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PASSIVE TRANSFER OF IMMUNITY TO JAPANESE ENCEPHALITIS AND WEST NILE VIRUSES FROM ACTIVELY IMMUNIZED MOTHERS TO INFANT MICE

A. B. KULKARNI, M. K. GOVERDHAN AND K. M. PAVRI
National Institute of Virology, Dr. Ambedkar Road, Pune 411 001, India.

ABSTRACT

The passive immunity to Japanese Encephalitis (JE) and West Nile (WN) viruses in the infant mice born to immune mothers was studied. The level of antibodies transferred across the foetal membranes or through colostrum or both was determined by challenging the infants with these viruses. The results indicate that the transmission of immunity occurs both before and after the birth. The cross-reactivity in JE and WN viruses confounds the serodiagnostic and epidemiological work. Therefore an attempt was made to understand the change in cross-reactivity amongst these viruses by passaging each in the heterologous immune infants. The cross reactivity remained unchanged even after five passages in these infants. Serological studies revealed that haemagglutinating and neutralizing antibodies were present in the sera of infant mice born and suckled by immune mothers.

INTRODUCTION

IMMUNOLOGICALLY competent organisms respond to a variety of antigens by synthesizing the specific antibodies for the elimination of antigens from the body. However, the immune system of the new born animal possesses little or no capacity to do so. The passively transferred antibodies from mother to the offspring offer protection to latter in overcoming some of the infections of new born. The transmission of such passive immunity may occur during ante and/or post partum period and the mode of transfer varies from species to species and is also dependent on the type of antigen. The present study was undertaken to determine the nature of passive immunity and the extent of cross protection, if any, in JE and WN viruses.

MATERIALS AND METHODS

(*a*) *Viruses*:

(i) Japanese Encephalitis Virus (JEV, VRC strain P 20778) originally isolated from the brain of a patient from Vellore, Tamilnadu[1] was used after it had undergone 18 passages in suckling mice.

(ii) West Nile Virus (WNV, VRC strain G 22886) originally isolated from a pool of *Culex vishnui* mos-

quitoes collected at Vellore, Tamilnadu[1] was used at 9th passage level in mice.

(*b*) *Animals*:

Swiss albino mice, originally imported from Rockefeller Laboratories, New York, maintained at this Institute (NIV strain) since 1960 were used in these experiments.

(*c*) *Immunization of mice*:

Adult females of NIV strain were immunized against JEV and WNV separately by giving intraperitoneal (ip) injections at weekly intervals for four weeks. These were mated and the fifth injections was given on the 7th day after mating.

The occurrence of transplacental transmission of JEV, if any, was tested by passaging the brains of randomly selected infants born to immune mothers by intracerebral (ic) route for three times.

The parameters of homologous and heterologous protection as well as the cross-reactivity were based on the following criteria:

(a) Infants born and suckled by JEV immune mother.
(b) Infants born to non-immune mother suckled by JEV immune mother.
(c) Infants born to JEV immune mother fed by non-immune mother.
(d) Infants born and suckled by WNV immune mother.
(e) Infants born to non-immune mother suckled by WNV immune mother.
(f) Infants born to WNV immune mother fed by non-immune mother.

Homologous titrations were performed by challenging the infants born to immune mother with the respective viruses independently, while heterologous titrations were carried out by challenging the infants born to JE and WN immune mothers with WNV and JEV respectively. All the infants were challenged on the 3rd day of life.

Cross reactivity studies with JEV and WNV:

To assess the change in cross-reactivity the brains of ip inoculated sick infants from heterologous protection studies were harvested and passaged in the similar way for five times. Quick complement fixation was tested[2] at each passage level using 10% saline suspension of the brain as a quick antigen.

Exchange of the litters born to immune mothers with those born to non-immune females and vice-versa was performed to allow foster-nursing to enable the studies on transfer of passive immunity through placenta as well as through milk.

Protective indices (PI) were the difference in the titres obtained in the infants born and/or suckled by immune females and that of non-immunized females.

In vitro studies:

The litters born to JE immune mothers were obtained as described earlier. The sera samples of randomly selected infants were collected before intake of colostrum and tested by haemagglutination inhibition (HI) test[3] performed in microtitre plates[4] to assess the transplacentally transmitted antibodies. The sera samples of mothers and infants and the stomach contents of the infants were collected on 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21 days post partum. The sera and the stomach contents of the infants were screened for HI antibodies with and without treatment of 2-Mercaptoethanol (2-ME) for JEV. Neutralization test (NT) was performed by ic and ip routes in the infant mice with the sera obtained from mothers and infants using approximately 100 $\log_{10}LD_{50}$ of JEV.

RESULTS

Incidence of stillbirth was not observed in the immunized mothers and no virus could be isolated from the brains of the new born infants of immunized mother. The results of homologous and heterologous protection in JEV and WNV are presented in table 1. The homologous protection was observed in JEV and WNV upto 2.4 $\log_{10}LD_{50}$ and 2.5 $\log_{10}LD_{50}$/0.03 ml respectively by ip route only. No heterologous protection was observed by either route in both the viruses. Similarly the cross-reactivity remained unchanged even after five passages in the heterologous immune infants.

In foster nursing experiment when JEV was given to non-immune infants fed by JE immune mother, the protection of 1.5 $\log_{10}LD_{50}$/0.03 ml was observed by ip route only. Similarly, when WNV was given to non-immune infants fed by WNV immune mother the protection was 1.6 $\log_{10}LD_{50}$/0.03 ml by ip route. However, when the infants born to JEV immune mother and fed by non-immune mother were challenged with JEV no protection was observed by either route, while in the similar event with WNV the protection upto 1.4 $\log_{10}LD_{50}$/0.03 ml was observed by ip route.

No cross protection was observed JEV and WNV in the foster nursing experiment.

In vitro studies:

HI antibodies could not be demostrated in the infant sera collected before suckling on the immune mother,

TABLE 1

Results of homologous and heterologous protection in Japanese Encephalitis and West Nile viruses

	a		b		c		d		e		f	
Route of challenge	ic	ip	ic	ip	ic	ip	ic	ip	ic	ip	ic	ip
P.I.** with JEV challenge $log_{10}LD_{50}$/0.02 or 0.03 ml	00	2.4	00	1.5	00	00	00*	00*	ND	00	ND	00
P.I.** with WNV challenge $log_{10}LD_{50}$/0.02 or 0.03 ml	00*	00*	ND	00	ND	00	00	2.5	00	1.6	00	1.4

ND = Not done
* = Heterologous titrations
P.I. = Protective indices
** = Average of two titrations

a) = Infants born and suckled by JEV immune mother.
b) = Infants born to non-immune mother suckled by JEV immune mother.
c) = Infants born to JEV immune mother fed by non-immune mother.
d) = Infants born and suckled by WNV immune mother
e) = Infants born to non-immune mother suckled by WNV immune mother.
f) = Infants born to WNV immune mother fed by non-immune mother.

suggesting that transplacental transfer of antibodies to JEV was absent.

HI antibodies could be demostrated in the sera samples of mother and infants treated with or without 2-ME on various days after delivery (table 2). Stomach contents of infants did not show HI antibodies. All the sera were able to neutralize about 2 $log_{10}LD_{50}$/0.02 or 0.03 ml of virus when tested by ic and ip routes respectively.

DISCUSSION

Transplacental infection of JEV and stillbirth has been reported in pregnant mice during different stages of gestation[5-7]. Miura *et al*[8]. have shown that the offsprings born to non-inoculated mothers but suckled by JEV infected mother withstood the virus challenge upto 180 days regardless of the stage at which their mothers had been infected during pregnancy. In the present study no protection to JEV was obtained in the infants of immune females which were foster-nursed by non-immune mothers, whereas in the case of WNV the protection was 1.4 log_{10}. Interestingly, the protection to JEV and WNV was 1.5 and 1.6 log_{10} respectively in those infants which were born to non-immune females but were suckled by immune mothers. Therefore it appears that in JEV, passive transfer of immunity occurs mainly through colostrum while in WNV both the systems are equally effective. However, in the homologous system higher protective indices with JEV, the cummulative effect of transfer of immunity through milk as well as maternal circulation was noticed. Brambell[9] suggested that although a sig-

TABLE 2

Resutls of HI test performed on sera of immune mothers and their offsprings collected on different post partum days

Days post delivery	HI titres in serum of			
	Mother		Infants	
	Without ME	With ME	Without ME	With ME
1	≥320*	≥320	20	<20
3	160	80	160	80
5	160	160	80	40
7	≥320	≥320	160	160
9	80	40	80	40
11	≥320	80	80	80
13	40	40	160	80
15	160	80	160	80
17	20	10	160	80
19	40	40	80	80
21	80	40	80	20

* = Reciprocal of HI titre

nificant amount of immunoglobulins is transmitted across the foetal membranes through the maternal circulation, the greater part is transmitted through colostrum. However, in the case of vaccinnia virus, Malkinson[10] has shown that the protection transferred across the foetal membrane is more effective. Since the protection obtained was only limited to ip challenge, one could speculate that blood-brain barrier restricts the diffusion of antibodies into the intracellular space.

Absence of alteration in the pattern of cross-reactivity despite five passages in the heterologous system indicates that there is no cross-protective passive immunity between these two viruses although antigenically they are known to exhibit cross-reactivity.

HI antibodies could be demonstrated in 2-ME treated and untreated sera of infants and their mothers when tested on various days after the delivery. In the studies with tritiated myeloma proteins, it was shown that only IgG molecules were transported across the epithelial barrier to blood while IgM and IgA were not absorbed in either monomeric or polymeric forms[11]. In the present study, therefore, HI titres in the infant sera could be due to IgG antibodies. Our inability to demonstrate HI antibodies in the stomach contents of the infants may be due to rapid absorption of antibodies across the gut[12]. It is of interest to study the persistence of these antibodies derived from maternal circulation and from colostrum separately so as to assess the effectiveness and duration of this protection.

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SYNTHESIS OF SOME FORMAZANS AND TETRAZOLIUM BROMIDES AS POTENTIAL ANTIVIRAL AGENTS

S. P. SINGH AND SURENDRA BAHADUR*
Division of Biophysics, Central Drug Research Institute, Lucknow 226 001, India.
*Department of Chemistry, Lucknow University, Lucknow 226 007, India.

ABSTRACT

1-Aryl-3-(3'-nitro-4'-methoxyphenyl)-5-phenyl formazans (II) were synthesized by the reaction of 3-nitro-4-methoxybenzaldehyde phenylhydrazone (I) with various aryl diazonium salts. Some of the formazans (II) on oxidation by H_2O_2/Fe^{++} were cyclized into their corresponding 3-aryl-5-(3'-nitro-4'-methoxyphenyl)-2-phenyl tetrazolium bromides (III). A majority of compounds II and III exhibited significant antiviral activity against ranikhet disease virus in a stationary culture of chorioallantoic membranes of chick embryos.

INTRODUCTION

FORMAZANS and tetrazolium salts have since long been found to possess antiviral[1] and antibacterial[2,3] properties. Recently Misra *et al*[4] and Mukherjee *et al*[5-7] have synthesized various formazans and tetrazolium salts, some of which have significantly inhibited both animal as well as plant viruses. Formazans on oxidation are converted into their tetrazolium salts which because of their polar nature possess