms. Treatment of cells or animals with IFN-α as few as 4 h after infection with dengue or SLE viruses resulted in almost a complete loss of antiviral activity (Brooks and Phillpotts, 1999; Diamond *et al*., 2000a). Similarly, IFN-α treatment of patients with documented JEV encephalitis had no significant effect on outcome (Solomon *et al*., 2003), despite anecdotal reports of its benefit (Harinasuta *et al*., 1985). The mechanisms by which the antiviral effect is avoided remain uncharacterized, but data suggest that it may act at a very early step in infection. Future studies should also help determine if the role of type I IFNs in these infections lies only in innate intracellular effector defenses or whether there are effects on the quality and magnitude of the subsequent cell-mediated immune response through their immunoregulatory effects.

2. Macrophages

Activation of macrophages and modulation of their effector functions are integral parts of flavivirus pathogenesis (Rothman and Ennis, 1999; Spain-Santana *et al*., 2001). In addition to their role in nonspecific defense, macrophages are targets of infection by some flaviviruses and have the potential to contribute to pathogenesis through antibody-dependent enhancement of infection mediated by Fc and complement receptors (Cardosa *et al*., 1986; Gollins and Porterfield, 1984; Hawkes, 1964; Peiris *et al*., 1981). The preponderance of data supports the protective role of macrophages in control of infection by means of cytokine production and antigen presentation to B and T cells (Kulkarni *et al*., 1991a; Marianneau *et al*., 1999). Classic studies have shown that abrogation of phagocytic activity of macrophages results in higher viremia, neuroinvasion, and more severe encephalitis (Ben-Nathan *et al*., 1996a; Khozinsky *et al*., 1985; Monath, 1971; Zisman *et al*., 1971). Some of the protective effect provided appears to be mediated by the stimulation of inducible nitric oxide synthetase (NOS-2) to produce nitric oxide (NO) and other reactive oxygen intermediates such as peroxynitrites (Saxena *et al*., 2000). Pretreatment of macrophages with agents that induce NO synthesis have been shown to inhibit JEV infection *in vitro* (Lin *et al*., 1997). Moreover, treatment of mice with a NOS-2 inhibitor increased mortality after JEV infection (Lin *et al*., 1997). However, other studies suggest that the inflammatory actions of NO and other reactive oxygen intermediates may, in some cases, contribute to flavivirus pathogenesis. The *in vivo* administration of a competitive inhibitor of NOS-2 improved survival in mice infected with TBEV (Kreil and Eibl, 1995, 1996). Activation of macrophages in response to flavivirus

infection promotes not only production of NO, but also release of TNF-α, IL-1β, IL-8, and other mediators of acute inflammation that may contribute to tissue damage where macrophages accumulate (Atrasheuskaya *et al*., 2003; Bosch *et al*., 2002; Raghupathy *et al*., 1998; Rothman and Ennis, 1999). These various studies indicate that the behavior of macrophages is fundamental to the pathogenesis of flavivirus disease, but the contribution to virus clearance versus deleterious effects driven by IFN-γ and other proinflammatory stimuli depends on regulation of their activity, and the properties of this innate defense may therefore vary from one context to another.

3. Natural Killer Cells

Natural killer (NK) lymphocytes lyse infected cells by releasing cytotoxic granules that contain perforin and granzymes or by binding to apoptosis-inducing receptors on target cells (Orange *et al*., 2002). NK cell activation is finely regulated through a balance of activating (Ly49D, Ly49H, and NKG2D) and inhibitory receptors [killer cell immunoglobulin-like receptors (KIR), immunoglobulin-like inhibitory receptors (ILT), and CD94-NKG2A] (Smith, *et al*., 2001). A decrease in expression of class I MHC molecules on a cell may prompt NK cell activation by attenuating the inhibitory signals. Thus NK cell target recognition occurs after ligation of activating receptors and repression of inhibitory receptors on the cell surface. NK responses have been analyzed in various experimental models of flavivirus infection, but as noted (Hill *et al*., 1993), the characterization of the responding cells has been limited. Studies of NK cell activity during WNV infection have revealed blunted cytolytic activity against virus-infected cells, associated with upregulation of MHC antigen and ICAM-1 expression on the target cells by interferon-independent mechanisms (Müllbacher *et al*, 1989). However, NK cell-dependent lysis of dengue virus-infected target cells by both natural killer and antibody-dependent cell-mediated cytotoxicity has been observed (Kurane *et al*., 1984). Infection of mice with Langat, WNV, and TBEV transiently activated and then suppressed NK cell activity (Vargin and Semenov, 1986). Despite these conflicting observations, the bulk of evidence currently suggests that flaviviruses have evolved a mechanism to evade NK cell responses through an augmentation of cell surface class I MHC expression (King and Kesson, 1988; King *et al*., 1989; Liu *et al*., 1988, 1989b), driven by a TAP-dependent process (Momburg *et al*., 2001; Müllbacher and Lobigs, 1995; Lobigs *et al*., 1999) and NF-κB-dependent transcriptional activation of MHC class I genes (Kesson and King, 2001). Thus, flaviviruses may overcome susceptibility to NK cell-mediated lysis at the

expense of increased class I MHC expression and later recognition by virus-specific cytotoxic lymphocytes (CTLs). Consistent with this hypothesis, splenocytes from WNV-immunized mice had poor NK cell lytic activity (Momburg *et al*., 2001), and mice that are genetically deficient in NK cells demonstrate no increased morbidity or mortality compared to wild-type controls in response to WNV infection (M. Engle, W. Yokoyama, and M. Diamond, unpublished results). Some residual NK cell function may still be important during flavivirus pathogenesis, as suggested by studies with perforin and Fas knockout mice, which are partially protected from encephalitic disease, through events that operate at the level of neuroinvasion. This may involve the cytotoxic activities of NK cells and/or CTLs (Lincon Luna *et al*., 2002). In addition, the lack of a substantial effect of IFN-α in the therapy of flavivirus encephalitis is also consistent with inhibition of the NK response, as type I interferons are normally potent activators of these cells.

4. Natural Antibody

Natural antibodies are primarily of the IgM class, although activity of IgG has also been described. They are secreted constitutively by $CD5^+$ B-1 cells without specific stimulation, have widely variable binding avidities, and represent an initial nonspecific defense against pathogens (Baumgarth *et al*., 2000; Casali and Notkins, 1989; Ochsenbein *et al*., 1999a) through direct neutralization of some bacteria and viruses in the circulation (Gobet *et al*., 1988; Ochsenbein *et al*., 1999a), enhancement of phagocytosis (Navin *et al*., 1989), and complement activation (Baumgarth *et al*., 2000). Although the role of natural antibody in flavivirus infection remains unexplored, mice that genetically lack secreted IgM, (sIgM −/−), but in which cell surface IgM and IgG responses are intact have increased mortality in certain viral infections, involving a defect in the antiviral IgG responses (Baumgarth *et al*., 2000; Boes *et al*., 1998). Such mice are also very susceptible to infection with WNV (Fig. 1; M. Engle and M. Diamond, unpublished data). This observation, along with other data, suggests an important role for natural antibody and complement during the early antiviral defense against flaviviruses, although a virus-specific IgM response is likely to be more important (see Section VII,A,5).

5. Complement

The complement system is an important innate defense for limiting infection by fungal, bacterial, and viral pathogens. Complement inhibits viruses by several mechanisms (Volanakis, 2002), including lysis

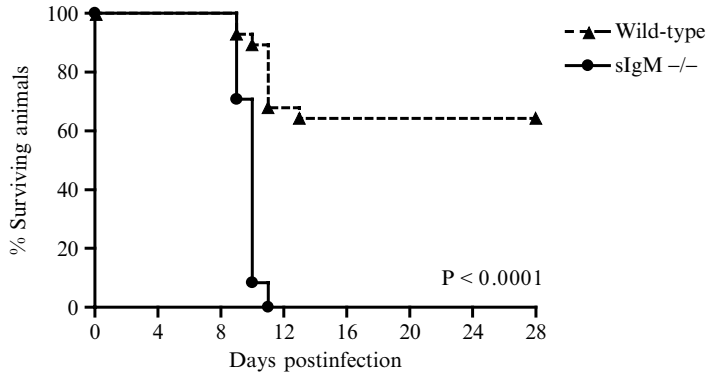

FIG 1. Soluble IgM-deficient mice are highly sensitive to peripheral infection with WNV. Mice were infected by subcutaneous inoculation of the footpad with 100 plaque-forming units of WNV NY 99 and monitored for mortality from CNS disease.

of enveloped viral particles and virus-infected cells by the C5–C9 membrane attack complex; recruitment and activation of monocytes and granulocytes by C3a and C5a; clearance of virus from circulation after opsonization by proteolytic fragments of C3, C3b, and C3bi, followed by uptake into cells that express complement receptor; and C3-facilitated uptake of antigen and presentation by macrophages and dendritic cells (Ochsenbein and Zinkernagel, 2000) during priming of T and B lymphocytes (Da Costa *et al*., 1999; Kopf *et al*., 2002; Ochsenbein *et al*., 1999b). Preliminary studies indicate that complement plays an essential role in limiting WNV infection. Mice that are genetically deficient in C3 uniformly succumb to infection even at low inoculating doses (Fig. 2; E. Mehlhop, M. Engle, and M. Diamond, unpublished data). Additional studies must be performed to determine which individual mechanisms are most critical for this complement-mediated control. A deficiency of C3 could exacerbate WNV infection because of depressed C5–C9 lytic or C3 opsonic activity that results in a failure to clear virus from the circulation. Alternatively, C3 may play important roles in linking the innate and adaptive immune responses (Barrington *et al*., 2001; Carroll, 1998; Ochsenbein and Zinkernagel, 2000) against WNV. C3 is required for normal IgG production and T-cell priming against influenza and herpes viruses (Da Costa *et al*., 1999; Fischer *et al*., 1996; Kopf *et al*., 2002; Ochsenbein *et al*., 1999), and a deficiency in C3 decreases opsonization and viral antigen presentation, leading to deficits in the adaptive B-and T-cell responses (Ochsenbein and Zinkernagel, 2000). Although the lytic and proinflammatory activity of complement

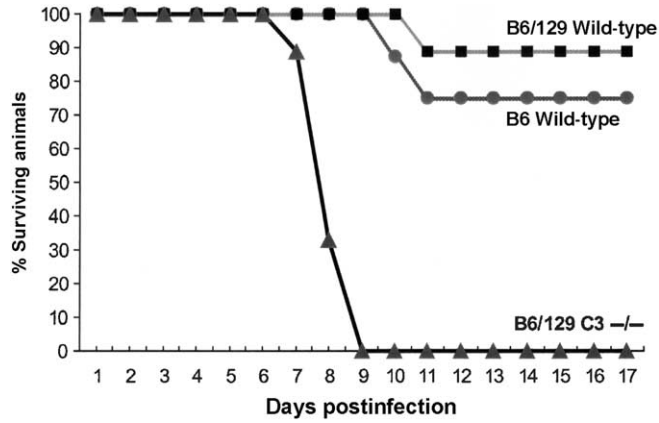

FIG 2. C3-deficient mice are highly sensitive to peripheral infection with WNV. Mice were infected as described in Fig. 1 and monitored for mortality from CNS disease.

may contribute to the defense against WNV, preliminary data indicate that a deficiency in either C3 compromises the antiviral B-cell immune response, as mice that lack C3 have markedly depressed titers of WNV-specific IgG (E. Mehlhop, M. Engle, and M. Diamond, unpublished data).

B. Adaptive Immunity

The roles of both humoral and cellular immunity during the pathogenesis of flavivirus disease have been studied in various models involving active and passive immunization in normal and immunodeficient animals, and understanding of the immunologic basis for protection against acute encephalitis is beginning to advance. B cells, $CD4^+$ cells, and $CD8^+$ T cells have all been implicated in contributing to protection, although as stated earlier, their relative importance seems to vary depending on the context of the experimental model under investigation.

1. B Cells and Antibody

Antibody responses to the E and NS1 proteins involve many epitopes, and both neutralizing and nonneutralizing antibodies (including those against NS1) can prevent fatal encephalitis, as demonstrated in many passive transfer and active immunization experiments in experimental animal models (Brandriss *et al.*, 1986; Diamond *et al.*, 2003; Gould *et al.*, 1987; Henchal *et al.*, 1988; Kimura-Kuroda and

Yasui, 1988; Putnak and Schlesinger, 1990; Schlesinger *et al*., 1985, 1987; Zhang *et al*., 1989). A protective effect can occur even after establishment of infection in the CNS. Mechanisms of antibody-mediated protection during flavivirus infection include direct neutralization of receptor binding (Crill and Roehrig, 2001), blocking of virus uncoating (Gollins and Porterfield, 1984), and Fc receptor-dependent virus clearance via the reticuloendothelial system. Most neutralizing antibodies recognize the structural E protein, and antibody epitopes appear to be broadly distributed over its surface (Heinz, 1986; Roehrig *et al*., 1989); however, these do not all represent potent sites for neutralization. A subset of neutralization epitopes is found on the prM protein (Colombage *et al*., 1998; Falconar, 1999; Pincus *et al*., 1992). The presence of nonneutralizing, yet protective antibodies against NS1 is also well documented (Cane and Gould, 1988; Després *et al*., 1991; Falgout *et al*., 1990; Henchal *et al*., 1988; Putnak and Schlesinger, 1990; Schlesinger *et al*., 1986, 1987). Antibodies to NS1 are proposed to mediate the lysis of virus-infected cells, which express this protein on their surface, by complement-mediated lysis and/or antibody-dependent cellular cytotoxicity (Hill *et al*., 1993; Kurane *et al*., 1984; Schlesinger *et al*., 1990). These humoral responses are believed to be important components of the protective immune response to flavivirus infection, but antibodies to NS1 are not often measured in experimental models of infection or immunization and challenge.

Although the neutralizing antibody responses are considered correlates of protection (Markoff, 2000), this process is probably also a function of additional innate and adaptive immune effector systems whose roles in the control of infection are less easily demonstrated. In this regard, challenge in the context of passively administered virus-specific antibodies is not necessarily associated with sterilizing immunity, indicating that antiviral defenses other than antibodies are involved in protection (Kreil *et al*., 1998a, 1998b). Definitive experiments to determine the extent of immune activation in this setting are likely to expand our understanding of the correlates of protection and the immunological basis for a successful antiviral response.

Mice that lack B cells are very vulnerable to flavivirus infections and encephalitis (Diamond *et al*., 2003; Liu and Chambers, 2001), purportedly as a consequence of lacking antibodies. However, these models must be explored further to determine whether T-cell responses are otherwise fully activated and effective, as it is possible that B cells could influence $CD4^+$ and $CD8^+$ T-cell responses through antigen presentation or other immunoregulatory events, as has been observed in other neurotropic viral infections (Bergmann *et al*., 2001).

Factors that drive B-cell activation and maturation during flavivirus infections are not well understood. Multiple T helper epitopes have been identified in the E protein of JEV and MVE viruses, some of which are dominant, broadly reactive among JE serogroup viruses, and prime for the neutralizing antibody (Kutubuddin *et al*., 1991; Mathews *et al*., 1991, 1992; Roehrig *et al*., 1992). Immunization with plasmid DNA encoding the E region is more effective than viral E antigen itself, presumably due to the induction of T-cell responses to the E protein (Chen *et al*., 1999). Immunization of HIV-infected individuals with inactivated flavivirus vaccines has suggested that $CD4^+$ T cells may not be critical for the induction of protective antibodies (Panasiuk *et al*., 2003). However, others have observed that antibody responses to inactivated or live viral vaccines are weak in these subjects (Rojanasuphot *et al*., 1998; Sibailly *et al*., 1997). Immunization of CD4 knockout or class II knockout mice with YFV markedly diminished or abrogated the neutralizing antibody response, respectively (Chambers and Liang, unpublished data), suggesting that there is dependence on functional $CD4^+$ T cells to generate long-lasting B-cell memory against flaviviruses. Differences in immunization schedules and numbers of residual $CD4^+$ lineage T cells in these various reports may explain the discrepancies. Studies on the role of dendritic cells in early B-cell activation and the nature of the toll-like receptor signals induced in response to viral antigens on these cells may provide new insights into the determinants that establish and drive the memory response to flavivirus antigens.

An IgM response to flaviviruses is a feature of most clinical and experimental infections (Martin *et al*., 2002) and has been reported to be a correlate of protection during clinical JEV encephalitis in some studies (Burke *et al*., 1985a, 1985b; Ravi *et al*., 1993). Flavivirus infections typically elicit IgM responses that can often persist for prolonged periods (Edelman *et al*., 1976; Monath, 1971; Roehrig *et al*., 2003). However, experimentally, the initial IgM response to encephalitic viruses may possess variable neutralization activity and protective capacity (Diamond *et al*., 2003; Hofmann *et al*., 1978; Ishii *et al*., 1968); in some studies, the complement-fixing activity was limited compared to that provided by the ensuing IgG response (Ishii *et al*., 1968; Lee and Scherer, 1961). This contrasts with what has been observed with YF 17D vaccination (Monath, 1971) and may reflect differences in early B-cell activation among these infections or differences between humans and mice with respect to the process. However, the role of a vigorous IgM response may be in providing temporary neutralizing activity, while more importantly activating complement-dependent pathways involved in

programming virus-specific B- and T-cell responses. Studies with the WNV model (see later) suggest a critical early function of both IgM and complement in the control of extraneural infection. The neutralizing activity of sera rises in conjunction with the appearance of IgG, and this response includes a variety of biological activities, including hemagglutination inhibition, complement fixation, and virus neutralization. The memory or "antigenic sin" response elicited by cross-reactive antigens involves all of these responses, but is weakest for the neutralization response (Innis, 1997), suggesting that there is some hierarchical pattern of B-cell epitopes or some selectivity in recognizing the most cross-reactive antigens. This phenomenon is probably evolutionarily adaptive for recurrent infections with heterologous flaviviruses, but is capitalized upon by dengue viruses during the pathogenesis of dengue hemorrhagic fever and shock syndrome. In any case, the anamnestic antibody response has been demonstrated to be critical to a defense against encephalitic viruses in the context of immunization and challenge models, which are surrogates for the efficacy of vaccines (Konishi *et al*., 1999; Lee and Sherer, 1961; Pan *et al*., 2001). It nevertheless remains unclear at the present time the extent to which antibody responses alone contribute to the control of acute infections.

2. T Cells

T-cell responses to flavivirus proteins have been best studied for members of the DEN and JEV serogroups. Both $CD4^+$ and $CD8^+$ T lymphocyte responses involve broad flavivirus cross-reactivity, although this varies significantly among different MHC haplotypes (Hill *et al*., 1992; Kulkarni *et al*., 1992; Kurane *et al*., 1991; Uren *et al*., 1987; reviewed in Hill *et al*., 1993). Multiple epitopes for T-cell responses have been identified on both structural and nonstructural proteins; however, genetic factors restrict the number of targets and fine specificities differ considerably. Determinants for class I responses are more frequent within the viral nonstructural region, particularly the NS3 protein, which contains dominant epitopes in both humans and mice. In contrast, viral structural protein antigens elicit class II responses more consistently. The role of virus-specific $CD4^+$ T cells in flavivirus encephalitis is not well understood, although experimental models indicate a requirement for such cells in protection against acute disease (see Section X). Some of the cytotoxic T-cell response to dengue and JE viruses is contained in the $CD4^+$ compartment and is probably mediated by Fas/FasL interactions (Aihara *et al*., 2000; Gagnon *et al*., 1999), which has implications for possible immunopathogenic responses both in the periphery and in the CNS.

Flavivirus-specific $CD8^+$ T cells have multiple effector functions, including cytotoxic activity and production of IFN-γ (Douglas *et al*., 1994; Kesson *et al*., 1987; Kulkarni *et al*., 1991b; Kurane *et al*., 1989, 1991, 1995; Liu *et al*., 1989a; Murali-Krishna *et al*., 1996; Takada *et al*., 2000), suggesting that polarization of the immune response toward a T_H1 phenotype is involved in control of these viruses. Although the importance of type I interferons in protection against these viruses would suggest its involvement in promoting this T-cell response, as has been described for other viruses (Cousens *et al*., 1999), the role of IL-12 in driving this process has received only limited study (Chen *et al*., 2001; Phillpotts *et al*., 2003), and its importance may vary from one context to another (Dalod *et al*., 2002). Cellular immunity clearly contributes to the control of virus infection in experimental animal models, but this varies depending on the context examined and with respect to the virulence of the challenge virus. T-cell-deficient mice fail to generate protective immunity after a sublethal challenge with YFV strains (Bradish *et al*., 1980). Moreover, animals that are treated with drugs that impair T-cell function develop a rapidly progressive flavivirus encephalitis (Camenga *et al*., 1974; Cole and Nathanson, 1968; Nathanson and Cole, 1970). The adoptive transfer of immune spleen cells can protect against encephalitis, but the lymphocyte subpopulations that mediate this protection have not been very well characterized in classic studies (Bradish *et al*., 1980; Camenga *et al*., 1974; Jacoby *et al*., 1980).

More recent studies have, to some degree, clarified the role of $CD8^+$ T cells in these infections. Quantitation of the $CD8^+$ T-cell response to YFV in experimental mice (van der Most *et al*., 2002) reveals activation by immunodominant epitopes and is supported by the observation that $CD8^+$ knockout mice exhibit a defect in the clearance of infectious YFV from the CNS (Liu and Chambers, 2001; T. J. Chambers, unpublished data) and also have increased mortality after WNV infection (B. Shrestha and M. Diamond, manuscript in preparation). Human recipients of YFV 17D vaccine exhibit an increase in $CD8^+$ T cells as well, and epitopes have been mapped to multiple proteins (Co *et al*., 2002). While data from most of these models would indicate that CTL responses are primarily protective *in vivo*, their potential for immunopathogenic effects requires further investigation. Some studies with MVE virus (Licon Luna *et al*., 2002) and dengue virus (Rothman and Ennis, 1999) suggest that the cytotoxicity of CTL may contribute to the disease pathogenesis. Studies with knockout mice continue to provide novel information on the role of T cells in flavivirus pathogenesis and immunity. However, an important limitation of these experiments

is that the effect of gene knockout on immunologic development and function is not known. Better attempts to assess the entire range of properties of the immune response in these types of experimental models are needed, as gene knockouts could have multiple effects beyond simply loss of the targeted function.

Human studies on immune responses to JEV or JEV structural protein antigens encoded in recombinant vaccinia virus revealed proliferation and induction of cytolytic activity in the $CD8^+$ T-cell compartment (Konishi *et al.*, 1995, 1998a), similar to what has been observed in mice (Konishi *et al.*, 1997, 1998b). The significance of these responses is uncertain, as another study found no correlation between T-cell proliferation and either the antibody response or the clinical outcome (Desai *et al.*, 1995a). Inactivated JEV vaccine induced $CD4^+$, class II-restricted T cells with cytolytic activity (Aihara *et al.*, 2000), suggesting that it is not capable of inducing high levels of $CD8^+$ CTLs; in mice, this vaccine elicts a T_H2 immune response (Ramakrishna *et al.*, 2003). As noted earlier, in the context of clinical infections with flaviviruses, the integrity of cellular immune function appears to be important (Iwamoto *et al.*, 2003; Neogi *et al.*, 1998; Okhuysen *et al.*, 1993; Szilak and Minamoto, 2000). However, better understanding of the effector properties of T cells and their role in protection in humans and in experimental animals is needed.

VIII. Neuroinvasion

Neuroinvasiveness is a critical step in the pathogenesis of flavivirus encephalitis and is affected by both viral and host factors. In terms of viral factors, characterization of various virulent and attenuated strains of JEV, TBEV, YFV, and WNV has revealed that viral determinants of neuroinvasiveness map principally the E protein (reviewed in McMinn, 1997). The mechanisms associated with these genetic determinants have not been completely determined, but are believed to relate to increased viral infectivity toward important target cells through enhanced binding and penetration. Entry into the CNS has been proposed to involve a number of potential processes, none of which has been definitively demonstrated *in vivo*. The proposed pathways include (1) transport across the cerebrovascular endothelium, or infection of these and other cells constituting the blood–brain barrier (Dropulic and Masters, 1990; Liou and Hsu, 1998); (2) access to the CNS after loss of blood–brain barrier integrity (Kobiler *et al.*, 1989; Lustig *et al.*, 1992); and (3) entry through the olfactory epithelium

(McMinn *et al*., 1996; Monath *et al*., 1983). There are regions of the CNS where lack of a blood–brain barrier may constitute sites of vulnerability to infection in the presence of viremia (i.e., the choroid plexus and the circumventricular organs). Some of these may be supported by nonspecific defenses that are sufficient to withstand a low level of viremia, as the choroid is a site of IFN-α expression and OAS induction (Asada-Kubota *et al*., 1997; Khan *et al*., 1989). Entry of virus into the CNS by passage across the small cerebrovascular vessels is consistent with the accumulation of perivascular infiltrates of inflammatory cells, which is a hallmark of flavivirus encephalitis (Johnson *et al*., 1985). However, access through the olfactory bulb is believed to occur either after infection by the aerosol route or intranasally (Hambleton *et al*., 1983; Myint *et al*., 1999; Nir *et al*., 1965; Raengsakulrach *et al*., 1999) or in the context of hematogenous dissemination of virus (McMinn, 1996; Monath, 1986; Monath *et al*., 1983). The olfactory bulb is especially vulnerable to infection because of the exposure of its nerve terminals within the olfactory mucosa, and this route is exploited by other neurotropic viruses (Fazakerley, 2002).

Disruption of the blood–brain barrier facilitates the entry of noninvasive flaviviruses into the CNS (Lustig *et al*., 1992), suggesting that neuroinvasion may be influenced by host factors that alter the permeability of this barrier as a result of systemic infection (Chaturvedi *et al*., 1991; Kaiser and Holzmann, 2000; Mathur and Chaturvedi, 1992), including IFN-γ, TNF-α, and possibly effector functions of CTLs and NK cells (Lincon Luna *et al*., 2002). Neuroinvasion is also influenced by physical stress and other agents, including inhalational anesthetics (Ben-Nathan *et al*., 1989, 1992, 1996b, 2000). Immunosuppression by corticosterone and other endogenous immunomodulators is an important factor, as involution of lymphoid tissue has been observed and probably facilitates the generation of viremia by attenuated viral strains. Perturbation of the blood–brain barrier may also be involved in this process (Ben-Nathan *et al*., 2000). Early viremia and sustained viremia are correlated with neuroinvasion in animal models, consistent with the belief that replication to high titers in peripheral tissues is an important property of invasive strains, at least in immunologically normal hosts (Albrecht, 1968; Huang and Wong, 1963; Monath, 1986). However, the timing of peak viremia is probably critical, as levels sufficient to cause neuroinvasion are dissipated concurrent with the appearance of adaptive immune responses in the periphery (Diamond *et al*., 2003; Halevy *et al*., 1994). This is also evident from studies of immunodeficient mice lacking T- and B-cell responses, which exhibit neuroinvasion in the presence of viremia (Chambers and

Nickells, 2001; Charlier *et al*., 2002; Diamond *et al*., 2003; Johnson and Roehrig, 1999; Halevy *et al*., 1994; Lin *et al*., 1998). At present it appears that the mechanisms responsible for entry into the CNS may vary, depending on a specific virus and the level of host immunocompetence. Continued research with the use of knockout strains harboring single and multiple defects in these redundant immune system defenses will improve the understanding of how this process occurs.

The mechanisms of flavivirus spread within the CNS are not established. Studies with alphaviruses in the mouse model suggest that this occurs in a circuit-specific manner among olfactory neurons that are undergoing developmental synaptogenesis (Oliver and Fazakerley, 1998). Spread of MVE virus in immature mice exhibited a pattern consistent with these observations, but occurred throughout the CNS (McMinn *et al*., 1996). An unusual accumulation of WNV particles in myelin lamellae was observed in spinal cord cultures (Shahar *et al*., 1990), suggesting the possibility of virus transport by mechanisms not only involving the axoplasm.

IX. Neuropathology

Flaviviruses, particularly members of the JEV and TBEV serogroups, cause viral encephalitis in many vertebrate species, a dead-end transmission pathway believed to reflect an evolutionarily conserved capacity of these viruses to grow in the CNS of arthropods and vertebrates (Monath, 1986). In contrast to the noncytopathic nature of infection in arthropods, a spectrum of acute and chronic CNS pathologic changes occur in vertebrates and have been documented extensively (Chu *et al*., 1999; Dominguez and Baruch, 1963; Hase *et al*., 1993; Levenbook *et al*., 1987; Manuelidis, 1956; Nathanson *et al*., 1966; Pogodina *et al*., 1983; Reyes *et al*., 1981; Vince and Grevic, 1969; Zlotnik *et al*., 1971, 1976). Virus can be demonstrated within neurons throughout the brain and spinal cord, but infection of other cell types has been less well characterized. In humans, neuroinvasive flaviviruses cause an acute, often fatal encephalomyelitis (Monath, 1986) associated with characteristic inflammatory changes and often targeted to specific regions (Johnson *et al*., 1985; Miyake, 1964; Suzuki and Phillips, 1966; Zimmerman, 1946). The pathological changes have been characterized in many experimental animal models, as well as in fatal human cases. These viruses can evoke inflammatory infiltrates extending from the meningeal layers into the brain substance, with features typical of other viral encephalitides, including leptomeningitis,

perivascular lymphocytic accumulation, parenchymal infiltrates, and microglial nodules associated with neuronophagia in regions of viral infection (Figs. 3, 4, and 5). The neuropathology can, in some cases, include the destruction of vascular structures with focal hemorrhage, suggesting a vasculitis. Loss of regional blood flow, as well as disruption of the blood–brain barrier, has been described in clinical cases of TBEV (Gunther *et al.*, 1998; Kaiser, 2002; Kaiser and Holzmann, 2000). The nature and degree of the inflammatory disease depend on many factors, including the virulence of the virus, the route of infection, and the age and immunocompetence of the host. Varying levels of CNS inflammation without frank evidence of neuronal damage have been described in some models, including human autopsy cases, suggesting that viral infection can induce lethal pathophysiology prior to or in the absence of recruitment of the peripheral immune response (reviewed in Monath, 1986). Although cytopathic effects have been observed primarily in virus-infected neurons, other noninfected cells can also exhibit pathologic changes, presumably through bystander injury (see later). A number of studies have documented the distribution of the neuropathology and the clinical manifestations that characterize flavivirus infection of the CNS. For instance, infection in cortical

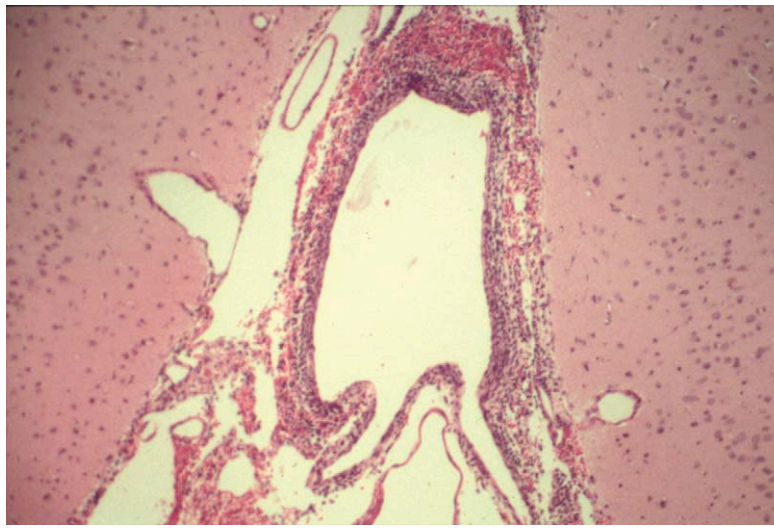

FIG 3. Yellow fever virus meningoencephalitis in the rhesus monkey showing leptomeningeal accumulation of acute inflammatory cells. Courtesy of the United States Army Medical Research Institute of Infectious Diseases (USAMRIID).

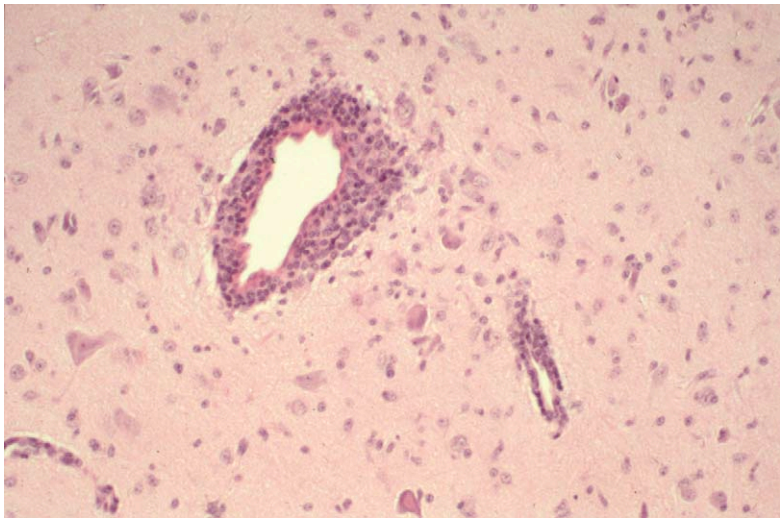

FIG 4. Yellow fever encephalitis in the rhesus monkey showing focus of perivascular infiltrate with mononuclear cells in the cerebral cortex. Courtesy of USAMRIID.

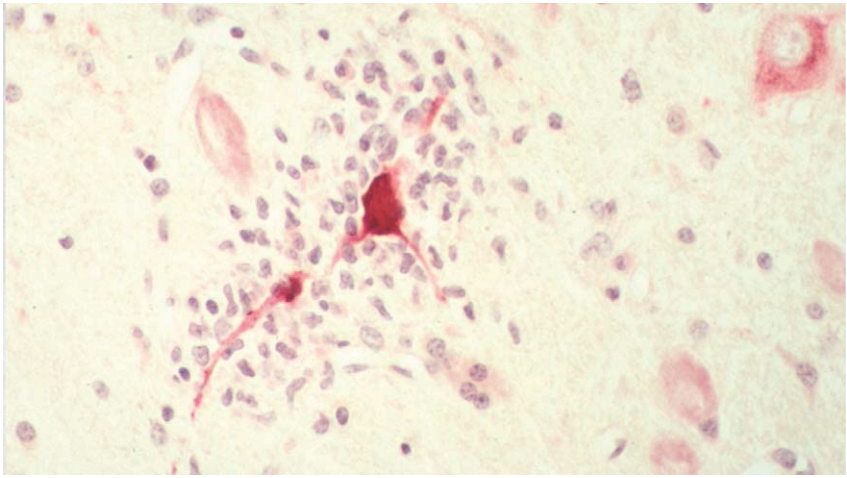

FIG 5. Yellow fever encephalitis in the rhesus monkey showing microglial nodule with neuronophagia of a cortical neuron stained for viral antigen. Courtesy of USAMRIID.

regions typically gives rise to depressed consciouness and seizures, but the involvement of subcortical regions, including the midbrain and brainstem, as well as thalamic and basal ganglial involvement, can give rise to a variety of movement disorders (Asher, 1975; Kalita and Misra, 2000; Misra and Kalita, 1997a; Murgod *et al*., 2001; Ogata *et al*., 2000; Pradhan *et al*., 1999). WNV encephalitis in the United States and JEV encephalitis have both been characterized by a poliomyelitis-like syndrome, suggesting infection of lower motor neurons in association with flaccid paralysis (Glass *et al*., 2002; Leis *et al*., 2002; Misra and Kalita, 1997b; Solomon *et al*., 1998). Lower motor neuron disease is also typical of TBEV infection. Factors governing the differences in neuronal susceptibility to flaviviruses are not known, but may be similar to those that operate in the case of other neurotropic arboviruses, such as Sindbis, where the neuronal response to viral injury may be variable (Griffin and Hardwick, 1999). Neuronal death associated with flavivirus infection has classically been ascribed to degenerative necrosis. Pathologic changes that accompany this process include vacuolization and proliferation of intracellular membranes, which produces a characteristic ultrastructural appearance (Fig. 6; Murphy *et al*., 1968). Whether there is a greater propensity to cause necrosis versus apoptosis requires further study.

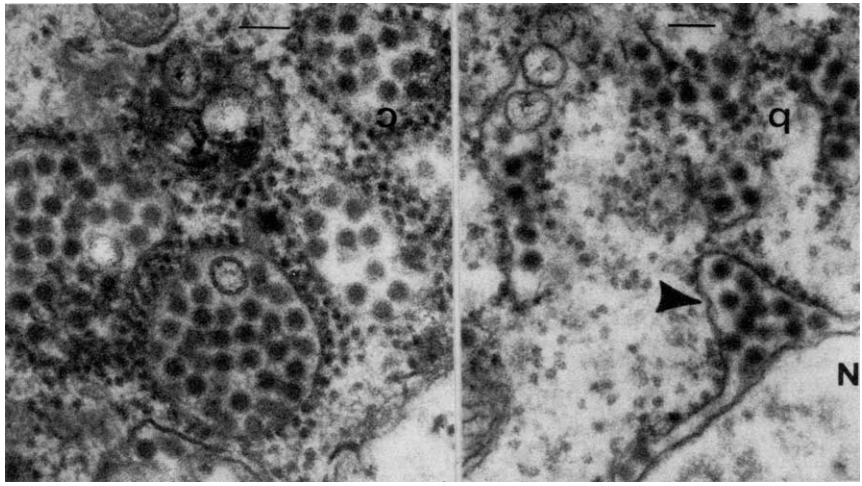

FIG 6. Electron micrograph of a mouse CNS neuron infected with SLE virus showing characteristic cytoplasmic pathology but integrity of the nuclear (N) envelope. Arrow indicates virions within inner and outer nuclear membranes. From Murphy *et al*. (1968), with permission.

Several flaviviruses have been shown to induce apoptosis, both *in vitro* (Isaeva *et al.*, 1998; Liao *et al.*, 1998; Parquet *et al.*, 2001; Prikhod'ko *et al.*, 2002) and *in vivo*, in the rodent CNS (Andrews *et al.*, 1999; Després *et al.*, 1998; Duarte dos Santos *et al.*, 2000; Isaeva *et al.*, 1998; Xiao *et al.*, 2001; Fig. 7). In this regard, reports describing primarily degenerative pathology at the light or electronmicroscopic

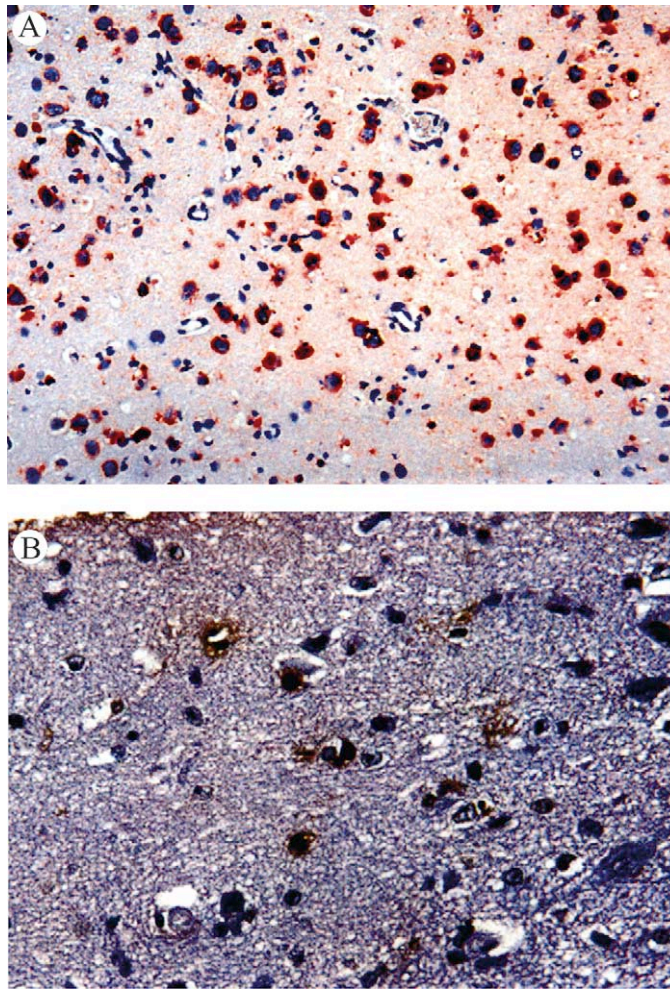

FIG 7. WNV infection in the Syrian golden hamster. (A) Viral antigen-positive neurons in the cerebral cortex. (B) TUNEL-positive apoptotic neurons in the cortex. Courtesy of Dr. Shu-Yan Xiao. From Xiao *et al.*, 2001.

level, typically involving cytoplasmic changes such as chromatolysis, swelling, and dissolution of Nissl substance, also mentioned nuclear pathology, including pyknosis, disruption of the nuclear envelope, and alterations in chromatin (Dominguez and Baruch, 1963; Manuelidis, 1956; Mathews *et al*., 2000). These findings are of interest given reports that neuronotropic viruses such as Sindbis virus may not induce typical apoptotic morphology in neurons (Griffin and Hardwick, 1999; Havert *et al*., 2000; Kerr *et al*., 2002; Sammin *et al*., 1999) and that cell death may occur through both apoptotic and necrotic mechanisms (Havert *et al*., 2000; Nargi-Aizenman and Griffin, 2001; Sammin *et al*., 1999), depending on the integrity of the apoptotic pathways of a given population of neurons, their profile of apoptotic modulators, and the presence of excitotoxic stimuli. The most detailed *in vivo* characterization of neuronal apoptosis by flaviviruses has been reported for dengue. Neuroadapted dengue virus induces apoptosis in infected neurons as well as in noninfected cells (Després *et al*., 1998), suggesting that indirect mechanisms of cellular injury occur in areas of heavy virus burden. It is important to note that these findings were demonstrated in very young mice, whose neurons are highly susceptible to apoptotic stimuli, and may not reflect the response of more mature cells in older mice. However, studies with WNV in the adult hamster model provide evidence that highly neurovirulent strains are potent inducers of apoptosis (Xiao *et al*., 2001; Fig. 7). A relationship between viral virulence and the extent of apoptosis has not been clearly established for flaviviruses; however, the general capacity of neuroadapted strains to produce high virus burdens in association with cytopathology suggests that the situation is likely to resemble that of alphaviruses and other neuronotropic viruses (Lewis *et al*., 1996; Oberhaus *et al*., 1997; Theerasurakarn and Ubul, 1998). Although the molecular details of this process are not fully known, the expression of some flavivirus proteins appears to directly induce apoptotic cell death of neurons, including the WNV capsid protein (Yang *et al*., 2002), the Langat virus NS3 protein, which causes apoptosis through the activation of caspases 3, 8, and 9 (Prikhod'ko *et al*., 2002), and the E proteins of neuroadapted dengue-2 virus (Duarte dos Santos *et al*., 2000) and JEV, which appear to stimulate an ER unfolded protein response (Su *et al*., 2002) and a component of oxidative stress (Raung *et al*., 2001). However, there are many other potential mechanisms for provoking apoptosis by neuronotropic RNA viruses, including signaling through interferon-α-dependent pathways, phospholipase A_2 activation, activation of NF-κB and p53-regulated genes (Fazakerley, 2001), and activation of apoptosis during

viral entry (Jan *et al.*, 2000). Some of these processes may also be involved in the pathogenesis of encephalitic flaviviruses.

The role of apoptotic regulators bcl-2, bax, bcl-X, and related gene products in modulating neuronal death has been only partially characterized for flaviviruses. Forced expression of bcl-2-related genes promotes the survival of neuronal cell lines infected with JEV and dengue virus and facilitates viral persistence, primarily by restricting virus-induced cytopathic effects and not viral replication (Liao *et al.*, 1997, 1998). These outcomes vary in neuronal versus nonneuronal cell lines, and differential effects of bcl-2 and bcl-X appear to operate. Such findings suggest that the effects of apoptosis modulators on flaviviruses are similar to those observed with alphaviruses, where it is known that the expression of these proteins can vary in their effects from one cell type to another. Conclusions about the role of these proteins in different *in vivo* models therefore require careful evaluation (Griffin and Hardwick, 1999; Levine, 2002). Furthermore, because neuronotropic viruses can induce both necrosis and apoptosis, neuronal death may require assessment by several criteria. Neuronal injury as a result of bystander effects may also be a factor during flavivirus neuropathogenesis given that microglial activation and elaboration of inflammatory mediators, including IL-1β and TNF-α, occur in the CNS during these infections (Andrews *et al.*, 1999; Liu and Chambers, 2001; Ravi *et al.*, 1997) and may accompany the production of nitric oxide and peroxynitrite, which can cause neurotoxicity. Other potential mechanisms include excitatory cell death due to the activation of NMDA receptors, which has been implicated in the pathogenesis of Sindbis virus and HIV (Nargi-Aizenman and Griffin, 2001). Thus, although it is likely that the neurovirulence phenotype of flaviviruses is linked to the extent of neuronal cell death caused during the encephalitis, there appear to be multiple independent mechanisms by which neuronotropic viruses cause cell death. This process can be affected by the region of the brain affected, the degree of neuronal maturity, the factors that regulate cell death signaling receptors and their pathways, and levels of apoptosis modulators and other innate responses of virus-infected neurons (Fazakerly, 2001; Griffin and Hardwick, 1999; Levine, 2002; Liang *et al.*, 1998). In some cases, changes in the expression of neurotrophins may also be involved in the CNS response to viral injury (Zocher *et al.*, 2000); however, the relevance of this phenomenon to other types of viral encephalitis has yet to be widely investigated. The process is also subject to additional influence by the properties of the immune response recruited into the CNS, including $CD4^+$ and $CD8^+$ T cells, which may be involved in

cytotoxicity toward virus-infected and, in the case of $CD4^+$ cells, perhaps even noninfected cells (Després *et al.*, 1998; Gagnon *et al.*, 1999), through Fas-dependent mechanisms under certain circumstances (Medana *et al.*, 2000). The effects of nonspecific inflammation, such as release of toxic substances from neutrophils (Andrews *et al.*, 1999), may also contribute to cellular injury.

X. The Central Nervous System Immune Response

Flavivirus infections induce a CNS inflammatory response of variable intensity. Data from most experimental models suggest that this inflammation is a requirement for protection from lethal infection with neurovirulent strains. The characteristics of the inflammation in experimental models have been shown to be affected by numerous factors, which include the endogenous CNS response, as well as the adequacy of the peripheral immune response and its timely recruitment into the CNS. In some cases of encephalitis, relatively scant inflammatory disease has been noted. This has also been observed experimentally as, for example, with TBEV under conditions where viral neuroinvasion and CNS involvement progress rapidly (Vince and Grevic, 1969). Immunosuppression also dramatically reduces the intensity of the CNS inflammation (Hirsch and Murphy, 1967; Leyssen *et al.*, 2003a). However, acute inflammation may become severe in response to a heavy antigen load and has been implicated in immunopathologic reactions in the CNS (reviewed in Monath, 1986).

A. Innate Responses

Viral infections of the CNS commonly result in the induction of innate responses, which include activation and proliferation of microglia and activation of astrocytes and cerebrovascular endothelium, with ensuing production of chemokines and proinflammatory cytokines (Benveniste, 1997). A consequence of this activation is the conditioning of cells in the CNS parenchyma and the blood–brain barrier to accommodate and modulate the influx of activated lymphocytes from the periphery by the upregulation of adhesion molecules and class I and II antigens. Activation of innate responses within the CNS during flavivirus encephalitis has been suggested by several human and experimental animal model studies in which the expression of chemokine and cytokine genes or their proteins has been analyzed. The production of IL-8 and macrophage inhibitory factor (MIF) has

been detected in cerebrospinal fluid (CSF) or brain tissue during early stages of encephalitis with JEV and MVE viruses (Andrews *et al.*, 1999; Singh *et al.*, 2000; Suzuki *et al.*, 2000), with levels of IL-8 correlating directly with the number of neutrophils in the CSF. TNF-α and IL-1β are also elicited in response to flavivirus infection of the CNS (Liu and Chambers, 2001; Ravi *et al.*, 1997; Suzuki *et al.*, 2000), presumably representing intrinsic responses of microglia and astrocytes to the acute injury (Benveniste, 1997). Thus the early stage of the encephalitis involves the endogenous expression of mediators that results in the recruitment of nonspecific acute inflammatory cells with the potential for the production of neurotoxic substances, such as reactive oxygen intermediates, and probably facilitates further stimuli that intensify the inflammation. For instance, IL-1β and TNF-α can also mediate the release of IL-8 from astrocytes (Aloisi *et al.*, 1992). The induction of the nonspecific acute inflammation may be a deleterious process because levels of IL-8 are predictive of fatal disease (Suzuki *et al.*, 2000) and treatment of infected mice with inhibitors of NOS-2 in the acute stage of encephalitis lessened mortality in association with reduced inflammation (Andrews *et al.*, 1999). Because viral infections of the CNS induce the expression of chemokines, which have been implicated in inflammatory cell recruitment, (Liu and Lane, 2001; Liu *et al.*, 2000, 2001), the intense level of inflammation observed in flavivirus encephalitis may be driven by the induction of one or more chemokines and their receptors. The expression of monocyte and T-cell chemokines has not been reported in these infections; however, studies with other models suggest that MCP-1, IP-10, RANTES, and other chemokines are involved in the trafficking of leukocytes into areas of virus infection in the CNS. In conjunction with the effects of TNF-α and IFN-γ, both of which can lead to loss of integrity of the blood–brain barrier, this collection of stimuli may be sufficient to drive the commitment phase of the inflammatory response, during which the unrestricted entry of T lymphocytes then proceeds (Hickey, 1999).

The intrinsic defensive response of the CNS to viral injury also includes the induction of other classes of genes likely to influence the antiviral activity of this compartment through direct effects and by shaping the virus-specific immune response to viral injury (Johnston *et al.*, 2001; Labrada *et al.*, 2002). These include IFN-α and IFN-regulated genes such as ISG12 (Labrada *et al.*, 2002). IFN-α itself does not seem to be strongly upregulated in acute encephalitis. However, IFN-α has been reported in the CSF and brain tissue of human cases of encephalitis with JEV serogroup viruses and, in such cases, appeared to represent a marker of severe infection with a fatal outcome (Burke and

Morill, 1987; Leport *et al*., 1984; Luby *et al*., 1969). The role of type I interferons in the CNS response to injury is complex, and differences in the activities of IRFs and IGSFs toward target response elements in neurons and glia can affect the range of genes involved in the response, including MHC antigens, the OAS1b protein, and antiapoptotic factors (Baron-Delage *et al*., 2000; Hirsch *et al*., 1986; Lucas *et al*., 2003; Massa *et al*., 1999; Njenga *et al*., 1997). Studies of viral infection in IFN-α and IFN-α-regulated gene knockout mice should help clarify the role of this defense system in directly controlling the viral infection of neurons versus effects on promoting the activity of the immune response recruited from the periphery. The importance of IL-12 in the innate CNS response to flavivirus encephalitis has not been investigated extensively; however, the outcome of CNS disease appears to vary among different viruses when this cytokine is used as an experimental therapy (Phillpotts *et al*., 2003). Because of the importance of IL-12 in antiviral defense against other CNS viral infections, through its ability to stimulate NOS (Reiss *et al*., 2002), more studies are needed to determine if it is implicated in protection against flaviviruses.

B. Virus-Specific Responses

Compared to information available on other neurotropic viruses, there has been relatively little work done to characterize the properties of virus-specific T cells recruited into the CNS in response to flavivirus infection (Scheider-Schaulies *et al*., 1997). This response is subject to a number of influences, including previous immunologic experience with related viruses, the level of immunocompetence, immunogenetic host factors, and the virulence of the infecting virus. Available information comes from a limited number of human and animal model experiments, which partially characterized lymphocytes or soluble markers of activated T cells and cytokines in the CNS (Burke *et al*., 1985b; Carson *et al*., 2003; Gunther *et al*., 1996; Iwasaki *et al*., 1993; Johnson *et al*., 1985, 1986; Kuno *et al*., 1993; Sampson *et al*., 2000), and other studies that have evaluated the requirements for these cells in the control of infection of this compartment in mouse models (Liu and Chambers, 2001; Liu *et al*., 1989a; Murali-Krishna *et al*., 1996; van der Most *et al*., 2000, 2003). T cells bearing both $CD4^+$ and $CD8^+$ surface markers have been visualized in perivascular infiltrates, in CSF, and in brain parenchyma during human flavivirus encephalitis (Johnson *et al*., 1985, 1986; Sampson *et al*., 2000). These cells include a larger proportion of $CD4^+$ to $CD8^+$ T cells and moderate numbers of B cells and macrophages. However, the composition of cells differed regionally

in the CNS, with macrophages and T cells more abundant in brain parenchyma and B cells more common within perivascular infiltrates. Factors governing the distribution of these cells and their effector functions are not well understood. Data from other experimental models suggest that $CD4^+$ T cells are important in directing the recruitment of lymphocytes (Hickey, 1999) and in maintaining the effector function of $CD8^+$ T cells within the brain parenchyma (Stohlman *et al.*, 1998). $CD8^+$ T cells have clearly been isolated from the brains of flavivirus-infected mice and, in some cases, demonstrated to have cytolytic activity (Liu *et al.*, 1989a); however, the contributions of such cells to both virus clearance and cellular injury within this compartment have not been defined. This remains a fundamental question, as the role of $CD4^+$ and $CD8^+$ T cells in different viral infections of the CNS and different experimental paradigms can vary considerably (Schneider-Schaulies *et al.*, 1997). For instance, virus clearance from mouse brains acutely infected with JEV has been reported by virus-specific $CD8^+$ T cells adoptively transferred into the CNS; however, $CD4^+$ T cells were also required (Murali-Krishna *et al.*, 1996). The relationship of this adoptive response to the natural immune response recruited from the periphery remains unclear, as the route of transfer may not reflect the normal pathway of lymphocyte trafficking. A requirement for CNS-associated $CD4^+$ and $CD8^+$ T cells was also observed in an immunization/challenge model of dengue virus (van der Most *et al.*, 2000). However, in the context of a memory response to YFV in the mouse model, $CD4^+$ T cells and B cells were required for the control of viral infection, whereas $CD8^+$ T cells were not required, although CD8-deficient mice exhibited a defect in virus clearance (Liu and Chambers, 2001; T. J. Chambers, unpublished data). Part of the effector activity of $CD8^+$ T cells may be mediated by the production of IFN-γ, which has a range of effects on the immunological properties of the CNS, including the upregulation of class I and II antigens on glial cells and injured neurons, activation of microglial, priming of astrocytes for cytokine production, and increasing permeability of the blood–brain barrier, as well as antiviral activity in the brain parenchyma (Benveniste, 1997; Kundig *et al.*, 1993; Popko *et al.*, 1997). IFN-γ knockout mice are defective in the clearance of YFV from the CNS and exhibit decreased inflammatory cell recruitment to this compartment (Liu and Chambers, 2001), indicating an important role of this cytokine in flavivirus encephalitis. IFN-γ may have a primary role in these processes because elimination of virus-infected neurons by CTLs is very tightly constrained by the absence of constitutive expression of MHC class I in these cells and the lack of susceptibility to perforin-mediated lysis (Medana *et al.*, 2000; Neumann *et al.*,

1995). Although killing can occur through Fas–FasL interactions (Medana *et al*., 2000), induction of FasL expression by neurons can also confer protection against CTL attack under certain conditions (Medana *et al*., 2001) and even induce apoptosis in infiltrating lymphocytes (Flugel *et al*., 2000). Virus-specific $CD8^+$ T cells have been shown to persist in the brains of mice recovering from dengue encephalitis for prolonged periods and differentiate into effector–memory cells that lose CTL activity while still producing IFN-γ (van der Most *et al*., 2003). Thus it appears that cellular immune-mediated mechanisms of neuronal cell death are subject to tight regulation in the presence of acute viral injury.

The role of antibody responses in the control of flavivirus encephalitis has been investigated in many classic studies, and it has been demonstrated repeatedly that immune serum can arrest viral infection in the CNS (Roehrig *et al*., 2001). Mechanisms responsible for this antibody-mediated control of infection remain unclear; however, studies with YFV encephalitis indicate that the Fc region and the IgG subclass are critical for protection in the mouse model (Schlesinger *et al*., 1993, 1995). These data suggest that direct effector functions associated with IgG, including interactions with cells bearing Fc receptors, such as microglia and recruited macrophages, are involved in this process. It is also conceivable that antibody–dependent cellular cytotoxicity directed against cell surface NS1 or even complement-mediated lysis also contributes to the protective effect. The antibody-mediated control of neuronal infection is a well-established mechanism for alphaviruses and coronaviruses, in which case the role of antibody in preventing reactivation of viral infection has been demonstrated (Griffin *et al*., 1997; Levine *et al*., 1991, 1992; Lin *et al*., 1999). In the case of Sindbis virus, there is inhibition of viral release and eventual sequestering of viral RNA within neurons without clearance. This process occurs preferentially at cortical sites of infection and obligates the local retention of virus-specific B cells (Tyor *et al*., 1992) in contrast to infection in the spinal cord where the cytokine-mediated (IFN-γ) elimination of viral RNA apparently predominates (Binder and Griffin, 2001). Antibody-mediated mechanisms may also operate in flavivirus encephalitis, as reactivation or recrudescence of CNS disease has been documented in some experimental animal systems and in clinical cases (Section XII). Factors responsible for the prolonged survival of B cells in the CNS are not known, particularly whether these cells represent a resident population of memory cells or require replenishment from peripheral sites (Tschen *et al*., 2002).

The beneficial role of antibody responses within the CNS is also supported by clinical studies of JEV and TBEV encephalitis (Burke *et al*.,

1985a, 1985c; Gunther *et al*., 1997; Han *et al*., 1988; Hoffman *et al*., 1978; Kaiser and Holzmann, 2000; Potula *et al*., 2003; Ravi *et al*., 1993). High levels of IgM correlate with an improved outcome in some cases, presumably reflecting the recruitment of virus-specific B cells into the CNS, as was reported in JEV cases (Burke *et al*., 1985a; Gunther *et al*., 1997), and the presence of IgG1 in particular is associated with the control of infection (Thakare *et al*., 1991), although IgM can be elevated in severe cases with a fatal outcome (Desai *et al*., 1994a). The functional activities provided by IgM in the CNS that result in clinical benefit remain unclear, as experimentally, IgM may have limited neutralization activity against encephalitic viruses at least early in infection (Diamond *et al*., 2003; Hoffman *et al*., 1978; Ishii *et al*., 1968). The contribution of complement-fixing activity could be important, as discussed earlier, in view of the fact that complement proteins may be upregulated in the CNS as a result of viral infection (Johnston *et al*., 2001).

The inflammatory response to flavivirus infection of the CNS may, in some cases, cause deleterious effects on neuronal function and survival. Immune complexes and autoantibodies to neurofilaments and myelin basic protein in the CSF and serum have been reported in severe JEV and TBEV infections (Desai *et al*., 1994a, 1994b; Fokina *et al*., 1991; Thakare *et al*., 1988) and may reflect an immunopathogenic process rather than nonspecific reactions to viral injury, particularly because of their association with poor outcome. Mechanisms leading to resolution of the acute inflammation in CNS viral infections remain undefined (Bradl and Flugel, 2002). At least three potential factors could be involved, including the expression of IL-4, IL-10, and TGF-β, which have been observed in the CNS of Sindbis virus-infected mice (Wesselingh *et al*., 1994) and are known to have immunomodulatory activities that reduce CNS inflammation; induction of apoptosis of infiltrating T cells; or possibly entry of NKT cells, which have been associated with the suppression of inflammatory responses. Further studies are needed to determine whether these or other factors are required to downregulate potentially harmful immune responses in cases where viral disease is eventually controlled.

XI. Neuropathogenesis: West Nile Virus as a Model

Similar to JEV, infections with WNV can be characterized as protean, involving an extraordinary host range, and an abundance of pathologic and virologic data has been obtained from experiments with

WNV in birds (Kramer and Bernard, 2001; Steele *et al*., 2000), horses (Bunning *et al*., 2002), and humans (Asnis *et al*., 2000; Hubalek and Halouzka, 1999). WNV has emerged as dimorphic in its clinical disease, with the apparent shift from typical WN fever to disease of greater severity, including more frequent cases of acute encephalitis in conjunction with the emergence of New World lineage I strains (Asnis *et al*., 2000; Cernescu *et al*., 1997). Studies with virulent strains such as New York 1999 WNV have demonstrated the neuropathogenic potential inherent in this virus. It is therefore important to note that lineage differences (type I versus type II) and perhaps genetic variation within lineages may influence the development of CNS disease.

After peripheral inoculation in the mouse model, WNV is believed to infect Langerhans dendritic cells (Johnston *et al*., 2000), which migrate to draining lymph nodes, and within 12 to 24 h of infection, viral replication is observed in secondary lymphoid tissue (Diamond *et al*., 2003; McMinn *et al*., 1996). Infectious virus is detected in serum within 24 to 48 h of infection (Diamond *et al*., 2003; Kramer and Bernard, 2001). The course of the early infection is slightly different in wild-type (WT) versus B-cell-deficient mice, as the peak of viremia occurs later in the latter case (day 2 versus days 4 to 6; Fig. 8). Shortly afterward, in WT mice, infectious virus is detected in visceral organs such as the spleen, kidney, and heart but not the liver (Diamond *et al*., 2003; Kramer and Bernard, 2001; Weiner *et al*., 1970), which may reflect restricted tropism or a high level of reticuloendothelial clearance in this organ. The levels of infectious virus in visceral tissues and serum peaked by day 4 after infection and thereafter diminished (Diamond *et al*., 2003; Kramer and Bernard, 2001; Xiao *et al*., 2001), concurrent with a rise in the titer of neutralizing antibodies (Fig. 9). In B-cell-deficient mice, replication in peripheral lymphoid tissue and visceral organs follows kinetics similar to those of WT mice; however, virus is not cleared from these sites. These data indicate the profound susceptibility of mice to WNV in the absence of antibody-producing B cells.

Virus can first be detected in the CNS by 4 days after peripheral subcutaneous infection in both WT and B-cell-deficient mice. Infectious virus is detected simultaneously in multiple sites in the brain, as well as in the inferior and superior spinal cord, suggesting a hematogenous route of dissemination and/or rapid spread within the CNS (Diamond *et al*., 2003). However, the route of peripheral inoculation influences the rate of spread to the brain and spinal cord. Infection via an intraperitoneal or intravenous route results in the spread of infectious virus to the brain within 2 days of infection (Kramer and Bernard, 2001), with these animals succumbing to infection several days earlier than

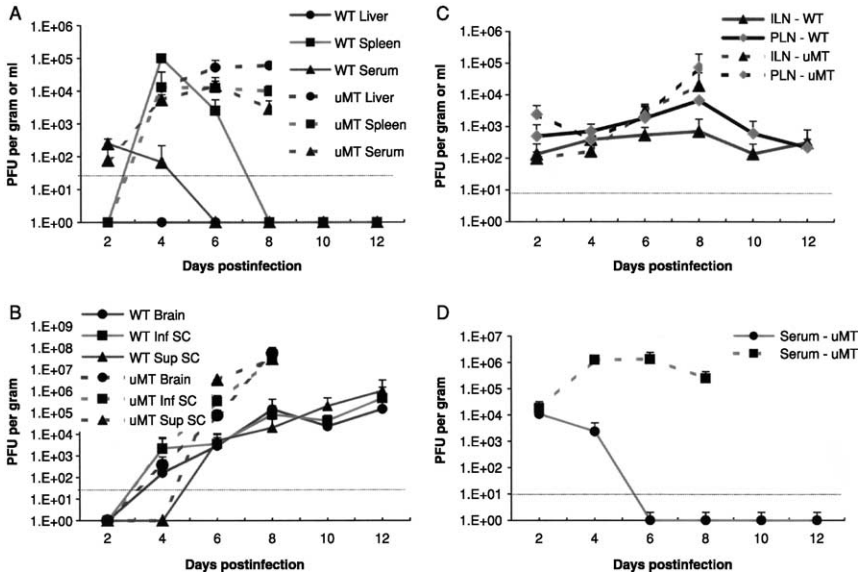

FIG 8. Wild-type or B-cell-deficient (uMT) mice were inoculated with WNV in the footpad, and the virus content in serum, peripheral tissues, and brain was measured serially using plaque assay or quantitative polymerase chain reaction. From Diamond *et al*. (2003), with permission.

those infected by a subcutaneous route. These differences probably reflect the fact that the interaction of virus with peripheral tissues results in the engagement of nonspecific and virus-specific defenses that have an impact on the course of disease. Models of infection that bypass the physiologic route of inoculation must be considered with this limitation in mind, as the balance of viral replication and dissemination versus immune activation can be skewed greatly by intraperitoneal or intravenous injection. Regardless of the route of administration, the course of disease after the onset of CNS infection is rapid and leads to fatal encephalitis beginning by days 9 to 10 postinfection, associated with high burdens of brain-associated virus.

Neutralizing activity associated with the IgM class is detectable by day 4 postinfection in the mouse model (Fig. 9), and this antibody response can confer partial protection against virus challenge in naïve mice (Diamond *et al*., 2003). Neutralizing IgG is detectable by day 8 and reaches levels of activity that are 10-fold higher than that of IgM by day 12 postinfection. The importance of secreted IgM in protection against disease is indicated from these data, as well as data presented

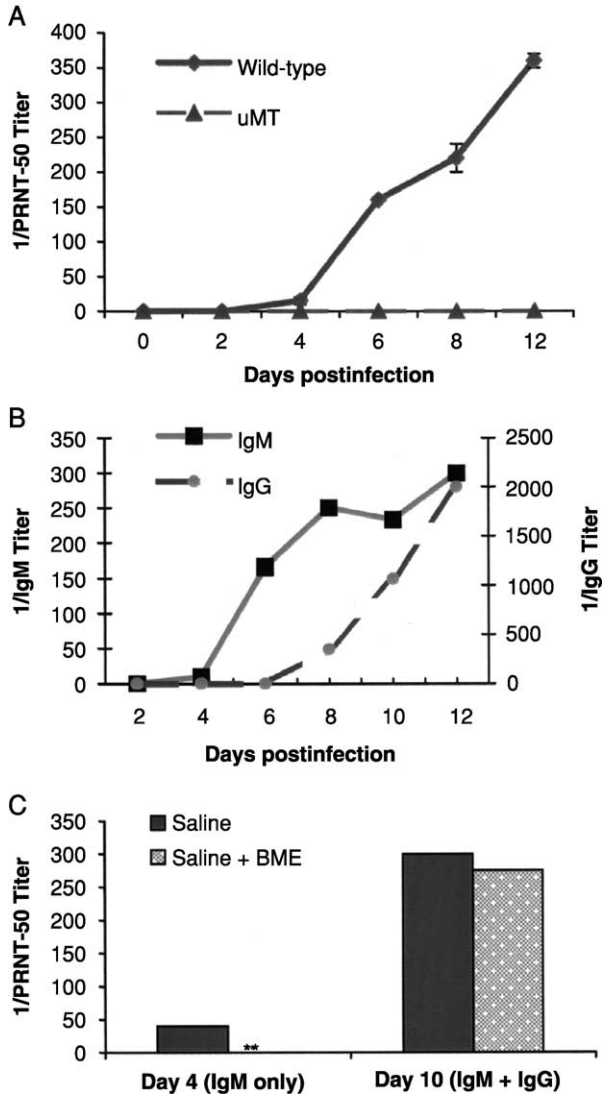

FIG 9. Neutralizing IgM and IgG antibody responses in acute WNV encephalitis in the mouse model. From Diamond *et al.* (2003), with permission.

earlier (Fig. 1). The relative importance of the mechanisms involved, including neutralizing activity per se or complement-assisted priming of the B-cell response to facilitate IgG production, is currently under investigation.

XII. Virus Persistence

Persistent infection *in vitro* and *in vivo* has been described in a number of experimental and clinical settings involving flaviviruses. The phenomenon of persistent infection in cell cultures of vertebrate and arthropod origin is well documented (Brinton, 1982; Chen *et al*., 1996; Jarman *et al*., 1968; Igarashi, 1979; Katz and Goldblum, 1968; Lancaster *et al*., 1998; Loginova *et al*., 1980; Poidinger *et al*., 1991; Randolph and Hardy, 1988a, 1988b; Schmaljohn and Blair, 1977, 1979; Shah and Gadkari, 1987; Vlaycheva and Chambers, 2002; Zhang *et al*., 1993), and the findings are analogous to many other models of viral infections *in vitro* where this process can be observed. Flavivirus persistence *in vitro* typically arises following a cytocidal infection, with survival of a residual population of cells that harbor low levels of replicating virus for long periods of time. The majority of cells in such cultures usually express viral antigen, but only a minority actually are productive of infectious virus. The cultures are generally resistant to superinfection with homologous but not heterologous virus, although in some cases, superinfection can drive virus production in quiescent antigen-positive cells (Schmaljohn and Blair, 1979). The persistence is not dependent on the expression of IFN-α, rather there is evidence for the involvement of host cell antiapoptotic pathways (see later). The infection is not necessarily deleterious for host cells, but in some cases, reduced growth efficiency occurs. Viruses detected in persistently infected cultures frequently undergo phenotypic alterations, including a reduction in plaque size, temperature sensitivity (Randolph and Hardy, 1988b; Shah and Gadkari, 1987), host-range restriction (Randolph and Hardy, 1988b), and loss of neurovirulence for mice (Igarashi, 1979). The genetic basis for the phenotypic change of viral variants has, in some cases, been demonstrated (Vlaycheva and Chambers, 2002). However, these infections are also associated with the emergence of defective viral particles or defective RNAs (Brinton, 1982; Debnath *et al*., 1991; Lancaster *et al*., 1998) that form the basis for superinfection resistance, and perhaps in part for the attenuation of mouse neurovirulence in flavivirus–resistant strains of mice. Some persistently infected cultures exhibit alterations in the composition of viral proteins or produce truncated forms of NS1 (Chen *et al*., 1996), which presumably are believed to reflect deletions in the viral genome associated with the generation of defective interfering viruses. The genome of defective MVE virus in Vero cells was shown to lack a large segment encompassing the prM-E region and a portion of the NS1 region, which leads to the production of truncated NS1 (Blitvich *et al*.,

1999; Lancaster *et al*., 1998). This finding is entirely consistent with the fact that replicons such as those engineered for Kunjin virus are designed to delete the corresponding region of the genome and are able to establish persistent replication in cell culture without any typical viral cytopathic effects (Varnavski and Khromykh, 1999). At least part of the explanation for the loss of cytocidal activity therefore may result from the deletion of structural proteins, which may provoke an ER overload response and/or trigger apoptosis (Duarte dos Santos *et al*., 2000; Prikhod'ko *et al*., 2001; Su *et al*., 2002). The production of infectious virus from persistently infected cultures can be reduced by treatment with a neutralizing antibody (Randolph and Hardy, 1988a), but it is not clear whether this shifts the cells to preferentially harbor defective viral genomes or whether these are actually eliminated.

The expression of apoptotic modulators is an important factor influencing the establishment and maintenance of persistent infection. In a model of JEV infection, the persistence in some cell lines was facilitated by the expression of bcl-2 (Liao *et al*., 1997), indicating that the mechanism is analogous to that of alphaviruses, where lytic infection can be converted to persistence in the presence of antiapoptotic proteins (Levine *et al*., 1993). However, the mechanisms that confer resistance to JEV-induced apoptosis must differ in some way from those of Sindbis and other viruses, as bcl-2 could fully protect N18 neuroblastoma cells against Sindbis, but not JEV. It is likely that the difference involves divergent pathways for activating the apoptotic process (Jan *et al*., 2000; Su *et al*., 2002). It is also possible that some cell lines represent variants that are defective in their apoptotic pathways, allowing virus to adapt in the absence of apoptotic events. At present, there is no evidence that flaviviruses encode proteins with antiapoptotic properties that might influence the process of the persistence.

The relevance of data on persistently infected cells *in vitro* to the issue of persistent infection in animal models and in apparent human cases is not straightforward. Although evidence for such clinical entities has been reported, the situation is complicated by the fact that there may be overlap between infections that have protracted convalescence and those that have frank neurological sequelae because flavivirus encephalitis is associated with neurologic complications that confer long-term disability among those who survive acute infection (Baruah *et al*., 2002; Finley and Riggs, 1980; Greve *et al*., 2002; Haglund and Gunther, 2003; Huy *et al*., 1994; Kumar *et al*., 1993; Richter *et al*., 1961; Vaneeva, 1969). In some cases, there is radiographic and pathologic evidence of permanent neurologic injury, including reduced cerebral blood flow, areas of abnormal signal density, and cellular dropout and gliosis

(Gunther *et al*., 1998; Ishii *et al*., 1977; Kumar *et al*., 1997; Shoji *et al*., 1990). Second, the possibility that a recrudescent latent infection really represents reinfection in a host with suboptimal immunity is a difficult question to evaluate. Also, the criteria for defining persistence are somewhat arbitrary. The presence of prolonged expression of IgM has been invoked as a basis for concluding persistence, but only infrequently has the presence of infectious virus been documented in situations where the duration of CNS disease exceeds that which is expected in acute uncomplicated flavivirus encephalitis (2 to 3 weeks).

Mechanisms of flavivirus persistence *in vivo* may theoretically also involve the formation of DI particles, which reduce infectious viral load, but like their *in vitro* counterparts, may not be sufficiently cytopathic to cause neuronal cell death. Such defective viruses may be capable of stimulating immune responses and thus be detected serologically. Experimentally, there is certainly evidence that DI particles can inhibit the production of infectious virus in the CNS (Atkinson *et al*., 1986; Barrett and Dimmock, 1984; Smith, 1981). The failure to detect infectious virus in both clinical and experimental situations where prolonged evidence of viral activity in the CNS was suspected is consistent with this hypothesis (Pogodina *et al*., 1983; Ravi *et al*., 1993). In a study of viral RNA in the brains of mice with the *Flv* resistance allele, a reduction in genome-length RNA rather than appearance of DI RNAs was observed (Urosevic *et al*., 1997), even though flavivirus–resistance in mice is associated with production of D1 virus (Smith, 1981). Thus, data implicating the role of DI particles in *in vitro* persistence may not correspond directly to an *in vivo* process.

Encephalitic flaviviruses that have been implicated in the occurrence of persistent infection of the CNS include members of the JEV serogroup and TBEV serogroup (Iliyenko *et al*., 1974; Ogawa *et al*., 1973; Pogodina *et al*., 1983; Slavin *et al*., 1943; Zlotnik *et al*., 1971), with the latter having a particular propensity to cause chronic infections. In humans, there have been reports of various types of persistent infection. These include (1) reactivation of latent disease, as in children with JEV encephalitis who experienced recurrent infection months after the primary infection and, in some cases, gave positive virus isolations (Sharma *et al*., 1991); (2) chronic progressive disease with cognitive and motor disturbances that resembled subacute sclerosing panencephalitis, years after a primary infection with Russian spring–summer encephalitis (RSSE) virus (Ogawa *et al*., 1973); (3) prolonged infection in primary cases of acute encephalitis, with elevation of CSF IgM for as long as 10 months and, in some cases, with virus isolated from spinal fluid (Ravi *et al*., 1993); and (4) evidence of prolonged

circulation of virus-infected cells without clear-cut CNS disease (Southam *et al.*, 1958). Protracted cases of TBEV encephalitis, with either cognitive dysfunction or spinal nerve paralysis, occur frequently (Haglund and Gunther, 2003), although the relationship of these outcomes to persistent viral infection is not known. These different types of clinical infections have been mimicked by various experimental models, and it has been documented that virus can be recovered after a protracted phase of infection. For instance, strains of WNV with differing neurovirulence properties were capable of causing prolonged encephalitis of variable severity, ranging up to 5 months in duration in monkeys (Pogodina *et al.*, 1981, 1983). Eventually the viruses were cleared or were rendered replication defective and detectable only by the presence of viral antigen. Some viruses recovered from brains of these animals had undergone attenuation of mouse neurovirulence, indicating a selection for genetic variants and/or defective interfering particles. Mechanisms preventing the efficient clearance of virus were not determined, but did not involve defects in the production of neutralizing antibodies. Immunosuppression, either by cytotoxic drugs or even that associated with pregnancy, has been demonstrated to cause prolonged flavivirus disease (Mathur *et al.*, 1986; Zlotnik *et al.*, 1971), and reactivation of infection can be elicited during or after the primary infection by immune depletion (Mathur *et al.*, 1986). In some cases, as in JEV infection of mice, viral latency was established in T lymphocytes, which could be subsequently activated by immunosuppression to produce infectious virus (Mathur *et al.*, 1989). These latter findings are consistent with reports of recurrent JEV infection observed in children, but the stimulus for reactivation in such cases is not known (Sharma *et al.*, 1991).

In the CNS, experimental persistent infections have been related to immune response factors, as well as the ability of neurons to survive viral infection by mechanisms involving apoptotic modulators. Immunological tolerance is also a potential factor that has been associated with other viruses such as LCMV that establish persistent CNS infection, but this does not seem to play any obvious role in flavivirus persistence. Failure to clear brain-associated virus may result from a limited effectiveness of the innate CNS defenses and the peripheral immune responses that are activated in response to flavivirus infections. In addition, elimination of viral RNA may require $CD8^+$ T-cell functions [as described for alphaviruses (Binder and Griffin, 2001)], which are severely restricted due to a lack of class I expression on neurons. The generally resistant state of differentiated neurons toward apoptotic stimuli is also likely to be an important factor (Griffin and Hardwick, 1999).

Together, these processes may facilitate the persistence of viral RNA and perhaps foster the evolution of DI particles and noncytopathic-attenuated viral variants. The host immune response, together with properties of the neuronal cellular environment, influences the likelihood of virus perisistence and may both contribute to differences in phenotypes of persisting viruses. For instance, WNV variants that emerged from persistent infection in monkeys tended to acquire attenuation phenotypes (Pogodina *et al*., 1983), whereas it has been observed in persistent infection of mice with Sindbis virus that neurovirulent mutants arise (Levine and Griffin, 1992, 1993). Selection pressures imposed by neutralizing antibodies that are produced intraparenchymally by virus-specific B cells may facilitate the emergence of viral variants in some cases, but tissue-specific adaptations are also involved. At present, it is reasonable to believe that the phenomenon of flavivirus persistence in the CNS is a function of many variables, which include the heterogeneity of the neuronal response to injury, encompassing innate interferon-regulated antiviral defenses (Johnston *et al*., 2001) and the competence for and propensity towards apoptosis. Interaction of virus–infected cells with virus-specific T cells possessing cytokine-mediated effector functions that can eliminate viral RNA and B cells that can provide antibodies capable of downregulating viral replication influences this process in a region-specific manner. The balance among these factors results in a spectrum of outcomes that may range from either clearance to merely suppression of viral infection, with variable consequences for long-term neurologic function.

References

Aihara, H., Takasaki, T., Toyosaki-Maeda, T., Suzuki, R., Okuno, Y., and Kurane, I. (2000). T-cell activation and induction of antibodies and memory T cells by immunization with inactivated Japanese encephalitis vaccine. *Viral Immunol.* **13:**179–186.

Albrecht, P. (1968). Pathogenesis of neurotropic arbovirus infections. *Curr. Top. Microbiol. Immunol.* **43:**44–91.

Aloisi, F., Care, A., Borsellino, G., Gallo, P., Rosa, S., Bassani, A., Cabibbo, A., Testa, U., Levi, G., and Peschle, C. (1992). Production of hemolymphopoietic cytokines (IL-6, IL-8, colony-stimulating factors) by normal human astrocytes in response to IL-1 beta and tumor necrosis factor-alpha. *J. Immunol.* **149:**2358–2366.

Andersen, A., A., and Hanson, R. P. (1974). Influence of sex and age on natural resistance to St. Louis encephalitis virus infection in mice. *Infect. Immun.* **9:**1123–1125.

Anderson, J. F., and Rahal, J. J. (2002). Efficacy of interferon alpha-2b and ribavirin against West Nile virus in vitro. *Emerg. Infect. Dis.* **8:**107–108.

Anderson, R., Wang, S., Osiowy, C., and Issekutz, A. C. (1997). Activation of endothelial cells via antibody-enhanced dengue virus infection of peripheral blood monocytes. *J. Virol.* **71:**4226–4232.

Andrews, D. M., Matthews, V. B., Sammels, L. M., Carrello, A. C., and McMinn, P. C. (1999). The severity of Murray Valley encephalitis in mice is linked to neutrophil infiltration and inducible nitric oxide synthase activity in the central nervous system. *J. Virol.* **73:**8781–8790.

Asada-Kubota, M., Ueda, T., Nakashima, T., Kobayashi, M., Shimada, M., Takeda, K., Hamada, K., Maekawa, S., and Sokawa, Y. (1997). Localization of 2′,5′-oligoadenylate synthetase and the enhancement of its activity with recombinant interferon-alpha A/D in the mouse brain. *Anat. Embryol. (Berl.)* **195:**251–257.

Asher, D. M. (1975). Movement disorders in rhesus monkeys after infection with tick-borne encephalitis virus. *Adv. Neurol.* **10:**277–289.

Asnis, D. S., Conetta, R., Teixeira, A. A., Waldman, G., and Sampson, B. A. (2000). The West Nile Virus outbreak of 1999 in New York: The Flushing Hospital experience. *Clin. Infect. Dis.* **30:**413–418.

Atkinson, T., Barrett, A. D. T., Mackenzie, A., and Dimmock, N. J. (1986). Persistence of virulent Semliki Forest virus in mouse brain following co-inoculation with defective interfering particles. *J. Gen. Virol.* **67:**1189–1194.

Atrasheuskaya, A. V., Fredeking, T. M., and Ignatyev, G. M. (2003). Changes in immune parameters and their correction in human cases of tick-borne encephalitis. *Clin. Exp. Immunol.* **131:**148–154.

Avirutnan, P., Malasit, P., Seliger, B., Bhakdi, S., and Husmann, M. (1998). Dengue virus infection of human endothelial cells leads to chemokine production, complement activation, and apoptosis. *J. Immunol.* **161:**6338–6346.

Azad, R., Gupta, R. K., Kumar, S., Pandey, C. M., Prasad, K. N., Husain, N., and Husain, M. (2003). Is neurocysticercosis a risk factor in coexistent intracranial disease? An MRI-based study. *J. Neurol. Neurosurg. Psychiatry* **74:**359–361.

Baron-Delage, S., Abadie, A., Echaniz-Laguna, A., Melki, J., and Beretta, L. (2000). Interferons and IRF-1 induce expression of the survival motor neuron (SMN) genes. *Mol. Med.* **6:**957–968.

Barrett, A. D. T., and Dimmock, N. J. (1984). Modulation of Semliki Forest virus-induced infection of mice by defective interfering virus. *J. Infect. Dis.* **150:**98–103.

Barrington, R., Zhang, M., Fischer, M., and Carroll, M. C. (2001). The role of complement in inflammation and adaptive immunity. *Immunol. Rev.* **180:**5–15.

Baruah, H. C., Biswas, D., Patgiri, D., and Mahanta, J. (2002). Clinical outcome and neurological sequelae in serologically confirmed cases of Japanese encephalitis patients in Assam, India. *Indian Pediatr.* **39:**1143–1148.

Baumgarth, N., Herman, O. C., Jager, G. C., Brown, L. E., Herzenberg, L. A., and Chen, J. (2000). B-1 and B-2 cell-derived immunoglobulin M antibodies are nonredundant components of the protective response to influenza virus infection. *J. Exp. Med.* **192:**271–280.

Ben-Nathan, D., Huitinga, I., Lustig, S., van Rooijen, N., and Kobiler, D. (1996a). West Nile virus neuroinvasion and encephalitis induced by macrophage depletion in mice. *Arch. Virol.* **141:**459–469.

Ben-Nathan, D., Kobiler, D., Rzotkiewicz, S., Lustig, S., and Katz, Y. (2000). CNS penetration by noninvasive viruses following inhalational anesthetics. *Ann. N. Y. Acad. Sci.* **917:**944–950.

Ben-Nathan, D., Lustig, S., and Feuerstein, G. (1989). The influence of cold or isolation stress on neuroinvasiveness and virulence of an attenuated variant of West Nile virus. *Arch. Virol.* **109:**1–10.

Ben-Nathan, D., Lustig, S., and Kobiler, D. (1996b). Cold stress-induced neuroinvasiveness of attenuated arboviruses is not solely mediated by corticosterone. *Arch. Virol.* **141:**1221–1229.

Ben-Nathan, D., Lustig, S., Kobiler, D., Danenberg, H. D., Lupu, E., and Feuerstein, G. (1992). Dehydroepiandrosterone protects mice inoculated with West Nile virus and exposed to cold stress. *J. Med. Virol.* **38:**159–166.

Benveniste, E. N. (1997). Cytokine expression in the nervous system. *In* "Immunology of the Nervous System" (R. W. Keane and W. F. Hickey, eds.), pp. 419–459. Oxford Univ. Press, New York.

Bergmann, C. C., Ramakrishna, C., Kornacki, M., and Stohlman, S. A. (2001). Impaired T cell immunity in B cell-deficient mice following viral central nervous system infection. *J. Immunol.* **167:**1575–1583.

Bernard, K. A., Klimstra, W. B., and Johnston, R. E. (2000). Mutations in the E2 glycoprotein of Venezuelan equine encephalitis virus confer heparan sulfate interaction, low morbidity, and rapid clearance from blood of mice. *Virology* **276:**93–103.

Bielefeldt-Ohmann, H. (1998). Analysis of antibody-independent binding of dengue viruses and dengue virus envelope protein to human myelomonocytic cells and B lymphocytes. *Virus Res.* **57:**63–79.

Binder, G., and Griffin, D. E. (2001). Interferon-gamma-mediated site-specific clearance of alphavirus from CNS neurons. *Science* **293:**303–306.

Blackwell, J. L., and Brinton, M. A. (1995). BHK cell proteins that bind to the 3′ stem-loop structure of the West Nile virus genome RNA. *J. Virol.* **69:**5650–5658.

Blackwell, J. L., and Brinton, M. A. (1997). Translation elongation factor-1 alpha interacts with the 3′ stem-loop region of West Nile virus genomic RNA. *J. Virol.* **71:**6433–6444.

Blitvich, B. J., Scanlon, D., Shiell, B. J., Mackenzie, J. S., and Hall, R. A. (1999). Identification and analysis of truncated and elongated species of the flavivirus NS1 protein. *Virus Res.* **60:**67–79.

Boes, M., Esau, C., Fischer, M. B., Schmidt, T., Carroll, M., and Chen, J. (1998). Enhanced B-1 cell development, but impaired IgG antibody responses in mice deficient in secreted IgM. *J. Immunol.* **160:**4776–4787.

Bonnevie-Nielsen, V., Heron, I., Monath, T. P., and Calisher, C. H. (1995). Lymphocytic 2′,5′-oligoadenylate synthetase activity increases prior to the appearance of neutralizing antibodies and immunoglobulin M and immunoglobulin G antibodies after primary and secondary immunization with yellow fever vaccine. *Clin. Diag. Lab. Immunol.* **2:**302–306.

Bonnevie-Nielsen, V., Larsen, M. L., Frifelt, J. J., Michelsen, B., and Lernmark, A. (1989). Association of IDDM and attenuated response of 2′,5′-oligoadenylate synthetase to yellow fever vaccine. *Diabetes* **38:**1636–1642.

Bosch, I., Xhaja, K., Estevez, L., Raines, G., Melichar, H., Warke, R. V., Fournier, M. V., Ennis, F. A., and Rothman, A. L. (2002). Increased production of interleukin-8 in primary human monocytes and in human epithelial and endothelial cell lines after dengue virus challenge. *J. Virol.* **76:**5588–5597.

Bradish, C. J., Fitzgeorge, R., and Titmuss, D. (1980). The responses of normal and athymic mice to infections by togaviruses: Strain differentiation in active and adoptive immunization. *J. Gen. Virol.* **46:**255–265.

Bradl, M., and Flugel, A. (2002). The role of T cells in brain pathology. *Curr. Top. Microbiol. Immunol.* **265:**141–162.

Brandriss, M. W., and Schlesinger, J. J. (1984). Antibody-mediated infection of P388D1 cells with 17D yellow fever virus: Effects of chloroquine and cytochalasin B. *J. Gen. Virol.* **65:**791–794.

Brandriss, M. W., Schlesinger, J. J., Walsh, E. E., and Briselli, M. (1986). Lethal 17D yellow fever encephalitis in mice. I. Passive protection by monoclonal antibodies to

the envelope proteins of 17D yellow fever and dengue 2 viruses. *J. Gen. Virol.* **67:**229–234.

Brandt, W. E., McCown, J. M., Gentry, M. K., and Russell, P. K. (1982). Infection enhancement of dengue type 2 virus in the U-937 human monocyte cell line by antibodies to flavivirus cross-reactive determinants. *Infect. Immun.* **36:**1036–1041.

Brinton, M. A. (1982). Characterization of West Nile virus persistent infection in genetically resistant and susceptible mouse cells. I. Generation of defective nonplaquing virus particles. *Virology* **116:**84–94.

Brinton, M. A. (2000). Host factors involved in West Nile virus replication. *Ann. N. Y. Acad. Sci.* **951:**207–219.

Brooks, T. J., and Phillpotts, R. J. (1999). Interferon-alpha protects mice against lethal infection with St. Louis encephalitis virus delivered by the aerosol and subcutaneous routes. *Antiviral Res.* **41:**57–64.

Broom, A. K., Wallace, M. J., Mackenzie, J. S., Smith, D. W., and Hall, R. A. (2000). Immunisation with gamma globulin to Murray Valley encephalitis virus and with an inactivated Japanese encephalitis virus vaccine as prophylaxis against Australian encephalitis: Evaluation in a mouse model. *J. Med. Virol.* **61:**259–265.

Bunning, M. L., Bowen, R. A., Cropp, C. B., Sullivan, K. G., Davis, B. S., Komar, N., Godsey, M. S., Baker, D., Hettler, D. L., Holmes, D. A., Biggerstaff, B. J., and Mitchell, C. J. (2002). Experimental infection of horses with West Nile virus. *Emerg. Infect. Dis.* **8:**380–386.

Burke, D. S., Lorsomrudee, W., Leake, C. J., Hoke, C. H., Nisalak, A., Chongswasdi, V., and Laorakpongse, T. (1985a). Fatal outcome in Japanese encephalitis. *Am. J. Trop. Med. Hyg.* **34:**1203–1210.

Burke, D. S., Nisalak, A., Lorsomrudee, W., Ussery, M. A., and Laorpongse, T. (1985b). Virus-specific antibody-producing cells in blood and cerebrospinal fluid in acute Japanese encephalitis. *J. Med. Virol.* **17:**283–292.

Burke, D. S., Nisalak, A., Usery, M. A., Laorakpongse, T., and Chantavibul, S. (1985c). Kinetics of IgM and IgG responses to Japanese encephalitis virus in human serum and cerebrospinal fluid. *J. Infect. Dis.* **151:**1093–1099.

Burke, D. S., and Morill, J. C. (1987). Levels of interferon in the plasma and cerebrospinal fluid of patients with acute Japanese encephalitis. *J. Infect. Dis.* **155:**797–799.

Burke, D. S., and Monath, T. P. (2001). Flaviviruses. *In* "Fields Virology" (D. M. Knipe and P. M. Howley, eds.), 4th Ed., Vol. 1, pp. 1043–1125. Lippincott Williams & Wilkins, Philadelphia.

Byrne, S. N., Halliday, G. M., Johnston, L. J., and King, N. J. (2001). Interleukin-1beta but not tumor necrosis factor is involved in West Nile virus-induced Langerhans cell migration from the skin in C57BL/6 mice. *J. Invest. Dermatol.* **117:**702–709.

Camenga, D. L., Nathanson, N., and Cole, G. A. (1974). Cyclophosphamide-potentiated West Nile viral encephalitis: Relative influence of cellular and humoral factors. *J. Infect. Dis.* **130:**634–641.

Cane, P. A., and Gould, E. A. (1988). Reduction of yellow fever virus mouse neurovirulence by immunization with a bacterially synthesized nonstructural protein (NS1) fragment. *J. Gen. Virol.* **69:**1241–1246.

Cardosa, M. J., Porterfield, J. S., and Gordon, S. (1983). Complement receptor mediates enhanced flavivirus replication in macrophages. *J. Exp. Med.* **158:**258–263.

Cardosa, M. J., Gordon, S., Hirsch, S., Springer, T. A., and Porterfield, J. S. (1986). Interaction of West Nile virus with primary murine macrophages: Role of cell activation and receptors for antibody and complement. *J. Virol.* **57:**952–959.

Carroll, M. C. (1998). The role of complement and complement receptors in induction and regulation of immunity. *Annu. Rev. Immunol.* **16:**545–568.

Carson, P. J., Steidler, T., Patron, R., Tate, J. M., Tight, R., and Smego, R. A. Jr. (2003). Plasma cell pleiocytosis in cerebrospinal fluid in patients with West Nile virus encephalitis. *Clin. Infect. Dis.* **37:**e12–e15.

Casali, P., and Notkins, A. L. (1989). $CD5^+$ B lymphocytes, polyreactive antibodies and the human B-cell repertoire. *Immunol. Today* **10:**364–368.

Cernescu, C., Ruta, S. M., Tardei, G., Grancea, C., Moldoveanu, L., Spulbar, E., and Tsai, T. (1997). A high number of severe neurologic clinical forms during an epidemic of West Nile virus infection. *Rom. J. Virol.* **48:**13–25.

Chambers, T. J., and Nickells, M. (2001). Neuroadapted yellow fever virus 17D: Genetic and biological characterization of a highly mouse-neurovirulent virus and its infectious molecular clone. *J. Virol.* **75:**10912–10922.

Charlier, N., Leyssen, P., Paeshuyse, J., Drosten, C., Schmitz, H., Van Lommel, A., De Clercq, E., and Neyts, J. (2002). Infection of SCID mice with Montana Myotis leukoencephalitis virus as a model for flavivirus encephalitis. *J. Gen. Virol.* **83:**1887–1896.

Chaturvedi, U. C., Dhawan, R., Khanna, M., and Mathur, A. (1991). Breakdown of the blood-brain barrier during dengue virus infection of mice. *J. Gen. Virol.* **72:**859–866.

Chen, C. J., Liao, S. L., Kuo, M. D., and Wang, Y. M. (2000). Astrocytic alteration induced by Japanese encephalitis virus infection. *Neuroreport* **11:**1933–1937.

Chen, H. W., Pan, C. H., Huan, H. W., Liau, M. Y., Chiang, J. R., and Tao, M. H. (2001). Suppression of immune response and protective immunity to a Japanese encephalitis virus DNA vaccine by coadministration of an IL-12-expressing plasmid. *J. Immunol.* **166:**7419–7426.

Chen, H. W., Pan, C. H., Liau, M. Y., Jou, R., Tsai, C. J., Wu, H. J., Lin, Y. L., and Tao, M. H. (1999). Screening of protective antigens of Japanese encephalitis virus by DNA immunization: A comparative study with conventional viral vaccines. *J. Virol.* **73:**10137–10145.

Chen, L. K., Liao, C. L., Lin, C. G., Lai, S. C., Liu, C. I., Ma, S. H., Huang, Y. Y., and Lin, Y. L. (1996). Persistence of Japanese encephalitis virus is associated with abnormal expression of the nonstructural protein NS1 in host cells. *Virology* **217:**220–229.

Chen, Y., Maguire, T., Hileman, R. E., Fromm, J. R., Esko, J. D., Linhardt, R. J., and Marks, R. M. (1997). Dengue virus infectivity depends on envelope protein binding to target cell heparan sulfate. *Nature Med.* **3:**866–871.

Chu, C. T., Howell, D. N., Morenlander, J. C., Hulette, C. M., McLendon, R. E., and Miller, S. E. (1999). Electron microscopic diagrosis of human flavivirus encephalitis: use of confocal microscopy as an aid. *Am. J. Surg. Path.* **23:**1217–1226.

Co, M. D., Terajima, M., Cruz, J., Ennis, F. A., and Rothman, A. L. (2002). Human cytotoxic T lymphocyte responses to live attenuated 17D yellow fever vaccine: Identification of HLA-B35-restricted CTL epitopes on nonstructural proteins NS1, NS2b, NS3, and the structural protein E. *Virology* **293:**151–163.

Cole, G. A., and Nathanson, N. (1968). Potentiation of experimental arbovirus encephalitis by immunosuppressive doses of cyclophosphamide. *Nature* **220:**399–401.

Colombage, G., Hall, R., Pavy, M., and Lobigs, M. (1998). DNA-based and alphavirus-vectored immunisation with prM and E proteins elicits long-lived and protective immunity against the flavivirus, Murray Valley encephalitis virus. *Virology* **250:**151–163.

Cousens, L. P., Peterson, R., Hsu, S., Dorner, A., Altman, J. D., Ahmed, R., and Biron, C. A. (1999). Two roads diverged: Interferon alpha/beta- and interleukin 12-mediated

pathways in promoting T cell interferon gamma responses during viral infection. *J. Exp. Med.* **189:**1315–1328.

Crill, W. D., and Roehrig, J. T. (2001). Monoclonal antibodies that bind to domain III of dengue virus E glycoprotein are the most efficient blockers of virus adsorption to Vero cells. *J Virol.* **75:**7769–7773.

Cypess, R. H., Lubiniecki, A. S., and Hammon, W. M. (1973). Immunosuppression and increased susceptibility to Japanese B encephalitis virus in *Trichinella spiralis*-infected mice. *Proc. Soc. Exp. Biol. Med.* **143:**469–473.

Da Costa, X. J., Brockman, M. A., Alicot, E., Ma, M., Fischer, M. B., Zhou, X., Knipe, D. M., and Carroll, M. C. (1999). Humoral response to herpes simplex virus is complement-dependent. *Proc. Natl. Acad. Sci. USA* **96:**2708–11272.

Dalod, M., Salazar-Mather, T. P., Malmgaard, L., Lewis, C., Asselin-Paturel, C., Briere, F., Trinchieri, G., and Biron, C. A. (2002). Interferon alpha/beta and interleukin 12 responses to viral infections: Pathways regulating dendritic cell cytokine expression in vivo. *J. Exp. Med.* **195:**517–528.

Debnath, N. C., Tiernery, R., Sil, B. K., Wills, M. R., and Barrett, A. D. (1991). In vitro homotypic and heterotypic interference by defective interfering particles of West Nile virus. *J. Gen. Virol.* **72:**2705–2711.

De Nova-Ocampo, M., Villegas-Sepulveda, N., and del Angel, RM. (2002). Translation elongation factor-1alpha, La, and PTB interact with the 3′ untranslated region of dengue 4 virus RNA. *Virology* **295:**337–347.

Desai, A., Ravi, V., Chandramuki, A., and Gourie-Devi, M. (1995a). Proliferative response of human peripheral blood mononuclear cells to Japanese encephalitis virus. *Microbiol. Immunol.* **39:**269–273.

Desai, A., Ravi, V., Chandramuki, A., and Gourie-Devi, M. (1994a). Detection of immune complexes in the CSF of Japanese encephalitis patients: Correlation of findings with outcome. *Intervirology* **37:**352–355.

Desai, A., Ravi, V., Guru, S. C., Shankar, S. K., Kaliaperumal, V. G., Chandramuki, A., and Gourie-Devi, M. (1994b). Detection of autoantibodies to neural antigens in the CSF of Japanese encephalitis patients and correlation of findings with the outcome. *J. Neurol. Sci.* **122:**109–116.

Despres, P., Dietrich, J., Girard, M., and Bouloy, M. (1991). Recombinant baculoviruses expressing yellow fever virus E and NS1 proteins elicit protective immunity in mice. *J. Gen. Virol.* **72:**2811–2816.

Despres, P., Frenkiel, M.-P., Ceccaldi, P.-E., Dos Santos, C. D., and Deubel, V. (1998). Apoptosis in the mouse central nervous system in response to infection with mouse-neurovirulent dengue viruses. *J. Virol.* **72:**823.

Diamond, M., Roberts, T., Edgil, D., Lu, B., Ernst, J., and Harris, E. (2000a). Modulation of dengue virus infection in human cells by alpha, beta, and gamma interferons. *J. Virol.* **74:**4957–4966.

Diamond, M. S., Edgil, D., Roberts, T. G., Lu, B., and Harris, E. (2000b). Infection of human cells by dengue virus is modulated by different cell types and viral strains. *J. Virol.* **74:**7814–7823.

Diamond, M. S., and Harris, E. (2001). Interferon inhibits dengue virus infection by preventing translation of viral RNA through a PKR-independent mechanism. *Virology* **289:**297–311.

Diamond, M. S., Shrestha, B., Marri, A., Mahan, D., and Engle, M. (2003). B cells and antibody play critical roles in the immediate defense of disseminated infection by West Nile encephalitis virus. *J. Virol.* **77:**2578–2586.

Dominguez, C., and Baruch, E. (1963). Histopathology of the central nervous system in Swiss mice intracerebrally inoculated with 17-D strain of yellow fever virus. *Am. J. Trop Med. Hyg.* **12:**815–819.

Dropulic, B., and Masters, C. L. (1990). Entry of neurotropic arboviruses into the central nervous system: An in vitro study using mouse brain endothelium. *J. Infect. Dis.* **161:**685–691.

Douglas, M. W., Kesson, A. M., and King, N. J. (1994). CTL recognition of West Nile virus-infected fibroblasts is cell cycle dependent and is associated with virus-induced increases in class I MHC antigen expression. *Immunology* **82:**561–570.

Duarte dos Santos, C. N., Frenkiel, M.-P., Courageot, M.-P., Rocja, C. F. S., Vazeille-Falcoz, M.-C., Wien, M. W., Rey, F. A., Deubel, V., and Despres, P. (2000). Determinants in the envelope E protein and viral RNA helicase NS3 that influence the induction of apoptosis in response to infection with dengue type 1 virus. *Virology* **274:**292–308.

Edelman, R., Schneider, R. J., Vejjajiva, A., Pornpibul, R., and Voodhikul, P. (1976). Persistence of virus-specific IgM and clinical recovery after Japanese encephalitis. *Am. J. Trop. Med. Hyg.* **25:**733–738.

Eldadah, A. H., Nathanson, N., and Sarsitis, R. (1967a). Pathogenesis of West Nile virus encephalitis in mice and rats. I. Influence of age and species on mortality and infection. *Am. J. Epidemiol.* **86:**765–775.

Eldadah, A. H., and Nathanson, N. (1967b). Pathogenesis of West Nile virus encephalitis in mice and rats. II. Virus multiplication, evolution of immunofluorescence, and development of histological lesions in the brain. *Am. J. Epidemiol.* **86:**776–790.

Falconar, A. K. (1999). Identification of an epitope on the dengue virus membrane (M) protein defined by cross-protective monoclonal antibodies: Design of an improved epitope sequence based on common determinants present in both envelope (E and M) proteins. *Arch. Virol.* **144:**2313–2330.

Falgout, B., Bray, M., Schlesinger, J. J., and Lai, C. J. (1990). Immunization of mice with recombinant vaccinia virus expressing authentic dengue virus nonstructural protein NS1 protects against lethal dengue virus encephalitis. *J. Virol.* **64**(9)**:**4356–4363.

Fazakerley, J. (2001). Programmed cell death in virus infections of the nervous system. *Curr. Top. Micro. Immunol.* **253:**95–119.

Fazakerley, J. (2002). Pathogenesis of Semliki Forest virus encephalitis. *J. Neurovirol.* **8**(S2)**:**66–74.

Finley, K., and Riggs, N. (1980). Convalescence and sequellae. *In* "St. Louis Encephalitis" (T. P. Monath, ed.), pp. 535–550. APHA, Washington, DC.

Fischer, M. B., Ma, M., Goerg, S., Zhou, X., Xia, J., Finco, O., Han, S., Kelsoe, G., Howard, R. G., Rothstein, T. L., Kremmer, E., Rosen, F. S., and Carroll, M. C. (1996). Regulation of the B cell response to T-dependent antigens by classical pathway complement. *J. Immunol.* **157:**549–556.

Fitzgeorge, R., and Bradish, C. J. (1980). The in vivo differentiation of strains of yellow fever virus in mice. *J. Gen. Virol.* **46:**1–13.

Flugel, A., Schwaiger, F. W., Neumann, H., Medana, I., Willem, M., Wekerle, H., Kreutzberg, G. W., and Graeber, M. B. (2000). Neuronal FasL induces cell death of encephalitogenic T lymphocytes. *Brain Pathol.* **10:**353–364.

Fokina, G. I., Roikhel, V. M., Magaznik, S. S., Volkova, L. I., Frolova, T. V., and Pogodina, V. V. (1991). Development of antibodies to axonal neurofilaments in the progression of chronic tick-borne encephalitis. *Acta Virol.* **35:**458–463.

Freestone, D. S. (1994). Yellow fever vaccine. *In* "Vaccines" (S. A. Plotkin and E. M. Mortimer, eds.), 2nd Ed., pp. 741–779. Saunders, Philadelphia.

Gagnon, S. J., Ennis, F. A., and Rothman, A. L. (1999). Bystander target cell lysis and cytokine production by dengue virus-specific human $CD4^+$ cytotoxic T-lymphocyte clones. *J. Virol.* **73:**3623–3629.

Germi, R., Crance, J. M., Garin, D., Guimet, J., Lortat-Jacob, H., Ruigrok, R. W., Zarski, J. P., and Drouet, E. (2002). Heparan sulfate-mediated binding of infectious dengue virus type 2 and yellow fever virus. *Virology* **292:**162–168.

Glass, J. D., Samuels, O., and Rich, M. M. (2002). Poliomyelitis due to West Nile virus. *N. Engl. J. Med.* **347:**1280–1281.

Gobet, R., Cerny, A., Ruedi, E., Hengartner, H., and Zinkernagel, R. M. (1988). The role of antibodies in natural and acquired resistance of mice to vesicular stomatitis virus. *Exp. Cell. Biol.* **56:**175–180.

Gollins, S. W., and Porterfield, J. S. (1984). Flavivirus infection enhancement in macrophages: Radioactive and biological studies on the effect of antibody on viral fate. *J. Gen. Virol.* **65:**1261–1272.

Gould, E. A., and Buckley, A. (1989). Antibody-dependent enhancement of yellow fever and Japanese encephalitis virus neurovirulence. *J. Gen. Virol.* **70:**1605–1608.

Gould, E. A., Buckley, A., Groeger, B. K., Cane, P. A., and Doenhoff, M. (1987). Immune enhancement of yellow fever virus neurovirulence for mice: Studies of mechanisms involved. *J. Gen. Virol.* **68:**3105–3112.

Gresikova, M., Sekeyova, M., Stupalova, S., and Necas, S. (1975). Sheep milk-borne epidemic of tick-borne encephalitis in Slovakia. *Intervirology* **5:**57–61.

Greve, K. W., Houston, R. J., Adams, D., Stanford, M. S., Bianchini, K. J., Clancy, A., and Rabito, F. J., Jr. (2002). The neurobehavioural consequences of St. Louis encephalitis infection. *Brain Injury* **16:**917–927.

Griffin, D. E., and Hardwick, J. M. (1999). Perspective: Virus infections and the death of neurons. *Trends Microbiol.* **7:**155–160.

Griffin, D. E., Levine, B., Tyor, W., Ubol, S., and Després, P. (1997). The role of antibody in recovery from alphavirus encephalitis. *Immunol. Rev.* **159:**155–161.

Grossberg, S. E., and Scherer, W. F. (1966). The effect of host age, virus dose and route of inoculation on inapparent infection in mice with Japanese encephalitis virus. *Proc. Soc. Exp. Biol. Med.* **123:**118–124.

Grubeck-Loebenstein, B., and Wick, G. (2002). The aging of the immune system. *Adv. Immunol.* **80:**243–284.

Gunther, G., Haglund, M., Lindquist, L., Skoldenberg, B., and Forsgren, M. (1996). Intrathecal production of neopterin and beta 2 microglobulin in tick-borne encephalitis (TBE) compared to meningoencephalitis of other etiology. *Scand. J. Infect. Dis.* **28:**131–138.

Gunther, G., Haglund, M., Lindquist, L., Skoldenberg, B., and Forsgren, M. (1997). Intrathecal IgM, IgA and IgG antibody response in tick-borne encephalitis: Long-term follow-up related to clinical course and outcome. *Clin. Diagn. Virol.* **8:**17–29. Erratum in *Clin. Diagn. Virol*. **8:**167–168.

Gunther, G., Haglund, M., Mesko, L., Bremmer, S., Lindquist, L., Forsgren, M., Skoldenberg, B., and Rudberg, U. (1998). Regional cerebral blood flow scintigraphy in tick-borne encephalitis and other aseptic meningoencephalitis. *J. Nuclear Med.* **39:**2055–2061.

Gupta, A. K., and Pavri, K. M. (1987). Alteration in immune response of mice with dual infection of *Toxocara canis* and Japanese encephalitis virus. *Trans. R. Soc. Trop. Med. Hyg.* **81:**835–840.

Haglund, M., and Gunther, G. (2003). Tick-borne encephalitis: Pathogenesis, clinical course and long-term follow-up. *Vaccine* **21**(Suppl. 1)**:**S11–S18.

Hajnicka, V., Kocakova, P., Slovak, M., Labuda, M., Fuchsberger, N., and Nuttall, P. A. (2000). Inhibition of the antiviral action of interferon by tick salivary gland extract. *Parasite Immunol.* **22:**201–206.

Halevy, M., Akov, Y., Ben-Nathan, D., Kobiler, D., Lachmi, B., and Lustig, S. (1994). Loss of active neuroinvasiveness in attenuated strains of West Nile virus: Pathogenicity in immunocompetent and SCID mice. *Arch. Virol.* **137:**355–370.

Halstead, S. B., and O'Rourke, E. J. (1977). Antibody-enhanced dengue virus infection in primate leukocytes. *Nature* **265:**739–741.

Halstead, S. B., Porterfield, J. S., and O'Rourke, E. J. (1980). Enhancement of dengue virus infection in monocytes by flavivirus antisera. *Am. J. Trop. Med. Hyg.* **29:**638–642.

Halstead, S. B., Venkateshan, C. N., Gentry, M. K., and Larsen, L. K. (1984). Heterogeneity of infection enhancement of dengue 2 strains by monoclonal antibodies. *J. Immunol.* **132:**1529–1532.

Hambleton, P., Stephenson, J. R., Baskerville, A., and Wiblin, C. N. (1983). Pathogenesis and immune response of vaccinated and unvaccinated rhesus monkeys to tick-borne encephalitis virus. *Infect. Imm.* **40:**995–1003.

Han, X. Y., Ren, Q. W., and Tsai, T. F. (1988). Serum and cerebrospinal fluid immunoglobulins M, A, and G in Japanese encephalitis. *J. Clin. Micro.* **26:**976–978.

Harinasuta, C., Nimmanitya, S., and Tisyakorn, U. (1985). The effect of interferon alpha on two cases of Japanese encephalitis in Thailand. *Southeast Asia J. Trop. Med. Pub. Health* **16:**332–336.

Harrington, D. G., Hilmas, D. E., Elwell, M. R., Whitmire, R. E., and Stephen, E. L. (1977). Intranasal infection of monkeys with Japanese encephalitis virus: Clinical response and treatment with a nuclease-resistant derivative of poly(I)-poly(C). *Am. J. Trop. Med. Hyg.* **26:**1191–1198.

Hase, T., Dubois, D. R., Summers, P. L., Downs, M. B., and Ussery, M. A. (1993). Comparison of replication rates and pathogenicities between the SA14 parent and SA14-14-2 vaccine strains of Japanese encephalitis virus in mouse brain neurons. *Arch. Virol.* **130:**131–143.

Havert, M. B., Schofield, B., Griffin, D. E., and Irani, D. N. (2000). Activation of divergent neuronal cell death pathways in different target cell populations during neuroadapted Sindbis virus infection in mice. *J. Virol.* **74:**5352–5356.

Hawkes, R. A. (1964). Enhancement of the infectivity of arboviruses by specific antisera produced in domestic fowls. *Aust. J. Exp. Biol. Med. Sci.* **42:**465–482.

Hayashi, K., and Arita, T. (1977). Experimental double infection of Japanese encephalitis virus and herpes simplex virus in mouse brain. *Jpn. J. Exp. Med.* **47:**9–13.

Heinz, F. X. (1986). Epitope mapping of flavivirus glycoproteins. *Adv. Vir. Res.* **31:**103–168.

Henchal, E. A., Henchal, L. S., and Schlesinger, J. J. (1988). Synergistic interactions of anti-NS1 monoclonal antibodies protect passively immunized mice from lethal challenge with dengue 2 virus. *J. Gen. Virol.* **69:**2101–2117.

Hickey, W. F. (1999). Leukocyte traffic in the central nervous system: The participants and their roles. *Semin. Immunol.* **11:**125–137.

Hilgard, P., and Stockert, R. (2000). Heparan sulfate proteoglycans initiate dengue virus infection of hepatocytes. *Hepatology* **32:**1069–1077.

Hill, A. B., Lobigs, M., Blanden, R. V., Kulkarni, A., and Müllbacher, A. (1993). The cellular immune response to flaviviruses. *In* "Viruses and the Cellular Immune Response" (D. Brian Thomas, ed.), pp. 363–388. Dekker, New York.

Hill, A. B., Müllbacher, A., Parrish, C., Coia, G., *et al.* (1992). Broad cross-reactivity with marked fine-specificity in the cytotoxic T cell response to flaviviruses. *J. Gen. Virol.* **73:**1115–1123.

Hirsch, M. R., Cailla, H., Wietzerbin, J., and Goridis, C. (1986). Interferon-alpha, -beta and -gamma induce (2′-5′) oligoadenylate synthetase in cultured mouse brain cells. *Neurosci. Lett.* **65:**139–144.

Hirsch, M. S., and Murphy, F. A. (1967). Effects of anti-thymocyte serum on 17D yellow fever infection in adult mice. *Nature* **216:**179–180.

Ho, L. J., Wang, J. J., Shaio, M. F., Kao, C. L., Chang, D. M., Han, S. W., and Lai, J. H. (2001). Infection of human dendritic cells by dengue virus causes cell maturation and cytokine production. *J. Immunol.* **166:**1499–1506.

Hofmann, H., Frisch-Niggemeyer, W., and Kunz, C. (1978). Protection of mice against tick-borne encephalitis by different classes of immunoglobulin. *Infection* **6:**154–157.

Huang, C. H., and Wong, C. (1963). Relation of the peripheral multiplication of Japanese B encephalitis virus to the pathogenesis of the infection in mice. *Acta Virol.* **7:**322–330.

Huang, Y. H., Lei, H. Y., Liu, H. S., Lin, Y. S., Liu, C. C., and Yeh, T. M. (2000). Dengue virus infects human endothelial cells and induces IL-6 and IL-8 production. *Am. J. Trop. Med. Hyg.* **63:**71–75.

Hubalek, Z., and Halouzka, J. (1999). West Nile fever—a reemerging mosquito–borne viral disease in Europe. *Emerg. Inf. Dis.* **5:**643–650.

Huy, B. V., Tu, H. C., Luan, T. V., and Lindquist, R. (1994). Early mental and neurological sequellae after Japanese B encephalitis. *Southeast Asia J. Trop. Med. Public Health* **25:**549–553.

Igarashi, A. (1979). Characteristics of *Aedes albopictus* cells persistently infected with dengue viruses. *Nature* **280:**690–691.

Iliyenko, V. I., Komandenko, N. I., Paltonov, V. G., Prozorova, I. N., and Panov, A. G. (1974). Investigation of the pathogenesis of chronic forms of tick-borne encephalitis. *Acta Virol.* **18:**341–346.

Innis, B. L. (1997). Antibody responses to dengue virus infection. *In* "Dengue and Dengue Hemorrhagic Fever" (D. J. Gubler and G. Kuno, eds.), pp. 221–243. CAB International, Wallingford, UK.

Isaeva, M. P., Leonova, G. N., Kozhemiako, V. B., Borisevich, V. G., Maistrovskaia, O. S., and Rasskazov, V. A. (1998). Apoptosis as a mechanism for the cytopathic action of tick-borne encephalitis virus. *Vopr. Virusol.* **43:**182–186.

Ishii, K., Matsunaga, Y., and Kono, R. (1968). Immunoglobulins produced in response to Japanese encephalitis virus infections of man. *J. Immunol.* **101:**770–775.

Ishii, T., Matsushita, M., and Hamada, S. (1977). Characteristic residual neuropathological features of Japanese B encephalitis. *Acta. Neuropathol. (Berl.)* **38:**181–186.

Iwamoto, M., Jernigan, D. B., Guasch, A., Trepka, M. J., Blackmore, C. G., Hellinger, W. C., Pham, S. M., Zaki, S., Lanciotti, R. S., Lance-Parker, S. E., DiazGranados, C. A., Winquist, A. G., Perlino, C. A., Wiersma, S., Hillyer, K. L., Goodman, J. L., Marfin, A. A., Chamberland, M. E., Petersen, L. R., and West Nile Virus in Transplant Recipients Investigation Team. (2003). Transmission of West Nile virus from an organ donor to four transplant recipients. *N. Engl. J. Med.* **348:**2196–2203.

Iwasaki, Y., Zhao, J.-X., Yamamoto, Y., and Konno, H. (1986). Immunohistochemical demonstration of viral antigens in Japanese encephalitis. *Acta Neuropathol.* **70:**79–81.

Iwasaki, Y., Sako, K., Tsunoda, I., and Ohara, Y. (1993). Phenotypes of mononuclear cell infiltrates in human central nervous system. *Acta. Neuropathol. (Berl.)* **85:**653–657.

Jacoby, R. O., Bhatt, P. N., and Schwartz, A. (1980). Protection of mice from lethal flavivirus encephalitis by adoptive transfer of splenic cells from donors infected with live virus. *J. Infect. Dis.* **141:**617–624.

Jan, J. T., Chatterjee, S., and Griffin, D. E. (2000). Sindbis virus entry into cells triggers apoptosis by activating sphingomyelinase, leading to the release of ceramide. *J. Virol.* **74:**6425–6432.

Jarman, R. V., Morgan, P. N., and Duffy, C. E. (1968). Persistence of West Nile virus in L-929 mouse fibroblasts. *Proc. Soc. Exp. Med. Biol.* **129:**633–637.

Johnson, A. J., and Roehrig, J. T. (1999). New mouse model for dengue virus vaccine testing. *J. Virol.* **73:**83–86.

Johnson, R. T., Burke, D. S., Elwell, M., Leake, C. J., Nisalak, *et al.* (1985). Japanese encephalitis: Immunocytochemical studies of viral antigen and inflammatory cells in fatal cases. *Ann. Neurol.* **18:**567–573.

Johnson, R. T., Intralawan, P., and Puapanwatton, S. (1986). Japanese encephalitis: Identification of inflammatory cells in cerebrospinal fluid. *Ann. Neurol.* **20:**691–695.

Johnston, L. J., Halliday, G. M., and King, N. J. (1996). Phenotypic changes in Langerhans' cells after infection with arboviruses: A role in the immune response to epidermally acquired viral infection. *J. Virol.* **70:**4761–4766.

Johnston, L. J., Halliday, G. M., and King, N. J. (2000). Langerhans cells migrate to local lymph nodes following cutaneous infection with an arbovirus. *J. Invest Dermatol.* **114:**560–568.

Johnston, C., Jiang, W., Chu, T., and Levine, B. (2001). Identification of genes involved in the host response to neurovirulent alphavirus infection. *J. Virol.* **75:**10431–10445.

Jordan, I., Briese, T., Fischer, N., Lau, J. Y., and Lipkin, W. I. (2000). Ribavirin inhibits West Nile virus replication and cytopathic effect in neural cells. *J. Infect. Dis.* **182:**1214–1217.

Kaiser, R., and Holzmann, H. (2000). Laboratory findings in tick-borne encephalitis: Correlation with clinical outcome. *Infection* **28:**78–84.

Kaiser, R. (2002). Tick-borne encephalitis (TBE) in Germany and clinical course of the disease. *Int. J. Med. Microbiol.* **291**(Suppl. 33)**:**58–61.

Kalita, J., and Misra, U. K. (2000). Markedly severe dystonia in Japanese encephalitis. *Mov. Disord.* **15:**1168–1172.

Kanesa-Thasan, N., Putnak, J. R., Mangiafico, J. A., Saluzzo, J. E., and Ludwig, G. V. (2002). Short report: Absence of protective neutralizing antibodies to West Nile virus in subjects following vaccination with Japanese encephalitis or dengue vaccines. *Am. J. Trop. Med. Hyg.* **66:**115–116.

Katz, E., and Goldblum, N. (1968). Establishment, steady state, and cure of a chronic infection of LLC cells with West Nile virus. *Arch. Ges. Virus Forsch.* **25:**69–82.

Kengsakul, K., Sathirapongsasuti, K., and Punyagupta, S. (2002). Fatal myeloencephalitis following yellow fever vaccination in a case with HIV infection. *J. Med. Assoc. Thai.* **85:**131–134.

Kerr, D. A., Larsen, T., Cook, S. H., Fanjiang, Y. R., Choi, E., Griffin, D. E., Hardwick, J. M., and Irani, D. N. (2002). BCL-2 and BAX protect adult mice from lethal Sindbis virus infection but do not protect spinal cord motor neurons or prevent paralysis. *J. Virol.* **76:**10393–10400.

Kesson, A. M., Blanden, R. V., and Müllbacher, A. (1987). The primary in vivo murine cytotoxic T cell response to the flavivirus, West Nile. *J. Gen. Virol.* **68:**2001–2006.

Kesson, A. M., and King, N. J. (2001). Transcriptional regulation of major histocompatibility complex class I by flavivirus West Nile is dependent on NF-kappaB activation. *J. Infect. Dis.* **184:**947–954.

Khan, N. U., Pulford, K. A., Farquharson, M. A., Howatson, A., Stewart, C., Jackson, R., McNicol, A. M., and Foulis, A. K. (1989). The distribution of immunoreactive interferon-alpha in normal human tissues. *Immunology* **66:**201–206.

Khozinsky, V. V., Semenov, B. F., Gresikova, M., Chunikhin, S. P., Sekeyova, M., and Kozuch, O. (1985). Role of macrophages in the pathogenesis of experimental tick-borne encephalitis in mice. *Acta Virol.* **29:**194–202.

Kimura-Kuroda, J., Ichikawa, M., Ogata, A., Nagashima, K., and Yasui, K. (1992). Specific tropism of Japanese encephalitis virus for developing neurons in primary rat brain culture. *Arch. Virol.* **130:**477–484.

Kimura-Kuroda, J., and Yasui, K. (1988). Protection of mice against Japanese encephalitis virus by passive administration with monoclonal antibodies. *J. Immunol.* **141:**3606–3610.

King, N. J., and Kesson, A. M. (1988). Interferon-independent increases in class I major histocompatibility complex antigen expression follow flavivirus infection. *J. Gen. Virol.* **69:**2535–2543.

King, N. J., Maxwell, L. E., and Kesson, A. M. (1989). Induction of class I major histocompatibility complex antigen expression by West Nile virus on gamma interferon-refractory early murine trophoblast cells. *Proc. Natl. Acad. Sci. USA* **86:**911–915.

Klimstra, W. B., Ryman, K. D., and Johnston, R. E. (1998). Adaptation of Sindbis virus to BHK cells selects for use of heparan sulfate as an attachment receptor. *J. Virol.* **72:**7357–7366.

Kobiler, D., Lustig, S., Gozes, Y., Ben-Nathan, D., and Akov, Y. (1989). Sodium dodecylsulphate induces a breach in the blood-brain barrier and enables a West Nile virus variant to penetrate into mouse brain. *Brain Res.* **496:**314–316.

Konishi, E., Kurane, I., Mason, P. W., Innis, B. L., and Ennis, F. A. (1995). Proliferative responses of human peripheral blood T lymphocytes against Japanese encephalitis antigens. *Am. J. Trop. Med. Hyg.* **53:**278–283.

Konishi, E., Kurane, I., Mason, P. W., Shope, R. E., and Ennis, F. A. (1997). Poxvirus-based Japanese encephalitis vaccine candidates induce JE virus-specific $CD8^+$ cytotoxic lymphocytes in mice. *Virology* **227:**353–360.

Konishi, E., Kurane, I., Mason, P. W., Shope, R. E., Kanesa-thasan, N., Smucny, J., Hoke, C. H., and Ennis, F. A. (1998a). Induction of Japanese encephalitis virus cytotoxic T lymphocytes in humans by poxvirus-based DNA vaccines. *Vaccine* **8:**842–849.

Konishi, E., Yamaoka, M., Win, K. S., Kurane, I., and Mason, P. W. (1998b). Induction of protective immunity against Japanese encephalitis in mice by immunization with a plasmid encoding JE virus premembrane and envelope genes. *J. Virol.* **72:**4925–4939.

Konishi, E., Yamaoka, M., Win, K.-S., Kurane, I., Takada, K., and Mason, P. W. (1999). The anamnestic neutralizing antibody response is critical for protection of mice from challenge following vaccination with a plasmid encoding the Japanese encephalitis virus premembrane and envelope genes. *J. Virol.* **73:**5527–5534.

Kopecky, J., Grubhoffer, L., Kovar, V., Jindrak, L., and Vokurkova, D. (1999). A putative host cell receptor for tick-borne encephalitis virus identified by anti-idiotypic antibodies and virus affinoblotting. *Intervirology* **42:**9–16.

Kopf, M., Abel, B., Gallimore, A., Carroll, M., and Bachmann, M. F. (2002). Complement component C3 promotes T-cell priming and lung migration to control acute influenza virus infection. *Nature Med.* **8:**373–378.

Korenberg, E. I., Gorban, L. Y., Kovalevskii, Y. V., Frizen, V. I., and Karavanov, A. S. (2001). Risk for human tick-borne encephalitis, borrelioses, and double infection in the pre-Ural region of Russia. *Emerg. Infect. Dis.* **7:**459–462.

Kozuch, O., Chunikhin, S. P., Gresikova, M., Nosek, J., Kurenkov, V. B., and Lysy, J. (1981). Experimental characteristics of viraemia caused by two strains of tick-borne encephalitis virus in small rodents. *Acta Virol.* **25:**219–224.

Kramer, L. D., and Bernard, K. A. (2001). West Nile virus infection in birds and mammals. *Ann. N. Y. Acad. Sci.* **951:**84–93.

Kreil, T. R., and Eibl, M. M. (1995). Viral infection of macrophages profoundly alters requirements for induction of nitric oxide synthesis. *Virology* **212:**174–178.

Kreil, T. R., and Eibl, M. M. (1996). Nitric oxide and viral infection: NO antiviral activity against a flavivirus in vitro, and evidence for contribution to pathogenesis in experimental infection in vivo. *Virology* **219:**304–306.

Kreil, T. R., Maier, E., Fraiss, S., Attakpah, E., Burger, I., Mannhalter, J. W., and Eibl, M. M. (1998a). Vaccination against tick-borne encephalitis virus, a flavivirus, prevents disease but not infection, although viremia is undetectable. *Vaccine* **16:**1083–1086.

Kreil, T. R., Maier, E., Fraiss, S., and Eibl, M. M. (1998b). Neutralizing antibodies protect against lethal flavivirus challenge but allow for the development of active humoral immunity to a nonstructural virus protein. *J. Virol.* **72:**3076–3081.

Kubes, M., Fuchsberger, N., Labuda, M., Zuffova, E., and Nuttall, P. A. (1994). Salivary gland extracts of partially fed *Dermacentor reticulatus* ticks decrease natural killer cell activity in vitro. *Immunology* **82:**113–116.

Kubes, M., Kocakova, P., Slovak, M., Slavikova, M., Fuchsberger, N., and Nuttall, P. A. (2002). Heterogeneity in the effect of different ixodid tick species on human natural killer cell activity. *Parasite Immunol.* **24:**23–28.

Kulkarni, A. B., Müllbacher, A., and Blanden, R. V. (1991a). Functional analysis of macrophages, B cells and splenic dendritic cells as antigen-presenting cells in West Nile virus-specific murine T lymphocyte proliferation. *Immunol. Cell. Biol.* **69:**71–80.

Kulkarni, A. B., Müllbacher, A., and Blanden, R. V. (1991b). In vitro T-cell proliferative response to the flavivirus, West Nile. *Viral Immunol.* **4:**73–82.

Kulkarni, A. B., Müllbacher, A., Parrish, C., *et al.* (1992). Analysis of murine major histocompatibility complex class II-restricted T-cell responses to the Kunjin virus using vaccinia virus expression. *J. Virol.* **66:**3583–3592.

Kumar, R., Mathur, A., Singh, K. B., *et al.* (1993). Clinical sequellae of Japanese encephalitis in children. *Ind. J. Med. Res.* **97:**9–13.

Kumar, S., Misra, U. K., Kalita, J., Salwani, V., Gupta, R. K., and Gujral, R. (1997). MRI in Japanese encephalitis. *Nueroradiology* **39:**180–184.

Kundig, T. M., Hengartner, H., and Zinkernagel, R. M. (1993). T cell-dependent IFN-gamma exerts an antiviral effect in the central nervous system but not in peripheral solid organs. *J. Immunol.* **150:**2316–2321.

Kuno, G., Hayes, C. G., and Chen, W. J. (1993). Cytokine concentrations in cerebrospinal fluid in flavivirus infections. *Southeast Asian J. Trop. Med. Public Health* **24:**781–782.

Kurane, I. (2002). Immune responses to Japanese encephalitis virus. *In* "Japanese Encephalitis and West Nile Viruses," (J. S. Mackenzie, A. D. T. Barrett, and V. Deubel, eds.), pp. 91–104. Springer-Verlag, Berlin.

Kurane, I., Hebblewaite, D., Brandt, W. E., and Ennis, F. A. (1984). Lysis of dengue virus-infected cells by natural cell-mediated cytotoxicity and antibody-dependent cell-mediated cytotoxicity. *J. Virol.* **52:**223–230.

Kurane, I., Innis, B. L., Nisalak, A., Hoke, C., Nimmannitya, S., Meager, A., and Ennis, F. A. (1989). Human T cell responses to dengue virus antigens: Proliferative responses and interferon gamma production. *J. Clin. Invest.* **83:**506–513.

Kurane, I., Brinton, M. A., Samson, A. L., and Ennis, F. A. (1991). Dengue virus-specific, human $CD4^+$ $CD8^-$ cytotoxic T-cell clones: Multiple patterns of virus cross-reactivity recognized by NS3-specific T-cell clones. *J. Virol.* **65:**1823–1828.

Kurane, I., Innis, B. L., Hoke, C. H., Jr., Eckels, K. H., Meager, A., Janus, J., and Ennis, F. A. (1995). T cell activation in vivo by dengue virus infection. *J. Clin. Lab. Immunol.* **46:**35–40.

Kurane, I., Janus, J., and Ennis, F. A. (1992). Dengue virus infection of human skin fibroblasts in vitro production of IFN-beta, IL-6 and GM-CSF. *Arch. Virol.* **124:**21–30.

Kutubuddin, M., Kolaskar, A. S., Galande, S., Gore, M. M., Ghosh, S. N., and Banerjee, K. (1991). Recognition of helper T cell epitopes in envelope (E) glycoprotein of Japanese encephalitis, West Nile and dengue viruses. *Mol. Immunol.* **28:**149–154.

Labrada, L., Liang, X. H., Zheng, W., Johnston, C., and Levine, B. (2002). Age-dependent resistance to lethal alphavirus encephalitis in mice: Analysis of gene expression in the central nervous system and identification of a novel interferon-inducible protective gene, mouse ISG12. *J. Virol.* **76:**688–703.

Labuda, M., Jones, L. D., Williams, T., and Nuttall, P. A. (1993). Enhancement of tick-borne encephalitis virus transmission by tick salivary gland extracts. *Med. Vet. Entomol.* **7:**193–196.

Lancaster, M. U., Hodgetts, S. I., Makenzie, J. S., and Urosevic, N. (1998). Characterization of defective viral RNA produced during persistent infection of Vero cells with Murray Valley encephalitis virus. *J. Virol.* **72:**2474–2482.

Lee, E., and Lobigs, M. (2000). 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.* **74:**8867–8875.

Lee, E., and Lobigs, M. (2002). Mechanism of virulence attenuation of glycosaminoglycan-binding variants of Japanese encephalitis virus and Murray Valley encephalitis virus. *J. Virol.* **76:**4901–4911.

Lee, H. W., and Scherer, W. F. (1961). The anamnestic antibody response to Japanese encephalitis virus in monkeys and its implications concerning naturally acquired immunity. *J. Immunol.* **86:**151–164.

Leis, A. A., Stokic, D. S., Polk, J. L., Dostrow, V., and Winkelmann, M. (2002). A poliomyelitis-like syndrome from West Nile virus infection. *N. Engl. J. Med.* **347:**1279–1280.

Leport, C., Janowski, M., Brun-Vezinet, F., Rouzioux, C., Rodhain, F., and Vilde, J. L. (1984). West Nile virus meningoencephalitis: value of inteferon assays in primary encephalitis. *Ann. Med. Interne (Paris)* **135:**460–463.

Levenbook, I. S., Pellen, L. J., and Ellisberg, B. L. (1987). The monkey safety test for neurovirulence of YF vaccines: The utility of quantitative clinical evaluation and histological examination. *J. Biol. Stand.* **15:**305–313.

Levine, B. (2002). Apoptosis in viral infections of neurons: A protective or pathologic host response. *Curr. Top. Micro. Immunol.* **265:**95–118.

Levine, B., and Griffin, D. E. (1992). Persistence of viral RNA in mouse brains after recovery from acute alphavirus encephalitis. *J. Virol.* **66:**6429–6435.

Levine, B., and Griffin, D. E. (1993). Molecular analysis of neurovirulent strains of Sindbis virus that evolve during persistent infection of scid mice. *J. Virol.* **67:**6872–6875.

Levine, B., Hardwick, J. M., Trapp, B. D., Crawford, T. O., Bollinger, R. C., and Griffin, D. E. (1991). Antibody-mediated clearance of alphavirus infection from neurons. *Science* **254:**856–860.

Levine, B., Huang, Q., Isaacs, J. T., Reed, J. C., Griffin, D. E., and Hardwick, J. M. (1993). Conversion of lytic to persistent alphavirus infection by the bcl-2 cellular oncogene. *Nature* **361:**739–742.

Lewis, J., Wesselingh, S. L., Griffin, D. E., and Hardwick, J. M. (1996). Alphavirus-induced apoptosis in mouse brains correlates with neurovirulence. *J. Virol.* **70:**1828–1835.

Leyssen, P., Drosten, C., Paning, M., Charlier, N., Paeshuyse, J., De Clercq, E., and Neyts, J. (2003b). Interferons, interferon inducers, and interferon-ribavirin in treatment of flavivirus-induced encephalitis in mice. *Antimicrob. Agents Chemother.* **47:**777–782.

Leyssen, P., Paeshuyse, J., Charlier, N., Van Lommel, A., Drosten, C., De Clercq, E., and Neyts, J. (2003a). Impact of direct virus-induced neuronal dysfunction and immunological damage on the progression of flavivirus (Modoc) encephalitis in a murine model. *J. Neurovirol.* **9:**69–78.

Li, W., Li, Y., Kedersha, N., Anderson, P., Emara, M., Swiderek, K. M., Moreno, G. T., and Brinton, M. A. (2002). Cell proteins TIA-1 and TIAR interact with the 3′ stem-loop of the West Nile virus complementary minus strand RNA and facilitate virus replication. *J. Virol.* **76:**11989–12000.

Liang, X. H., Kleeman, L. K., Jiang, H. H., Gordon, G., Goldman, J. E., Berry, G., Herman, B., and Levine, B. (1998). Protection against fatal Sindbis virus encephalitis by beclin, a novel Bcl-2-interacting protein. *J. Virol.* **72:**8586–8596.

Liao, C.-L., Lin, Y.-L., Shen, S.-C., Shen, Y.-Y., Su, H.-L., Huang, Y.-L., Ma, S.-H., Sun, Y.-C., Chen, K.-P., and Chen, L.-K. (1998). Antiapoptotic but not antiviral function of human bcl-2 assists establishment of Japanese encephalitis virus persistence in cultured cells. *J. Virol.* **72:**9844–9854.

Liao, C. L., Lin, Y. L., Wang, J. J., Huang, Y. L., Yeh, C. T., Ma, S. H., and Chen, L. K. (1997). Effect of enforced expression of human bcl-2 on Japanese encephalitis virus-induced apoptosis in cultured cells. *J. Virol.* **71:**5963–5971.

Libraty, D. H., Pichyangkul, S., Ajariyakhajorn, C., Endy, T. P., and Ennis, F. A. (2001). Human dendritic cells are activated by dengue virus infection: Enhancement by gamma interferon and implications for disease pathogenesis. *J. Virol.* **75:**3501–3508.

Licon Luna, R. M., Lee, E., Müllbacher, A., Blanden, R. V., Langman, R., and Lobigs, M. (2002). Lack of both Fas ligand and perforin protects from flavivirus-mediated encephalitis in mice. *J. Virol.* **76:**3202–3211.

Lin, M. T., Hinton, D. R., Marten, N. W., Bergmann, C. C., and Stohlman, S. A. (1999). Antibody prevents virus reactivation within the central nervous system. *J. Immunol.* **162:**7358.

Lin, Y. L., Huang, Y. L., Ma, S. H., Yeh, C. T., Chiou, S. Y., Chen, L. K., and Liao, C. L. (1997). Inhibition of Japanese encephalitis virus infection by nitric oxide: Antiviral effect of nitric oxide on RNA virus replication. *J. Virol.* **71:**5227–5235.

Lin, Y. L., Liao, C. L., Chen, L. K., Yeh, C. T., Liu, C. I., Ma, S. H., Huang, Y. Y., Huang, Y. L., Kao, C. L., and King, C. C. (1998). Study of dengue virus infection in SCID mice engrafted with human K562 cells. *J. Virol.* **72:**9729–9737.

Liou, M. L., and Hsu, C. Y. (1998). Japanese encephalitis virus is transported across the cerebral blood vessels by endocytosis in mouse brain. *Cell Tissue Res.* **293:**389–394.

Liu, M. T., Armstrong, D., Hamilton, T. A., and Lane, T. E. (2001). Expression of Mig (monokine induced by interferon gamma) is important in T lymphocyte recruitment and host defense following viral infection of the central nervous system. *J. Immunol.* **166:**1790–1795.

Liu, M. T., Chen, B. P., Oertel, P., Buchmeier, M. J., Armstrong, D., Hamilton, T. A., and Lane, T. E. (2000). The T cell chemoattractant IFN-inducible protein 10 is essential in host defense against viral-induced neurological disease. *J. Immunol.* **165:**2327–2330.

Liu, M. T., and Lane, T. E. (2001). Chemokine expression and viral infection of the central nervous system: Regulation of host defense and neuropathology. *Immunol. Res.* **24:**111–119.

Liu, T., and Chambers, T. J. (2001). Yellow fever virus encephalitis: Properties of the brain-associated T-cell response during virus clearance in normal and gamma interferon-deficient mice and requirement for $CD4^+$ lymphocytes. *J. Virol.* **75:**2107–2118.

Liu, Y., Blanden, R. V., and Müllbacher, A. (1989a). Identification of cytolytic lymphocytes in West Nile virus-infected murine central nervous system. *J. Gen. Virol.* **70:**565–573.

Liu, Y., King, N., Kesson, A., Blanden, R. V., and Müllbacher, A. (1988). West Nile virus infection modulates the expression of class I and class II MHC antigens on astrocytes in vitro. *Ann. N. Y. Acad. Sci.* **540:**483–485.

Liu, Y., King, N., Kesson, A., Blanden, R. V., and Müllbacher, A. (1989b). Flavivirus infection up-regulates the expression of class I and class II major histocompatibility antigens on and enhances T cell recognition of astrocytes in vitro. *J. Neuroimmunol.* **21:**157–168.

Lobigs, M., Müllbacher, A., Blanden, R. V., Hammerling, G. J., and Momburg, F. (1999). Antigen presentation in Syrian hamster cells: Substrate selectivity of TAP controlled by polymorphic residues in TAP1 and differential requirements for loading of H2 class I molecules. *Immunogenetics* **49:**931–941.

Lobigs, M., Müllbacher, A., Wang, Y., Pavy, M., and Lee, E. (2003a). Role of type I and type II interferon responses in recovery from infection with an encephalitic flavivirus. *J. Gen. Virol.* **84:**567–572.

Lobigs, M., Pavy, M., and Hall, R. (2003b). Cross-protective and infection-enhancing immunity in mice vaccinated against flaviviruses belonging to the Japanese encephalitis virus serocomplex. *Vaccine* **21:**1572–1579.

Loginova, N. V., Deryabin, P. G., Mikhailova, G. R., Tsareva, A. A., and Buinitskaya, O. B. (1980). Chronic infection of HeLa cells with Japanese encephalitis virus: General characteristics of the system. *Acta. Virol.* **24:**399–405.

Loke, H., Bethell, D. B., Phuong, C. X., Dung, M., Schneider, J., White, N. J., Day, N. P., Farrar, J., and Hill, A. V. (2001). Strong HLA class I-restricted T cell responses in dengue hemorrhagic fever: A double-edged sword? *J. Infect. Dis.* **184:**1369–1373.

Lubiniecki, A. S., Cypess, R. H., and Lucas, J. P. (1974). Synergistic interaction of two agents in mice: Japanese B encephalitis virus and *Trichinella spiralis*. *Am. J. Trop. Med. Hyg.* **23:**235–241.

Luby, J. P., Miller, G., Gardner, P., Pigford, C. A., Henderson, B. E., and Eddins, D. (1967). The epidemiology of St. Louis encephalitis in Houston, Texas, 1964. *Am. J. Epidemiol.* **86:**584–597.

Luby, J. P., Stewart, W. E., 2nd, Sulkin, S. E., and Sanford, J. P. (1969). Interferon in human infections with St. Louis encephalitis virus. *Ann. Intern. Med.* **71:**703–709.

Lucas, M., Mashimo, T., Frenkiel, M. P., Simon-Chazottes, D., Montagutelli, X., Ceccaldi, P. E., Guenet, J. L., and Despres, P. (2003). Infection of mouse neurones by West Nile virus is modulated by the interferon-inducible 2′-5′ oligoadenylate synthetase 1b protein. *Immunol. Cell Biol.* **81:**230–236.

Ludewig, B., Ehl, S., Karrer, U., Odermatt, B., Hengartner, H., and Zinkernagel, R. M. (1998). Dendritic cells efficiently induce protective immunity. *J. Virol.* **72:**3812–3818.

Lustig, S., Danenberg, H. D., Kafri, Y., Kobiler, D., and Ben-Nathan, D. (1992). Viral neuroinvasion and encephalitis induced by lipopolysaccharide and its mediators. *J. Exp. Med.* **176:**707–712.

Malkova, D., and Frankova, V. (1959). The lymphatic system in the development of experimental tick-bone encephalitis in mice. *Acta Virol.* **3:**210–214.

Mandl, C. W., Kroschewski, H., Allison, S. L., Kofler, R., Holzmann, H., Meixner, T., and Heinz, F. X. (2001). Adaptation of tick-borne encephalitis virus to BHK-21 cells results in the formation of multiple heparan sulfate binding sites in the envelope protein and attenuation in vitro. *J. Virol.* **75:**5627–5637.

Manuelidis, E. E. (1956). Neuropathology of experimental West Nile virus infection in monkeys. *J. Neuropathol Exp. Neurol.* **15:**448–460.

Marianneau, P., Steffan, A. M., Royer, C., Drouet, M. T., Jaeck, D., Kirn, A., and Deubel, V. (1999). Infection of primary cultures of human Kupffer cells by dengue virus: No viral progeny synthesis, but cytokine production is evident. *J. Virol.* **73:**5201–5206.

Markoff, L. (2000). Points to consider in the development of a surrogate for efficacy of novel Japanese encephalitis virus vaccines. *Vaccine* **18**(Suppl. 2)**:**26–32.

Martin, D. A., Biggerstaff, B. J., Allen, B., Johnson, A. J., Lanciotti, R. S., and Roehrig, J. T. (2002). Use of immunoglobulin M cross-reactions in differential diagnosis of human flaviviral encephalitis infections in the United States. *Clin. Diagn. Lab. Immunol.* **9:**544–549.

Martinez-Barragan, J. J., and del Angel, R. M. (2001). Identification of a putative coreceptor on Vero cells that participates in dengue 4 virus infection. *J. Virol.* **75:**7818–7827.

Mashimo, T., Lucas, M., Simon-Chazottes, D., Frenkiel, M.-P., Montagutelli, X., Ceccaldi, P.-E., Deubel, V., Guenet, J.-L., and Despres, P. (2002). A nonsense mutation in the gene encoding 2′-5′-oligoadenylate synthetase/L1 isoform is associated with West Nile virus susceptibility in laboratory mice. *Proc. Natl. Acad. Sci. USA* **99:**11311–11316.

Massa, P. T., Whitney, L. W., Wu, C., Ropka, S. L., and Jarosinski, K. W. (1999). A mechanism for selective induction of 2′-5′ oligoadenylate synthetase, anti-viral state, but not MHC class I genes by interferon-beta in neurons. *J. Neurovirol.* **5:**161–171.

Mathews, J. H., Allan, J. E., Roehrig, J. T., Brubaker, J. R., Uren, M. F., and Hunt, A. R. (1991). T-helper cell and associated antibody response to synthetic peptides of the E glycoprotein of Murray Valley encephalitis virus. *J. Virol.* **65:**5141–5148.

Mathews, J. H., Roehrig, J. T., Brubaker, J. R., Hunt, A. R., and Allan, J. E. (1992). A synthetic peptide to the E glycoprotein of Murray Valley encephalitis virus defines multiple virus-reactive T- and B-cell epitopes. *J. Virol.* **66:**6555–6562.

Mathews, V., Robertson, T., Kendrick, T., Abdo, M., Papadimitrou, J., and McMinn, P. (2000). Morphological features of Murray Valley encephalitis virus infection in the central nervous system of Swiss mice. *Int. J. Exp. Pathol.* **81:**31–40.

Mathur, A., Arora, K. L., Rawat, S., and Chaturvedi, U. C. (1986). Persistence, reactivation and latency of Japanese encephalitis virus infection in mice. *J. Gen. Virol.* **67:**381–385.

Mathur, A., and Chaturvedi, U. C. (1992). Breakdown of the blood-brain barrier by virus-induced cytokine during Japanese encephalitis virus infection. *Int. J. Exp. Pathol.* **73:**603–611.

Mathur, A., Kulshreshtha, R., and Chaturvedi, U. C. (1989). Evidence of latency of Japanese encephalitis virus in T lymphocytes. *J. Gen. Virol.* **70:**461–465.

McMinn, P. C. (1997). The molecular basis of virulence of the encephalitogenic flaviviruses. *J. Gen. Virol.* **78:**2711–2722.

McMinn, P. C., Dalgarno, L., and Weir, R. C. (1996). 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* **220:**414–423.

Medana, I., Li, Z., Flugel, A., Tschopp, J., Wekerle, H., and Neumann, H. (2001). Fas ligand (CD95L) protects neurons against perforin-mediated T lymphocyte cytotoxicity. *J. Immunol.* **167:**674–681.

Medana, I. M., Gallimore, A., Oxenius, A., Martinic, M. M. A., Wekerle, H., and Neumann, H. (2000). MHC class I-restricted killing of neurons by virus-specific $CD8^+$ T lymphocytes is effected through the Fas/FasL, but not the perforin pathway. *Eur. J. Immunol.* **30:**3623–3633.

Misra, U. K., and Kalita, J. (1997a). Movement disorders in Japanese encephalitis. *J. Neurol.* **244:**299–303.

Misra, U. K., and Kalita, J. (1997b). Anterior horn cells are also involved in Japanese encephalitis. *Acta Neurol. Scand.* **96:**114–117.

Miyake, M. (1964). The pathology of Japanese encephalitis. *Bull World Health Org.* **30:**153–160.

Momburg, F., Müllbacher, A., and Lobigs, M. (2001). Modulation of transporter associated with antigen processing (TAP)-mediated peptide import into the endoplasmic reticulum by flavivirus infection. *J. Virol.* **75:**5663–5671.

Monath, T. P. (1971). Neutralizing antibody responses in the major immunoglobulin classes to yellow fever 17D vaccination of humans. *Am. J. Epidemiol.* **93:**122–129.

Monath, T. P. (1986). Pathobiology of the flaviviruses. *In* "The Togaviridae and the Flaviviridae" (S. Schlesinger and M. J. Schlesinger, eds.), pp. 375–440. Plenum Press, New York.

Monath, T. P., and Borden, E. C. (1971). Effects of thorotrast on humoral antibody, viral multiplication and interferon during infection with St. Louis encephalitis virus in mice. *J. Infect. Dis.* **123:**297–300.

Monath, T. P., Cropp, C. B., and Harrison, A. K. (1983). Mode of entry of a neurotropic arbovirus into the central nervous system: Reinvestigation of an old controversy. *Lab. Investigation* **48:**399–410.

Monath, T. P., and Heinz, F. X. (1996). Flaviviruses. *In* "Fields Virology" (B. N. Fields, D. M. Knipe, P. M. Howley, *et al.*, eds.), pp. 961–1034. Lippincott-Raven, Philadelphia.

Müllbacher, A., and King, N. J. C. (1989). Natural killer cell lysis of target cells is influenced by β_2-microglobulin expression. *Scand. J. Immunology* **30:**21–29.

Müllbacher, A., and Lobigs, M. (1995). Up-regulation of MHC class I by flavivirus-induced peptide translocation into the endoplasmic reticulum. *Immunity* **3:**207–214.

Munoz, M. L., Cisneros, A., Cruz, J., Das, P., Tovar, R., and Ortega, A. (1998). Putative dengue virus receptors from mosquito cells. *FEMS Microbiol. Lett.* **168:**251–258.

Murali-Krishna, K., Ravi, V., and Manjunath, R. (1996). Protection of adult but not newborn mice against lethal intracerebral challenge with Japanese encephalitis virus by adoptively transferred virus-specific cytotoxic T lymphocytes: Requirement for $L3T4^+$ T cells. *J. Gen. Virol.* **77:**705–714.

Murgod, U. A., Muthane, U. B., Ravi, V., Radhesh, S., and Desai, A. (2001). Persistent movement disorders following Japanese encephalitis. *Neurology* **57:**2313–2315.

Murphy, F. A., Harrison, A. K., Gary, G. W., Jr., Whitfield, S. G., and Forrester, F. T. (1968). St. Louis encephalitis virus infection in mice: Electron microscopic studies of central nervous system. *Lab. Invest.* **19:**652–662.

Myint, K. S., Raengsakulrach, B., Young, G. D., Gettyayacamin, M., Ferguson, L. M., Innis, B. L., Hoke, C. H., Jr., and Vaughn, D. W. (1999). Production of lethal infection that resembles fatal human disease by intranasal inoculation of macaques with Japanese encephalitis virus. *Am. J. Trop. Med. Hyg.* **60:**338–342.

Nargi-Aizenman, J. L., and Griffin, D. E. (2001). Sindbis virus-induced neuronal death is both necrotic and apoptotic and is ameliorated by N-methyl-D-aspartate receptor antagonists. *J. Virol.* **75:**7114–7121.

Nathanson, N., and Cole, G. A. (1970). Fatal Japanese encephalitis virus infection in immunosuppressed spider monkeys. *Clin. Exp. Immunol.* **6:**161–166.

Nathanson, N., Davis, M., Thind, I. S., *et al.* (1966). Histologic studies of the neurovirulence of group B arboviruses. II. Selection of indicator centers. *Am. J. Epidemiol.* **84:**524–535.

Navarro-Sanchez, E., Altmeyer, R., Amara, A., Schwartz, O., Fieschi, F., Virelizier, J. L., Arenzana-Seisdedos, F., and Despres, P. (2003). Dendritic-cell-specific ICAM3-grabbing non-integrin is essential for the productive infection of human dendritic cells by mosquito-cell-derived dengue viruses. *EMBO Rep.* **4**(Suppl)**:**1–6.

Navin, T. R., Krug, E. C., and Pearson, R. D. (1989). Effect of immunoglobulin M from normal human serum on *Leishmania donovani* promastigote agglutination, complement-mediated killing, and phagocytosis by human monocytes. *Infect. Immun.* **57:**1343–1346.

Neogi, D. K., Bhattacharya, N., Chakrabarti, T., and Mukherjee, K. K. (1998). Detection of HIV seropositivity during an outbreak of Japanese encephalitis in Manipur. *J. Commun. Dis.* **30:**113–116.

Neumann, H., Cavalie, A., Jenne, D. E., and Wekerle, H. (1995). Induction of MHC class I genes in neurons. *Science* **269:**549–552.

Ni, H., and Barrett, A. D. T. (1998). Attenuation of Japanese encephalitis virus by selection of its mouse brain membrane receptor preparation escape mutants. *Virology* **241:**30–36.

Ni, H., Ryman, K. D., Wang, H., Saeed, M. F., Hull, R., Wood, D., Minor, P. D., Watowich, S. J., and Barrett, A. D. T. (2000). Interaction of yellow fever virus French neurotropic vaccine strain with monkey brain: Characterization of monkey brain membrane receptor escape variants. *J. Virol.* **74:**2903–2906.

Nir, Y., Beemer, A., and Goldwasser, R. A. (1965). West Nile virus infection in mice following exposure to a viral aerosol. *Br. J. Exp. Pathol.* **46:**443–449.

Njenga, M. K., Pease, L. R., Wettstein, P., Mak, T., and Rodriguez, M. (1997). Interferon alpha/beta mediates early virus-induced expression of H-2D and H-2K in the central nervous system. *Lab. Invest.* **77:**71–84.

Oberhaus, S. M., Smith, R. L., Clayton, G. H., Dermody, T. S., and Tyler, K. L. (1997). Reovirus infection and tissue injury in the mouse central nervous system are associated with apoptosis. *J. Virol.* **71:**2100–2106.

Ochsenbein, A. F., Fehr, T., Lutz, C., Suter, M., Brombacher, F., Hengartner, H., and Zinkernagel, R. M. (1999a). Control of early viral and bacterial distribution and disease by natural antibodies. *Science* **286:**2156–2159.

Ochsenbein, A. F., Pinschewer, D. D., Odermatt, B., Carroll, M. C., Hengartner, H., and Zinkernagel, R. M. (1999b). Protective T cell-independent antiviral antibody responses are dependent on complement. *J. Exp. Med.* **190:**1165–1174.

Ochsenbein, A. F., and Zinkernagel, R. M. (2000). Natural antibodies and complement link innate and acquired immunity. *Immunol. Today* **21**(12)**:**624–630.

Odeola, H. A., and Oduye, O. O. (1977). West Nile virus infection of adult mice by oral route. *Arch. Virol.* **54:**251–253.

Ogata, A., Nagashima, K., Hall, W. W., Ichikawa, M., Kimura-Kuroda, J., and Yasui, K. (1991). Japanese encephalitis virus neurotropism is dependent on the degree of neuronal maturity. *J. Virol.* **65:**880–886.

Ogata, A., Tashiro, K., and Pradhan, S. (2000). Parkinsonism due to predominant involvement of substantia nigra in Japanese encephalitis. *Neurology* **55:**602.

Ogawa, M., Okubo, H., Tsuji, Y., *et al.* (1973). Chronic progressive encephalitis occurring 13 years after Russian spring-summer encephalitis. *J. Neurol. Sci.* **19:**363–373.

Okhuysen, P. C., Crane, J. K., and Pappas, J. (1993). St. Louis encephalitis in patients with human immuodeficiency virus infection. *Clin. Infect. Dis.* **17:**140–141.

O'Leary, J. L., Smith, M. G., and Reames, H. R. (1942). Influence of age on susceptibility of mice to St. Louis encephalitis virus and on the distribution of lesions. *J. Exp. Med.* **75:**233–247.

Oliver, K. R., and Fazakerley, J. K. (1998). Transneuronal spread of Semliki Forest virus in the developing mouse olfactory system is determined by neuronal maturity. *Neuroscience* **82:**867–877.

Oliver, K. R., Scallan, M. F., Dyson, H., and Fazakerley, J. K. (1997). Susceptibility to a neurotropic virus and its distribution in the developing brain is a function of CNS maturity. *J. Neurovirol.* **3:**38–48.

Orange, J. S., Fassett, M. S., Koopman, L. A., Boyson, J. E., and Strominger, J. L. (2002). Viral evasion of natural killer cells. *Nature Immunol.* **3:**1006–1012.

Pan, C.-H., Chen, H.-W., Huang, H.-W., and Tao, M.-H. (2001). Protective mechanisms induced by a Japanese encephalitis virus DNA vaccine: Requirement for antibody but not $CD8^+$ cytotoxic T-cell responses. *J. Virol.* **75:**11457–11463.

Panasiuk, B., Prokopowicz, D., and Panasiuk, A. (2003). Immunological response in HIV-positive patients vaccinated against tick-borne encephalitis. *Infection* **31:**45–46.

Parquet, M. C., Kumatori, A., Hasebe, F., Morita, K., and Igarashi, A. (2001). West Nile virus-induced bax-dependent apoptosis. *FEBS Lett.* **500:**17–24.

Pavri, K. M., Ghalsasi, G. R., Dastur, D. K., Goverdhan, M. K., and Lalitha, V. S. (1975). Dual infections of mice: Visceral larva migrans and sublethal infection with Japanese encephalitis virus. *Trans. R. Soc. Trop. Med. Hyg.* **69:**99–110.

Peiris, J. S. M., Gordon, S., Unkeless, J. C., and Porterfield, J. S. (1981). Monoclonal anti-Fc receptor IgG blocks antibody enhancement of viral replication in macrophages. *Nature* **289:**189–191.

Perelygin, A. A., Scherbik, S. V., Zhulin, I. B., Stockman, B. M., Li, Y., and Brinton, M. A. (2002). Positional cloning of the murine flavivirus resistance gene. *Proc. Natl. Acad. Sci. USA* **99:**9322–9327.

Phillpotts, R. J., Jones, L. D., Lukaszewski, R. A., Lawrie, C., and Brooks, T. J. (2003). Antibody and interleukin-12 treatment in murine models of encephalitogenic flavivirus (St. Louis encephalitis, tick-borne encephalitis) and alphavirus (Venezuelan equine encephalitis) infection. *J. Interferon Cytokine Res.* **23:**47–50.

Pincus, S., Mason, P. W., Konishi, E., Fonseca, B. A., Shope, R. E., Rice, C. M., and Paoletti, E. (1992). Recombinant vaccinia virus producing the prM and E proteins of yellow fever virus protects mice from lethal yellow fever encephalitis. *Virology* **187:**290–297.

Pogodina, V. V., Frolova, M. P., Malenko, G. V., Fokina, G. I., Koreshkova, G. V., Kiseleva, L. L., Bochkova, N. G., and Ralph, N. M. (1983). Study on West Nile virus persistence in monkeys. *Arch. Virol.* **75:**71–86.

Pogodina, V. V., Levina, L. S., Fokina, G. I., *et al.* (1981). Persistence of tick-borne encephalitis virus in monkeys. III. Phenotypes of the persisting virus. *Acta Virol.* **25:**352–360.

Poidinger, M., Coelen, R. J., and Mackenzie, J. S. (1991). Persistent infection of Vero cells by the flavivirus Murray Valley encephalitis virus. *J. Gen. Virol.* **72:**573–578.

Popko, B., Corbin, J. G., Baerwald, K. D., Dupree, J., and Garcia, A. M. (1997). The effects of interferon-gamma on the central nervous system. *Mol. Neurobiol.* **14:**19–35.

Potula, R., Badrinath, S., and Srinivasan, S. (2003). Japanese encephalitis in and around Pondicherry, South India: A clinical appraisal and prognostic indicators for the outcome. *J. Trop. Pediatr.* **49:**48–53.

Powell, K. E., and Kappus, K. D. (1978). Epidemiology of St. Louis encephalitis and other acute encephalitides. *Adv. Neurol.* **19:**197–213.

Pradhan, S., Pandey, N., Shashank, S., Gupta, R. K., and Mathur, A. (1999). Parkinsonism due to predominant involvement of substantia nigra in Japanese encephalitis. *Neurology* **53:**1781–1786.

Prikhod'ko, G. G., Prikhod'ko, E. A., Cohen, J. I., and Pletnev, A. G. (2001). Infection with Langat flavivirus or expression of the envelope protein induces apoptotic cell death. *Virology* **286:**328–335.

Prikhod'ko, G. G., Prikhod'ko, E. A., Pletnev, A. G., and Cohen, J. I. (2002). Langat flavivirus protease NS3 binds caspase-8 and induces apoptosis. *J. Virol.* **76:**5701–5710.

Putnak, J. R., and Schlesinger, J. J. (1990) Protection of mice against yellow fever virus encephalitis by immunization with a vaccinia virus recombinant encoding the yellow fever virus non-structural proteins, NS1, NS2a and NS2b. *J. Gen. Virol.* **71:** 1697–1702.

Raengsakulrach, B., Nisalak, A., Gettayacamin, M., Thirawuth, V., Young, G. D., Myint, K. S., Ferguson, L. M., Hoke, C. H. Jr., Innis, B. L., and Vaughn, D. W. (1999). An intranasal challenge model for testing Japanese encephalitis vaccines in rhesus monkeys. *Am. J. Trop. Med. Hyg.* **60:**329–337.

Raghupathy, R., Chaturvedi, U. C., A1-Sayer, H., Elbishbishi, E. A., Agarwal, R., Nagar, R., Kapoor, S., Misra, A., Mathur, A., Nusrat, H., Azizieh, F., Khan, M. A., and Mustafa, A. S. (1998). Elevated levels of IL-8 in dengue hemorrhagic fever. *J. Med. Virol.* **56:**280–285.

Ramakrishna, C., Ravi, V., Desai, A., Subbakrishna, D. K., Shankar, S. K., and Chandramuki, A. (2003). T helper responses to Japanese encephalitis virus infection are dependent on the route of inoculation and the strain of mouse used. *J. Gen. Virol.* **84:**1559–1567.

Ramos-Castaneda, J., Imbert, J. L., Barron, B. L., and Ramos, C. (1997). A 65-kDa trypsin-sensible membrane cell protein as a possible receptor for dengue virus in cultured neuroblastoma cells. *J. Neurovirol.* **3:**435–440.

Randolph, V. A., and Hardy, J. L. (1988a). Establishment and characterization of St. Louis encephalitis virus persistent infections in *Aedes* and *Culex* mosquito cell lines. *J. Gen. Virol.* **69:**2189–2198.

Randolph, V. A., and Hardy, J. L. (1988b). Phenotypes of St. Louis encephalitis virus mutants produced in persistently infected mosquito cell cultures. *J. Gen Virol.* **69:**2199–2207.

Raung, S. L., Kuo, M. D., Wang, Y. M., and Chen, C. J. (2001). Role of reactive oxygen intermediates in Japanese encephalitis virus infection in murine neuroblastoma cells. *Neurosci. Lett.* **315:**9–12.

Ravi, V., Desai, A. S., Shenoy, P. K., Satishchandra, P., Chandramuki, A., and Gourie-Devi, M. (1993). Persistence of Japanese encephalitis virus in the human nervous system. *J. Med. Virol.* **40:**326–329.

Ravi, V., Parida, S., Desai, A., Chandramuki, A., Gourie-Devi, M., and Grau, G. E. (1997). Correlation of tumor necrosis factor levels in the serum and cerebrospinal fluid with clinical outcome in Japanese encephalitis patients. *J. Med. Virol.* **51:**132–136.

Receveur, M. C., Thiebaut, R., Vedy, S., Malvy, D., Mercie, P., and Bras, M. L. (2000). Yellow fever vaccination of human immunodeficiency virus-infected patients: Report of 2 cases. *Clin. Infect. Dis.* **31:**E7–E8.

Reiss, C. S., Chesler, D. A., Hodges, J., Ireland, D. D. C., and Chen, N. (2002). Innate immune responses in viral encephalitis. *Curr. Top. Microbiol. Immunol.* **265:**63–84.

Reyes, M. G., Gardner, J. J., Poland, J. D., and Monath, T. P. (1981). St. Louis encephalitis: Quantitative histologic and immunofluorescent studies. *Arch. Neurol.* **38:**329–334.

Richter, R. W. (1961). Neurologic sequellae of Japanese B encephalitis. *Neurology* **11:**553–559.

Roehrig, J. T., Hunt, A. R., Johnson, A. J., and Hawkes, R. A. (1989). Synthetic peptides derived from the deduced amino acid sequence of the E-glycoprotein of Murray Valley encephalitis virus elicit antiviral antibody. *Virology* **171:**49–60.

Roehrig, J. T., Johnson, A. J., Hunt, A. R., Beaty, B. J., and Mathews, J. H. (1992). Enhancement of antibody response to flavivirus B-cell epitopes by using homologous or heterologous T-cell epitopes. *J. Virol.* **66:**3385–3390.

Roehrig, J. T., Nash, D., Maldin, B., Labowitz, A., Martin, D. A., Lanciotti, R. S., and Campbell, G. L. (2003). Persistence of virus-reactive serum immunoglobulin M antibody in confirmed West Nile virus encephalitis cases. *Emerg. Infect. Dis.* **9:**376–379.

Roehrig, J. T., Staudinger, L. A., Hunt, A. R., Mathews, J. H., and Blair, C. D. (2001). Antibody prophylaxis and therapy for flaviviral encephalitis infections. *Ann. N.Y. Acad. Sci.* **951:**286–297.

Rojanasuphot, S., Shaffer, N., Chotpitayasunondh, T., Phumiamorn, S., Mock, P., Chearskul, S., Waranawat, N., Yuentrakul, P., Mastro, T. D., and Tsai, T. F. (1998). Response to JE vaccine among HIV-infected children, Bangkok, Thailand. *Southeast Asian J. Trop. Med. Public Health* **29:**443–450.

Rothman, A. L., and Ennis, F. A. (1999). Immunopathogenesis of dengue hemorrhagic fever. *Virology* **257:**1–6.

Sammin, D. J., Butler, D., Atkins, G. J., and Sheahan, B. J. (1999). Cell death mechanisms in the olfactory bulb of rats infected intranasally with Semliki Forest virus. *Neuropathol. Appl. Neurobiol.* **25:**236–243.

Sampson, B. A., Ambrosi, C., Charlot, A., Reiber, K., Veress, J. F., and Armbrustmacher, V. (2000). The pathology of human West Nile Virus infection. *Hum. Path.* **31:**527–531.

Samuel, C. E. (2002). Host genetic variability and West Nile virus susceptibility. *Proc. Natl. Acad. Sci. USA* **99:**11555–11557.

Sangster, M. K., Urosevic, N., Mansfield, J. P., Mackenzie, J. S., and Shellam, G. R. (1994). Mapping the *Flv* locus controlling resistance to flaviviruses on mouse chromosome 5. *J. Virol.* **68:**448–452.

Saxena, S. K., Singh, A., and Mathur, A. (2000). Antiviral effect of nitric oxide during Japanese encephalitis virus infection. *Int. J. Exp. Pathol.* **81:**165–172.

Schlesinger, J. J., and Brandriss, M. W. (1983). 17D yellow fever virus infection of P388D1 cells mediated by monoclonal antibodies: Properties of the macrophage Fc receptor. *J. Gen. Virol.* **64:**1255–1262.

Schlesinger, J. J., Brandriss, M. W., Cropp, C. B., and Monath, T. P. (1986). Protection against yellow fever in monkeys by immunization with yellow fever virus nonstructural protein NS1. *J. Virol.* **60:**1153–1155.

Schlesinger, J. J., Brandriss, M. W., Putnak, J. R., and Walsh, E. E. (1990). Cell surface expression of yellow fever virus non-structural glycoprotein NS1: Consequences of interaction with antibody. *J. Gen. Virol.* **71:**593–599.

Schlesinger, J. J., Brandriss, M. W., and Walsh, E. E. (1985). Protection against 17D yellow fever encephalitis in mice by passive transfer of monoclonal antibodies to the nonstructural glycoprotein gp48 and by active immunization with gp48. *J. Immunol.* **135:**2805–2809.

Schlesinger, J. J., Brandriss, M. W., and Walsh, E. E. (1987). Protection of mice against dengue 2 virus encephalitis by immunization with the dengue 2 virus non-structural glycoprotein NS1. *J. Gen. Virol.* **68:**853–857.

Schlesinger, J. J., and Chapman, S. (1995). Neutralizing F(ab′)2 fragments of protective monoclonal antibodies to yellow fever virus (YF) envelope protein fail to protect mice against lethal YF encephalitis. *J. Gen. Virol.* **76:**217–220.

Schlesinger, J. J., Chapman, S., Nestorowicz, A., Rice, C. M., and Chambers, T. J. (1996). Replication of yellow fever virus in the mouse central nervous system: Comparison of neuroadapted and nonneuroadapted virus and partial sequence analysis of the neuroadapted strain. *J. Gen. Virol.* **77:**1277–1285.

Schlesinger, J. J., Foltzer, M., and Chapman, S. (1993). The Fc portion of antibody to yellow fever virus NS1 is a determinant of protection against YF encephalitis in mice. *Virology* **192:**132–141.

Schmaljohn, C., and Blair, C. D. (1977). Persistent infection of cultured mammalian cells by Japanese encephalitis virus. *J. Virol.* **24:**580–589.

Schmaljohn, C. S., and Blair, C. D. (1979). Clonal analysis of mammalian cell cultures persistently infected with Japanese encephalitis virus. *J. Virol.* **31:**816–822.

Schneider-Schaulies, J., Liebert, U. G., Dorries, R., and ter Meullen, V. (1997). Establishment and control of viral infections in the central nervous system. *In* "Immunology of the Nervous System" (R. W. Keane and W. F. Hickey, eds.), pp. 576–616. Oxford Univ. Press, New York.

Shah, P. S., and Gadkari, D. A. (1987). Persistent infection of porcine kidney cells with Japanese encephalitis virus. *Ind. J. Med Res.* **85:**481–491.

Shahar, A., Lustig, S., Akov, Y., David, Y., Schneider, P., Friedmann, A., and Levin, R. (1990). West Nile virions aligned along myelin lamellae in organotypic spinal cord cultures. *J. Neurosci. Res.* **26:**495–500.

Sharma, S., Mathur, A., Prakash, V., Kulshreshta, R., Kumar, R., and Chaturvedi, U. C. (1991). Japanese encephalitis virus latency in peripheral blood lymphocytes and recurrence of infection in children. *Clin. Exp. Immunol.* **85:**85–91.

Shoji, H., Murakami, T., Murai, I., Kida, H., Sato, Y., Kojima, K., Abe, T., and Okudera, T. (1990). A follow-up study by CT and MRI in 3 cases of Japanese encephalitis. *Neuroradiology* **32:**215–219.

Sibailly, T. S., Wiktor, S. Z., Tsai, T. F., Cropp, C. B., Ekpini, E. R., Adjorlolo-Johnson, G., Gnaore, E., DeCock, K. M., and Greenberg, A. E. (1997). Poor antibody response to yellow fever vaccination in children infected with human immunodeficiency virus type 1. *Pediat. Infect. Dis.* **16:**1177–1179.

Singh, A., Kulshreshta, R., and Mathur, A. (2000). Secretion of the chemokine interleukin-8 during Japanese encephalitis virus infection. *J. Med. Micro.* **49:**607–612.

Slavin, H. B. (1943). Persistence of the virus of St. Louis encephalitis in the central nervous system of mice for over five months. *J. Bacteriol.* **46:**113–116.

Smith, A. L. (1981). Genetic resistance to lethal flavivirus encephalitis: Effect of host age and immune status and route of inoculation on production of interfering Banzi virus in vivo. *Am. J. Trop. Med. Hyg.* **30:**1319–1323.

Smith, H. R., Idris, A. H., and Yokoyama, W. M. (2001). Murine natural killer cell activation receptors. *Immunol. Rev.* **181:**115–125.

Solomon, T., Dung, N. M., Wills, B., Kneen, R., Gainsborough, M., Diet, T. V., Thuy, T. T., Loan, H. T., Khanh, V. C., Vaughn, D. W., White, N. J., and Farrar, J. J. (2003). Interferon alpha-2a in Japanese encephalitis: A randomised double-blind placebo-controlled trial. *Lancet* **361:**821–826.

Solomon, T., Kneen, R., Dung, N. M., Khanh, V. C., Thuy, T. T., HA, D. Q., Day, N. P., Nisalak, A., Vaughn, D. W., and White, N. J. (1998). Poliomyelitis-like illness due to Japanese encephalitis virus. *Lancet* **351:**1094–1097.

Solomon, T., and Vaughn, D. W. (2002). Pathogenesis and clinical features of Japanese encephalitis and West Nile infections. *In* "Japanese Encephalitis and West Nile Viruses" (J. S. Mackenzie, A. D. T. Barrett, and V. Deubel, eds.), pp. 171–194. Springer-Verlag, Berlin.

Southam, C. M., Nojes, W. F., and Mellors, R. (1958). Virus in human cancer cells in vivo. *Virology* **5:**395–400.

Spain-Santana, T. A., Marglin, S., Ennis, F. A., and Rothman, A. L. (2001). MIP-1 alpha and MIP-1 beta induction by dengue virus. *J. Med. Virol.* **65:**324–330.

Steele, K. E., Linn, M. J., Schoepp, R. J., Komar, N., Geisbert, T. W., Manduca, R. M., Calle, P. P., Raphael, B. L., Clippinger, T. L., Larsen, T., Smith, J., Lanciotti, R. S., Panella, N. A., and McNamara, T. S. (2000). Pathology of fatal West Nile virus infections in native and exotic birds during the 1999 outbreak in New York City, New York. *Vet. Pathol.* **37:**208–224.

Stephens, H. A., Klaythong, R., Sirikong, M., Vaughn, D. W., Green, S., Kalayanarooj, S., Endy, T. P., Libraty, D. H., Nisalak, A., Innis, B. L., Rothman, A. L., Ennis, F. A., and Chandanayingyong, D. (2002). HLA-A and -B allele associations with secondary dengue virus infections correlate with disease severity and the infecting viral serotype in ethnic Thais. *Tissue Antigens* **60:**309–318.

Stohlman, S. A., Bergmann, C. C., Lin, M. T., Cua, D. J., and Hinton, D. R. (1998). CTL effector function within the central nervous system requires $CD4^+$ T cells. *J. Immunol.* **160:**2896–2904.

Su, C. M., Liao, C. L., Lee, Y. L., and Lin, Y. L. (2001). Highly sulfated forms of heparin sulfate are involved in Japanese encephalitis virus infection. *Virology* **286:**206–215.

Su, H. L., Liao, C. L., and Lin, Y. L. (2002). Japanese encephalitis virus infection initiates endoplasmic reticulum stress and an unfolded protein response. *J. Virol.* **76:**4162–4171.

Suri, N. K., and Banerjee, K. (1995). Growth and cytopathic effect of Japanese encephalitis virus in astrocyte-enriched cell cultures from neonatal mouse brains. *Acta Virol.* **39:**143–148.

Suzuki, M., and Phillips, C. A. (1966). St. Louis encephalitis: A histopathologic study of the fatal cases from the Houston epidemic in 1964. *Arch. Pathol.* **81:**47–54.

Suzuki, T., Ogata, A., Tashiro, K., Nagashima, K., Tamura, M., Yasui, K., and Nishihira, J. (2000). Japanese encephalitis virus up-regulates expression of macrophage migration inhibitory factor (MIF) mRNA in the mouse brain. *Biochim. Biophys. Acta* **1517:**100–106.

Szilak, I., and Minamoto, G. Y. (2000). West Nile viral encephalitis in an HIV-positive woman in New York. *N. Engl. J. Med.* **42:**59–60.

Ta, M., and Vrati, S. (2000). Mov34 protein from mouse brain interacts with the 3′ noncoding region of Japanese encephalitis virus. *J. Virol.* **74:**5108–5115.

Takada, K., Masaki, H., Konishi, E., Takahashi, M., and Kurane, I. (2000). Definition of an epitope on Japanese encephalitis virus (JEV) envelope protein recognized by JEV-specific murine $CD8^+$ cytotoxic T lymphocytes. *Arch. Virol.* **145:**523–534.

Tassaneetrithep, B., Burgess, T. H., Granelli-Piperno, A., Trumpfheller, C., Finke, J., Sun, W., Eller, M. A., Pattanapanyasat, K., Sarasombath, S., Birx, D. L., Steinman, R. M., Schlesinger, S., and Marovich, M. A. (2003). DC-SIGN (CD209) mediates dengue virus infection of human dendritic cells. *J. Exp. Med.* **197:**823–829.

Tesh, R. B., Travassos da Rosa, A. P., Guzman, H., Araujo, T. P., and Xiao, S. Y. (2002). Immunization with heterologous flaviviruses protective against fatal West Nile encephalitis. *Emerg Infect. Dis.* **8:**245–251.

Thakare, J. P., Wadia, R. S., Banerjee, K., and Ghosh, S. N. (1988). Increased level of myelin basic protein in cerebrospinal fluid of patients of Japanese encephalitis. *Indian J. Med. Res.* **88:**297–300.

Thakare, J. P., Gore, M. M., Risbud, A. R., Banerjee, K., and Ghosh, S. N. (1991). Detection of virus specific IgG subclasses in Japanese encephalitis patients. *Indian J. Med. Res.* **93:**271–276.

Theerasurakarn, S., and Ubul, S. (1998). Apoptosis induction in brain during the fixed strain of rabies virus infection correlates with onset and severity of illness. *J. Neurovirol.* **4:**407–414.

Tschen, S.-I., Bergmann, C. C., Ramakrishna, C., Morales, S., Atkinson, R., and Stohlman, S. A. (2002). Recruitment kinetics and composition of antibody-secreting cells within the central nervous system following viral encephalomyelitis. *J. Immunol.* **168:**2922–2929.

Turell, M. J., and Spielman, A. (1992). Nonvascular delivery of Rift Valley fever virus by infected mosquitoes. *Am. J. Trop. Med. Hyg.* **47:**190–194.

Turell, M. J., Tammariello, R. F., and Spielman, A. (1995). Nonvascular delivery of St. Louis encephalitis and Venezuelan equine encephalitis viruses by infected mosquitoes (Diptera: Culicidae) feeding on a vertebrate host. *J. Med. Entomol.* **32:**563–568.

Tyor, W. R., Wesselingh, S., Levine, B., and Griffin, D. E. (1992). Long term intraparenchymal Ig secretion after acute viral encephalitis in mice. *J. Immunol.* **149:**4016–4020.

Uren, M. F., Doherty, P. C., and Allan, J. E. (1987). Flavivirus-specific murine $L3T4^+$ T cell clones: Induction, characterization and cross-reactivity. *J. Gen. Virol.* **68:**2655–2663.

Urosevic, N., van Maanen, M., Mansfield, J. P., Mackenzie, J. S., and Shellam, G. R. (1997). Molecular characterization of virus-specific RNA produced in the brains of flavivirus-susceptible and -resistant mice after challenge with Murray Valley encephalitis virus. *J. Gen. Virol.* **78:**23–29.

van der Most, R. G., Harrington, L. E., Giuggio, V., Mahar, P. L., and Ahmed, R. (2002). Yellow fever virus 17D envelope and NS3 proteins are major targets of the antiviral T cell response in mice. *Virology* **296:**117–124.

van der Most, R. G., Murali-Krishna, K., and Ahmed, R. (2003). Prolonged presence of effector-memory CD8 T cells in the central nervous system after dengue virus encephalitis. *Int. Immunol.* **15:**119–125.

van der Most, R. G., Murali-Krishna, K., Ahmed, R., and Strauss, J. H. (2000). Chimeric yellow fever/dengue virus as a candidate dengue vaccine: Quantitation of the dengue virus-specific CD8 T cell response. *J. Virol.* **74:**8094–8101.

Vaneeva, G. G. (1969). Long-term observation over children having sustained tick-borne encephalitis. *Pediatrics* **48:**46–48.

Vargin, V. V., and Semenov, B. F. (1986). Changes of natural killer cell activity in different mouse lines by acute and asymptomatic flavivirus infections. *Acta Virol.* **30:**303–308.

Varnavski, A. N., and Khromykh, A. A. (1999). Noncytopathic flavivirus replicon RNA-based system for expression and delivery of heterologous genes. *Virology* **255:**366–375.

Vince, V., and Grevic, N. (1969). Development of morphological changes in experimental tick-borne meningoencephalitis induced in mice by different virus doses. *J. Neurol. Sci.* **9:**109–130.

Vlaycheva, L., and Chambers, T. J. (2002). Neuroblastoma cell-adapted yellow fever 17D: Characterization of a viral variant associated with persistent infection and decreased virus spread. *J. Virol.* **75:**10912–10922.

Volanakis, J. E. (2002). The role of complement in innate and adaptive immunity. *Curr. Top. Microbiol. Immunol.* **266:**41–56.

Waldvogel, K., Bossart, W., Huisman, T., Boltshauser, E., and Nadal, D. (1996). Severe tick-borne encephalitis following passive immunization. *Eur. J. Pediatr.* **155:**775–779.

Wang, J. J., Liao, C. L., Chiou, Y. W., Chiou, C. T., Huang, Y. L., and Chen, L. K. (1997). Ultrastructure and localization of E proteins in cultured neuron cells infected with Japanese encephalitis virus. *Virology* **238:**30–39.

Wasay, M., Diaz-Arrastia, R., Suss, R. A., Kojan, S., Haq, A., Burns, D., and Van Ness, P. (2000). St Louis encephalitis: A review of 11 cases in a 1995 Dallas, Tex, epidemic. *Arch. Neurol.* **57:**114–118.

Weiner, L. P., Cole, G. A., and Nathanson, N. (1970). Experimental encephalitis following peripheral inoculation of West Nile virus in mice of different ages. *J. Hyg.* **68:**435–446.

Wesselingh, S. L., Levine, B., Fox, R. J., Choi, S., and Griffin, D. E. (1994). Intracerebral cytokine mRNA expression during fatal and nonfatal alphavirus encephalitis suggests a predominant type 2 T cell response. *J. Immunol.* **152:**1289–1297.

Wu, S. J., Grouard-Vogel, G., Sun, W., Mascola, J. R., Brachtel, E., Putvatana, R., Louder, M. K., Filgueira, L., Marovich, M. A., Wong, H. K., Blauvelt, A., Murphy, G. S., Robb, M. L., Innes, B. L., Birx, D. L., Hayes, C. G., and Frankel, S. S. (2000). Human skin Langerhans cells are targets of dengue virus infection. *Nature Med.* **6:**816–820.

Xiao, S. Y., Guzman, H., Zhang, H., Travassos da Rosa, A. P., and Tesh, R. B. (2001). West Nile virus infection in the golden hamster (*Mesocricetus auratus*): A model for West Nile encephalitis. *Emerg. Infect. Dis.* **7:**714–721.

Yang, J. S., Ramanathan, M. P., Muthumani, K., Choo, A. Y., Jin, S. H., Yu, Q. C., Hwang, D. S., Choo, D. K., Lee, M. D., Dang, K., Tang, W., and Kim, J. J. (2002). Induction of inflammation by West Nile virus capsid through the caspase-9 apoptotic pathway. *Emerg. Infect. Dis.* **8:**1379–1384.

Yazi Mendoza, M., Salas-Benito, J. S., Lanz-Mendoza, H., Hernandez-Martinez, S., and del Angel, R. M. (2002). A putative receptor for dengue virus in mosquito tissues: Localization of a 45-kDa glycoprotein. *Am. J. Trop. Med. Hyg.* **67:**76–84.

Yocupicio-Monroy, R. M., Medina, F., Reyes del Valle, J., and del angel, R. M. (2003). Cellular protein from human monocytes bind to dengue 4 virus minus strand 3′ untranslated RNA. *J. Virol.* **77:**3067–3076.

Zeidner, N. S., Higgs, S., Happ, C. M., Beaty, B. J., and Miller, B. R. (1999). Mosquito feeding modulates Th1 and Th2 cytokines in flavivirus susceptible mice: An effect mimicked by injection of sialokinins, but not demonstrated in flavivirus resistant mice. *Parasite Immunol.* **21:**35–44.

Zhang, M. J., Wang, M. J., Jiang, S. Z., and Ma, W. Y. (1989). Passive protection of mice, goats, and monkeys against Japanese encephalitis with monoclonal antibodies. *J. Med. Virol.* **29:**133–138.

Zhang, P. F., Klutch, M., Muller, J., and Marcus-Sekura, C. J. (1993). St. Louis encephalitis virus establishes a productive, cytopathic and persistent infection of Sf9 cells. *J. Gen. Virol.* **74:**1703–1708.

Zimmerman, H. M. (1946). The pathology of Japanese B encephalitis. *Am. J. Pathol.* **22:**965–991.

Zisman, B., Wheelock, E. F., and Allison, A. C. (1971). Role of macrophages and antibody in resistance of mice against yellow fever virus. *J. Immunol.* **107:**236–243.

Zlotnik, I., Carter, G. B., and Grant, D. P. (1971). The persistence of louping ill virus in immunosuppressed guinea-pigs. *Br. J. Exp. Pathol.* **52:**395–407.

Zlotnik, I., Grant, D. P., and Carter, G. B. (1976). Experimental infection of monkeys with viruses of the tick-borne encephalitis complex: Degenerative cerebellar lesions following inapparent forms of the disease or recovery from clinical encephalitis. *Br. J. Exp. Pathol.* **57:**200–210.

Zocher, M., Czub, S., Schulte-Monting, J., de La Torre, J. C., and Sauder, C. (2000). Alterations in neurotrophin and neurotrophin receptor gene expression patterns in the rat central nervous system following perinatal Borna disease virus infection. *J. Neurovirol.* **6:**462–467.