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Treatment of West Nile virus-infected mice with reactive immunoglobulin reduces fetal titers and increases dam survival

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Abstract

The objectives of this study were to determine if injection of West Nile virus (WNV) into timed-pregnant mice would result in fetal infection and if administration of WNV-reactive immunoglobulin would increase dam survival and reduce fetal viral titers. Dams injected on 7.5 days post-coitus (dpc) had detectable viral titers in the placenta 10.5 dpc with a mean titer of $10^{4.9}$ 50% cell-culture infectious doses per gram of tissue ($CCID_{50}$/g tissue). The mean placental titer increased to $10^{8.6}$ $CCID_{50}$/g tissue at 12.5 dpc. Infectious virus was detectable 12.5 dpc in 10 of 10 fetuses with a mean titer of $10^{7.5}$ $CCID_{50}$/g tissue. Treatment of dams (challenged with WNV on 7.5 dpc) with WNV-reactive human immunoglobulin (Ig) on 8.5 and 9.5 dpc resulted in a significant reduction of virus in fetuses as compared with non-reactive human Ig-treated females on 12.5 dpc ($P \leq 0.001$). Treatment also resulted in survival of dams to term. Treatment of dams with WNV-reactive human Ig on 12.5 and 13.5 dpc also resulted in reduction of viral titer on 14.5 dpc, indicating that later treatment may also be efficacious. This suggests that Ig treatment may be useful in treating fetal WNV infection in women.

Keywords: West Nile virus; Pregnancy; Fetus; Placenta; Immunoglobulin therapy; Flavivirus

1. Background

West Nile Virus (WNV) has spread across the United States after an initial outbreak in New York. The outbreak has resulted in many deaths from WNV-associated disease, and efforts have been focused on diagnosis and prevention, although treatment is also important for individuals who contract the disease. West Nile virus has been implicated in intrauterine infection in human patients (Anonymous, 2002; Shi and Wong, 2003). One case of congenital chorioretinal scarring and central nervous system malformation was observed in a child born to a WNV-infected mother (Alpert et al., 2003). There were also 70 cases of women who contracted WNV illness during pregnancy in 2003 (Hayes and O'Leary, 2004). Other concerns with congenital flavivirus infections, especially those that cause brain pathology, are potential mental disorders associated with disease in babies born to infected mothers.

The placenta generally serves as an effective barrier to viruses and other infectious organisms (Zeichner and Plotkin, 1988), but this barrier is sometimes breeched during maternal infection. Viral infections of the placenta generally pass through the hematogenous route and are associated with maternal viremia (Zeichner and Plotkin, 1988). Hematogenous viral infection can occur when virus present in the maternal blood reaches the intervillous space, transfers across the placenta, and enters the fetal blood supply. There are multiple potential modes of transfer across the placenta, including direct and contiguous infection of the cell layers, passage through a breech in the placenta, or carriage by mobile cells (Kaplan, 1993). Examples of viruses crossing the placenta are human immunodeficiency virus, hepatitis B virus, hepatitis C virus, some herpesviruses, rubella virus, and parvovirus

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B19 (Koi et al., 2001). Alternatively, infection might occur at a point in gestation before the placenta has been formed (Kaplan, 1993).

Various flaviviruses are known to cause fetal infection. Japanese encephalitis virus (JEV) has been isolated from placental tissues, the brain, and the liver of aborted fetuses from JEV-infected mothers (Chaturvedi et al., 1980). Mouse models of intrauterine JEV infection have also been developed (Mathur et al., 1981; Mathur et al., 1982). Infection of JEV in pregnant mice results in spontaneous abortion and stillbirths (Mathur et al., 1981). Out of 24 infants born to mothers with $>10^6$/ml of the flavivirus-like GB-C virus, 23 (96%) were positive for the virus (Ohto et al., 2000). Infection with WNV, Banzi virus, and an unidentified virus related to Banzi (AR 5189) in sheep resulted in abortion, stillbirth, and neonatal death, characterized by congenital abnormalities of the brain (Barnard and Voges, 1986). St. Louis encephalitis virus (SLEV) also crossed the placenta of mice when injected intravenously on d 8 of gestation (Andersen and Hanson, 1975). In utero flaviviral infections are often associated with fetal and infant morbidity and mortality (Andersen and Hanson, 1975; Barnard and Voges, 1986; Mathur et al., 1981).

Anti-WNV therapies have been largely supportive in human patients, although some animal studies have suggested the possible use of antiviral agents. Anti-WNV antibodies (Ben-Nathan et al., 2003), and interferons (Morrey et al., 2004a) have been shown to be effective in reducing morbidity and mortality in animal models when administered before or early after viral challenge. Antibody treatment up to 5 days (d) post-viral challenge has been shown to be effective in reducing titers of mice infected with WNV (Engle and Diamond, 2003). The prevention and treatment of congenital WNV infections, however, have not been investigated.

Pregnant women will likely become infected with WNV in the future, so a model for in utero WNV infection would be useful in determining the mode of infection as well as treatment options to inhibit or eliminate WNV infection of the fetus. In this study, intrauterine infection was demonstrated in mice and hamsters infected subcutaneously with WNV, and the virus load could be reduced in fetal tissues by treating the dams with human WNV-reactive immunoglobulin up to 5 days after viral challenge.

2. Materials and methods

2.1. Animals

Timed-pregnant Swiss Webster mice were used in initial intrauterine infection studies. Because of their reproductive capacity, FVB/N mice were used in all other intrauterine infection studies and were obtained from Charles River Laboratories (Wilmington, MA). Animals were housed in the Biosafety Level 3 (BL-3) area of the AAALAC-accredited Laboratory Animal Research Center (LARC) at Utah State University (USU). Animals were also bred in-house and potential pregnancy was identified according to the presence of vaginal plugs, which indicated 0.5 days post-coitus (dpc). Animal use and care was in compliance with the USU Institutional Animal Care and Use Committee.

2.2. Virus

A New York WNV crow brain stock (NY, CDC 996625, V1 D3 11/10/1999) was grown on MA-104 cells and had a titer of $10^{6.75}$ $\log_{10}$ 50% cell-culture infectious doses ($CCID_{50}$). Virus was diluted in Minimal Essential Media (MEM) with no fetal bovine serum (FBS) for injection into timed-pregnant mice. Virus was administered subcutaneously (s.c.) at $10^{5.3}$ $CCID_{50}$/mouse.

2.3. Compounds

WNV-reactive and non-reactive human immunoglobulins (Ig) (OMRIX Biopharmaceuticals, Israel) were obtained as aqueous suspensions (50 mg/ml) and will be referred to human Ig and non-reactive human Ig, respectively. The neutralizing antibody titers for the Igs were: human Ig G24191: ELISA, 1/900; PRNT 1/100; non-reactive human Ig: ELISA, <1/10; PRNT, <1/10 (Engle and Diamond, 2003). WNV-reactive horse IgM, referred to in the text as horse IgM, was purified from serum taken 9 days after immunization with 1 ml of Fort Dodge WNV horse vaccine (Fort Dodge Animal Health, Overland Park, KS). The horse IgM was isolated by ammonium sulfate precipitation and purified by dialysis and Sephacryl 300 size-exclusion chromatography. The horse IgM was then concentrated using a Pellicon XL 50 filtration device (Millipore, Billerica, MA). The human Igs were administered via intraperitoneal (i.p.) injection at 1 g/kg in a volume of 0.5 ml on 1 and 2 days post-maternal viral challenge. A second experiment was conducted with administration of antibody on 5 and 6 days post-maternal viral challenge. The horse IgM was administered in a volume of 0.1 ml, i.p. on 1 and 2 days post-maternal challenge.

2.4. Infectious cell-culture assay

The virus titers in tissues or plasma were assayed using an infectious cell-culture assay (Morrey et al., 2002) where a specific volume of either tissue homogenate or plasma was added to the first tube of a series of dilution tubes. Serial dilutions were made and added to Vero 76 cells. Six to seven days later cytopathic effect (CPE) was used to identify the endpoint of infection (Sidwell and Huffman, 1971). Four replicates were used to calculate the infectious doses per milliliter of plasma or gram of tissue (Reed and Muench, 1938).

2.5. Quantitative real-time (RT)-PCR (QRT-PCR)

Primer-pairs (forward TCAGCGATCTCTCCACCAAAG, reverse GGGTCAGCACGTTTGTCCATTG) and Taqman probe (5′-6-carboxyfluorescein-TGCCCGACCATG-

GGAGAAGCTC-6-carboxy-*N*,*N*,*N*′,*N*′-tetramethylrhodamine-3′) specific for the envelope protein of WNV (Shi et al., 2001) were used (Qiagen, Valencia, CA). The one-step Brilliant QRT-PCR kit (Stratagene, La Jolla, CA) was used for RT and amplification of WNV RNA with primers and probe at 1.0 and 0.2 μM, respectively. One microliter of total cellular RNA (from a total of 50 μl), extracted from infected or control tissues was used. Samples were run on a DNA Engine Opticon 2 (MJ Research Inc., Waltham, MA). Reverse transcription of cellular RNA was performed for 30 min at 50 °C followed by PCR, which consisted of 40 cycles of 15 s at 95 °C and 60 s at 61 °C. Results are given in terms of genome equivalents (ge), reflecting the amount of WNV genomic copies present in the sample as extrapolated from a standard curve obtained from amplification of a known amount of positive control WNV RNA.

2.6. Experimental design

Timed-pregnant female FVB/N or Swiss Webster mice were randomly assigned to groups and challenged with WNV on 7.5 or 11.5 dpc. Mice were euthanized and the uteruses were removed and washed in a 70% ethanol/Betadyne™ mixture and rinsed in physiologic saline. The conceptus was then removed and washed as above. The placentas (after 10.5 dpc) or primitive placentas (before 10.5 dpc) were removed from the fetal membranes and washed again as above. The fetuses were removed from the amniotic sac and washed as above. Five fetuses and placentas were taken from each dam if available. Brain, kidney, or spleen samples were taken from the dam to detect maternal infection. Tissues were homogenized in 1 ml of MEM 2% fetal bovine serum for culture. In antiviral experiments, timed-pregnant females were challenged with WNV 7.5 dpc, and treated either 8.5 and 9.5 dpc or 12.5 and 13.5 dpc. Tissues samples were taken and processed as above on 13.5 dpc for 8.5 and 9.5 dpc treatment or on 13.5 dpc for 12.5 and 13.5 dpc treatment. QRT-PCR was used to verify infectious cell-culture assay results. Some females treated with human Ig and horse IgM were followed to term to determine the effects of Ig treatment on the survival of dams to term. Student's *t*-tests were run on data to determine statistical significance between experimental and control samples. The computer program JMP (SAS Institute Inc.) was used to perform these analyses.

3. Results

3.1. Infection 11.5 days post-coitus (dpc)

Seventeen timed-pregnant females were injected with $10^{4.0}$ $CCID_{50}$ of WNV 11.5 days post-coitus, and samples were taken from two females at 14.5 dpc, from four females at 15.5 dpc, and from seven females on 16.5 dpc. Multiple (3–5) fetuses and placentas were taken from each female. Virus was detected in six of eight placentas starting 14.5 dpc with a mean titer in positive samples of $10^{6.1}$ $CCID_{50}$/g tissue, and titers increased on 16.5 dpc to a mean of $10^{6.9}$ $CCID_{50}$/g tissue with virus present in all placentas (Table 1). Three out of 28 fetuses had virus titers just above the limit of detection 16.5 dpc with a mean titer of $10^{5.8}$ $CCID_{50}$/g tissue. Infected females had brain and kidney titers of $10^{6.8}$ and $10^{5.6}$ $CCID_{50}$/g tissue 16.5 dpc, respectively. Virus was present at higher titer in the placentas of infected dams than in the brain and kidney on 14.5 and 16.5 dpc.

Two dams infected on 11.5 dpc delivered pups prematurely on d 17.5 of gestation (6 days post-viral challenge), some of which survived to weanling age when raised by surrogate uninfected dams. The infected dams died within hours to days of giving birth. Some pups were stillborn or died shortly after birth (data not shown). Two other dams died before delivery. Another group of eight timed-pregnant females were infected as above to determine the morbidity and mortality of pups born to infected dams, but all females died before or during delivery. All of the pups that were delivered just before death of the females were stillborn.

3.2. Infection 7.5 dpc

To determine if the frequency of intrauterine infection and titer of fetal tissues would change if dams were injected with

Table 1
Average West Nile virus (WNV) titers in whole fetus, placenta, maternal brain, and maternal kidney at various time-points after infection

Tissue	Average WNV titer of positive samples (log_{10} $CCID_{50}$/g tissue)[a]					QRT-PCR (ge eq[b])
	Infected 11.5 days post-coitus (dpc)		Infected 7.5 days post-coitus (dpc)			
	14.5 dpc[c] (pos/total[d])	16.5 dpc (pos/total)	10.5 dpc (pos/total)	12.5 dpc (pos/total)	13.5 dpc (pos/total)	13.5 dpc (pos/total)
Fetus	<3.7 (0/8)	6.8 (3/28)	3.7 (1/8)	7.5 (10/10)	6.0 (24/31)	7.9(3/3)
Placenta	6.1 (6/8)	6.9 (28/28)	4.9 (7/8)	8.6 (10/10)	7.8 (31/31)	9.4(3/3)
Maternal brain	<3.6 (0/2)	6.8 (2/7)	<3.6 (0/2)	NT[e]	5.6 (2/4)	NT
Maternal kidney	4.2 (1/2)	5.6 (3/5)	5.1 (2/3)	6.8 (2/2)	<3.6 (0/5)	NT

[a] Average WNV viral titers determined by infectious cell-culture assay and expressed as log_{10} cell-culture infectious doses/gram tissue.
[b] Genome equivalents determined by extrapolation of standard curve obtained by quantitative reverse-transcriptase PCR (QRT-PCR) of control WNV RNA of known quantity.
[c] Days post-coitus.
[d] Tissue samples positive for WNV per total tissue samples tested.
[e] Not tested.

WNV earlier in gestation, 15 timed-pregnant FVB/N mice were injected with $10^{5.3}$ $CCID_{50}$ of WNV 7.5 dpc in three different experiments. Samples were taken from one female on 8.5 dpc, three females on 10.5 dpc, and five females on 13.5 dpc. Multiple (3–5) fetuses and placentas were taken from each dam in all experiments. Virus was detected in seven out of eight placentas by infectious cell-culture assay 10.5 dpc with an average titer of $10^{4.9}$ $CCID_{50}$/g tissue. On 13.5 dpc, virus was detected in 31 of 31 placentas and increased to an average titer of $10^{7.8}$ $CCID_{50}$/g tissue. WNV was detected in 24 of 31 fetuses 13.5 dpc with an average titer of $10^{6.0}$ $CCID_{50}$/g tissue (Table 1). Virus was also present in 10 out of 10 fetuses and placentas on 12.5 dpc with titers of $10^{7.5}$ and $10^{8.6}$ $CCID_{50}$/g tissue, respectively (Table 1). Viral titers were detected by QRT-PCR in fetal and placental RNA samples 13.5 dpc with average titers of $10^{7.9}$ and $10^{9.4}$ $CCID_{50}$/g tissue, respectively (Table 1).

3.3. Immunoglobulin treatment 1 and 2 days post-maternal challenge

To determine if treatment with human Ig, known to be effective in the treatment of mice (Ben-Nathan et al., 2003), would inhibit or prevent WNV passage from infected dam to fetuses, seven timed-pregnant female FVB/N mice injected with WNV 7.5 dpc were treated 8.5 and 9.5 dpc, five with human Ig and two with non-reactive human Ig placebo control. Four sham-infected females were treated with both immunoglobulin preparations as toxicity controls. No toxicity was apparent by fur ruffling, altered behavior, or mortality up to 13.5 dpc. Treatment with human Ig reduced fetal titers to an average of $10^{4.2}$ $CCID_{50}$/g tissue in 24 fetuses on 13.5 dpc as compared to an average titer of $10^{7.4}$ $CCID_{50}$/g tissue in 14 fetuses from mice treated with non-reactive human Ig, which was a 3-log reduction ($P \leq 0.001$). There was also a significant reduction in titers of placental samples of human Ig-treated females that had an average of $10^{5.2}$ $CCID_{50}$/g tissue from 24 placentas as compared with an average of $10^{7.9}$ $CCID_{50}$/g tissue from 14 placentas from non-reactive human Ig-treated mice ($P \leq 0.001$) (Fig. 1). Virus was also significantly reduced in maternal brain and spleen ($P \leq 0.001$ and $P \leq 0.01$, respectively). Viral infection was inhibited in human Ig-treated females, and treatment prevented detectable infection in 7 out of 19 fetuses.

A subsequent study was conducted to determine the effect of Ig treatment on the survival of dams to term, and survival of pups born to treated dams. Groups of pregnant females were injected with $10^{5.3}$ $CCID_{50}$ on 7.5 dpc, and treated on 8.5 and 9.5 dpc with human Ig and non-reactive human Ig as above. Horse IgM was isolated from serum obtained 9 days after inoculation with WNV vaccine. Dams were treated with horse IgM to determine the effects on dam and fetal survival. Infected and uninfected untreated controls were included for comparison. One dam from each Ig treatment group was necropsied on 13.5 dpc to obtain samples for infectious cell-culture assay. Fetal and placental titers were not detected in dams treated with human Ig or horse IgM, but virus was detected in fetus and placenta samples from non-reactive human Ig-treated dams. All untreated, infected females died by 14.5 dpc. One of the four females treated with non-reactive human Ig survived to term, but died shortly after giving birth to three stillborn pups. Three out of four dams treated with human Ig and two out of four dams treated with horse IgM survived to term and beyond to give birth and raise pups normally (Table 2). Some stillborn pups were observed in litters of females treated with human Ig (Table 2). Pups born to dams treated with human Ig and horse IgM had some mortality in the first week after birth, which was not observed in uninfected controls (Table 2). Pups that died in the first week after birth had strongly positive WNV titers with averages

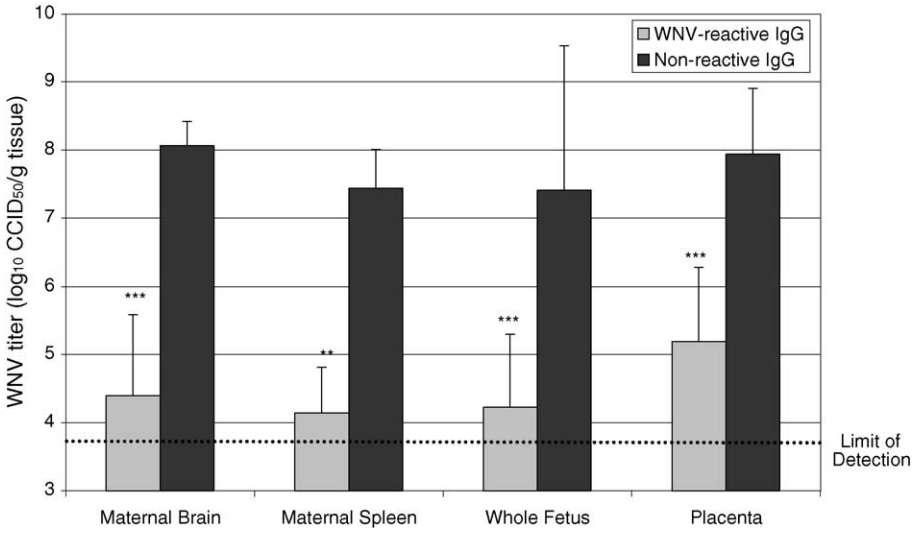

Fig. 1. West Nile virus titers of tissues from dams injected subcutaneously with WNV on 7.5 days post-coitus (dpc) and treated with human WNV-reactive Ig 8.5 and 9.5 dpc. Tissues were obtained 13.5 dpc ($^{**}P < 0.01$, $^{***}P < 0.001$).

Table 2
Survival and birthrate of dams injected with West Nile virus (WNV) on 7.5 days post-coitus (dpc) and treated on 8.5 and 9.5 dpc with WNV-reactive human Ig, non-reactive human Ig, or WNV-reactive horse IgM as compared to survival and birthrate of untreated controls and uninfected controls. Pup survival is also shown for the first week after birth

Treatment	Surviving dams/total dams treated	Total pups born	Pups stillborn	Pups dead 1 week after birth	Pups surviving beyond 1 week[a]
WNV-reactive human Ig	3/4	24	3	7	14
WNV-reactive horse IgM	2/4	17	0	3	14
Non-reactive human Ig	0/4	3	3	N/A[b]	N/A
Uninfected control	4/4	36	0	0	36
Untreated infected control	0/6	0	N/A	N/A	N/A

[a] Total pups alive at one week after birth.
[b] Not applicable, no live pups were born.

of $10^{10.1}$ and $10^{9.0}$ CCID50/g in human Ig-treated and horse IgM-treated dams, respectively.

3.4. Immunoglobulin treatment 5 and 6 days post-maternal challenge

Seven timed-pregnant females injected with WNV 7.5 dpc were treated 12.5 and 13.5 dpc, three with human Ig and four with non-reactive human Ig to determine if treatment at later stages of maternal infection can reduce fetal titer. At this stage of infection, the virus is entering the brain and anti-WNV antibodies have nearly cleared viremia (Morrey et al., 2004b). Treatment on 12.5 dpc also coincides with viral infection of the fetus (Table 1). Two out of three human Ig-treated dams had fetal viral titers substantially below the titers of fetuses of the four dams treated with the non-reactive human Ig (Fig. 2). Mean virus titer in fetuses from two females treated with human Ig was $10^{3.7}$ $CCID_{50}$/g tissue. In these two females, virus was not detected in 7 out of 10 fetuses. The non-reactive human Ig-treated animals had a mean fetal titer of $10^{7.8}$ $CCID_{50}$/g tissue, and 16 of 16 fetuses had detectable virus. The reduction of fetal viral titer in the two females treated with human Ig was verified by QRT-PCR. Of those animals that had reduced infectious cell-culture titers, a 2-log reduction in fetal WNV-titer was observed by QRT-PCR from an average of $10^{8.3}$ genome equivalents in placebo-treated animals to $10^{6.3}$ ge in human Ig-treated animals.

4. Discussion

These experiments showed that WNV infection of the dam can result in fetal infection in mice. This was not wholly unexpected in light of other mouse models of flavivirus intrauterine infection (Aaskov et al., 1981; Andersen and Hanson, 1975; Mathur et al., 1981). It was observed that females challenged with WNV on 7.5 dpc had a higher rate

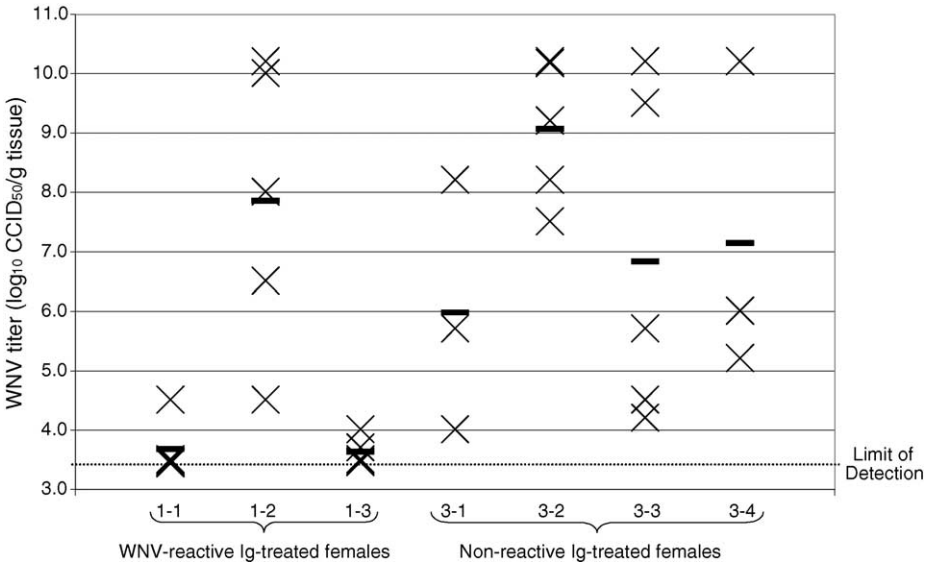

Fig. 2. Fetal viral titers from females infected 7.5 days post-coitus (dpc) and treated with WNV-reactive or non-reactive human Ig on 12.5 and 13.5 dpc. Individual fetal titers for each female are shown with an X and mean viral titers are represented with a – for all fetuses from each female. Samples were taken on 14.5 dpc. Fetal titers from WNV-reactive Ig-treated dams were statistically different non-reactive Ig-treated controls ($P \leq 0.05$).

of viral transmission to the fetus than females injected on 11.5 dpc (Table 1). The high infection rate of fetuses with maternal challenge on 7.5 as compared with day 11.5 dpc may suggest that infection of the fetus with WNV may occur more readily at different stages of placental development. Viral infections of the fetus are heavily influenced by the time of gestation and the condition of the placenta (Kaplan, 1993). Infection of women with rubella during the first 12 weeks and the final weeks of gstation results in a fetal infection rate of 90–100%, while infection between 23 and 30 weeks of gestation results in 30% fetal infection rate (Freij et al., 1988). Infection of the placenta with cytomegalovirus will vary in disease severity depending on the gestational time of infection and villous state of the placenta (Garcia et al., 1989). Intrauterine infection with JEV in mice also occurred at a greater rate when dams were challenged earlier (d 5 or 8) in gestation as compared with later challenge (d 13) (Mathur et al., 1981). Therefore, the timing of infection in regard to gestation may be an important aspect to fetal infection with WNV in pregnant women.

The placenta may be involved in fetal infection, and some viruses are known to infect the fetus through the placenta by a hematogenous route (Kaplan, 1993). Virus was present 3 days after injection of the dam in the placenta at higher titers than those of the brain and kidney of the infected dam. This observed difference in titers between tissues could be due to high tropism of the virus for placental tissues. Secondary HCMV infection of guinea pigs may occur after initial clearance due to virus in the placenta that evaded the immune system (Goff et al., 1987). This secondary maternal viremia suggests that the tropism of the virus for the placenta allows the virus to persist and cause recurrent maternal infection. Another reason for WNV tropism for the placenta is supported by the observation that other RNA viruses downregulate the immune system in specific tissues (Lyles, 2000). The increased tropism of WNV for the placenta could also be due to a relatively high amount of receptors for the virus on the surface of the placental cells. Trophoblast cells lining the maternal blood space of the placenta may efficiently support entry and replication of WNV accounting for higher titers in that organ.

Pre-term delivery, spontaneous abortion, and post-partum death have been observed in mouse models of other flavivirus infections, including JEV, SLEV, and Murray Valley encephalitis virus (Aaskov et al., 1981; Andersen and Hanson, 1975; Mathur et al., 1981, 1982), which correlated with similar outcomes in WNV infection of timed-pregnant mice. In mice injected on 11.5 dpc, pre-term delivery on 17.5 dpc was observed. Some of these pre-term delivered pups were stillborn, or died shortly after birth while the majority of pups developed normally to weanling age. In a subsequent experiment, some mice infected on 11.5 dpc died shortly after birth, and all babies born to these females were stillborn (data not shown).

Human placentation is similar to rodent placentation in several ways. Rodents and humans both have interstitial implantation of the embryo. Placental attachment is discoidal in both rodent and man. The placenta of the guinea pig shows the most structural homology of all rodents with that of humans. The placenta of other rodents, including mice, hamsters, and rats, differ from that of the guinea pig in the number of trophoblast layers (Enders, 1965). Mice have been used as models of human placentation (Abram et al., 2003; Georgiades et al., 2002; Sapin et al., 2001). Since guinea pigs have more structural similarity with human placentation and also have a longer gestation than mice, infection experiments were conducted with adult and weanling guinea pigs to determine if they could be used as models for WNV transplacental infection. Productive WNV infection could not be achieved in the guinea pig, even under immunosuppression with cobra venom factor (reduction of complement), cyclophosphamide, or cyclosporin. A transient viremia was observed in the serum 2 days post-viral injection and one guinea pig challenged with WNV by intracranial injection had virus present in the brain (data not shown). This could be partly due to possible genetic factors that may be present in guinea pigs, similar to the Flv (Oas1b) gene of WNV-resistant mouse strains (Perelygin et al., 2002). Because guinea pigs were not productively infected with WNV, mice were used in these experiments.

It was not unexpected that treatment of WNV early in the course of disease with Ig would result in a reduction in fetal viral titers. Neutralization of virus in the maternal blood would likely result in less virus exposure of the placenta. Despite the possible utility of maternal antibody administration, there may be a risk of maternal or fetal drug toxicity. Consequently, therapies for intrauterine infections in human patients are generally administered in the final weeks of pregnancy, which is aimed primarily at reduction of maternal viremia to lower the incidence of passage at parturition (Money, 2003; Sheffield et al., 2003). The potential problem is that treatment just before parturition may be too late to affect some intrauterine viral infections. In regards to intrauterine WNV infection in mice, antibody treatment of dams as late as 5 days after viral challenge reduced fetal titers in only some of the treated females, but not in all. Treatment with Ig as early as one day after viral challenge reduced viral titers in fetuses of all WNV-reactive Ig-treated animals and also resulted in some females surviving to deliver and raise pups. It is unknown if circulating levels of maternal antibody or administered Ig correlated with fetal titer reduction. Virus was able to rebound after cessation of treatment as observed with no detectable virus in fetuses 4 days after cessation of treatment as compared to high viral titers in pups that died during the first week after birth (14–21 days after treatment). Clearance of reactive Ig may be a reason for the rebound of virus, but we did not monitor the level of Ig after treatment. Neonatal mortality might be reduced by modifying the treatment regimen or by treating of the pups shortly after birth. If fetal morbidity and mortality from WNV infection does occur in human patients, these data suggest that reactive Ig might be used for treatment.

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