Available online at www.sciencedirect.com
SCIENCE DIRECT
Virology 347 (2006) 175 – 182
VIROLOGY
www.elsevier.com/locate/yviro

West Nile virus infection of the placenta

Justin G. Julander [a], Quinton A. Winger [b], Lee F. Rickords [b], Pei-Yong Shi [c], Mark Tilgner [c], Iwona Binduga-Gajewska [c], Robert W. Sidwell [a], John D. Morrey [a,*]

[a] The Institute for Antiviral Research, Utah State University, Logan, UT 84322, USA
[b] Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, UT 84322, USA
[c] Wadsworth Center, New York State Department of Health, Albany, NY 12208, USA

Received 23 September 2005; returned to author for revision 17 October 2005; accepted 19 November 2005
Available online 9 January 2006

Abstract

Intrauterine infection of fetuses with West Nile virus (WNV) has been implicated in cases of women infected during pregnancy. Infection of timed-pregnant mice on 5.5, 7.5, and 9.5 days post-coitus (dpc) resulted in fetal infection. Infection of dams on 11.5 and 14.5 dpc resulted in little and no fetal infection, respectively. Pre-implantation embryos in culture were also infected with WNV after the blastocyst stage and the formation of trophectoderm. Green fluorescent protein (GFP) expression was observed in a trophoblast stem (TS) cell line after infection with a GFP-expressing WNV construct. However, no fluorescence was observed in differentiated trophoblast giant cell (TGC) cultures. GFP fluorescence was present in TGC cultures if infected TS cells were induced to differentiate. These results suggest that embryos are susceptible to WNV infection after the formation of the trophectoderm around 3.5 dpc through the formation of the functional placenta around 10.5 dpc.

Keywords: West Nile virus; Placenta; Trophoblast; Intrauterine; Vertical transmission; Pregnancy

Introduction

West Nile virus (WNV) causes disease in man, including encephalitis, paralysis, and death (Anderson et al., 2004). WNV may also infect horses, dogs, cats, and alpacas, as well as other species such as alligators (Abutarbush et al., 2004; Austgen et al., 2004; Kutzler et al., 2004; Miller et al., 2003; Yaeger et al., 2004). The primary vector for human transmission is the mosquito, however, other modes of infection have been observed (Sardelis et al., 2001; Turell et al., 2001). Virus has been transferred in human patients by blood and organ transplantation, as well as by accidental laboratory infection (Laboratory-acquired West Nile Virus, 2002; Macedo de Oliveira et al., 2004; Wadei et al., 2004). Some animal species have become infected after ingestion of infected materials or contact, such as feather picking or grooming, with infected individuals (Banet-Noach et al., 2003; Miller et al., 2003; Odelola and Oduye, 1977).

Intrauterine infection of fetuses with WNV has been implicated (Intrauterine West Nile virus, 2002), but other reports of maternal infection with WNV during pregnancy have shown no evidence for morbidity of the fetus (Bruno et al., 2004). Many other WNV cases of maternal infection during pregnancy are under investigation (Interim Guidelines, 2004). A woman infected with WNV during pregnancy gave birth to a WNV-seropositive baby with chorioretinal scarring and some brain abnormalities that may have been due to maternal infection with WNV during the second trimester of gestation (Alpert et al., 2003). Fetal viral infections are generally transmitted from maternal viremia across the placenta to fetal circulation, so an understanding of viral interactions with the placenta is important (Kaplan, 1993).

Infection of mouse fetuses was recently demonstrated in our laboratory (Julander et al., 2005). In that study, dams infected with WNV 7.5 days post-coitus (dpc) had a high rate of passage of maternal virus to fetuses as compared to low frequency of fetal infection when dams were infected 11.5 dpc.

* Corresponding author. Mailing address: Biotechnology Center 305, Utah State University, 4700 Old Main Hill, Logan, UT 84322-4700, USA. Fax: +1 435 797-2766.
E-mail address: jmorrey@cc.usu.edu (J.D. Morrey).

0042-6822/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2005.11.040

The placenta had elevated viral titer compared to other maternal organs regardless of the gestational time point of infection. Dams had high mortality and generally died prior to, or during, delivery unless treated with WNV-specific immunoglobulin. Immunoglobulin treatment allowed dams to conceive and raise pups.

Placental development is a dynamic process involving the interaction between invasive fetal-derived trophoblast cells and maternal decidual cells of the uterus (Fazleabas et al., 2004). At the blastocyst stage (3.5 dpc), just prior to implantation, surface cells of the embryo will differentiate into trophectodermal cells that will eventually give rise to the placenta and other extraembryonic structures (Cross et al., 1994). Trophoblast cells invade the maternal decidua during development and establish an interface between maternal and fetal blood for the transfer of nutrients to the developing fetus. The placental barrier between maternal and fetal blood is established in mice around 10.5 dpc and consists of one layer of mononuclear trophoblast cells (cytotrophoblast) and two layers of differentiated syncytial trophoblast (syncytiotrophoblast) (Georgiades et al., 2002). The placental barrier functions to allow selective transfer of nutrients and to inhibit the transfer of harmful materials, but this barrier may be breached by different chemicals or microorganisms (Koi et al., 2001a, 2001b).

A trophoblast stem (TS) cell line has been established by culturing blastocysts or early post-implantation trophoblasts in media containing fetal growth factor-4 (FGF-4), haprin, and fibroblast conditioned media (Tanaka et al., 1998). Upon removal of these components, the TS cells differentiate into other trophoblast cell types including trophoblast giant cells. The TS cell line serves as a model for the replicative and differentiated trophoblast cells of the placenta.

An understanding of the mechanism of WNV intrauterine infection may be important for preventing clinical cases as well as for the development of therapies to reduce fetal disease and associated symptoms. The objectives of this study were to delineate the timing of viral passage from infected dam to fetus and to identify the placental cell types susceptible to viral infection in vitro.

Results

Timing of fetal infection

To determine the gestational timing of fetal infection with WNV, timed-pregnant dams were challenged with WNV on 5.5, 7.5, 9.5, 11.5, and 14.5 dpc. Whole fetus, placenta, and maternal brain, kidney, and spleen were titered for WNV by infectious cell culture assay (Table 1). Virus was present in fetuses 6 days post-maternal challenge when dams were challenged on 5.5, 7.5, and 9.5 dpc. Fetuses from dams challenged 9.5 dpc had higher WNV titers than fetuses from dams challenged 7.5 dpc (Table 1). Little or no virus was present in fetuses from dams challenged 11.5 or 14.5 dpc. High WNV titers were present in the placenta regardless of gestational state at the time of infection. Maternal tissues had some detectable virus, but titers in maternal organs were much lower than titers in fetuses and placentas.

Infection of pre-implantation embryos

Groups of embryos were infected 1.5 dpc or 3.5 dpc with WNV-GFP and fixed 2 or 4 days post-infection (dpi). M16 media, used for culturing embryos, supported development of the embryos to blastocyst stage, but not further. Around 10% of the embryos died in culture (data not shown), and the remaining 90% were observed for fluorescence. A representative embryo from each time point is shown (Fig. 1). When embryos were infected 1.5 dpc, fluorescence was detected in embryos 5.5 dpc (Fig. 1B), but not 3.5 dpc (A) or in sham-infected controls (C). If embryos were cultured for 2 days and then infected on 3.5 dpc, fluorescence was observed in embryos 2 days post-infection on 5.5 (D), respectively, but not in sham-infected embryos (E). The fluorescence appeared in the trophectoderm of the blastocyst stage embryo (B, D). The trophectoderm, including some stem cells, differentiates around 3.5 dpc, therefore, infection with WNV-GFP coincided with the formation of the trophectoderm.

Table 1
West Nile virus (WNV) titers recovered from tissues and fetuses from mice infected at various times during gestation

Infected[d]	Necropsy[f]	Mean[a] virus titer ± SD[b] in maternal tissue samples (pos/total[c])				Mean virus titer ± SD in fetal samples (pos/total)	
(dpc[e])	(dpc)	Brain	Kidney	Spleen	Uterus	Fetus	Placenta
5.5	11.5	7.0 ± 1.8 (3/6)	5.5 ± 0.9 (2/6)	5.6 ± 0.3 (6/6)	6.5 ± 0.8 (6/6)	5.0 ± 0.8 (21/24)	8.4 ± 1.0 (24/24)
7.5	13.5	7.7 ± 2.0 (2/4)	<3.6 ± 0 (0/4)	6.2 ± 1.4 (3/4)	N/T[g]	7.0 ± 2.1 (23/31)	7.8 ± 1.1 (31/31)
9.5	15.5	6.7 ± 2.8 (2/5)	5.5 ± 0.4 (2/5)	6.6 ± 0.3 (5/5)	6.9 ± 0.4 (5/5)	7.6 ± 2.3 (15/15)	9.5 ± 0.5 (15/15)
11.5	16.5	6.8 ± 2.8 (2/7)	5.6 ± 0.5 (3/5)	N/T	N/T	5.8 ± 1.2 (3/28)	6.9 ± 1.2 (28/28)
14.5	19.5	<3.6 ± 0 (0/6)	5.7 ± 0.1 (3/6)	N/T	N/T	<3.6 ± 0 (0/30)	6.9 ± 0.8 (30/30)

[a] Mean virus titer is the average $TCID_{50}$/g tissue titer from positive samples that had viral titers above the levels of detection.
[b] Standard deviation.
[c] Tissue samples with detectable WNV titers per total samples tested.
[d] Day of gestation on which dam was challenged with WNV.
[e] Days post-coitus.
[f] Day on which tissue samples were harvested from infected dams.
[g] Not tested.