*J. Hyg., Camb.* (1984), **92**, 297–301
*Printed in Great Britain*



# Antibody against viruses in maternal and cord sera: non-specific inhibitors are found to higher titre on the maternal side of the circulation

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(*Received* 23 *December* 1983; *accepted* 27 *January* 1984)

## SUMMARY

Pregnancies were identified in which maternal IgG antibodies against rubella virus were not detectable by single radial haemolysis. Twenty paired maternal/cord sera were then tested for haemagglutination-inhibiting (HI) activity against rubella virus without kaolin pretreatment of the sera. In the absence of specific antibody, the HI activity observed could thus be ascribed to the effect of non-specific inhibitors. The HI activity in maternal sera was significantly ($P < 0{\cdot}001$) higher than that in cord sera. The 20 pairs of sera were similarly tested against a bunyavirus, an alphavirus and a flavivirus, both with and without kaolin pretreatment. The results showed non-specific inhibitors were found to higher titre in maternal sera, with the difference being statistically significant ($P < 0{\cdot}001$) for each of the three viruses.

## INTRODUCTION

Antibodies of IgG class are concentrated across the placenta so that the neonate is passively immunized with this immunoglobulin (Longsworth, Curtis & Pembroke, 1945; Kohler & Farr, 1966). It would therefore be expected that the titres of antiviral antibodies found in maternal and cord sera would reflect this difference, but reports in the literature have given conflicting results, especially when haemagglutination-inhibition (HI) assays have been employed (Brouwer, De Groot & Verheij, 1974; Ventura, Ehrenkranz & Rosenthal, 1975; Masurel *et al.* 1978; Sarateanu, Ehrengut & Fofana, 1980).

We have previously studied this phenomenon and have reported that complement-fixing antibodies specific for herpes simplex, measles and respiratory syncytial viruses and HI antibodies against rubella virus were indeed concentrated on the fetal side of the circulation (Griffiths *et al.* 1982). In the same sera, antibodies against strains of influenza A and influenza B would only produce similar patterns

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when tested by assays which are unaffected by non-specific inhibitors (NSI) of haemagglutination. We therefore suggested that the conflicting results in the literature could be explained if NSI were found to higher titre in maternal sera than in cord sera, or if NSI were more readily destroyed or removed from the latter specimen by the methods routinely employed (Griffiths *et al.* 1982; Grint, Argent & Heath, 1982). In this paper we have addressed these questions directly by devising assays which allow the titration of NSI in maternal and cord sera.

## MATERIALS AND METHODS

### *Collection of serum samples*

Twenty pairs of matched cord and maternal sera were obtained from pregnancies being investigated as part of another study (Griffiths & Baboonian, 1984). These samples were chosen because the mothers had been shown to lack rubella-specific antibodies when tested by single radial haemolysis. Since these sera did not therefore contain antibodies against the virus, they could be titrated by HI without kaolin pretreatment, to provide an assay for NSI. In parallel experiments the sera were also tested after absorption with kaolin to ensure that rubella-specific antibodies were not interfering with the assay.

The same 20 sera were also titrated, with and without kaolin pretreatment, against three arboviruses. It was expected that most women residing in London would not have encountered these exotic infections, so that NSI could be assayed without interference from virus-specific antibodies.

Similar experiments were conducted to measure factors which inhibit haemagglutination induced by influenza A. Three strains of virus were chosen which are not recognized human pathogens so that virus-specific antibodies should not be found in human sera. For the studies reported here, sera were selected from a further six women from the same population for this purpose.

### *Serological techniques*

For rubella virus, the techniques of single radial haemolysis (Morgan-Capner *et al.* 1979) and HI (Doherty *et al.* 1975) were performed as described previously. The initial serum dilution for the HI test was 1 in 8.

For bunyamwera (a bunyavirus), sindbis (an alphavirus) and dengue type I (a flavivirus), antibodies were measured by HI using a standard technique with 8 HA units of each virus and kaolin to absorb NSI (Clarke & Casals, 1958). These experiments were performed at the Special Pathogens Reference Laboratory, Porton, using an initial serum dilution of 1 in 10.

A variety of strains of influenza A viruses was kindly provided by Dr G. Schild. Each virus was propagated in the allantoic cavity of fertile hen's eggs. The allantoic fluid was clarified by centrifugation at 500 *g* for 10 min and the supernatant was used as HA antigen. Serum HI activity was measured starting at a serum dilution of 1 in 8, as described by Griffiths, Ronalds & Heath (1980) after treatment with receptor-destroying enzyme (Wellcome Reagents, Ltd).

### *Statistical methods*

The significance of differences between distributions of HI titres in cord and maternal sera was assessed by *t*-test (Armitage, 1971).

Table 1. *Results of titrating, without kaolin pretreatment, 20 matched maternal and cord sera for haemagglutination-inhibiting activity against four viruses*

| Virus | Number of serum pairs analysed | Haemagglutination-inhibition titres ($log_2$) Maternal Range | Maternal GMT (S.D.) | Cord Range | Cord GMT (S.D.) | Statistical analysis* $t$ | $P$ |
|---|---|---|---|---|---|---|---|
| Rubella | 20 | 5·00–8·00 | 6·35 (0·79) | 3·00–6·00 | 4·35 (0·91) | 7·42 | < 0·001 |
| Bunyamwera | 20 | 6·32–8·32 | 7·22 (0·44) | 4·32–8·32 | 5·37 (0·97) | 7·76 | < 0·001 |
| Sindbis | 20 | 5·32–7·32 | 6·77 (0·67) | 3·32–7·32 | 4·52 (0·98) | 8·48 | < 0·001 |
| Dengue | 17† | 6·32–7·32 | 6·79 (0·50) | 3·32–7·32 | 4·79 (0·85) | 8·38 | < 0·001 |

* For comparison between maternal and cord sera for each virus.

† Three pairs contained HI activity after kaolin treatment and so are excluded from analysis.

Table 2. *Titration of maternal sera, with or without receptor-destroying enzyme treatment, for haemagglutination-inhibiting activity against influenza A virus strains*

| Virus strain | Haemagglutination-inhibition titres* in serum nos. 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| A/Texas/77 (H3N2) | 256/16* | 256/16 | 256/16 | 512/64 | 128/32 | 128/16 |
| A/Equine/Prague/56 (H7N7) | < 8/< 8 | < 8/< 8 | < 8/< 8 | < 8/< 8 | < 8/< 8 | < 8/< 8 |
| A/Duck/England/56 (H11N6) | | | | | | |
| A/Duck/Alberta/76 (H12N5) | | | | | | |

* Shown as titres without/with RDE treatment.

## RESULTS

### *Rubella serology*

Twenty pairs of maternal and cord sera were tested by HI after kaolin absorption; as expected, none of the sera had rubella-specific antibodies detected by HI (data not shown). However, all of the maternal sera had detectable HI activity when kaolin absorption was omitted, with a geometric mean titre of 6·35. All of the cord sera also had HI activity under the same circumstances but their geometric mean titre (GMT) (4·35) was significantly ($P < 0·001$) less than that of the corresponding maternal sera (see Table 1).

### *Arbovirus serology*

The 20 pairs of sera were tested, after kaolin absorption, for antibodies against bunyamwera, sindbis and dengue type I. Three pairs of sera contained low level HI activity against dengue, but the remaining 57 pairs of titrations gave clearly negative reactions. These three sera had no HI activity after acetone extraction, showing that true antibody against dengue was not present. Nevertheless, the whole serum HI results of these three pairs of sera were excluded from the subsequent analysis of NSI activity.

Table 1 shows the results of testing, without kaolin absorption, the 20 pairs of sera against all three viruses by HI. All sera contained detectable levels of HI activity which, in 57 cases, could be ascribed to activity of NSI. For each virus, the maternal GMT was significantly ($P < 0{\cdot}001$ for each comparison) greater than the cord GMT. In none of the 57 pairs was the cord HI titre greater than the corresponding maternal HI titre.

Inspection of the individual results showed that, for each maternal serum, the titre of NSI against each of the three viruses was identical, or varied by only one well This suggests that identical NSI were being measured by each viral assay. More variation was seen in the results for the cord sera, with four sera in particular showing up to three-well differences when tested against the three viruses.

*Influenza serology*

Four strains of influenza A virus were titrated against six maternal sera, both with and without serum pre-treatment with receptor-destroying enzyme (RDE), and the HI results obtained are shown in Table 2.

All six sera contained antibodies against a recently circulating H3N2 human strain (A/Texas/77). As expected, no serum antibody was found in the six sera when tested against three animal strains of influenza A which are not recognized human pathogens. Since these three viruses do not share antigenic determinants with human strains of influenza A, we predicted that they would provide an assay system for NSI against influenza A. In practice, as shown in Table 2, no HI activity was detected in any serum sample against the three antigenically distinct virus strains.

## DISCUSSION

This work has unequivocally demonstrated that NSI of virus-induced haemagglutination are found to higher titre in the maternal than in the fetal circulation. This was shown to be true for a togavirus (rubella), a bunyavirus (bunyamwera), an alphavirus (sindbis), and a flavivirus (dengue). In each of these cases the NSI are known to be lipoproteins, which are readily absorbed by treatment with kaolin. This finding emphasizes that, if erroneous results are to be avoided, an excess of kaolin must be added to maternal and cord sera to ensure that all traces of NSI are absorbed before titration is performed. In all cases, NSI were found in cord sera, albeit at a lower titre, so it remains to be shown whether the lipoprotein NSI in cord sera have been transferred across the placenta from the mother or whether they have been produced by the fetus *in utero*.

In contrast, it was not possible to determine whether the factors which affect HI tests for influenza type A are differentially concentrated on one side of the placenta. Six maternal sera were examined and all contained antibodies against a recently circulating H3N2 strain, showing that these women had been exposed to type A influenza virus. However, no HI activity against three animal strains of influenza A virus was found either before or after RDE treatment of the same six sera. We did not expect to find true serum antibody against the three viruses with novel antigenic structures, but our inability to demonstrate serum HI activity before RDE treatment fails to provide evidence for the existence of NSI acting

in the way that NSI interfere with rubella or arbovirus HI assays. This suggests that the factors which affect the HI test for influenza do so either in conjunction with true antibody or that the factors are more specific than has been assumed and are induced only when an individual is infected by a particular strain of influenza virus. At present, the chemical nature of the factors involved is poorly defined, but work is currently under way in this laboratory in an attempt to clarify the situation.

We wish to thank the medical and nursing staff of the Department of Obstetrics for their co-operation and the Wellcome Trust for financial support.

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