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Complement fixation and neutralization RS antibodies in maternal and neonatal sera

By R. A. HEIJTINK, G. BACKX, J. M. VAN DER HORST and N. MASUREL

Department of Virology, Erasmus University Rotterdam, Rotterdam, The Netherlands

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SUMMARY

RSV complement fixation antibodies were established in 200 paired maternal and cord blood sera. Geometric mean titres in cord sera were significantly higher than in maternal sera. The differences did not depend on the virus strain used. Half of the paired sera (taken at random) were also submitted to microneutralization tests. No differences were found between geometric mean titres in maternal and cord sera.

INTRODUCTION

Respiratory syncytial virus (RSV) is currently considered as a major cause of acute respiratory illness in early childhood. Serum antibodies measured in neutralization tests do not protect against RSV disease (Parrott, Kim, Brandt & Chanock, 1975). On the contrary serum antibodies, presumably of maternal origin, are thought to play a part in the pathogenesis of the disease, as serious RSV respiratory tract disease most commonly occurs in newborn infants who possess the highest titre of passively transferred serum neutralization antibodies (Chanock *et al.* 1968).

Serum neutralization antibodies may be responsible for an impaired immunologic response due to immunologic immaturity and/or immunologic suppression (Parrott *et al.* 1975). In former studies the possible role of pre-existing antibodies in a type III immune reaction (Arthus type) as a cause of severe RS disease has been suggested. In this connection depletion of serum complement and complement fixation in the lung in cases of acute bronchiolitis has been studied, yielding no conclusive results (Gardner, McQuillin & Court, 1970; Ana *et al.* 1970).

The purpose of our study was to analyse the transplacental passage of complement fixation antibodies in relation to serum neutralization antibodies in sera of mothers and newborns.

MATERIALS AND METHODS

Sera

The sera used in complement fixation (CF) and neutralization tests were kindly supplied by Professor J. I. de Bruijne from the Department of Neonatology, University of Amsterdam. They were collected during the period from February

1970 to December 1971. Maternal sera were obtained by venepuncture on the day of delivery. Two hundred umbilical cord sera and corresponding maternal sera were submitted to a comparative investigation of CF titres. Half of the paired sera were tested for neutralization antibodies. CF antibodies of an additional group of 31 paired neonatal and maternal sera collected during delivery and 6 months later were set up in one test.

A small number of sera were tested for RS specific IgM antibodies, using the sucrose density gradient centrifugation method of Field & Murphy (1972). Confirmation of the correct separation of IgG and IgM antibodies was obtained after testing the fractions by using immunodiffusion plates for quantitative determination of globulines IgG and IgM (Tri-Partigen®-IgG and S-Partigen®-IgM, Behringwerke, Germany). Possible contamination of cord serum by maternal serum was checked using IgA (Diffu-gen, Oxford Laboratories, USA) as a marker. All sera were frozen immediately after sampling and stored at −20 °C.

CF test

Complement fixation was carried out in a microtitre test as described by Lennette & Schmidt (1969), using two units of complement and four units of antigen. Two hundred paired sera were tested with antigen prepared from Long strain RSV grown in HeLa cell cultures. The antigen consisted of infected cells and tissue culture fluid harvested when the entire cell sheet showed CPE. The material was frozen and thawed once and then stored at −70 °C. Fifty of the two hundred paired sera were tested with a RSV strain isolated in 1959 by Professor F. Dekking (Laboratory for Hygiene, University of Amsterdam). We named this strain the DA59 strain. CF antigen preparation was as described for the Long strain. The paired maternal and neonatal sera were tested simultaneously after coding. Standard reference sera were used as controls. HeLa cell controls were included in the test and were found to be negative. Titres were expressed as reciprocals. In calculations and plotting a titre of < 7 was considered as 6.

Neutralization test

Neutralization antibody titres were determined by a microtitre method. Sera were heat-inactivated at 56 °C for 30 min. Serum and virus dilutions were prepared in Medium 199 with 5% fetal bovine serum (FBS), 0·2 mM-$MgCl_2$, 0·3% glucose, 0·15% sodium bicarbonate and antibiotics. Twofold serum dilutions (0·025 ml) were made in microplates in replicates of four. An equal volume of virus suspension (RSV Long strain) containing 100 TCD 50/0·025 ml was added to each well. The plates were covered and kept at room temperature for one hour. A virus titration and serum and tissue controls were included in the test. After 1 h 0·1 ml HeLa cell suspension (100 000 cells/ml) was added to each well. Cells were suspended in Eagle's Minimal Essential Medium with 5% FBS, 0·2 mM-$MgCl_2$, 0·3% glucose, 1% glutamine, 0·15% sodium bicarbonate and antibiotics. The plates were covered, incubated at 37 °C and checked for CPE after 7 days. Fifty % serum neutralization end-points were calculated by the Kärber formula (Grist, Ross & Bell, 1974).

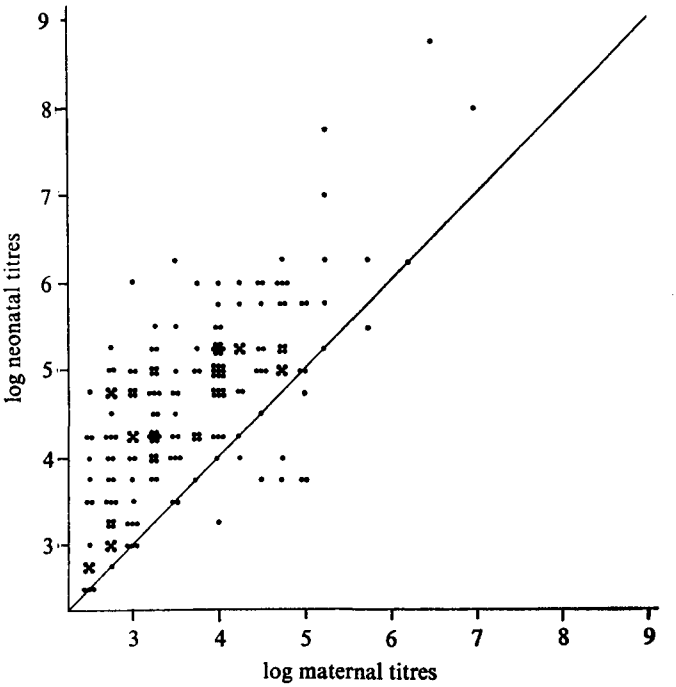

Fig. 1. Complement fixation antibodies in 200 maternal and neonatal sera. Tests were performed using Long strain CF antigen. Titres are expressed as $\log_2$.

A number of investigators use fresh guinea-pig serum to enhance the neutralizing activity. We have investigated 20 paired sera with and without guinea-pig serum. Although a slight enhancement was observed, it occurred in maternal as well as in neonatal sera. The ratio of the antibody titres in maternal and neonatal sera was not affected. Guinea-pig serum was therefore not used in our experiments.

RESULTS

Figure 1 illustrates the relation between CF titres of 200 pairs of maternal and neonatal sera (Long strain). Neonatal sera showed significantly more high titres than maternal sera ($P < 0{\cdot}001$ by the sign test, Siegel, 1956), both in high and in low titre regions. In 174 pairs (87·0%) titres were higher in neonatal than in maternal sera, in 17 pairs (8·5%) they were identical and in 9 pairs (4·5%) they were lower in neonatal than in maternal sera.

No detectable titres (< 7) were found in 4% of the maternal and in 1·5% of the neonatal sera. Geometric means of neonatal and maternal titres were 24·9 and 13·3 respectively.

Contamination of neonatal sera by maternal sera was checked by testing the former for IgA antibodies. No IgA antibodies could be demonstrated.

To establish the maternal origin of neonatal titres we investigated the presence of CF antibodies against RSV six months after delivery. Maternal antibodies are

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known to decline to undetectable levels in about 6–8 months (Beem, Egerer & Anderson, 1964). Sera of 31 mothers and children obtained during delivery (May, 1971) and 6 months later were compared in one test. These sera were the least likely to be influenced by RSV infections, since this is the period of lowest incidence. The maternal titres showed no significant change during this period (9·4 to 10·0), whereas the geometric mean titre of the infants' sera decreased sharply from 19·0 to 6·3 (titre < 7 considered 6). Only 5 of 31 children showed detectable antibody to RSV at the age of 6 months (titre 7 or 8). Even if the initial antibody titre was high (titre 56–112), no measurable antibodies were found in the infants' sera 6 months later. The relationship between maternal and neonatal titres of this group of 31 mothers and newborns at the time of delivery was similar to that presented in Fig. 1.

The high antibody titres in maternal sera could be the result of a recent infection. As for the high antibody titres in neonatal sera it would be interesting to know the subclass (IgG or IgM). Eight maternal and seven neonatal sera with titres $\geqslant 32$ were fractionated after sucrose density centrifugation. Specific RS antibodies were only found in fractions containing IgG antibodies. In the seven neonatal sera no IgM-containing fractions could be detected. Complement fixation activity of RS-specific antibodies was restricted to the IgG-containing fractions.

Until now, antigenic differences between RSV strains have only been observed in animal sera (Coates, Alling & Chanock, 1966). However, to exclude the possibility that strain differences might influence our results, we have randomly taken 50 paired sera from the 200 pairs mentioned before and tested these for CF antibodies with the DA59 strain of RSV. The relation between neonatal and maternal antibody titres was similar to that illustrated in Fig. 1 for Long strain. Geometric mean titres in neonatal and maternal sera were 24·6 and 11·1 respectively. In comparison, the geometric mean titres of the same sera tested with Long strain antigen were 24·4 and 13·1 respectively.

One hundred serum pairs, taken at random from the 200 pairs mentioned before, were tested for neutralization antibodies. Fig. 2 shows the result of one hundred neonatal sera in comparison with the corresponding maternal sera. All individuals had detectable neutralization antibody titres. Negative controls were included in the test. As the graph indicates, neonatal and maternal titres were generally similar. Differences were not significant (P value is 0·03 in the sign test, Siegel, 1956). Geometric mean titres were 79·1 for neonatal and 74·6 for maternal sera.

Comparison of neutralization antibody titres in maternal and neonatal sera (Fig. 2) showed that while the geometric mean titres were equal, 56 % of the sera showed higher titres in neonatal than in maternal sera; in 36 % of the serum pairs higher titres were found in maternal sera.

Serum pairs, showing a higher titre in neonatal than in maternal serum in the neutralization test, were observed in the complement fixation test. Geometric mean titres of this group of sera were 26·5 and 13·4 for neonatal and maternal sera respectively. These values resemble the means of the whole group of 200 paired sera.

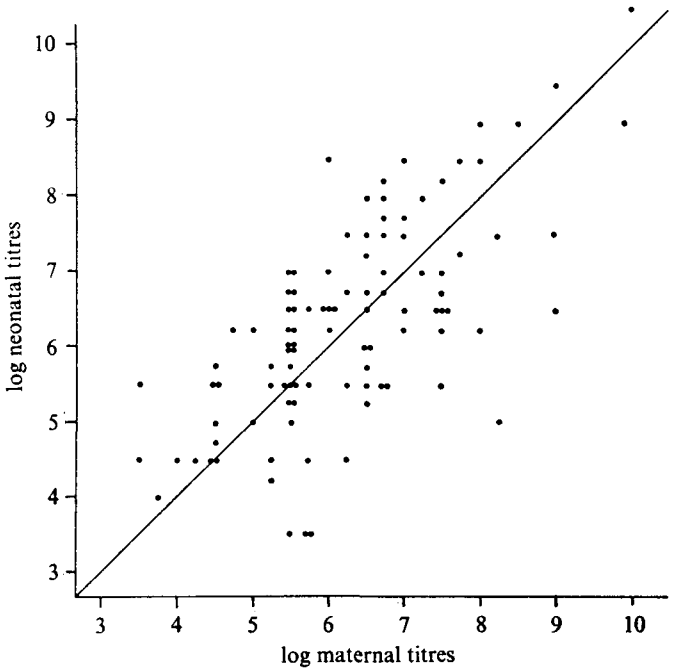

Fig. 2. Comparison of neutralization antibody titres (log_2) of 100 maternal and neonatal sera.

DISCUSSION

In former studies the serum titres of a number of viral antibodies have been investigated in cord blood and maternal sera by means of complement fixation or haemagglutination inhibition or neutralization tests. CF antibodies were significantly higher in neonatal than in maternal sera for influenza A, adeno, parainfluenza 1 and 3, herpes simplex and measles virus. No significant differences could be demonstrated for RS, mumps and cytomegalo virus (Toivanen, Mäntyjärvi & Hirvonen, 1968; Mäntyjärvi, Hirvonen & Toivanen, 1970; Stelzner *et al.* 1973).

Antibody titres in maternal and cord sera were determined by haemagglutination inhibition tests for measles, rubella, and influenza A and B. HI antibody titres to measles and rubella virus were significantly higher in neonatal than in maternal sera (Brouwer, de Groot & Verheij, 1974). Sera used in our study were also applied in another investigation and showed a higher concentration of antibodies against influenza A and a lower concentration against influenza B virus for neonates than for the corresponding mothers (Masurel, de Bruijne, Beuningh & Schouten, 1977).

In another study no significant differences were found between neutralization antibody titres against RSV in maternal and neonatal sera (Beem *et al.* 1964).

We have measured RSV complement fixation and neutralization antibodies simultaneously for maternal and neonatal sera. Equal titres of neutralization

antibodies were detected in maternal and neonatal sera. Complement fixation antibody titres, however, differed considerably. About twice as much antibody was present in neonatal as in maternal sera. Results were similar if recently obtained sera (1–2 weeks old) were used (unpublished results).

High titres of viral antibodies found in neonatal sera in comparison with maternal sera are sometimes ascribed to a high IgG concentration in neonatal sera, but this has not been definitely confirmed by literature data (Longworth, Curtis & Pembroke, 1945; Gitlin, Kumate, Urrusti & Morales, 1964; Fulginiti, Sieber, Claman & Merrill, 1966; Kohler & Farr, 1966; Michaux, Heremans & Hitzig, 1966).

To link viral antibody titres to the IgG concentration in neonatal sera might be relevant except for the observation that the extent of placental passage is different for antibodies evoked by different antigens. Moreover, our results show that even functionally different antibodies against one antigen may pass the placenta to a different extent. The clinical and epidemiological significance of our observation is still to be substantiated.

Maternally derived serum antibodies are supposed to offer protection against viral infections in early childhood. However, in a number of studies it was concluded that an interaction of RSV antigen with the immune system might be the cause of severe illness (Chanock *et al.* 1970). In former studies much attention has been paid to the hypothesis that a type III (Arthus type) reaction might occur (Gell & Coombs, 1968). Usually complement is fixed in an Arthus type reaction, suggesting a role for complement fixation antibodies. Recent literature is focused on immuno-suppression (Parrott, Kim, Brandt and Chanock, 1974) although cell-mediated immunity (Kim *et al.* 1976) or an age dependent factor (Prince & Porter, 1976) may also play a role in the pathogenesis of RSV disease. Immunosuppression may be the result of the presence of serum neutralization antibodies in newborns (Parrott *et al.* 1975). Whether any of these hypotheses is preferable, remains a matter of discussion. The postulated involvement of serum neutralization antibodies in an Arthus type reaction or in immuno-suppression may be valid as much for complement fixation antibodies (Pyhälä & Kleemola, 1976). Considering the high transfer through the placenta of complement fixation in relation to neutralization antibodies, the role of complement fixation antibodies in the pathogenesis of RSV disease needs further study.

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