Immunoglobulin concentration in mothers with leprosy and in healthy controls and their babies at the time of birth

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Summary Immunoglobulins were quantitated in sera from 52 matched mothers at delivery and in the corresponding cord blood samples. The cord IgA concentration was significantly increased in babies from mothers with active lepromatous leprosy compared to a control group, and a group where the mothers suffered from tuberculoid leprosy. The cord IgM concentration was normal both in babies from mothers with active lepromatous leprosy, the control group and the group of mothers suffering from tuberculoid leprosy. Since IgA does not cross the placenta, this increase reflects an active increased production of IgA in the foetus of mothers suffering from active lepromatous leprosy. This could indicate transfer of M. leprae or M. leprae antigens across the placenta into the foetus.

Introduction

In some maternal infections, namely rubella, cytomegalovirus infection, toxoplasmosis and syphilis, transplacental transfer of pathogens occurs (Alford, 1962; Scotti and Logan, 1968; McCracken and Shinefield, 1965) causing a severe generalized infection in the foetus. Under these circumstances, the foetus can start to produce antibodies in utero. This antibody production is sometimes indicated by increased IgM and/or IgA concentration in cord blood (Stiehm et al., 1966), and may be proven by specific antibodies of the IgM or IgA class against the infectious agent (Scotti and Logan, 1968; Reimer et al., 1975).

Up to the present time leprosy has not been demonstrated to cause intrauterine infection. The first clinical signs of manifest leprosy have been

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demonstrated at 1.5 to 2 years of age (Soul, 1958;6 Worth, 1960).7 Based upon these observations, it was generally thought that leprosy bacilli do not infect the foetus. The long incubation period before the appearance of definite clinical signs and symptoms has made the subject difficult to study.

A prospective study on the effect of leprosy on pregnancy, parturition and of the baby was carried out in Addis Ababa, Ethiopia, from 1975 to 1978. As part of this study, IgA, IgM and IgG were quantitated during pregnancy and at birth. We were especially interested in the increase of IgM and/or IgA concentration in cord blood as an indication of foetal exposure to M. leprae antigen(s).

Materials and methods

Patients

Fifty-two pregnant women were selected for this study. All of them attended the outpatient clinic at the Addis Ababa Leprosy Hospital. The patients were clinically and histologically classified according to the Ridley-Jopling scale (Ridley and Jopling, 1966)8 and were divided into four groups.

Group 1

Seventeen mothers who suffered from lepromatous leprosy (LL-BL) with a positive bacterial index (BI), i.e. with acid fast bacilli in one or several skin smears taken from 6 different sites. All of them were treated with 100 mg DDS daily.

Group 2

Five mothers who suffered from lepromatous leprosy with a negative BI (LL-BL). Four of these patients received DDS, 50 to 100 mg daily, and had been on continuous treatment for at least 5 years. One patient had stopped treatment prior to the study.

Group 3

Sixteen mothers who suffered from tuberculoid leprosy (BT). Six patients still received DDS treatment (50–100 mg daily) and 10 patients were released from control before the trial started and did not receive any treatment.

Group 4

Fourteen mothers without any clinical sign of leprosy (NL), but with the same socio-economic background as the leprosy patients.
The patients were divided into these 4 groups to separate the mothers with highly bacilliferous disease from the others, since the foetus in the first group would have the greatest chance of being exposed to *M. leprae* or their antigens. All the patients were Ethiopians living in the villages around the leprosy hospital under poor socio-economic conditions. Serum samples were obtained during the last trimester of pregnancy, and from the mother at delivery and from cord blood. The serum samples were stored at $-20^\circ C$ and freeze dried before transportation to Norway. Prior to estimation of the immunoglobulin concentration, the sera were reconstituted with distilled water, and 0.01% NaN$_3$ was added as a preservative. They were subsequently stored at $+4^\circ C$.

**IMMUNOLOGICAL METHODS**

Immunoglobulin concentrations were determined by the single radial diffusion technique (Mancini *et al.*, 1964; Mancini *et al.*, 1965; Fahey and McKelvey, 1965) with specific anti-IgG, anti-IgA and anti-IgM (Dakopatts a/s, Copenhagen, Denmark) in 1% Litex agarose gel (Litex a/s, Glostrup, Denmark) containing 0.05 M barbiturate buffer of pH 8.6. The antisera were tested for specificity by immunoelectrophoresis and single radial diffusion using sera and isolated immunoglobulins from patients with myeloma and macroglobulinaemia, and sera from individuals with isolated lack of IgA. The sera were found to be monospecific by these methods.

Immunoglobulins in the maternal sera were quantitated by the single radial diffusion method routinely used in our laboratory. A 1.5 mm thick agarose gel was made on a glass plate of $11 \times 20$ cm. The total volume of agarose gel on the plate was 44 ml and the amount of anti-IgG 3.7 ml, i.e. 0.17 ml anti-IgG/cm$^2$. In this plate, 66 wells were punched out with a diameter of 2 mm. A volume of 5 μl of either standard or sera to be tested was filled in the wells. Locally prepared IgG standard solutions were used and controlled at regular intervals against the Behringwerke's IgG standard (Behringwerke AG, Marburg, Frankfurt/M, Germany). The plates were left in a moist chamber at room temperature for 24 hrs. The precipitin rings had a sharply defined edge and were measured directly on the unstained plates.

The IgA and IgM concentrations in maternal sera were determined in the same way using 0.04 ml anti-IgA or anti-IgM per cm$^2$ gel. The Behringwerke IgA and IgM standards were used to prepare the standard curves.

The amount of IgA and IgM in cord blood is so low that it is difficult to determine the concentration by single radial diffusion methods (Papadatou *et al.*, 1969; Evans *et al.*, 1971). The technique was modified to ensure that minute amounts of IgA and IgM could be detected. The concentration of anti-IgA was lowered to 0.35 μl anti-IgA/cm$^2$ in the agarose gel. At this point, weak but definite precipitin rings with sharply defined edges could be seen after staining with Coomasie brilliant blue when minute amounts of IgA were put in the wells. This concentration of anti-IgA in the gel was therefore chosen
to detect IgA in the cord sera. Wells with a diameter of 2 mm were made and filled with 5 μl either standard or test sera. The plates were left in a moist chamber for 48 h, washed and pressed 4 times, left for a final wash overnight, dried and stained with Coomassie brilliant blue. In this way distinct precipitin rings were obtained demonstrating IgA in concentrations down to $4 \times 10^{-3}$ g/l, and the IgA concentration could be determined if above $8 \times 10^{-3}$ g/l.

The concentration of IgM in the cord blood is 5 to 10 times higher than IgA (Faulkner and Borella, 1970; Hardy et al., 1969). It was therefore easier to determine the IgM concentration in cord blood. The agarose gel contained 0.7 μl anti-IgM/cm². The plates were left at room temperature for 48 h, washed and stained as for the IgA plates. The standard used was diluted Behringwerke IgM standard.

For calculation of the statistical significance of difference between groups, Wilcoxon's modified ranking test was used (Documenta Geigy, 1962).

Results

IMMUNOGLOBULIN CONCENTRATION IN MATERNAL SERA AT DELIVERY

The median IgG concentration in maternal sera at delivery was 8 g/l with a range of 3 g/l to 16 g/l. Figure 1 shows that there was no significant difference between the four groups of patients.

The median IgA concentration was 1.24 g/l with a range of 0.25 to 2.6 g/l.

Figure 1. IgG, IgA and IgM concentration in maternal serum. Each point represents one individual and the top of the columns the median value.
The median IgM concentration was 0.82 g/l with a variation between 0.3 and 2.0 g/l. Neither IgA nor IgM concentration showed any significant difference between the four groups of patients, as shown in Fig. 1.

The IgG concentration in sera obtained from the mothers during the last three months of pregnancy was higher than at delivery. This fall of IgG concentration from pregnancy to delivery was observed in 24 out of 36 women. The 3 women with IgG concentration below 6 g/l at delivery had IgG concentration below 6 g/l when tested in the 3rd trimester.

**IMMUNOGLOBULIN CONCENTRATION IN CORD BLOOD**

Figure 2 shows the IgG concentration in the cord sera. The highest concentration was found in group 1 with a median value of 9.5 g/l, but there was no significant difference between the four groups. There was a good correlation between the IgG concentration in cord blood and maternal sera taken at delivery in each mother–baby pair. Out of 52 pairs, only 12 pairs showed a difference greater than 25% between the IgG concentration in cord blood and maternal blood taken at delivery.

The IgA concentration could be measured by single radial diffusion methods at levels above $8 \times 10^{-3}$ g/l. IgA could be detected if the concentration was above $4 \times 10^{-3}$ g/l, but it could not be quantitated at levels between 4 and $8 \times 10^{-3}$ g/l. These two limits are indicated on Fig. 3 with horizontal dotted lines. Out of 17 cord sera in group 1, two fell below the detection limit of $4 \times 10^{-3}$ g/l, while 12 out of 35 in group 2, 3 and 4 fell below this limit. The median value of each group is indicated on Fig. 3 with a horizontal bar. Group 1 had a median value of $9.5 \times 10^{-3}$ g/l while the median value of group 2, 3

![Figure 2. IgG concentration in cord serum, otherwise as for Figure 1.](image-url)
and 4 fell below $8 \times 10^{-3}$ g/l. This difference is significant using Wilcoxon’s ranking test. The cord serum labelled 110 on Fig. 3 is excluded from the series because of possible leakage of maternal blood into this cord blood sample. The IgM concentration of this cord blood sample was $110 \times 10^{-3}$ g/l, and both the IgA and the IgM concentration was lower in a sample taken 6 weeks after birth from the same baby. In the other cord sera, there was no correlation between high concentration of IgA and IgM, nor between IgA and IgM concentration in matched maternal and cord blood samples.

The IgM concentration in cord sera is shown in Fig. 4. The control group had the highest concentration with a median value of $74 \times 10^{-3}$ g/l, while the median value of the three other groups varied from 40 to $54 \times 10^{-3}$ g/l. These differences were not statistically significant ($p > 0.1$).

Discussion

Leprosy has not yet been described in patients below 1.5 yr (Noussitou et al., 1976), in children it is still uncommon below the age of four, thus it is generally thought that leprosy is not transferred to the foetus.

Leprosy has an incubation time of 2 to 5 yrs (Newell, 1966) which can be partially explained by the slow multiplication rate of *M. leprae* (Shepard and McRae, 1965). After experimental inoculation of armadillo, more than 9

![Figure 3. IgA concentration in cord serum. See text for explanation of the two horizontal dotted lines. The horizontal bars show the median value for the 4 groups.](image-url)
months elapsed before the leprosy bacilli had multiplied sufficiently to cause any clinical sign of leprosy (Kirchheimer and Storrs, 1971; Storrs, 1973). The absence of published reports of leprosy in humans occurring within the first 1.5 years of life could be explained by the long incubation time even though the infection was acquired in utero.

Patients with active lepromatous leprosy, can have up to $10^5$ leprosy bacilli per ml of blood (Drutz et al., 1972). In pregnant women with active lepromatous leprosy, the exposure of the placenta to M. Leprae bacilli is intense and leprosy bacilli could cross the placenta and infect the foetus. However, soon after birth the newborn baby will be exposed to a heavy dose of M. leprae. The lepromatous leprosy mother can shed up to $2.4 \times 10^8$ leprosy bacilli from her nose in 24 h (Davey and Rees, 1974) and leprosy bacilli can be present in breast milk (Pedley, 1967). The long incubation period of leprosy makes it impossible to decide if the baby was infected with leprosy before or after birth.

IgG crosses the placenta, therefore most of the IgG present in the cord blood has been made by the mother. The lower IgG concentration at parturition compared with that of the last trimester of pregnancy has been documented previously (Wagner and Knobloch, 1973; Maroulis et al., 1971). It is probably caused partially by active transport of IgG across the placenta and by increased catabolism in the mother around delivery.

IgM and IgA do not cross the placenta. Increased concentration of these
immunoglobulins in cord blood could be taken as an indication of stimulation of the immune system of the foetus by transfer of antigen(s).

Increased IgM concentration in cord blood may be found in congenital infections such as syphilis, toxoplasmosis and cytomegalovirus infections but even in these diseases heavy congenital infections are not always associated with increased IgM concentration in cord blood (Reimer et al., 1975; McCraken et al., 1965; Hardy et al., 1969). Several workers have determined the IgM concentration in large numbers of cord sera and babies with abnormal IgM concentration were followed for clinical and serological signs of congenital infections. In many instances (from 60 to 80%), babies with increased IgM concentration in cord blood could not be associated with any sign of congenital infection or disease during the first year of life (Hardy et al., 1969; Miller et al., 1969). Furthermore, intra-uterine infections such as syphilis and rubella do not always cause an increase in foetal IgM, therefore increased foetal IgM must be regarded as a non-specific and poor indicator of intra-uterine infection.

Little information is available regarding the IgA concentration in cord blood. In many instances, IgA has only been demonstrated in 5 to 10% of the cord sera examined. This has been due to insufficient sensitivity of the assay, the detection limit being 50–200 × 10⁻³ g/l (Stiehm et al., 1966; Evans et al., 1971; Seth et al., 1971). Faulkner and Borelli (1970) developed a radioimmunoassay for quantitation of IgA in cord blood samples. They found that IgA was present in all cord sera tested in concentrations ranging from 1.5 to 25.5 × 10⁻³ g/l with a mean value of 8 × 10⁻³ g/l. In our sera, we could determine IgA concentration down to 8 × 10⁻³ g/l, and detect but not accurately quantitate down to 4 × 10⁻³ g/l. IgA could be detected in 43 out of 52 cord sera we examined, and the concentration could be determined in 20 out of 52 sera. The median IgA cord concentration in our series is between 4 & 8 × 10⁻³ g/l. Our IgA cord blood concentrations are in agreement with the concentrations found by Faulkner and Borelli.

Increased IgA concentrations were demonstrated in cord blood from babies of mothers with active lepromatous leprosy (group 1). It is significant that at least 9 out of the 16 women in group 1 had an active relapse or were diagnosed as having lepromatous leprosy during this pregnancy. These women would have a large quantity of M. leprae bacilli in their blood stream throughout the pregnancy thus exposing the placenta, and possibly the foetus, to massive antigenic stimulation. The median cord IgA concentration in group 1 was 9.6 × 10⁻³ g/l, while the median cord IgA concentrations in the three other groups (2, 3 and 4) was below 8 × 10⁻³ g/l. Cord IgA concentration was above 8 × 10⁻³ g/l in 9 samples out of 16 in group 1, while the cord IgA concentration was above 8 × 10⁻³ g/l in only 5 samples out of 35 from groups 2, 3 and 4. These differences are significant using Wilcoxon's ranking test. These results indicate that the immune system of the foetus was often stimulated when the mother suffered from active lepromatous leprosy.
Other possibilities should also be considered. Leakage through the placenta could occur due to damage of the placenta in mothers with active lepromatous leprosy. The difference in IgA concentration in maternal and cord serum is great, about 500 times higher in the mother. A small placental leakage would lead to a marked increase in cord IgA concentration. Placental leakage ought to lead to simultaneous leakage of IgM and IgA. IgM concentrations in cord sera were normal, also in group 1. Except for one cord serum, marked 110 on Fig. 3, we have found no indication that the increased IgA in cord blood from group 1 could be caused by leakage. The cord serum marked 110 has been excluded from the calculation due to possibility of placental leakage. The increased IgA concentrations in the other cord sera in group 1 must have been produced by the foetus before birth. This may have been caused by transfer of \textit{M. leprae} or \textit{M. leprae} antigen(s) across the placenta. Studies of the antigenic specificities of these babies' IgA will be studied later.

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