

Haemagglutination-inhibition antibodies against influenza A and influenza B in maternal and neonatal sera

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SUMMARY

Haemagglutination inhibition (HI) antibodies against the influenza viruses A/Hong Kong/8/68 (H3N2) and B/Nederland/77/66 were determined in 420 paired sera from mothers and newborns (umbilical cord sera), sampled in 1970–1.

A higher concentration of antibodies against influenza A virus was found more frequently in neonatal than in maternal sera. By contrast, low titres against influenza B virus were more frequently observed in neonatal than in maternal sera. Maternal age, duration of pregnancy, and birth-weight did not affect the results of the tests.

It is suggested that the titre of the newborn against an epidemic influenza virus can be predicted from that of the mother. Furthermore, the maternal titre may be an indication of the susceptibility of the newborn infant to influenza infections.

INTRODUCTION

The relation between the immunoglobulin concentration and the titre of specific antibodies present in the serum of mothers and newborns has been studied by other investigators (Kohler & Farr, 1966; Toivanen, Mäntyjärvi & Hirvonen, 1968; Mäntyjärvi, Hirvonen & Toivanen, 1970; Cloonan, Hawkes & Stevens, 1971; Papadatos *et al.* 1974).

Mäntyjärvi *et al.* (1970) found that newborns of mothers with a low or normal antibody titre showed a higher antibody titre than their mothers in 33%, and a lower titre in 11% of the sera tested. If the maternal titre was high, these percentages were 12 and 41, respectively. This phenomenon was first reported by

* Deceased.

Michaux, Heremans & Hitzig (1966) in a comparative study of IgG concentrations in mothers and infants.

Recently, a relation between the circulating antibody titre against influenza A virus (HI titre above 100) or influenza B virus (HI titre above 50) and protection against these viruses has been established in adults and children during epidemics and in challenge studies (Hobson, Curry, Beare & Ward-Gardner, 1972; Wesseliuss-de Casparis, Masurel & Kerrebijn, 1972).

The purpose of our study was to find a relation between maternal and neonatal antibodies against influenza A as well as influenza B virus, measured in the HI test. Furthermore, the role in this relationship of maternal age, duration of pregnancy, birth weight, and maternal antibody titre was studied. A small fraction of the sera were tested in an animal model for neutralizing antibodies to compare the protective value of maternal and neonatal HI antibodies.

MATERIALS AND METHODS

Volunteers

Maternal and neonatal sera were collected from 420 mothers and corresponding newborns from February 1970 to June 1971. The maternal ages varied between 15 and 42 years; expressed as percentages they were as follows: 19.5% under 20 years, 65.5% between 20 and 29 years, 13.5% between 30 and 39 years, 1.5% above 40 years. The percentages of full-term, premature and post-mature deliveries were 87, 9, and 4, respectively. The birth weight of 84% of the newborns was between 2500 and 4000 g, of 10% below 2500 g, and of 6% above 4000 g. Among the newborns 103 were hospitalized for a short or long period, because of artificial delivery (1–2 days), or premature or dysmature birth (several weeks). There were no clinical indications of intrauterine infections, such as rubella and cytomegalovirus infection, or toxoplasmosis.

HI test, sera and antigens

Umbilical cord blood was collected immediately after birth. Maternal blood was obtained by venipuncture on the day of delivery. Maternal and neonatal sera were immediately frozen and stored at -20°C until titration.

The HI test was carried out as described by Van der Veen & Mulder (1950). Sera of mothers and newborns were first treated with *Vibrio cholerae* filtrate and incubated at 56°C , to eliminate non-specific inhibitors (Masurel, 1962). No demonstrable non-specific inhibition occurred against the test strains in any of the normal ferret sera, or in the human sera without specific antibody included in the test, if treated with the filtrate.

The antigens used in the HI test were the influenza viruses A/Hong Kong/8/68 (H3N2) and B/Nederland/77/66. About 20 pairs of maternal and corresponding neonatal sera were set up in one HI test against the influenza strains on each consecutive day. In all tests ferret antisera were used as homologous standard reference sera. To eliminate biased interpretation, all sera were coded before the beginning of the test.

Complement fixation (CF) test

CF was performed in a microtitre test as described by Lennette & Schmidt (1969). Twenty-five maternal sera with a high initial HI titre against the Hong Kong/1968 virus (270–2200) were tested by the soluble antigen of influenza A. No recent infection by influenza A could be demonstrated. The CF titres were < 7 (8 sera) or 7–16 (17 sera). During the trial no influenza B virus was isolated in the Netherlands.

Neutralization test

Fifteen neonatal and corresponding maternal sera were tested for neutralizing antibodies in mice with the mouse-adapted A/1968 virus. The sera were inactivated for half an hour at 56 °C. Equal volumes of virus suspensions containing 100 LD 50 were added to each serum dilution. The mixtures remained at 20 °C for one hour. Each serum-virus mixture was then administered intranasally to 6 mice (0.05 ml/mouse). The mouse experiments also included serum and virus controls. Readings for dead mice were made during 10 days. Titres were calculated according to Reed & Muench (1938).

RESULTS

Figure 1 shows that the 420 neonatal sera frequently produced higher antibody titres to the influenza A/Hong Kong/1968 strain than the respective maternal sera ($P < 0.001$ by the sign test) (Siegel, 1956). The titre of 138 (33 %) newborns was higher and of 56 (13 %) lower than the maternal titre. A group of 21 (5 %) neonates showed a fourfold higher titre than their mothers, while 13 (3 %) newborns possessed a fourfold lower titre. Of 198 mothers showing a titre above 100, 13 (7 %) corresponding newborns had a titre below 100, and 4 (2 %) a titre below 50.

Table 1 shows the HI titres of maternal and neonatal sera in comparison with the neutralization titres in mice. The neonatal neutralization titres were equal to or higher than the maternal titres in all but one serum. The neonatal titres that were equal to or lower than the maternal titres in the HI test were higher than or equal to the maternal titres in the neutralization test.

The results of the HI test with the B/Nederland/1966 virus, presented in Fig. 2, indicate that titres against this virus were more frequently higher in the maternal than in the neonatal sera ($P < 0.001$). The neonatal titre was lower than the maternal titre in 132 (31 %) sera and higher in 65 (15 %) sera. The titre was fourfold lower in 28 (7 %) and fourfold higher in 12 (3 %) neonatal sera. Of 198 mothers with an antibody titre above 50, 53 (27 %) corresponding neonates had a titre below 50 and 7 (4 %) a titre below 25.

Table 2 presents the correlation matrix. No correlation appeared to exist between maternal age, duration of pregnancy, or birth weight, and the antibody titres of mothers and newborns. A high correlation was observed between maternal and neonatal sera if tested against the A/1968 virus and the B/1966 virus. No correlation was found between the titres against influenza A virus and those against influenza B virus.

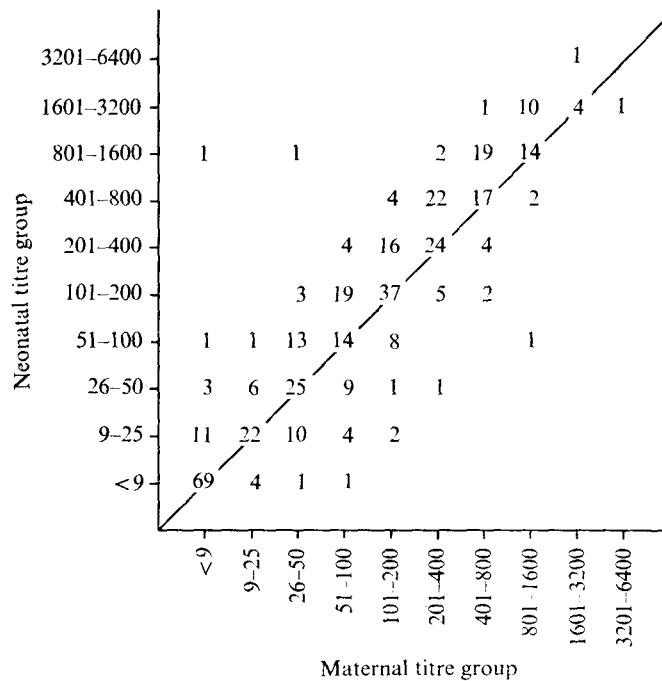


Fig. 1. HI antibodies against A/Hong Kong/8/68 (H3N2) influenza virus in 420 neonatal and maternal sera. Figures represent the number of serum pairs.

Table 1. Serum neutralization test for influenza A virus in mice, in comparison to the HI test, carried out in paired maternal and neonatal sera

Serum no.	HI titre (mother-child)	Neutralization titre (mother-child)
108	180-55	5-5
78	540-380	90-218
335	<9-<9	<2-<2
347	95-95	11-22
92	135-135	13-16
345	170-170	8-14
312	270-270	22-72
276	540-540	22-32
100	1100-1100	55-178
393	<9-15	<2-<2
378	<9-35	<2-4
270	<9-70	<2-11
418	17-60	3-2
263	35-1100	3-178
407	270-480	5-6

DISCUSSION

The study was set up with sera of people for whom no indication of recent infection by influenza A virus was given by the results of the CF test. Our observation that the HI titre against influenza A virus was higher in the neonatal than in the corresponding maternal serum, resembles the results of Mäntyjärvi *et al.*

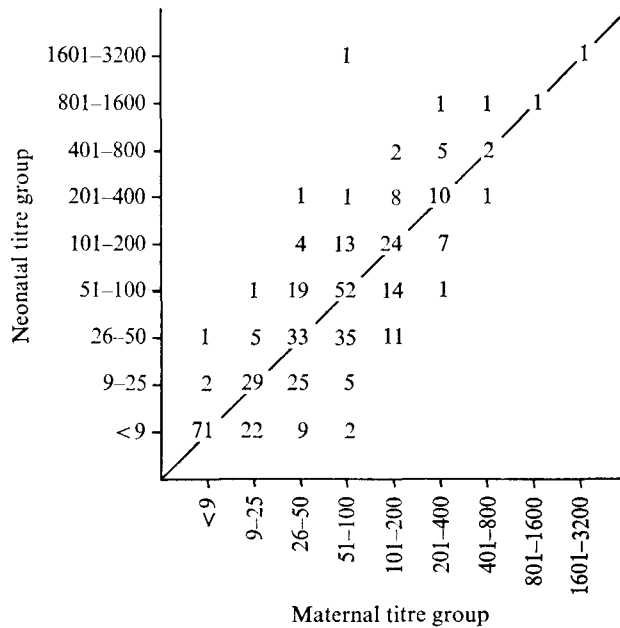


Fig. 2. HI antibodies against B/Nederland/77/66 influenza virus in 420 neonatal and maternal sera. Figures represent the number of serum pairs.

Table 2. Correlation matrix of maternal age, duration of pregnancy, birth weight, and antibody titres of mother and baby to the influenza viruses A/Hong Kong/8/68 and B/Nederland/77/66*

	Maternal age	Duration of pregnancy	Birth weight	Titres† against			
				Hong Kong mother	Hong Kong baby	B mother	B baby
Maternal age	1	0.01	0.07	-0.06	-0.09	-0.13	-0.20
Duration of pregnancy	0.01	1	0.53	-0.02	0.07	-0.01	0.06
Birth weight	0.07	0.53	1	0.02	0.08	-0.01	0.04
Titre Hong Kong mother	-0.06	-0.02	0.02	1	0.92	0.27	0.24
Titre Hong Kong baby	-0.09	0.07	0.08	0.92	1	0.22	0.26
Titre B mother	0.13	-0.01	-0.01	0.27	0.22	1	0.85
Titre B baby	-0.20	0.06	0.04	0.24	0.26	0.85	1

* Product-moment correlation according to Pearson.

† Before computing the correlation coefficients the titres were scored from 1 to 10, in accordance with the subdivision in groups, used in Figs. 1 and 2.

(1970) in the CF test. However, while they found lower neonatal titres mainly if the maternal titres were high, we observed that neonatal titres were higher in all maternal titre groups.

Toivanen *et al.* (1968) found that a series of antibodies to influenza A, adeno, parainfluenza 1 and 3, herpes simplex, and measles viruses, were more frequently present in a high concentration in cord blood than in maternal blood. Similar

Table 3. *Subclasses of serum pairs*

HI titre against A/Hong Kong/1968	HI titre against B/Nederland/1966		
	M < B*	M = B	M > B
M < B	38	72	26
M = B	26	129	71
M > B	2	20	36

* M = mother; B = baby.

results were obtained in CF tests for antibodies to ornithosis, mumps, cytomegalo, and RS virus. Brouwer, de Groot & Verheij (1974) assessed higher titres of measles and rubella antibodies in cord blood.

By contrast, in our study titres against influenza B virus were lower in neonatal than in maternal sera. However, as Table 3 indicates, we may have to do with two subclasses of serum pairs. Higher titres against influenza A in neonatal than in maternal sera do not seem to correspond with the reciprocal value for influenza B (upper horizontal row). Furthermore, lower titres against influenza B in neonatal than in maternal sera do not correspond with the reciprocal value for influenza A (right vertical row). This finding requires further investigation of the IgG subclass of the maternal and neonatal antibodies (Morell, Skvaril, van Loghem & Kleemola, 1971; Virella, Silveira Nunes & Tamagnini, 1972), since Hitzig (1961) suggested that the placental transport of antibodies within the various IgG immunoglobulins is a selective and active process.

Analysing the maternal and neonatal antibodies to influenza A and influenza B virus, we concluded that the titre of the unborn child against these viruses might be predicted from the titre in the maternal serum: if maternal age, duration of pregnancy, and weight of the newborn child were included in the analysis, the coefficient of determination (R^2) increased by only 0.01. This finding could be of special importance if the mother possesses a protective HI titre against influenza A of above 100 (Wesselijs-de Casparis *et al.* 1972). Our results show that only 7% of the newborns' sera had a titre below 100 to the influenza A virus if the corresponding maternal serum had a titre above 100. For the influenza B virus the results were different: 27% of the neonatal sera showed a titre below 50 if the mothers' possessed a protective titre of above 50.

The results of the neutralization test carried out with the A/Hong Kong/1968 virus in a number of paired sera illustrate that the neonatal sera may be expected to possess a higher neutralizing antibody titre to influenza A than the corresponding maternal sera. In our opinion, from this last finding and the results of the HI test, the conclusion can be made that the newborn may be considered to be protected against the epidemic influenza virus if the maternal titre is above the protective level.

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