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Antibody against viruses in maternal and cord sera: specific antibody is concentrated on the fetal side of the circulation

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SUMMARY

Paired maternal and cord sera from 100 pregnancies were tested for antibodies against herpes simplex virus, measles virus and respiratory syncytial virus by complement fixation and for antibodies against rubella virus, influenza A virus and influenza B virus by haemagglutination-inhibition. For four viruses (herpes simplex, measles, respiratory syncytial and rubella) higher levels of antibody were found in cord than in maternal sera. There was no difference between maternal and cord serum titres against influenza B virus but significantly higher levels of antibody against influenza A virus were found in maternal sera than in cord sera. This discrepancy was investigated by measuring antibodies against the surface antigens of influenza A by a complement fixation technique, and by single radial haemolysis. Both methods showed a preponderance of virus-specific antibody in cord sera. We conclude that IgG antibodies against most, if not all, viruses are concentrated on the fetal side of the circulation, but that conventional haemagglutination-inhibition techniques may fail to detect this difference.

INTRODUCTION

Several investigators have measured the total immunoglobulin G (IgG) concentration in maternal and corresponding cord sera and have found the IgG concentration to be higher on the fetal side of the circulation (Longsworth, Curtis & Pembroke, 1945; Kohler & Farr, 1966). This phenomenon has been attributed to active transport of IgG molecules (Kohler & Farr, 1966) and is presumably of teleological importance in providing passive immunization of the neonate. Accordingly, several workers have investigated the antiviral function of the transferred antibodies by means of a variety of conventional techniques.

Complement fixing (CF) antibodies against influenza A virus, adenovirus, parainfluenza viruses 1 and 3, herpes simplex virus (HSV), measles virus (MV) and respiratory syncytial virus (RSV) were found to higher titre in cord sera than in maternal sera (Toivanen, Mantyjarvi & Hirvonen, 1968; Mantyjarvi, Hirvonen & Toivanen, 1970; Heijtink *et al.* 1977). Likewise, haemagglutination-inhibiting (HI) antibody titres against MV, rubella virus (RV), dengue, a coronavirus and a strain

of influenza A were found to be higher in cord than in maternal sera (Brouwer, DeGroot & Verheij, 1974; Ventura, Ehrenkranz & Rosenthal, 1975; Masurel *et al.* 1978; Sarateanu, Ehrengut & Fofana, 1980). However, the opposite trend was noted when further strains of influenza A (Sarateanu, Ehrengut & Fofana, 1980) or of influenza B (Masurel *et al.* 1978; Sarateanu, Ehrengut & Fofana, 1980) were employed in the HI tests. When measured by neutralization, influenza A antibody titres were higher in cord sera (Masurel *et al.* 1978). However, neutralizing antibodies against RSV were found to similar titre in both maternal and cord sera (Heijtink *et al.* 1977).

Several of these earlier studies looked at many different viruses, often using small numbers of paired serum samples. We decided to investigate the phenomenon of passive immunization of the fetus by testing a large number of paired cord and maternal serum samples. For these studies virus-specific antibodies which have previously been reported to be preferentially sequestered on either side of the placental barrier were measured.

MATERIALS AND METHODS

Collection of serum samples

Cord sera were obtained at the time of delivery by expressing the umbilical cord contents into a sterile container. Maternal sera were obtained by venepuncture 3 days after delivery.

Cord and maternal sera obtained in October and November 1978 as part of another study (Griffiths, Campbell-Benzie & Heath, 1980) from 100 patients were employed initially. More detailed experiments were conducted later with a further 100 paired samples collected during August and September 1980.

Serological techniques

CF antibodies against HSV, MV and RSV were measured by a standard technique (Hawkes, 1979) using antigens kindly provided by the Public Health Laboratory Service.

Antibodies against RV were measured by HI after kaolin treatment as previously described (Doherty *et al.* 1975).

Antibodies against influenza A/Texas/1/77 (H3N2) and influenza B/Hong Kong/8/73 were measured by HI after treatment with receptor-destroying enzyme (Wellcome Reagents Ltd) as previously described (Griffiths, Ronalds & Heath, 1980) using viruses propagated in embryonated hens' eggs. In one experiment, haemagglutinin prepared from a recombinant influenza strain (A/Alaska/5/77 [H3N2] and A/PR/8/34 [H0N1]), known to be relatively resistant to the action of serum non-specific inhibitors, was employed.

Influenza A/Texas/1/77 was partially purified for use in the single radial haemolysis (SRH) test, as follows. Allantoic fluid containing the virus was clarified by centrifugation at 500 *g* for 10 min. The supernatant was then centrifuged in a fixed angle rotor at 53000 *g* for 90 min. The resulting pellets were resuspended in a small volume of phosphate-buffered saline (PBS) and were ultrasonicated for

60 s (MSE ultrasonic disintegrator). The resulting virus preparation was stored in aliquots at −70°C and was shown to contain 5000 haemagglutinating doses/ml. The SRH test was otherwise performed exactly as described by Oxford *et al.* (1979) except that 8% (v/v) sheep erythrocytes were used. Sera (2·5 μl) were added to wells of 2 mm diameter cut in the gels and the diameters of the zones of haemolysis were measured after overnight incubation at 37 °C.

Antibodies against the surface antigens of influenza A/Texas/1/77 were measured by CF as follows. Infected allantoic fluid (200 ml) was clarified by centrifugation at 500 ***g*** for 10 min. The supernatant was then centrifuged in a fixed angle rotor at 78000 ***g*** for 90 min and the pellets were resuspended in 40 ml PBS. Washed chicken erythrocytes (4 ml packed cells) were added and the mixture was held overnight at 4 °C. Soluble antigens were removed from the mixture by two cycles of low-speed centrifugation at 4 °C (500 ***g*** for 10 min) followed by gentle resuspension of the erythrocyte–virus complex in barbitone buffer (Oxoid BR16). After the second centrifugation, the suspension was shaken gently at 37 °C for 60 min to permit dissociation of virus. The suspension was then centrifuged at 500 ***g*** for 10 min at 37 °C to pellet the erythrocytes, and the supernatant, containing eluted virus, was removed. This supernatant was first tested in a CF chessboard titration and two CF units were then used as antigen in the standard CF test to detect antibodies in sera.

Statistical analysis

The significance of each observed difference between the geometric mean titres of virus-specific antibodies in paired cord and maternal sera was assessed by *t* test (Armitage, 1971).

RESULTS

The titres of antibodies against six viruses found in maternal and cord sera are depicted graphically in Fig. 1. It will be seen that for the viruses represented in the upper four panels of this figure, CF or HI antibodies were found to higher titre more frequently in cord than in corresponding maternal sera. However, the reverse trend was evident in the case of influenza A since, in general, higher titres were found on the maternal side of the circulation. For influenza B, no trend towards concentration of HI activity on either side of the placenta was evident. Fig. 1 also shows that, when higher concentrations of antibody specific for a particular virus were found in cord sera, this occurred with all levels of antibody. Thus, although the best-fit regression lines for two viruses (RSV; HSV) appear to cross the central axis, inspection of the individual data points reveals that the active transport mechanism for IgG did not cease to operate once a particular IgG concentration had been provided for the fetus.

The differences between the geometric mean titres (GMTs) found in matched maternal and cord sera were next analysed statistically and the results are presented in Table 1. The preponderance of antibody on the fetal side of the circulation was statistically highly significant for the four viruses previously

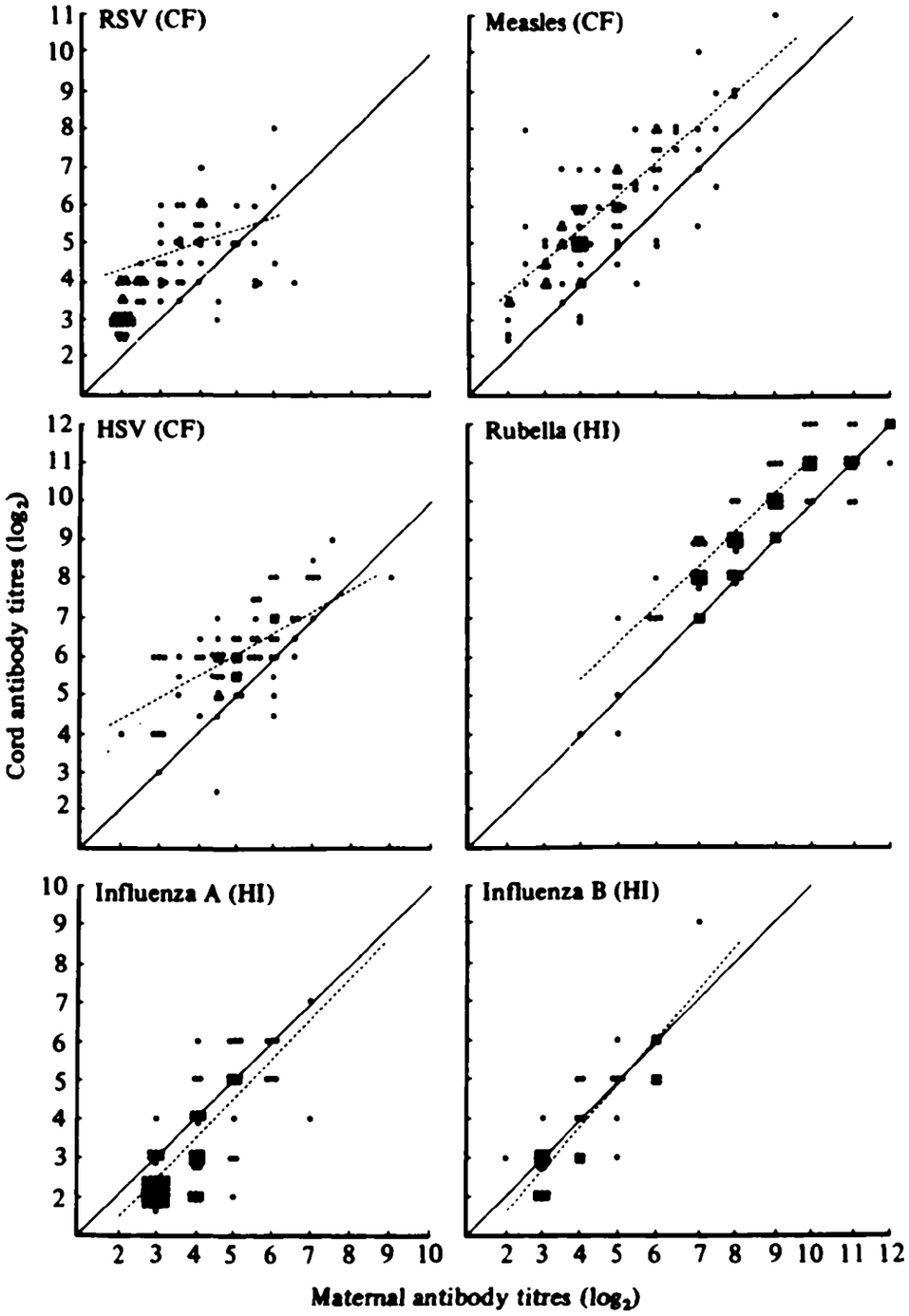

Fig. 1. Distributions of antibody titres against six viruses found by complement fixation or by haemagglutination-inhibition in 100 paired maternal and cord sera. Each point represents the titres found in a pair of maternal cord sera. Pairs without detectable antibody in either sample are not shown. The broken lines represent the best-fit linear regressions for each set of data.

described (HSV, RSV, MV, RV). However, the contrasting higher mean HI titre against influenza A found in maternal sera was also statistically highly significant.

It was considered probable that the apparent anomalous distribution of HI activity against influenza A had resulted from the influence of non-immunoglobulin moieties such as non-specific inhibitors of haemagglutination that had not been removed by treatment with receptor-destroying enzyme. Accordingly, antibodies against influenza A were next measured by a series of techniques which are less

Table 1. *Geometric mean antibody titres against each of six different viruses found by complement fixation or haemagglutination-inhibition in 100 paired maternal and cord sera*

Virus	Test	Number of seropositives	Geometric mean titre (log_2) Cord	Maternal	Difference	Significance of difference (P)
Herpes simplex	CF	74	6·05	5·09	+0·96	<0·001
Measles	CF	95	5·77	4·60	+1·17	<0·001
Respiratory syncytial	CF	80	4·24	3·27	+0·97	<0·001
Rubella	HI	93	9·42	8·66	+0·76	<0·01
Influenza A	HI	87	3·22	3·91	−0·69	<0·001
Influenza B	HI	42	3·93	4·10	−0·17	>0·5

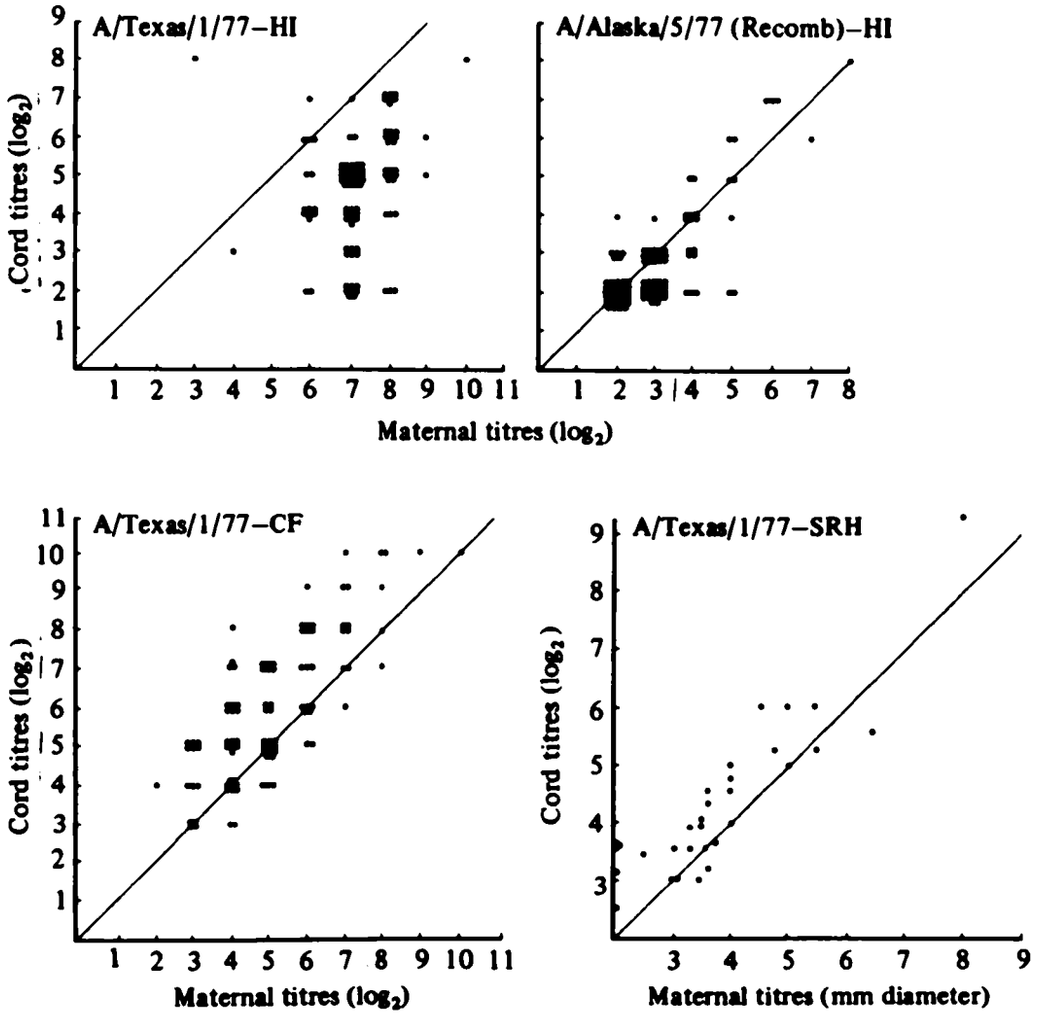

Fig. 2. Antibodies against influenza A viruses detected in 100 paired maternal and cord sera by conventional haemagglutination-inhibition and by three other techniques less affected by the presence of non-specific inhibitors of haemagglutination. Sufficient serum was available to test only 87 of the 100 pairs of sera by SRH.

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affected by the activity of such non-specific factors. For these experiments, a further group of 100 paired cord and maternal sera were employed. Fig. 2 shows that, as with the original batch of sera, higher HI titres against influenza A/Texas were found in maternal sera. However, when the sera were tested against a recombinant H3N2 strain of influenza A virus, which is known to be relatively insensitive to the action of non-specific inhibitors, higher HI titres were no longer found in the maternal samples. Furthermore, when antibodies against surface antigens were measured by CF, higher antibody levels were found in cord rather than maternal sera, in a pattern reminiscent of that depicted previously in Fig. 1 for four other viruses. Finally, when measured by SRH, larger zones of haemolysis were given by more cord sera than by their corresponding maternal sera (Fig. 2).

DISCUSSION

This study has confirmed previous work showing that higher levels of antibodies against many viruses are found on the fetal side of the circulation (Toivanen, Mantyjarvi & Hirvonen, 1968; Brouwer, DeGroot, & Verheij, 1974; Ventura, Ehrenkranz & Rosenthal, 1975; Heijtink *et al.* 1977; Sarateanu, Ehrengut & Fofana, 1980). It is known that IgG antibodies are preferentially concentrated by an active transport mechanism, (Kohler & Farr, 1966), so that the neonate is passively immunized against many infectious agents. It was therefore surprising to find in the present study that antibodies against influenza A virus were of lower titre on the fetal side of the circulation. Discordant results have also previously been reported for influenza A (Sarateanu, Ehrengut & Fofana, 1980) and for influenza B (Sarateanu, Ehrengut & Fofana, 1980; Masurel *et al.* 1980) by workers who found higher levels of antibodies against these viruses on the maternal side of the circulation. Several possible explanations for such a phenomenon can be envisaged.

First, it is possible that the fetus has been provided with a means of procuring antibodies against some viruses but not against others. This possibility seems rather unlikely since the Fc portion of the IgG molecule is the part responsible for placental transport rather than the antigen-specific Fab portion. Secondly, antibodies capable of fixing complement might be more preferentially concentrated than those mediating inhibition of haemagglutination. This explanation also seems unlikely since, in the present study, higher levels of HI antibodies against rubella were found in cord sera. Furthermore, although IgG 2 and IgG 4 fix complement poorly or not at all, it has recently been shown that all four subclasses of IgG are equally transferred across the placenta (Pitcher-Wilmott, Hinducha & Wood, 1980) so that a sub-class specific immune response to a particular virus cannot be invoked to explain the phenomenon.

Thirdly, it is possible that the unexpected results obtained for influenza are not mediated solely by immunoglobulins. Various non-specific inhibitors have been described that affect the results obtained when the HI test is used to measure antibodies against influenza A (Hawkes, 1979). The results of the present invest-

igation clearly suggest that this last explanation is correct since, when measured by a CF technique which is thought not to be affected by non-specific inhibitors, an apparent preponderance of antibodies in maternal sera reverted to the expected pattern. Furthermore, antibodies against influenza A were detected to higher titre in cord sera when measured by SRH, another technique which is unaffected by non-specific inhibitors (Russell, McCahon & Beare, 1975). Presumably these factors are present to higher titre in maternal than in cord sera, or are less readily removed from the former. Haemagglutination-inhibition techniques appear to give reliable results for rubella (Brouwer, DeGroot & Verheij, 1974), dengue virus (Ventura, Ehrenkranz & Rosenthal, 1975) and a coronavirus (Sarateanu, Ehrengut & Fofana, 1980) which suggests that the types of non-specific inhibitors active in these assays are either not found predominantly in maternal sera or are being effectively absorbed. Experiments are currently underway in this laboratory to evaluate these various possibilities.

We conclude that a system has evolved whereby the fetus becomes passively immunized with IgG antibodies of maternal origin. Such antibodies may be active against a wide variety of viruses and are concentrated on the fetal side of the circulation to provide protection for the first few months of infancy. Earlier reports suggesting that this mechanism might not protect the neonate against all viruses to which the mother has become immune could not be substantiated.

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