IMMUNOLOGICAL REACTIONS OF THE COXSACKIE VIRUSES

III. Cross-Protection Tests in Infant Mice Born of Vaccinated Mothers. Transfer of Immunity Through the Milk*

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Newborn mice are highly susceptible to infection with C viruses, but in a matter of days develop a natural resistance to the disease. Thus, cross-protection tests in which virus is inoculated into vaccinated animals cannot be employed, because by the time an immune status would be expected to be reached the animal would have become naturally resistant. However, by challenging baby mice born of mothers vaccinated with different strains of active virus, it has been possible to work out the immunologic relationships of these strains. In addition, it has been found that type-specific immunity may be transferred through the milk.

Although this phenomenon has not been previously used as a method of typing virus strains, the passive transfer of immune substances *via* placenta and milk has been recognized since Ehrlich's work (1). The finding of Aycock and Kramer (2) that neutralizing antibodies to poliomyelitis virus when present in the maternal blood may also be found in the blood of infants for the first months of life is undoubtedly an important consideration in accounting for the absence of the disease in infancy. Passive transfer has been experimentally demonstrated in mice for St. Louis encephalitis (3), for MM strain of encephalomyocarditis virus (4, 5), for the TO type of mouse encephalomyelitis virus (6), and in cotton rats for the Lansing strain of poliomyelitis virus (7).

Materials and Methods

Adult mice were immunized with the following strains: High Point, Texas, Conn.-5, and Ohio. In the early part of this work, 10 per cent suspensions of brain were employed, but this was soon abandoned in favor of suspensions of skinned, eviscerated torsos of infected infant mice when it was found that the latter yield uniformly higher titers of virus. Mice were inoculated either subcutaneously (0.3 ml.) or intraabdominally (0.5 ml.), receiving about 8 inoculations in the course of 3 weeks. Thereafter injections were carried out about once each

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month. Mice were bled from the heart about 10 days after each booster vaccination and these samples had neutralizing antibody titers of about 1:1000 against 100 ID_{50} of virus (8) and complement-fixing titers of about 1:64 against 8 units of antigen (9). The mice were kept in groups of 5 females per male and observed daily for appearance of young. Within 48 hours of birth, the newborn mice received 100 to 1000 ID_{50} doses of virus by the intraperitoneal or subcutaneous route.

In some of the experiments mice were nursed by their own mothers following the inoculation of virus, but in the majority they were nursed by foster mothers. However, in practically all instances, the infant mice received at least one feeding of colostrum from their own mother before the inoculation of virus. Whether or not such mice were nursed by their vaccinated mothers or by non-vaccinated foster mothers did not influence the results. In some experiments to be described, young mice born of non-vaccinated mothers were nursed by vaccinated foster mothers to study the transfer of immunity by milk alone.

TABLE I

Immunity in Mice Born of or Nursed by Vaccinated Mother

Group 1 From vaccinated mother, nursed on vaccinated mother	*Group 2* From vaccinated mother, nursed on non-vaccinated foster mother	*Group 3* From non-vaccinated mother, nursed on vaccinated foster mother	*Group 4* From non-vaccinated mother, nursed on non-vaccinated mother	*Group 5* From non-vaccinated mother, nursed 48 hrs. on vaccinated foster mother, challenged with virus, and then placed with a non-vaccinated foster mother
13 litters	16 litters	12 litters	11 litters	5 litters
0/52	1/65(1)	2/43(2)	30/32(30)	0/12

Challenge dose of virus = 100 ID_{50}.

Numerator = number of deaths; denominator = number of mice used.

Number in parentheses denotes mice showing paralysis before death.

Experiment 1. Transfer of Immunity by Colostrum and Milk.—For these experiments, mice, not older than 24 hours, and born of both vaccinated and non-vaccinated mothers were used. Vaccination of the mothers and challenge were carried out with one strain of virus, the Ohio. Mice were placed in the following groups, each litter from vaccinated mothers being divided into groups 1 and 2, and each litter from non-vaccinated mothers being divided into groups 3 and 4, or into groups 3, 4, and 5.

No. 1. Born of vaccinated mother, inoculated within 24 hours of birth, and returned to vaccinated mother for nursing.

No. 2. Born of vaccinated mother, inoculated with virus, and placed with non-vaccinated foster mother for nursing.

No. 3. Born of non-vaccinated mother, inoculated with virus, and placed with vaccinated foster mother for nursing.

No. 4. Born of non-vaccinated mother, inoculated with virus, and returned to non-vaccinated mother for nursing (control group).

No. 5. Born of non-vaccinated mother, suckled for 24 to 48 hours on a vaccinated foster mother, challenged with virus, and then returned to a non-vaccinated lactating female.

The results are shown in Table I. Non-specific deaths resulting from failure of lactating females to nurse their foster young are not included. From these

results it is clear that immunity to C virus can be passively transferred by colostrum and milk. The fact that all the mice probably had a meal of colostrum before challenge does not permit evaluating the role of the placenta in the transfer of this immunity.

Fifty-two mice in group 1 were completely protected against the challenge dose of virus, 100 ID_{50}. Group 2 also showed practically complete protection with only 1 of 65 mice developing the disease. This group was originally designed to demonstrate placental transfer of immunity, but in view of the colostrum which most of the mice ingested soon after birth and the results obtained with group 5, this result with group 2 cannot be interpreted as for or against such transfer. All 12 mice in group 5—to which can be added over 100 mice in which this part of the experiment was repeated—were completely protected against the challenge dose of virus, indicating that nursing for only 48 hours will confer this degree of immunity. Subsequent experiments showed that a 24 hour nursing period would also suffice. This result was borne out with group 3 in which 43 mice were nursed on vaccinated foster mothers and only 2 developed the disease, again proving this to be an efficient means of conferring immunity. That the newborn mice used in these experiments were uniformly susceptible is shown by the results with the 32 mice in group 4 in which 30 developed paralysis and died.

Experiment 2. Duration of Transfer of Immunity through the Milk.—Three vaccinated pregnant females were placed in separate cages. Their infants were separated from them at birth and replaced with normal babies. These babies were allowed to nurse for varying periods of time on the immune female, removed, and challenged with 100 ID_{50} of the homologous Ohio strain, or of the heterotypic Texas strain. They were then transferred to a normal lactating female. Newborn normal babies were again placed with the vaccinated female for nursing. This cycle was repeated as long as the vaccinated mothers continued to lactate. The results of such foster suckling experiments are shown in Table II.

These experiments demonstrate that as long as a vaccinated mother lactates, sufficient protection is afforded by a short nursing period to prevent mice from being infected by 100 ID_{50} of homologous virus. The immunity conferred is type-specific, as evidenced by the following experiments. 32 newborn mice were nursed on Ohio-vaccinated mothers for a period sufficient to produce immunity to the Ohio strain. When challenged with the heterologous Texas strain (100 ID_{50}) all the mice succumbed to the disease. As shown in Table II, these lactating vaccinated mothers were subsequently still capable of conferring homotypic (Ohio strain) immunity to newborn mice, in the case of mouse A for at least 20 days, mouse B at least 40 days, and mouse C for at least 32 days.

Experiment 3. Strain Typing by Cross-Protection Tests in Babies Born of Vaccinated Mothers.—Mice were vaccinated with 4 strains of C virus and mated as indicated above under Methods. Within 48 hours of birth the offspring were inoculated with the homologous or heterol-

TABLE II

Duration of Transfer of Immunity through the Milk

Day of lactation	Mouse A		Mouse B		Mouse C	
	Challenge strain	Result of test	Challenge strain	Result of test	Challenge strain	Result of test
1		↓				
2		↓	Ohio	0/8		Inc.
3	Ohio	0/7	Ohio	0/4		↓
4		↓		↓		↓
5		Inc.	Ohio	0/8		↓
6		↓		↓		↓
7	Ohio	0/7	Ohio	0/4		↓
8		↓		↓		↓
9	Ohio	0/3	Ohio	0/7		↓
10		↓		↓		n.t.
11		Inc.	Ohio	0/4		↓
12		↓		↓		Inc.
13		↓		↓		↓
14		↓		↓	Ohio	0/8
15		↓		↓		↓
16		↓		↓		↓
17		↓		↓		↓
18		Inc.		n.t.		↓
19		↓		↓		↓
20	Ohio	0/5	Ohio	0/8		↓
21		↓		↓	Texas	6/6(6)
22		↓	Ohio	0/5		↓
23		↓		↓	Ohio	0/6
24		↓		↓		↓
25		↓		↓		↓
26		↓		↓		↓
27	Texas	5/5(5)		↓		↓
28		↓		↓		↓
29		Inc.	Texas	5/5(4)		↓
30		↓		↓	Texas	8/8(6)
31		Inc.	Ohio	0/8		↓
32				↓	Ohio	0/7
33				↓		↓
34	Lactation ceased			↓		↓
35				↓		↓
36				↓		↓
37				↓		↓
38			Texas	8/8(2)		n.t.
39					Lactation ceased	
40			Ohio	0/10		
41				↓		
42			Lactation ceased			

Inc. = incomplete test, mice destroyed by non-vaccinated foster mother.

n.t. = not tested.

Arrows indicate the period of nursing on the vaccinated females before the mice were challenged with 100 ID_{50} of the indicated strain.

ogous strain and returned to their own mother or placed with non-vaccinated foster mothers. The results are shown in Table III.

In confirmation of Experiments 1 and 2, immunity was conferred from vaccinated mother to young. This immunity was type-specific, and did not exist at all when mice were challenged with heterotypic viruses. Strains which fall into the same immunological type by other tests (neutralization and complement fixation) also fall into the same type with these cross-protection tests. Thus mice vaccinated with the High Point or Texas viruses had babies immune

TABLE III

*Maternal Transmission of Homotypic Immunity**

Challenge virus	Babies born of mothers vaccinated with			
	Hi.Pt.	Texas-1	Conn.-5	Ohio-1
Conn.-5	73/92(21)	27/29(11)	0/36	
JLM(C)	6/6(1)	16/16(9)	1/27(0)	
NL (C)	29/33(2)	7/7(0)		13/13(2)
Greensboro-4	13/13(3)	16/16(1)		
Winston-Salem-1	12/12(6)	11/11(6)		16/16(8)
Ohio-1	22/22(19)	24/24(24)	17/17(14)	3/172(3)
Texas-1	31/69(25)	0/47	42/42(14)	41/41(29)
Hi.Pt.	8/74(5)	10/63(3)	65/65(21)	8/8(7)
NHF-'43	0/25			
Easton-2 (D-1)	34/34(22)	62/62(53)	46/50(41)	67/67(58)
NY-HA (D-1)		25/31(20)		16/16(13)
NM	17/17(12)	10/10(10)		

* Related strains are boxed together.

to each strain and to the related NHF-'43 strain. Also when a series of strains related to the Conn.-5 were used for challenge, only mice from Conn.-5 vaccinated mothers were protected. When two strains related to Dalldorf's type 1 were used, none of the offspring from mothers vaccinated with any of the four types used were protected.

Experiment 4. Transfer of Complement-Fixing Antibodies.—In two instances mice born of mothers vaccinated with the High Point strain were kept with their mother until each litter was 7 days old. At that time, the baby mice were bled from the heart and the serum examined for complement-fixing antibodies by the plate complement fixation test as adapted to C viruses (9). Sera from each litter had a titer of 1:16 or over. Serum pools obtained from groups of 30 or more vaccinated adults had titers of about 1:64.

Together with a transfer of immunity to mice, there is a simultaneous transfer of complement-fixing antibodies from mother to young. It is of interest that in the human population a similar transfer of neutralizing and complement-fixing antibodies has been found for the High Point strain of C virus (8).

DISCUSSION

It is apparent from the results presented that maternal antibodies to the C viruses are transmitted to the offspring through the colostrum and milk. Transplacental passage has not been proved, and it would be interesting to study the resistance to challenge of mice obtained close to term by Caesarian section from vaccinated mothers.

It may at first seem impracticable to use litters born to immune mothers as a means of screening strains of C virus. However, when numbers of mature mice are undergoing immunization to known types of virus, it becomes feasible to carry on such a program. Care must, of course, be taken not to overwhelm the immunity of the infant mice; 100 ID_{50} doses of virus appear to be satisfactory from our results.

When typing a new strain it is essential to use three groups of mice:—

1. Mice from non-vaccinated mothers to be inoculated with 100 ID_{50} of the strain in question. All should succumb to infection.

2. Groups of mice from vaccinated mothers with known types, each group to be inoculated with 100 ID_{50} of its homotype. No mice should die of C virus infection.

3. The test mice; *viz.*, litters from the same groups of mothers as in Item 2 (vaccinated against known types of C virus). Mice with homotypic immunity should not succumb to C virus infection.

It is important that the maternal antibodies should be maintained by means of booster vaccinations at 3 to 4 week intervals.

SUMMARY

Maternal antibodies to the Coxsackie viruses (C virus) are conveyed to newborn mice through the colostrum and milk of vaccinated mothers. No evidence for or against placental transmission of immunity was obtained.

The immunity conferred on the young is type-specific.

Immunity may be conferred to infants born of non-immune mice by allowing a suckling period of 24 to 48 hours with an immune mother.

Immunity appears to be transferred through the milk for the duration of lactation.

Strains of C virus can be typed by challenging infant mice born to mothers vaccinated with known types according to the outline presented above.

Complement-fixing antibodies are also transferred from vaccinated mother mice to their offspring.

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