Antibody Prophylaxis and Therapy against West Nile Virus Infection in Wild-Type and Immunodeficient Mice

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West Nile virus (WNV) is a mosquito-borne *Flavivirus* that causes encephalitis in a subset of susceptible humans. Current treatment for WNV infections is supportive, and no specific therapy or vaccine is available. In this study, we directly tested the prophylactic and therapeutic efficacy of polyclonal antibodies against WNV. Passive administration of human gamma globulin or mouse serum prior to WNV infection protected congenic wild-type, B-cell-deficient (μ MT), and T- and B-cell-deficient (*RAG1*) C57BL/6J mice. Notably, no increased mortality due to immune enhancement was observed. Although immune antibody completely prevented morbidity and mortality in wild-type mice, its effect was not durable in immunocompromised mice: many μ MT and *RAG1* mice eventually succumbed to infection. Thus, antibody by itself did not completely eliminate viral reservoirs in host tissues, consistent with an intact cellular immune response being required for viral clearance. In therapeutic postexposure studies, human gamma globulin partially protected against WNV-induced mortality. In μ MT mice, therapy had to be initiated within 2 days of infection to gain a survival benefit, whereas in the wild-type mice, therapy even 5 days after infection reduced mortality. This time point is significant because between days 4 and 5, WNV was detected in the brains of infected mice. Thus, passive transfer of immune antibody improves clinical outcome even after WNV has disseminated into the central nervous system.

A member of the *Flavivirus* genus of the *Flaviviridae* family, West Nile virus (WNV) is a neurotropic enveloped virus with a single-stranded, positive-polarity 11-kb RNA genome. WNV cycles primarily between mosquitoes and birds but also infects humans, horses, and a variety of other vertebrate species. It is endemic in parts of Africa, Europe, the Middle East, and Asia, and outbreaks throughout the United States during the past 4 years indicate that it has established its presence in the Western hemisphere. Humans develop a febrile illness that can progress rapidly to a meningitis or encephalitis syndrome (32). Infants, the elderly, and patients with impaired immune systems are at greatest risk for severe neurological disease (5, 32, 63).

At present, treatment for all flavivirus infections, including WNV, is supportive. Based on studies in cell culture, ribavirin (33) and alpha interferon (4) have been proposed as candidate antiviral agents against WNV, yet neither has demonstrated efficacy in vivo. Although antibody has been used for therapy against several viral infections (53, 67), with the exception of its prophylactic use against tick-borne encephalitis virus (52), it has not been used against flaviviral infections in humans. Although few data are available with respect to WNV infection, animal studies have provided information on how antibodies mediate protection against flavivirus infections. Most neutralizing antibodies recognize the structural E protein, although a subset against another virion-associated protein, the prM or membrane protein (13, 19, 48, 64), have also been described. Several groups also have generated nonneutralizing yet pro-

tective monoclonal antibodies against NS1 (14, 20, 31, 50, 54, 55, 57, 58), a protein that is absent from the virion. Thus, protection against flavivirus infections in vivo does not necessarily correlate with neutralizing activity in vitro (8, 51, 56). The ability to cure mice of flavivirus infection with immune serum or monoclonal antibodies depends on the dosage and time of administration (12, 34, 47, 52), and polyclonal antibodies that prevent infection against one flavivirus do not provide durable cross-protection against heterologous flaviviruses (9, 52).

Although these studies suggest that antibodies could have a potential therapeutic role, there are concerns that treatment could exacerbate flavivirus infection. Subneutralizing concentrations of antibody enhance flavivirus replication in myeloid cells in vitro (10, 11, 21, 22, 44–46) and thus could complicate the therapeutic administration of antibodies. This phenomenon of antibody-dependent enhancement of infection (ADE) may contribute to a pathological cytokine cascade that occurs during secondary dengue virus infection and causes a severe hemorrhagic syndrome (27, 28, 36, 41); despite its extensive characterization in vitro, the significance of ADE in vivo with WNV or other flaviviruses remains uncertain.

Apart from or perhaps related to ADE, an “early-death” phenomenon (41) has been reported that could also limit the utility of antibody therapy against WNV. According to this model, animals that have existing humoral immunity but do not respond well to viral challenge may succumb to infection more rapidly than animals without existing immunity. Although it has been described after passive acquisition of antibodies against yellow fever and Langkat encephalitis viruses (6, 23, 24, 65), this phenomenon was not observed after transfer of monoclonal or polyclonal antibodies against Japanese encephalitis virus (34) or tick-borne encephalitis virus (35).

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Because of the expanding WNV epidemic, it is critical to evaluate novel therapeutic strategies, such as immunotherapy, in a well-defined model of WNV encephalitis. Individual case reports have suggested that administration of pooled gamma globulin to humans may improve outcome after WNV infection (30, 61). In this study, we evaluated the efficacy of polyclonal antibodies as immune prophylaxis and treatment of WNV infection in immunocompetent and immunocompromised mice. Passive administration of immune antibodies prior to WNV infection protected wild-type and T- and B-cell-deficient C57BL/6J mice. Although antibody completely prevented morbidity in wild-type mice, many immunocompromised mice eventually succumbed to infection at later time points. Thus, immune antibody, by itself, limited infection but did not completely eliminate viral reservoirs in host tissues. In therapeutic studies, immune gamma globulin partially protected against WNV-induced mortality. Initiation of antibody therapy even after WNV had spread to the central nervous system (CNS) still improved clinical outcome.

MATERIALS AND METHODS

Cells and viruses. BHK-21, Vero, and C6/36 *Aedes albopictus* cells were cultured as previously described (15, 16). The WNV strain 3000.0259 was isolated in New York in 2000 (18) and obtained from Laura Kramer (Albany, N.Y.). The initial isolate was harvested after inoculating a mosquito homogenate into Vero cells (passage 0). All cell culture and in vivo studies used a stock (2.6×10^8 PFU/ml) of this virus that was propagated (passage 1) once in C6/36 cells. Viruses were diluted and injected into mice as previously described (17).

Mouse experiments. All mice used in these experiments were derived from the inbred C57BL/6J strain (*H-2^b*). The wild-type C57BL/6J and congenic B-cell-deficient (μ MT) mice (strain B6 *Igh6-6^{mt1Cgn}*) were purchased from Jackson Laboratories (Bar Harbor, Maine). The congenic *RAG1* mice (strain B6 *RAG1^{tm1Mom}*) were a gift from E. Unanue, Washington University School of Medicine. Mice were used between 5 and 8 weeks of age, depending on the particular experiment, and inoculated subcutaneously with WNV by footpad injection after anesthetization with xylazine and ketamine. Fur ruffling, hunchback posture, and weight loss were used as markers for WNV-associated morbidity. Mouse experiments were approved and performed according to the guidelines of the Washington University School of Medicine Animal Safety Committee.

Serum and antibody preparations. To obtain immune sera, wild-type mice that had survived primary WNV infection were maintained for 28 days, anesthetized, and phlebotomized. Serum was collected, and after heat inactivation (30 min at 56°C), aliquoted, and stored at -80°C. Purified human gamma globulin was obtained commercially (Omrix Biopharmaceuticals Ltd.) from a region of WNV endemicity (Israel) which has had repeated outbreaks over the past few years. Approximately 10 to 20% of Israeli blood donors have antibodies that react with WNV proteins. Several batches of pooled human gamma globulin (50 mg of IgG per ml) were tested for reactivity by enzyme-linked immunosorbent assay (ELISA) against WNV antigens and by plaque reduction neutralization (PRNT) assay (17). Nonimmune human gamma globulin (50 mg of IgG per ml) was obtained from a region where WNV is not endemic (Midwestern United States, 2000) and lacked immunoreactivity as judged by enzyme-linked immunosorbent assays (ELISAs) and PRNT assays.

Antibody administration experiments. For passive transfer of sera, wild-type and immunodeficient mice were administered the indicated doses of serum by intraperitoneal injection 1 day prior to and after inoculation with 10^2 PFU of WNV. In experiments with human gamma globulin, mice were administered a single dose of immune or nonimmune purified IgG by intraperitoneal injection at the same time as or at different times after footpad inoculation of WNV.

Quantitation of viral burden in mice. For analysis of virus in tissues of infected mice, titrations were performed by plaque assay on BHK-21 cells as described previously (16, 17). Viral RNA from serum and tissues was harvested and quantitated with real-time fluorogenic reverse transcription-PCR as described previously (17).

Quantitation of antibodies. The titer of neutralizing antibodies was determined by a standard PRNT assay (17, 29). Results were plotted and the titer for 50% inhibition (PRNT₅₀) was calculated. To determine the immune IgG titers,

a WNV antigen ELISA was used (17, 62). The human gamma globulin preparations used in this study had the following titers: nonimmune gamma globulin F43312: ELISA, <1/10; PRNT₅₀, <1/10; immune gamma globulin G12101: ELISA, 1/900; PRNT₅₀, 1/50; G24191: ELISA, 1/900, PRNT₅₀, 1/100.

Statistical analysis. Differences in outcome were assessed after Kaplan-Meier survival curves with a log rank test. Differences in average survival time were determined by a two-tailed Mann-Whitney test.

RESULTS

Previously, we demonstrated that antibody prevented dissemination of WNV infection in C57BL/6J mice (17). Passive administration of immune mouse serum protected wild-type and congenic B-cell-deficient (strain μ MT) mice against lethal infection. At 21 days after infection, all μ MT mice that received immune serum remained alive (17) and showed no evidence of morbidity (fur ruffling, hunchback posture, or weight loss). Although these studies demonstrated that antibody was required for protection, it remained unclear whether it was sufficient for viral clearance. To evaluate this, the time courses were extended (Fig. 1).

Passive transfer of pooled mouse immune serum (titer by ELISA, 1/10,000) completely protected μ MT mice against lethal infection for approximately 30 days. However, protection waned over time and depended on the initial dose of immune serum; 89% of μ MT mice that were administered 0.1 ml of immune serum survived for greater than 35 days, but only 11% survived after 50 days. In contrast, all μ MT mice that received 1.0 ml of immune serum were alive at 40 days, and 75% were alive after 60 days (Fig. 1A). In congenic *RAG1* mice that lacked both B and T cells, the duration of protection was shorter. Although 100% of *RAG1* mice that were administered 1.0 ml of immune serum were alive at 30 days, all mice succumbed to WNV infection within 52 days of the initial infection (Fig. 1B). Although infectious virus was recovered when immunocompromised mice became morbid, no virus was recovered at 35 days after infection, when mice appeared healthy (data not shown). In contrast, immunocompetent wild-type C57BL/6 mice that received immune serum did not develop WNV-associated disease even months after the initial infection (data not shown) (17). Although neutralizing antibodies effectively controlled WNV infection, in the absence of a functional B- and T-cell response, virus infection was not completely eradicated.

Prophylaxis studies with gamma globulin. Passive transfer of immune serum against WNV provided long-lasting protection against infection in wild-type and immunodeficient mice. To confirm that antibodies mediated this protection and explore the possibility for antibody therapy against WNV, we evaluated the efficacy of purified immune human gamma globulin against WNV infection in mice. Human gamma globulin with immunoreactivity against WNV was obtained from donors in Israel. Over the past several years, a series of WNV epidemics have occurred in Israel, so that 10 to 20% of the population has antibodies against WNV (61).

For this study, two immune human gamma globulin lots were used: G12101 and G24191 had significant in vitro immunoreactivity (ELISA titers of 1/900 against WNV antigen) and neutralizing (PRNT₅₀ of 1/50 to 1/100) potential. Nonimmune human gamma globulin was obtained from a region where WNV was nonendemic and lacked neutralizing activity or im-

FIG. 1. (A) Passive administration of mouse serum to μ MT mice. Serum was collected from immune (day 28 postinfection; titer $\approx 1/10,000$) wild-type mice and pooled. After heat inactivation, the indicated amounts of serum were administered in a divided dose to μ MT mice 1 day prior to and after infection with 10^2 PFU of WNV. Data reflect between 5 and 10 mice per condition. (B) Passive administration of mouse serum to *RAG1* mice. Immune serum (1 ml) or PBS was administered in a divided dose to *RAG1* mice 1 day prior to and after infection with 10^2 PFU of WNV. Data reflect between 5 and 10 mice per condition.

munoreactivity. In vivo studies with human gamma globulin were performed in 8-week-old wild-type and μ MT B-cell-deficient C57BL/6J mice (Fig. 2). Administration of a single dose of 0.2 mg (10 mg/kg) or greater of immune gamma globulin completely protected wild-type mice against infection with 10^2 PFU of WNV (Fig. 2A). Doses of 0.02 mg (1.0 mg/kg) and 0.002 mg (0.1 mg/kg) were less effective. In contrast, administration of nonimmune gamma globulin did not protect against WNV infection. Because subneutralizing concentrations of antibodies can facilitate enhanced WNV infection in myeloid cells (10, 21, 22), we investigated the effect of very low doses of immune gamma globulin on WNV infection in mice. Notably, treatment of wild-type mice with the lowest dose (0.0002 mg or less) did not induce excess mortality.

In general, passive transfer of immune gamma globulin to μ MT mice provided significant yet lower levels of protection compared to wild-type mice (Fig. 2B). Although doses greater than 0.2 mg increased the average survival time of μ MT mice after infection, only those that received 10 mg (500 mg/kg) survived beyond 30 days. This was even more apparent with *RAG1* mice. Although survival was prolonged, even a high dose of immune gamma globulin (10 mg) did not protect against WNV-induced mortality for greater than 20 days (data not shown). Overall, these results were similar to that obtained with immune serum (Fig. 1) and confirmed that antibodies

against WNV were necessary but not sufficient to eliminate WNV infection in vivo.

To better understand the mechanism of inhibition by antibody, we evaluated the effects of immune and nonimmune human gamma globulin on the viral load in serum and the CNS during the early stages after infection of μ MT mice.

(i) **Viremia.** The administration of a single dose (10 mg) of immune gamma globulin to μ MT mice completely abolished viremia after subcutaneous inoculation with 10^2 PFU of WNV (Fig. 3A): at days 2, 4, 6, and 8 after infection, neither infectious virus nor viral RNA (data not shown) was detected in the serum of μ MT mice. In contrast, in μ MT mice that were administered a single dose of nonimmune gamma globulin, after day 2, $\approx 10^2$ to 10^4 PFU of infectious virus per ml was recovered from serum (Fig. 3A).

(ii) **CNS.** Passive transfer of 10 mg of immune gamma globulin had more variable effects on viral burden in the brain and spinal cord of μ MT mice (Fig. 3B and C). Although most mice demonstrated no detectable infectious virus in the CNS within the first 6 days of infection, by day 8 a subset of mice had 10^4 to 10^5 PFU of infectious virus per g in the brain and spinal cord. In contrast, by day 8 after infection, μ MT mice that received nonimmune gamma globulin uniformly had high levels (10^6 to 10^9 PFU/g) of infectious virus at several independent sites in the CNS. Thus, although prophylaxis with immune

FIG. 2. Prophylaxis studies with human gamma globulin. (A) Passive administration of human immune gamma globulin to 8-week-old wild-type mice. The indicated amounts of purified immune gamma globulin (lot G12101) were administered as a single dose via an intraperitoneal route immediately prior to administration of 10^2 PFU of WNV via footpad inoculation. Data reflect at least 20 mice per condition. Statistical differences compared to the PBS control were as follows: 0.2 μ g, 2 μ g, and 20 μ g, $P > 0.3$; 200 μ g, 1,000 μ g, 5,000 μ g, and 10,000 μ g, $P \leq 0.0002$. (B) Passive administration of human immune gamma globulin to 8-week-old μ MT mice. The indicated amounts of purified immune gamma globulin (lot G12101) were administered as a single dose via an intraperitoneal route immediately prior to administration of 10^2 PFU of WNV via footpad inoculation. Statistical differences compared to the PBS control were as follows: immune IgG: 200 μ g, $P > 0.3$; 1,000 μ g, $P = 0.0002$; 5,000 μ g, $P = 0.009$; 10,000 μ g, $P < 0.0001$; and nonimmune IgG: 10,000 μ g, $P > 0.7$.

gamma globulin appeared to completely block WNV viremia in μ MT mice, it did not prevent dissemination to the CNS in a subset of mice.

Therapeutic studies with gamma globulin. Because of the lack of specific treatment against WNV infection, we evaluated the therapeutic potential of immune gamma globulin. Mice were inoculated with 10^2 PFU of WNV at day 0 and then administered a single dose (15 mg; 750 mg/kg) of immune or nonimmune gamma globulin at a particular day after infection and followed clinically. Initial studies were performed with the immunodeficient μ MT mice. As expected, nonimmune gamma globulin demonstrated no clinical improvement compared to the PBS controls: all mice succumbed to infection (Fig. 4A). Immune gamma globulin, by contrast, had a modest therapeutic effect. μ MT mice treated at day 1 or 2 after infection had an 80 and 20% survival rate, respectively. Treatment of μ MT mice with immune gamma globulin after day 2 had no significant effect on mortality or average survival time (Fig. 4B).

Preliminary therapeutic studies were also performed in 8-week-old wild-type C57BL/6J mice. Because the survival rate after subcutaneous inoculation with WNV was $\approx 70\%$ (17),

although immune gamma globulin therapy (10 mg) at days 1, 2, and 3 after infection improved survival rates, the absolute benefit was small (data not shown), and large numbers of mice would be required to attain statistical power. Instead, therapeutic trials with gamma globulin were conducted with 5-week-old wild-type mice; because only 13% of these mice survived infection with WNV in the absence of therapy (Fig. 5A), the possibility for mortality benefit was greater. Treatment of 5-week-old mice with PBS or nonimmune gamma globulin had no significant effect on average survival time or mortality (Fig. 5B). In contrast, treatment with immune gamma globulin 1, 2, 3, 4, or 5 days after infection increased the average survival time and decreased mortality rates (Fig. 5C and Table 1).

Because therapy with immune gamma globulin provided a beneficial effect even 5 days after initial infection, we hypothesized that antibody limited disease even after WNV had spread to the CNS. To confirm this, the levels of infectious virus were measured in the brain of 5-week-old mice after infection with 10^2 PFU of WNV (Fig. 5D). When nonimmune gamma globulin was administered, 33 and 100% of 5-week-old mice developed measurable viral burdens in the brain at days

FIG. 3. WNV burden in serum and the CNS in μ MT mice after treatment with immune or nonimmune human gamma globulin. (A) Infectious virus levels in peripheral serum. μ MT mice were treated immediately prior to infection with 10 mg of immune (lot G24191) or nonimmune (lot F43312) human gamma globulin. Serum was harvested at the indicated days, and virus levels were measured with a viral plaque assay in BHK21 cells. Data are shown as the average number of PFU per milliliter of serum and reflect at least three mice per time point. The dotted line represents the limit of sensitivity of the assay. (B) Infectious virus levels in the brain. Virus levels were determined in the brain after homogenization by plaque assay and normalized per gram of tissue. (C) Infectious virus levels in the spinal cord. Virus levels were determined from the spinal cord as described above.

4 and 5 postinfection, respectively. In contrast, if mice were treated with immune gamma globulin, no virus was detected in the brain at days 4 and 5 after infection. Collectively, these data indicate that passive transfer of immune antibody improved clinical outcome even after WNV had disseminated into the CNS.

DISCUSSION

The efficacy of pre- and postexposure protection by homologous and heterologous polyclonal antibodies against WNV was evaluated in immunocompetent and immunocompromised mice. These experiments were undertaken because of the lack of available therapy against WNV and because recent human case reports suggest that gamma globulin administration may improve clinical outcome (30, 61). We found that passive administration of immune human gamma globulin or mouse serum prior to WNV infection protected wild-type, μ MT, and

RAG1 mice from morbidity and mortality. Although immune antibody completely prevented morbidity in wild-type mice, its effect was not durable in mice that lacked T and B cells, as they ultimately succumbed to infection at delayed times. In postexposure therapeutic studies, heterologous human gamma globulin partially protected mice against WNV-induced mortality. For μ MT mice, therapy had to be initiated within 2 days of infection to gain a survival benefit. In contrast, for wild-type mice, therapy that was initiated even 5 days after infection reduced mortality; this time point is significant because between days 4 and 5 WNV was produced in the CNS.

Preexposure prophylaxis with homologous or heterologous antibodies protected wild-type mice against WNV-induced morbidity and mortality. The degree of protection was associated with the amount of antibody transferred and no mortality was observed above a dose of 10 mg/kg. These results are consistent with other studies that demonstrate protection against flaviviruses after passive acquisition of antibodies (8, 9,

FIG. 4. Therapeutic studies with human gamma globulin in 8-week-old μ MT mice. A single 15-mg dose of purified nonimmune (panel A, lot F43312) or immune (panel B, lot G24191) gamma globulin was administered via an intraperitoneal route immediately prior to (day 0) or at the indicated day after (day 1, 2, or 3) administration of 10^2 PFU of WNV via footpad inoculation. Data reflect between 5 and 10 mice per condition. Statistical differences compared to the PBS control were as follows: immune IgG: day 0, $P < 0.005$; day 1, $P < 0.005$; day 2, $P = 0.06$, day 3, $P = 0.12$; and nonimmune IgG: days 0, 1, 2, and 3, $P > 0.4$.

12, 34, 35, 40, 52, 56, 59). Notably, immune enhancement was not observed after passive transfer of low doses of immune gamma globulin; even when 200 ng (10 μ g/kg) of antibody was administered, there was no evidence of increased lethality or reduced average survival time. This dose is significant because it readily facilitated ADE of the U937 myeloid cell line in cell culture (M. S. Diamond, unpublished observations). Thus, as has been observed for tick-borne encephalitis virus (35), ADE in cell culture did not necessarily predict clinical outcome in vivo.

The prophylaxis experiments with immunocompromised mice also provide insight into the pathogenesis of WNV infection. Although passive transfer of high-titer immune serum against WNV prior to infection delayed the onset of disease, most μ MT and *RAG1* mice ultimately succumbed to WNV infection. Thus, antibody by itself was not sufficient to prevent and/or eliminate WNV infection from all cellular compartments; an intact cellular immune response was required for viral eradication. These results are consistent with our recent experiments that demonstrate that mice that lack CD8⁺ T cells have excess viral burden in the CNS and increased mortality (B. Shrestha and M. S. Diamond, manuscript in preparation). These studies also agree with experiments with a neuroadapted Sindbis alphavirus in which passive administration of immune

serum abolished viral replication in SCID mice; however, when these antibody titers declined infectious virus was again produced (25, 37, 39). Thus, antibody-mediated protection against WNV likely occurs through a mechanism that does not cause lysis of infected cells (25, 38, 39).

μ MT mice that received nonimmune antibody prior to infection developed a sustained viremia within 4 days of infection, results that agree with our published studies (17). In contrast, after the acquisition of immune gamma globulin, at the limits of detection of our assays (1 PFU/ml by fluorogenic reverse transcription-PCR, 20 PFU/ml by plaque assay) viremia was never observed. Nonetheless, within 6 days of infection, a subset of μ MT mice that received immune gamma globulin had evidence of infectious virus in the brain and spinal cord. These data suggest that antibody alone efficiently blocked the hematogenous phase of viral infection but was less effective at controlling spread to the CNS. It is possible that some WNV enters the CNS by a nonhematogenous route, through a process that is not accessible to antibody-mediated inhibition, such as retrograde axonal transport from infected peripheral neurons (2, 42). Alternatively, very low levels of WNV, which were below the limit of detection by fluorogenic reverse transcription-PCR, may be sufficient for CNS dissemination in immunocompromised mice.

FIG. 5. Therapeutic studies with human gamma globulin in 5-week-old wild-type mice. (A) Survival curve of 5-week-old wild-type mice. Mice were inoculated via footpad with diluent (mock) or 10^2 PFU of WNV. The survival curves were constructed with data from two independent experiments. The number of animals for each viral dose ranged from 6 (mock) to 23 (10^2 PFU). The difference in survival curves was statistically significant: $P < 0.001$. (B) A single 15-mg dose of purified nonimmune (lot F43312) gamma globulin was administered via an intraperitoneal route to 5-week-old wild-type mice immediately prior to (day 0) or at the indicated day after (day 1, 2, 3, 4, or 5) administration of 10^2 PFU of WNV via footpad inoculation. Data reflect approximately 20 mice per condition. Significance values for mortality and average survival times are indicated in Table 1. (C) A single dose of 15 mg of purified immune (lot G24191) gamma globulin was administered to 5-week-old wild-type mice via an intraperitoneal route immediately prior to (day 0) or at the indicated day after (day 1, 2, 3, 4, or 5) administration of 10^2 PFU of WNV via footpad inoculation. Data reflect approximately 20 mice per condition. Significance values for mortality and average survival times are indicated in Table 1. (D) WNV burden in the brain of 5-week-old wild-type mice. Five-week-old mice were treated with a single 15-mg dose of immune (lot G24191) or nonimmune (lot F43312) human gamma globulin immediately prior to infection with 10^2 PFU of WNV. At days 4 and 5 after infection, brains were harvested and viral burdens were determined by plaque assay after tissue homogenization. The data are expressed as PFU per gram. The following percentage of mice had viral burdens below detection (<20 PFU/g): immune IgG: day 4, 100%; day 5, 100%; nonimmune IgG: day 4, 66%; day 5, 0%.

Our studies with immunodeficient mice suggest that under certain conditions WNV can establish a state of persistence. Treatment of μ MT and *RAG1* mice with homologous high-titer immune serum facilitated long-term but not permanent protection against WNV. Given the lack of evidence for latency with flaviviruses, it is likely that in the presence of antibodies but absence of an adaptive cellular immune response, low-level WNV infection was ongoing. Persistence of WNV infection has been described in the brains of experimentally infected monkeys (49) and hamsters (66). Similar viral persistence has been reported in animals infected with Japanese and tick-borne encephalitis viruses (43, 60). In contrast, we did not observe WNV persistence in wild-type mice that survived infection; virus could not be cultivated from wild-type mouse

brain or spinal cord homogenates weeks or months after infection (B. Shrestha and M. S. Diamond, unpublished observations). Based on the observation of flavivirus persistence in brain tissues, neurons may act as reservoirs for persistent infection. Alternatively, other cells in the CNS (e.g., microglia, astrocytes, or oligodendrocytes) could sustain low-level WNV replication and facilitate dissemination in immunocompromised mice as antibody titers waned.

Therapeutic trials in 5-week-old wild-type mice demonstrated that heterologous and relatively low-titer immune gamma globulin against WNV significantly depressed mortality after infection. As expected, greater intervals between infection and treatment were associated with decreased clinical benefit. Nonetheless, even when antibody was administered 4

TABLE 1. Results of therapeutic trials with human gamma globulin^a

Day postinfection	Human IgG treatment	Avg survival time in days (<i>P</i>)	% Survival (<i>P</i>)	No. of mice
0	None	12.1 ± 0.7	15.1	33
	Nonimmune	11.4 ± 0.7 (0.8)	10.0 (0.7)	20
	Immune	19.3 ± 0.9 (0.0001**)	83.3 (0.0001**)	18
1	Nonimmune	11.6 ± 0.8 (0.9)	14.2 (0.7)	19
	Immune	19.6 ± 0.8 (0.0001**)	84.2 (0.0001**)	19
2	Nonimmune	11.4 ± 1.0 (0.13)	20.0 (0.5)	20
	Immune	18.8 ± 0.9 (0.0001**)	73.7 (0.0001**)	20
3	Nonimmune	11.1 ± 0.8 (0.15)	5.0 (0.06)	20
	Immune	17.2 ± 1.2 (0.008**)	60.0 (0.002**)	20
4	Nonimmune	10.9 ± 0.7 (0.2)	6.0 (0.2)	18
	Immune	15.1 ± 1.0 (0.01**)	34.6 (0.02**)	19
5	Nonimmune	11.9 ± 0.9 (0.6)	15.0 (0.7)	20
	Immune	15.5 ± 1.3 (0.08)	50.0 (0.04**)	20

^a The endpoint of the study was 21 days after initial infection. For average survival times, *P* values were calculated with a two-tailed Mann-Whitney test. For survival analyses, *P* values were calculated with a log-rank test. *P* values were compared to those for the PBS-saline control. Asterisks indicate statistical significance.

to 5 days after subcutaneous infection, a 20 to 35% improvement in mortality rate and average survival time was observed. Clinical improvement after day 5 administration of antibody was significant because virologic analysis indicated that WNV disseminated to the brain of 5-week-old mice as early as day 4 but uniformly by day 5 after infection. Thus, passive transfer of immune antibody improved clinical outcome even after WNV had spread into the CNS; these results agree with prior studies with yellow fever, St. Louis encephalitis, and Sindbis viruses (8, 26, 39, 52). In contrast, protection against tick-borne encephalitis with antibodies was possible only before infection of the brain was established (35). Clearly, the issue of treatment after dissemination to the CNS is complex; the results may be influenced by additional factors including the age and genetic background of the mice, the route of inoculation, and the relative titers of the antibodies.

The lack of available therapy and the expanding WNV epidemic necessitate the evaluation of agents that inhibit infection and can be rapidly transferred into the clinical setting. Polyclonal antibody preparations have been used sparingly against flaviviruses because of concerns about ADE (27, 41). In the present study, clinically significant immune enhancement was not observed. Although antibody therapy against WNV could be complicated in regions where heterologous flaviviruses cocirculate, because homologous IgG is cleared with a half-life of approximately 20 days (3), this risk is temporary. In wild-type mice, prophylaxis was effective across a broad range of doses. For immunocompromised mice, higher doses of antibody were required and even then, protection was incomplete. In theory, sustained protection could be generated in the immunocompromised with repeated dosing regimens. In post-exposure therapeutic trials in mice, gamma globulin therapy decreased mortality rates, results that agree with those recently reported by another group (7). Clearly, trials in primates and/or humans are the next step to confirm the efficacy and safety of passive antibody protection and treatment against WNV infection (1).

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