

## JAPANESE ENCEPHALITIS VIRUS INFECTION IN FETAL MICE AT DIFFERENT STAGES OF PREGNANCY. II. RESISTANCE TO JAPANESE ENCEPHALITIS VIRUS INFECTION

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*Summary.* — The relationship between the stage of pregnancy of mice at the time of Japanese encephalitis (JE) virus inoculation and the resistance to JE virus infection of their offsprings was investigated. It was found that there was a stronger resistance to JE virus infection in offsprings born of mothers inoculated with JE virus at nine to sixteen days before parturition than in offsprings of mothers inoculated at one to eight days or at seventeen to twenty days before parturition. Resistance of the offsprings to JE virus infection lasted up to the age of 180 days after birth.

*Key words:* Japanese encephalitis virus; mice; pregnancy; resistance to infection

### Introduction

Stillbirth and malformations resulting from Japanese encephalitis (JE) virus infection during pregnancy have been reported in mice (Sugamata and Miura, 1982), but the rate of stillbirth differed according to the stage of pregnancy at the time of JE virus inoculation. The highest stillbirth rate was observed when mothers were inoculated at nine to sixteen days before parturition. This indicated that the effects of maternal JE virus infection during pregnancy of fetuses differed according to the stages of fetal development at the time of inoculation. Therefore, it was assumed that the immunizing effect may also differ with the stage of pregnancy at the time of exposure to JE virus.

The relationship between the stage of pregnancy at the time of JE virus inoculation and the resistance to JE virus infection of offsprings born of those mothers was investigated.

### Materials and Methods

*Mice.* ICR mice reared at our laboratory were used.

*Virus.* The JaGAR-01 strain of JE virus used has undergone five intracerebral passages in suckling mice.

*Virus inoculation.* The virus was inoculated intravenously to pregnant mice in a single dose of  $10^{2.7}$  LD<sub>50</sub>/0.2 ml.

Table 1. Experimental groups

Group	Route of offspring immunization	Status of the mother	Status of the foster mother
U	Uterus	Inoculated	Non-inoculated
M	Mammary	Non-inoculated	Inoculated
J	Both	Inoculated	None
Control	Neither	Non-inoculated	None

*Stage of pregnancy at the time of inoculation.* The day of parturition of each mouse was taken as day 0, and the number of days between parturition and the virus inoculation was counted. The pregnant mice inoculated with JE virus were further divided into three categories in which inoculation was carried out from one to eight (A), nine to sixteen (B) and seventeen to twenty (C) days before parturition. One control group and three experimental groups consisted of offsprings born of mothers inoculated with JE virus during pregnancy but suckled by non-inoculated foster mothers (group U) and of offsprings born of uninoculated mothers but suckled by foster mothers inoculated with JE virus during pregnancy (group M) in addition to those born of infected mothers (group J). The experimental groups are summarized in Table 1.

*Resistance to JE virus infection.* The offsprings in the control group and each experimental group were inoculated intravenously at the age from eleven up to 180 days with the same single dose of JE virus as the mothers had been inoculated during pregnancy. The challenged offsprings were observed for 21 days and their mortality was determined.

## Results

### Control group mortality rates

As much as 99 % of the offsprings in the control group inoculated at ages of eleven to twenty days died from JE virus infection, while the rate was 43 % among those inoculated at ages of 91 to 180 days (Table 2).

### Group J mortality rates

These offsprings showed a lower mortality rate than those in the control group, regardless of which stage their mothers had been inoculated in pregnancy (Table 2). When challenged up to the age of 35 days, the offsprings of all categories (A, B and C) showed an equally low mortality rate. Those inoculated after age of 36 days belonging to categories A and C had a mortality rate higher than those belonging to category B. Furthermore, category B offsprings inoculated at ages of 91 to 180 days also showed a lower mortality rate than those in categories A and C.

### Group U mortality rates

In this group, there were great differences between mortality rates of offsprings from categories A, B and C (Table 3). Among those inoculated at age of eleven to twenty days, the category A offsprings demonstrated a higher mortality rate (79 %) than those in categories B (0) and C (0).

**Table 2. Comparison of mortality rates of offsprings born from and suckled by mothers inoculated with JE virus during pregnancy (group J) and challenged with JE virus at various age**

Category	JE virus inoculation to the pregnant mother before parturition	Age of offsprings at JE virus challenge (in days)				
		11-20	21-35	36-60	61-90	91-180
A	1-8 days	2/66 ( 3)*	0/75 ( 0)	6/119( 5)	9/51 (18)	23/82 (28)
B	9-16 days	0/54 ( 0)	0/15 ( 0)	0/19 ( 0)	0/41 ( 0)	3/72 ( 4)
C	17-20 days	ND**	0/8 ( 0)	1/8 (13)	ND	10/65 (15)
D	Control	180/181(99)	199/260(77)	115/210(55)	85/163(52)	113/260(43)

\* Number of dead offsprings out of offsprings inoculated with JE (%).

\*\* Not done.

**Table 3. Comparison of mortality rates of offsprings born to JE virus inoculated mothers, but suckled by non-inoculated foster mothers (group U) and challenged with JE virus at various age**

Category	JE virus inoculation to pregnant mother before parturition	Age of offsprings at JE virus challenge (in days)		
		11-20	21-35	91-180
A	1-8 days	52/66 (79)*	21/43 (49)	30/82 (37)
B	9-16 days	0/26 (0)	5/18 (28)	16/59 (27)
C	17-20 days	0/20 (0)	ND**	19/37 (51)
D	Control	180/181 (99)	199/260 (77)	113/260 (43)

\* No of dead offsprings out of offsprings inoculated (with JEV (%)).

\*\* Not done.

Among those inoculated at age of 91 to 180 days, the mortality rate in the category B was lower than in other categories.

#### *Group M mortality rates*

These offsprings showed a lower mortality rate than those in the control group regardless of which stage their mothers had been inoculated during pregnancy (Table 4). There was a little increase in the mortality rate among offsprings inoculated at age of 91 to 180 days.

#### *Discussion*

Offsprings, the mother of which had been inoculated with JE virus during pregnancy, acquired a resistance to infection with JE virus. From the age of eleven to 180 days the offsprings received the same dose of JE virus with which their mothers were inoculated. In our cross-fostering experiments, the degree of the resistance acquired within the uterus, differed according to

**Table 4. Comparison of mortality rates of offsprings born from non-inoculated mothers but suckled by JE virus inoculated mothers (group M) and challenged with JE virus**

Category	JE virus inoculation to the pregnant mother before parturition	Age of offsprings at JE virus challenge (in days)		
		11-20	21-25	91-180
A	1-8 days	0/34 (0)*	0/9 (0)	ND**
B	9-16 days	0/25 (0)	1/17 (6)	8/70 (11)
C	17-20 days	0/14 (0)	ND	5/37 (14)
D	Control	180/181 (99)	199/260 (77)	113/260 (43)

\* No. of dead offsprings/out of offsprings inoculated with JEV (%).

\*\* Not done.

the stage of pregnancy at which the virus inoculation was made. The resistance acquired by the suckling, however, showed little difference regardless of the stage of pregnancy when the virus inoculation had been done. These results indicate that the immunizing effects of infected mothers on their offspring differ depending on the stage of fetal development when their mothers were inoculated with JE virus.

There is a report on vaccinia virus in mice, showing that if pregnant mouse had been inoculated at ten to eighteen days before parturition, her offsprings acquired the strongest resistance to the same virus (Malkinson, 1967). His observation, however, was made in newborns within 24 hours after birth.

In our preceding experiments (Sugamata and Miura, 1982), the transmission of JE virus from the infected mother mice to their offsprings occurred most frequently when the mothers were inoculated with JE virus at nine to sixteen days before parturition. This fact corresponds to the finding in presented experiments that mothers inoculated with JE virus at nine to sixteen days before parturition endowed their offsprings with the strongest resistance to JE virus infection after birth.

#### References

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