Serum immunoglobulins in multiple pregnancy

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Bryan, E. M., Slavin, B., and Nicholson, E. (1976). *Archives of Disease in Childhood*, 51, 354. **Serum immunoglobulins in multiple pregnancy.** The concentrations of immunoglobulins (Ig) G, A, M, and E were determined in paired umbilical cord and maternal sera in 50 twin pregnancies. Mean IgG levels were higher in cord than maternal sera and in most cases the cord IgG level related more closely to that of the other twin than to either maternal level or birthweight, and was in the range for singletons of the same gestational age.

The three cases of fetofetal transfusion syndrome were exceptional in the large difference between IgG concentrations in recipient and donor twins. The discrepancy was much greater than that found between the levels of proteins produced by the fetus, suggesting a disturbance in maternofetal placental transfer.

IgM was detected in all cord sera, with one exception, and the level was not related to order of birth. IgA was detected in 16% of cord sera, 13% in sera from first borns. IgE was detected in only 8% of cord sera and there was no evidence of placental transfer.

For several centuries it has been recognized that the human infant has a surprising resistance to some infections during the first months of life. That this is due to the transfer of immunity from mother to fetus was first shown by Fischl and Wundtcheim (1895) when they isolated diphtheria antitoxin from human umbilical cord blood. Since then the subject of maternofetal transfer of immunity has been extensively studied and it is now well known that it is the immunoglobulin G (IgG) antibodies which cross the placenta and that, in the human newborn, IgG is almost entirely of maternal origin. Much is still unknown about the regulation of maternofetal transfer of IgG and we hoped that further light might be shed on the subject by studying the pattern of immunoglobulins in multiple pregnancy. Our interest was further stimulated on finding a large intrapair difference in cord serum IgG concentration in 2 male infants suffering from the fetofetal transfusion syndrome (Bryan and Slavin, 1974).

Materials and methods

In addition to the first case of fetofetal transfusion syndrome, a series of 49 unselected pairs of twins born between September 1973 and August 1974 at Hammersmith and Queen Charlotte's Maternity Hospitals (London) and the Maternity Hospital (York) were studied prospectively. Venous umbilical cord blood was collected through a wide bore needle immediately after delivery of the placenta, and in 46 cases venous blood was collected from the mother within 24 hours of delivery. The serum was separated and stored at -10°C .

Serum IgG, IgA, and IgM were determined by radial immunodiffusion (Wootton, 1974). Plates for IgG were prepared in the laboratory using commercial antisera and standards for immunoglobulins. Pools of known value were included in each run. Pools and standards were checked against the MRC 67/69 standard. All specimens were measured in duplicate and corresponding maternal and twin pairs were included in the same plate. Cord serum IgA and IgM were determined using the commercial S-Partigen plates (Hoechst). Serum IgE was measured by a double antibody procedure using a modification of the method described by Gleich, Averbek, and Swedlund (1971). Serum total protein was determined by the density column (Lowry and Hunter, 1945). Serum protein electrophoresis was carried out on cellulose acetate membrane and stained with amidoschwarz 10 B (Wootton, 1974). The strips were scanned on a Zeiss Absorbance recorder with an

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integrating recorder and the albumin concentration calculated using the total protein figure obtained from the density column.

Zygoty was determined by study of sex and a number of genetic markers at the Galton Laboratory, University College London. The markers included red cell antigens (ABO, Rhesus, MNSs, Kell, and Duffy) and enzymes (phosphoglucomutase (PGM₁), adenylate kinase (AK), adenosine deaminase (ADA), phosphogluconate dehydrogenase (PGD), esterase-D (ES-D) and glutamic-pyruvic transaminase (GPT) (Harris, Hopkinson, and Robson, 1974). Of the serum protein markers only α_1 -antitrypsin (Pi) was regularly typed. This range of genetic markers gives a high degree of efficiency for determination of zygoty and thus all pairs alike in both sex and markers were classified as monozygotic. In many instances a monochorionic placenta gave additional evidence in favour of monozygoty.

Cord blood haemoglobin and haematocrit levels were measured and the blood film examined in 40 pairs. In the remaining 10 there was neither clinical nor placental evidence of the fetofetal transfusion syndrome.

Results

Table I shows the sex and zygoty of the infants. Among the monozygotic group three pairs fulfilled the criteria as defined by Rausen, Seki, and Strauss (1965) for the diagnosis of the fetofetal transfusion syndrome in having a cord blood haemoglobin difference of > 5 g/100 ml.

TABLE I

Sex and zygoty of 50 pairs of newborn twins

50 twin pairs						
Monozygotic 23		Dizygotic 25			Unknown 2	
MM	FF	MM	FF	MF	MM	FF
9	14	6	6	13	1	1

M, male; F, female.

IgG. In Fig. 1 the IgG levels of the 100 twin infants are plotted against gestational age and the results superimposed on the normal range for singletons as established in this laboratory by Hobbs and Davis (1967). All calculations were performed on log mg/100 ml. Table II shows that birth order has no effect on the IgG level. The mean level of IgG was higher in the total firstborn group, but, on exclusion of the 3 cases of fetofetal transfusion syndrome, there is no significant difference.

FIG. 1.—Cord sera IgG levels (on logarithmic scale) in 100 twin infants plotted against gestational age. Hatched area indicates normal range for singletons (Hobbs and Davis, 1967).

TABLE II

Cord serum IgG (mg/100 ml) in 50 pairs of twins

	Twin 1 (n=50)	Twin 2 (n=50)
Mean	831 (813)	741 (815)
1 SD range	585–1210 (564–1169)	449–1222 (563–1178)
Range	370–1520 (370–1520)	110–1590 (290–1590)

Parentheses represent fetofetal transfusion syndrome excluded.

The geometric mean level of maternal serum IgG was 656 mg/100 ml (1SD, range 444–948). Comparison of paired maternal and mean intrapair cord IgG (Fig. 2) showed a significantly higher level of IgG in cord than maternal sera ($P < 0.001$). Fig. 3 shows the intrapair comparison of cord IgG levels. Fig. 4 shows that there was no intrapair correlation between birthweight and IgG level, and in the 8 pairs of twins with a weight discrepancy of over 500 g the lighter twin had a higher IgG level in three instances. There is no significant difference between monozygotic and dizygotic twins in the mean intrapair IgG differences after exclusion of the cases of fetofetal transfusion (monozygotic 145 mg/100 ml, dizygotic 134 mg/100 ml).

Four infants—all members of monozygotic pairs—had major congenital malformations and included a pair of conjoined twins and pairs discordant for oesophageal atresia and agenesis of the right lung. None of the affected infants showed

FIG. 2.—Comparison of paired maternal and mean cord sera IgG levels in multiple pregnancy.

FIG. 3.—Intrapair comparison of cord sera IgG levels in twins.

FIG. 4.—Cord sera IgG levels (on logarithmic scale) in twins plotted against birthweight. Twin pairs are joined by lines.

any abnormality in their cord serum immunoglobulin profiles. Likewise, a pair of infants concordant for Coxsackie meningitis had normal serum immunoglobulins. In a dizygous pair discordant for neonatal hepatitis, the discrepancy in IgG levels was one of the largest found in the dizygous group (normal twin 1205 mg/100 ml, twin with hepatitis 862 mg/100 ml). In each of the cases of fetofetal transfusion syndrome, the diagnosis was supported by the characteristic histology of the monochorionic placenta (Aherne, Strong, and Corney, 1968) and an erythroblastosis in the blood film of the donor twin. Table III shows the cord haemoglobin, serum IgG, total protein, and albumin levels in these infants.

IgM and IgA. Serum IgM and IgA concentrations were measured in 48 pairs of twins. IgM was detected in all except one sample and the results are shown in Fig. 5. IgA (up to 5 mg/100 ml) was detected in 17 cord sera—12 first-born and 5 second-born twins—only two pairs were concordant for IgA.

TABLE III
Cord blood results in the fetofetal transfusion syndrome

Case no.		Haemoglobin (g/dl)	Serum IgG (mg/100 ml)	Serum total proteins (g/100 ml)	Serum albumin (g/100 ml)
1	Donor	7.9	180	3.4	2.0
	Recipient	21.5	1450	7.4	3.7
2	Donor	11.4	110	3.0	2.1
	Recipient	21.8	490	6.1	4.3
3	Donor	15.3	640	4.2	3.0
	Recipient	20.9	1250	5.9	4.3

FIG. 5.—Cord sera IgM levels (on logarithmic scale) in first- and second born twins. Showing mean and 1SD range.

IgE. IgE concentrations were measured in 41 pairs of cord and 36 maternal sera. IgE was detected in the cord sera of only 6 infants (range 2–9 IU/ml). 4 were concordant and 2 discordant, IgE being present in the second born in both cases. The geometric mean level of maternal serum IgE was 31.5 IU/ml (1SD, range 6.1–163). There was no relation between the presence of IgE in cord sera and the level of maternal IgE.

Discussion

During the past decade many reports on immunoglobulins in the newborn have appeared, and have been reviewed by Gitlin (1971), Adinolfi and Lessof (1972), and Wood (1971–72). However, there has been scant reference to immunoglobulin levels in multiple pregnancy. Several workers, in the course of wider studies, have specifically mentioned their findings in twins (McFarlane and Udeozo, 1968; McKay, Thom, and Gray, 1968; Yeung and Hobbs, 1968; Burdea *et al.*, 1970; Hautala and Kunnas, 1970) but in most cases the numbers have been small.

As a result of our study, it appears that, in general, twin infants are at no disadvantage to singletons in the amount of passive immunity received from the mother, and that there is the same evidence of active placental transfer (Fig. 2) as has been found by Hitzig (1961), Kohler and Farr (1966), Allansmith *et al.* (1968), and Mendenhall (1970). It was interesting to find that, like Yeung and Hobbs (1968), within a pair of twins IgG was unrelated

to birthweight. Though most workers are agreed that gestational age is the more important determinant, several have found some correlation with birthweight in singletons (Hobbs and Davis, 1967; Berg, 1968; Jones, 1969; Hautala and Kunnas, 1970; and Papadatos *et al.*, 1970) and particularly in those infants whose weight lay below the 10th centile for gestational age (Yeung and Hobbs, 1968; Papadatos *et al.*, 1969). But this has not been a constant finding (Addy, 1970). Our findings suggest that it is not the lower birthweight itself that is responsible for the reduced concentration of IgG, but rather an influence common to both infants such as maternal pre-eclamptic toxæmia which may result in low birthweight and reduction of both maternal and cord serum IgG concentrations (Studd, Shaw, and Bailey, 1972). This could explain the lack of correlation with birthweight in twin infants where, obviously, maternal health is a factor common to both.

In the majority of infants the most important factor determining the level of IgG seems to be that of the co-twin (Fig. 3), but the 3 cases of fetofetal transfusion syndrome present a unique picture. One of these cases has been reported previously in greater detail (Bryan and Slavin, 1974). In each case, the donor twin has a much lower level of IgG than the recipient and the percentage discrepancy is considerably greater than in any of the 47 unaffected pairs of twins. 2 further cases of this condition not included in this study have also shown a similar picture (unpublished data). As the discrepancy in concentration of IgG, a protein of maternal origin, is so much greater than that of the fetally synthesized albumin, it suggests that there may be a disturbance in maternofetal transfer across the donor's portion of the placenta in addition to the transfer of protein from twin to twin. Furthermore, it is remarkable that the mother, with her comparatively vast reservoir of IgG, is unable to compensate for any amount of fetal loss, especially when that loss from donor to recipient twin is in most cases occurring very gradually. The only mention of IgG levels in the fetofetal transfusion syndrome is that made by Yeung and Hobbs (1968). Interestingly, their findings in a single case was in total contradiction to ours, and we have no explanation for this. Several authors have reported an occasional pair of twins with large IgG discrepancies at birth, and as no haemoglobin levels are given, some of these could have been cases of this syndrome (McKay *et al.*, 1968; Burdea *et al.*, 1970) though the dizygotic cases cannot be explained in this way (McFarlane and Udeozo, 1968), as arterio-venous anastomoses found in the fetofetal trans-

fusion syndrome occur exclusively in monochorionic placenta (Rausen *et al.*, 1965).

A raised IgM level in cord serum is regarded as a useful indicator of intrauterine infection (Stiehm, Ammann, and Cherry, 1966; Alford *et al.*, 1967) and as there have been several reports of twins discordant for intrauterine infections (Forrester, Lees, and Watson, 1966; Satge *et al.*, 1966; Henriksen, Flusgrud, and Orstavik, 1968; Shearer *et al.*, 1972) we were interested to see whether any information might be gleaned from the intrapair comparison of cord IgM concentrations. Of particular interest was the possibility of a higher IgM level in the first-born twin on the premise that the presenting fetus would be more likely to receive antigenic stimulation from organisms in the lower genital tract. Benirschke and Driscoll (1967) found that chorioamnitis was invariably associated with the first twin when only one sac was involved. However, in paired twin specimens we found that the IgM level in the first born was not significantly higher than that of the second born, but it may be that some changes in IgM level, while still a reflection of the intrauterine environment, do not occur until sometime after birth. Certainly in extrauterine life there is usually a delay of several days between the onset of an infection and a significant rise in IgM level. One such case occurred within the study. A pair of monozygous twins were born to a mother whose membranes had been ruptured for 48 hours. She was pyrexial at birth but the babies remained clinically well. The cord IgM levels were not raised at 8 mg and 6 mg/100 ml but when they were measured at the age of 10 days the IgM in the first born had risen to 44 mg and in the second to 28 mg/100 ml. Similarly, it was interesting to find that there was a larger number of first-born infants with detectable IgA in their cord sera, an immunoglobulin usually absent in fetal serum, and the presence of which may also represent evidence of antigenic stimulation. However, the numbers are small and this point obviously requires confirmation.

We found no evidence of placental transfer of IgE in that the concentration of this immunoglobulin in the cord sera was either low or undetectable and in the four instances where the mother's IgE level was above 300 units/ml, no IgE was detected in the infants. These findings agree with those of Johansson (1968) and Bazaral, Orgel, and Hamburger (1971).

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