Immunoglobulin and anti-*Escherichia coli* antibody in lower respiratory tract secretions from infants weighing less than 1500 g at birth

F H Sennhauser, A Balloch, M J Shelton, L W Doyle, V Y H Yu, D M Robertson

Abstract

Concentrations of immunoglobulins and anti-*Escherichia coli* antibody were studied longitudinally in tracheobronchial aspirates from 33 premature intubated neonates, median gestational age 27 weeks. Aspirates collected at birth contained IgG, IgA, and IgM in 100%, 93%, and 79% of samples, respectively. The median IgA concentration at birth was 0.7 µg/mg total protein and increased to 5.8 µg/mg protein by the sixth week. IgG and IgM antibodies to *E coli* were present in 90% and 30%, respectively, of tracheobronchial aspirates collected at birth. Samples from three of 28 neonates (11%) contained IgA anti-*E coli* antibody at birth, and the proportion with IgA antibody rose to 50% during the sixth week. Secretory component associated IgA and IgM were detectable in samples tested at birth and at 4 weeks of age, and secretory component associated anti-*E coli* antibody was present in aspirates from three of nine neonates studied at 4 weeks of age, but had not been detectable at birth.

Premature neonates often require respiratory support, and during this time the respiratory tract rapidly becomes colonised.¹ Bacterial colonisation of the respiratory tract is associated with an increased incidence of systemic infection,² which contributes to the high morbidity and mortality of very low birthweight infants.^{3,4} Antibodies in secretions play an important part in protecting the infant from infection by preventing adherence of antigen and by neutralising organisms or their products.⁵ Although the immune system of the airways and lungs in adults and older children has been well studied,⁶⁻⁹ little is known of the respiratory defence mechanisms in neonates and infants.^{10,11} Forsyth *et al* have shown in premature neonates that concentrations of IgA and IgM in tracheobronchial lavage increase with increasing postnatal age.¹¹ Intubated premature neonates have also been shown to produce IgM antibody in serum to bacteria present in the lower respiratory tract.¹ The presence of specific antibody in lower respiratory tract secretions, however, and the association of the appearance of specific antibody in secretions and postnatal age in very premature neonates are unknown.

In this prospective study we investigated the origin and development of immunoglobulin and specific antibodies in tracheobronchial aspirates from very premature neonates. Total IgG, IgA, and IgM concentrations and anti-*Escherichia coli*

IgG, IgA, and IgM antibody concentrations in tracheobronchial aspirates were determined longitudinally up to 5.5 weeks after birth in a cohort of 33 intubated premature neonates whose gestational ages ranged from 25 to 30 weeks.

Patients and methods

Thirty three premature infants (13 boys, 20 girls) who required immediate postnatal intubation and ventilation were enrolled in a prospective study. The birth weights ranged from 674 to 1500 g (median 1001) and the median gestational age assessed by serial ultrasonography and clinical examination was 27.0 weeks (range 25-30). The indications for artificial ventilation in all 33 neonates were prematurity and hyaline membrane disease. The duration of intubation ranged from 14 to 39 days (median 27).

The position of the endotracheal tube was determined by chest radiographs, the tip of the tube being positioned 1-2 cm above the tracheal carina. Tracheobronchial aspirates were collected at the time of intubation and at weekly intervals (within one day) thereafter. Samples were collected through the endotracheal tube by gentle suction after instillation of up to 0.2 ml of sterile normal saline followed by ventilation for 15 to 30 seconds. The mean volume of aspirate collected was 200 µl (range 50-400 µl/sample). Aspirates containing blood were discarded. Samples were stored in polypropylene containers at -70°C until analysed. Cord blood was collected from 23 of the 33 preterm infants at delivery. The serum samples were also stored in polypropylene containers at -70°C until analysed.

PREPARATION OF ASPIRATES

Before analysis, all aspirates were thawed and sonicated (Branson Sonifier 450) at 100% output for at least one minute. The samples were then centrifuged at 1500 g for 10 minutes, and the supernatant collected and used in the assays described below.

QUANTITATION OF TOTAL CONCENTRATIONS OF IGG, IGA, AND IGM

Enzyme immunoassays for the measurement of total concentrations of IgG, IgA, and IgM in serum samples from cord blood were carried out as previously described.¹² All samples were tested in duplicate at four dilutions. A pooled human serum reference preparation containing 8.064 g IgG/l, 1.449 g IgA/l, and 0.966 g IgM/l

Departments of Immunology and Paediatrics, University of Melbourne
F H Sennhauser
D M Robertson

Department of Immunology, Royal Children's Hospital, Melbourne
A Balloch
M J Shelton

Department of Obstetrics and Gynaecology, University of Melbourne
L W Doyle

Department of Paediatrics, Monash University, Melbourne
V Y H Yu

Correspondence to:
Dr D M Robertson,
Department of Paediatrics, Adelaide Children's Hospital, North Adelaide, South Australia 5006, Australia.

Accepted 1 September 1989

was tested at tenfold dilutions ranging from $1/10^3$ to $1/10^8$ on each plate.¹³ With this assay IgG was detectable to 2×10^{-7} g/l, IgA to 4×10^{-7} g/l, and IgM to 3×10^{-7} g/l.

Enzyme immunoassays for the estimation of total concentrations of IgG, IgA, and IgM in tracheobronchial secretions were carried out as previously described.¹² All samples were tested in duplicate at three dilutions. A reference preparation of pooled multiple tracheobronchial aspirates from two patients aged 6 years and 8 years (who had permanent tracheostomies for management of high spinal injuries) was included on each assay plate in fivefold dilutions ranging from 1/500 to 1/1562500 so that a standard curve for the determination of relative units for each test sample could be derived. The concentrations of IgG, IgA, and IgM/mg total protein in this reference preparation were 58.2 µg, 159.1 µg, and 20.9 µg, respectively. The lower limit of sensitivity was 3.9×10^{-7} g/l, 3.6×10^{-7} g/l, and 1.4×10^{-7} g/l for IgG, IgA, and IgM, respectively.

Enzyme immunoassays for the determination of secretory component associated immunoglobulin were carried out by modifications of the above assays in which a murine monoclonal antisecretory component antibody (Australian Monoclonal Developments, Sydney) was used to detect secretory component associated IgM or IgA bound to the solid phase by affinity isolated anti-IgM or anti-IgA precoating antibody (Tago, Burlingame, California). Affinity isolated peroxidase conjugated antimouse IgG was used to develop the substrate reaction.

ANTIBODY TO POOLED *E COLI* ANTIGENS

Enzyme immunoassays were used to measure concentrations of IgG, IgA, and IgM antibodies specific to *E coli* polysaccharide in all samples of tracheobronchial fluid and cord serum. A pool of eight strains of *E coli* representing strains found most frequently in faecal isolates or diseases (serotypes 01, 02, 04, 06, 07, 016, 018, and 075) was prepared by a heat extraction technique.^{14 15}

Ninety six well microtitre plates (Nunc) were incubated with 100 µl of *E coli* antigen in 0.05 M bicarbonate buffer (pH 9.5; final concentration 0.09 µg *E coli* antigen/ml) in each test well for two hours at 37°C and then overnight at 4°C. Residual binding sites were blocked with 50 µl/well 2.5% skim milk powder in 0.05 M bicarbonate buffer (pH 9.5) for two hours at 37°C.¹⁶ After washing, 50 µl/well of sample diluted in phosphate buffered saline containing 0.05% Tween 20 (PBS-T) (pH 7.2) and containing 2.5% skimmed milk powder (SMP) was added and incubated for two hours at 37°C. After washing, 50 µl of a 1:1000 dilution of peroxidase conjugated, affinity isolated, goat antihuman IgG, or IgA, or IgM (Tago), in PBS-T/SMP was added and incubated for two hours at 37°C. The plates were then washed and 50 µl of orthophenylene diamine substrate was added. The reaction was stopped after incubation for 15 minutes in the dark by adding 25 µl of 8N hydrochloric acid solution to each well. Absor-

bance values were read at 492 nm using a Titertek Multiscan (Flow Laboratories).

All samples were tested in duplicate and at three dilutions ranging from 1/10 to 1/250 for tracheobronchial fluid samples, and from 1/10 to 1/1250 for cord samples. The pooled human reference serum¹³ and the tracheobronchial fluid reference preparation were each included in six serial dilutions on every test plate. Each test sample was quantitated by reading from interpolated points generated from the linear portion of the appropriate reference standard curve. The concentration of anti-*E coli* antibodies in the reference preparations was estimated using a modification of a method previously described.¹⁷ The pooled reference serum contained 0.42×10^{-3} g IgA anti-*E coli* antibody/l, 3.43×10^{-3} g IgG antibody/l, and 6.98×10^{-3} g IgM antibody/l, whereas the tracheobronchial fluid reference preparation contained 31.01 ng IgA anti-*E coli* antibody/mg total protein, 6.25 ng IgG antibody/mg total protein, and 51.14 ng IgM antibody/mg total protein.

Specificity of the assays for IgG, IgA, and IgM antibodies to the *E coli* antigens was illustrated by appropriate liquid phase absorption experiments using the reference sample preparations and the pooled *E coli* antigen preparation. The lower limit of detection was taken at optical density values of more than twice the background (sample free) optical density readings. The lower limit of IgG, IgA, and IgM anti-*E coli* antibody detection (g/l) for the serum reference preparation was 1.2×10^{-7} , 1.0×10^{-7} , and 0.9×10^{-7} , respectively, and 0.3×10^{-7} , 0.1×10^{-7} , and 0.1×10^{-7} for the tracheobronchial fluid reference preparation.

Secretory component associated antibody to the pooled *E coli* antigens was determined by appropriate modification of a previously described assay.¹²

TOTAL PROTEIN QUANTITATION

Measurements of total protein concentration were made by the method of Lowry *et al.*¹⁸ To correct for any errors introduced by sample dilution during endotracheal tube aspiration, the concentration of tracheobronchial immunoglobulin or specific antibody in samples was expressed as a ratio of µg of immunoglobulin or ng of antibody/mg of total protein in the sample.

STATISTICAL ANALYSIS AND ETHICS

Results in the tables are given as medians for all samples. As age increased after birth fewer neonates remained intubated. Statistical comparisons of results at differing postnatal ages were made using paired samples only. Analyses of immunoglobulin concentrations in paired samples were done by the Wilcoxon matched pairs signed rank test and the Spearman rank order correlation analysis. Longitudinal comparisons of proportions of study samples with detectable anti-*E coli* antibody were done with the McNemar test for the significance of changes.

The study was approved by the institutional human ethics committees for each of the three participating neonatal intensive care units. Informed written consent was obtained from the parents of all neonates before enrolment in the study.

Results

TOTAL IGG, IGA, AND IGM CONCENTRATIONS

All samples of cord serum contained IgG, IgA, and IgM. The median concentrations for IgG, IgA, and IgM were 4.87 g/l (range 1.82–9.52 g/l), 0.004 g/l (range 0.001–0.04 g/l), and 0.07 g/l (range 0.04–0.58 g/l).

A total of 144 tracheobronchial aspirates from 33 premature neonates were collected and analysed. The median immunoglobulin concentrations/mg total protein in tracheobronchial fluid samples at each postnatal collection time are shown in table 1. All aspirates contained IgG, with the highest concentrations present at birth (table 1). Two weeks later, the median IgG concentration had decreased significantly (Wilcoxon matched pairs signed rank test, 24 pairs, $p < 0.03$), and the median IgG concentration at 5.5 weeks remained significantly lower than at the time of perinatal intubation (10 pairs, $p < 0.02$).

The median IgA concentrations in the tracheobronchial aspirates are shown in table 1. IgA was detectable in 26 of the 28 aspirates collected at birth (93%), although the median IgA concentration was low (0.7 µg/mg total protein). The median IgA concentration remained low during the first three weeks, and then showed a progressive increase (Wilcoxon: between three and four weeks after birth, 16 pairs, $p < 0.04$; between 3 and 5.5 weeks after birth, nine pairs, $p < 0.03$). The median concentration of IgA in tracheobronchial aspirates at 5.5 weeks of age was 5.8 µg/mg total protein (range 0.3–13.0 µg/mg total protein, $n = 10$).

IgM was detectable in samples from 22 of the 28 neonates studied at birth (79%). Within the first week the median IgM concentration increased significantly (Wilcoxon; 24 pairs, $p < 0.001$), but there was no further significant change thereafter.

The ratio of the median concentration of total IgA: total IgM in tracheobronchial aspirates increased significantly after the second week (Wilcoxon: week 2 to week 3, 17 pairs, $p < 0.01$), reaching a value of 1.0 at about five weeks (Wilcoxon: week 2 to 5.5, eight pairs, $p < 0.02$).

Using the Spearman rank order correlation analysis, there were no significant correlations between IgG, IgA, and IgM concentrations in samples of cord serum (mg/100 ml) and in tracheobronchial fluids (µg/mg total protein) collected at the time of perinatal intubation ($n = 23$, each $r_s < 0.38$, each $p > 0.05$). Similarly, there were no significant correlations between concentrations of tracheobronchial IgG, IgA, and IgM (µg/mg total protein) at delivery and the gestational age at birth ($n = 28$, each $r_s < 0.21$, each $p > 0.14$).

ANTIBODY TO POOLED *E COLI* ANTIGENS

The median concentrations of IgG, IgA, and IgM antibodies to the pooled *E coli* antigens in cord samples were 0.91×10^{-3} g/l (range 0.10–5.08), 0×10^{-3} g/l (range 0–0.01), and 0.03×10^{-3} g/l (range 0–0.46) respectively.

The median concentrations of anti-*E coli* antibody in tracheobronchial fluid at differing postnatal ages are shown in table 2. Table 3 shows the percentage of analysed tracheobronchial fluid samples with detectable anti-*E coli* antibody within the three isotypes IgG, IgA, and IgM at differing postnatal ages.

Almost all samples contained IgG anti-*E coli* antibody. At the time of intubation, IgG anti-*E coli* antibody was detected in 25 of 28 samples (89%) and the median concentration was 19.1

Table 1 Median (range) concentrations of IgG, IgA, and IgM in samples of tracheobronchial fluid (µg/mg total protein)

	Birth (n=28)	Week 1 (n=27)	Week 2 (n=29)	Week 3 (n=21)	Week 4 (n=18)	Week 5 (n=12)	Week 5.5 (n=10)
IgG	61.5 (25.1–381.5)	57.8 (13.3–238.0)	46.1 (16.4–156.9)	37.1 (12.2–90.5)	27.1 (11.8–81.5)	22.9 (13.4–47.5)	22.6 (5.6–33.5)
IgA	0.7 (0.0–3.5)	0.9 (0.0–6.94)	0.6 (0.0–5.8)	0.7 (0.2–8.4)	1.4 (0.1–10.5)	3.4 (0.3–18.1)	5.8 (0.3–13.0)
IgM	0.6 (0.0–4.3)	2.1 (0.2–21.6)	2.1 (0.2–13.1)	2.6 (0.3–38.8)	2.8 (0.3–8.2)	3.3 (0.2–15.0)	2.9 (0.2–8.5)

Table 2 Median (range) concentrations of IgG, IgA, and IgM anti-*E coli* antibody in samples of tracheobronchial fluid (ng/mg total protein)

	Birth (n=28)	Week 1 (n=27)	Week 2 (n=29)	Week 3 (n=21)	Week 4 (n=18)	Week 5 (n=12)	Week 5.5 (n=10)
IgG	19.1 (0–189.5)	8.7 (0–218.7)	6.2 (0–72.9)	5.6 (0–34.2)	3.8 (0–22.5)	2.9 (0–16.8)	3.2 (0–16.4)
IgA	0.0 (0–1.6)	0 (0–1.2)	0 (0–10.5)	0 (0–0.6)	0 (0–0.5)	0 (0–19.8)	0.1 (0–54.6)
IgM	0 (0–7.7)	0 (0–5.7)	0.3 (0–8.9)	0.3 (0–1.6)	0.3 (0–1.0)	0.2 (0–2.1)	0.2 (0–2.6)

Table 3 Percentage of tracheobronchial aspirates with detectable amounts of IgG, IgA, and IgM anti-*E coli* antibody at increasing postnatal ages

	Birth (n=28)	Week 1 (n=27)	Week 2 (n=29)	Week 3 (n=21)	Week 4 (n=18)	Week 5 (n=12)	Week 5.5 (n=10)
IgG	90.2	81.4	83.0	76.4	89.1	75.2	70.3
IgA	11.1	18.5	21.4	23.8	33.3	41.7	50.0
IgM	30.4	40.7	62.1	66.7	61.1	58.3	60.0

ng/mg total protein (range 0–189.5). There was an insignificant decrease to a median value of 3.2 ng/mg total protein (range 0–16.4) at 5.5 weeks (McNemar test for significance of changes, nine pairs, $p > 0.1$).

Concentrations of IgA anti-*E coli* antibody ranged between 0 and 19.8 ng/mg total protein during the first five weeks, with median values of 0 ng/mg total protein at each time of sampling up to and including 5 weeks of age. The median value at 5.5 weeks was 0.13 ng/mg total protein (range 0–54.6 ng/mg total protein). The percentage of samples with detectable IgA anti-*E coli* antibody increased as postnatal age increased. IgA antibody was present in 11.1% of samples at birth, and in 50.0% of samples at 5.5 weeks after delivery (McNemar: eight pairs, $p < 0.04$).

IgM anti-*E coli* antibody was detectable in 30% of samples at the time of intubation. At two weeks postpartum significantly more neonates (62%) had detectable IgM anti-*E coli* antibody than at birth (McNemar: 28 pairs, $p < 0.03$). The median IgM antibody concentration at two weeks was 0.3 ng/mg total protein (range 0.0–8.9 ng/mg total protein); it remained at this value at 3 and 4 weeks after birth, and then fell to a median concentration of 0.17 ng IgM antibody/mg total protein ($n = 10$) at 5.5 weeks.

There was a significant correlation between the concentration of IgG anti-*E coli* antibody in paired cord samples and tracheobronchial aspirates at the time of immediate postnatal intubation ($n = 21$, $r_s = 0.43$, $p = 0.02$) but no similar correlation was found for IgA and IgM antibodies. There was no significant correlation between gestational age and concentrations of IgG, IgA, or IgM anti-*E coli* antibodies in tracheobronchial fluids at the time of delivery ($n = 28$, all $r_s < 0.18$, $p > 0.17$).

Concentrations of IgA and IgM anti-*E coli* antibodies did not correlate with total concentrations of IgA or IgM in tracheobronchial aspirates at any of the sampling times. Concentrations of IgG anti-*E coli* antibody in tracheobronchial aspirates correlated significantly with total concentrations of IgG in tracheobronchial fluid during the first two weeks of postnatal life ($r_s = 0.65$, $r_s = 0.61$, $r_s = 0.48$, at intubation, and at weeks one and two, respectively, all $p < 0.005$) but not thereafter (all $r_s < 0.33$, all $p > 0.05$).

SECRETORY COMPONENT ASSOCIATED IMMUNOGLOBULINS AND ANTIBODY IN TRACHEOBRONCHIAL ASPIRATES

Enough of the sample remained from collections at birth and at 4 weeks of age from 12 patients with detectable IgA and IgM at each of these times to measure secretory component associated IgA and IgM concentrations. Secretory component associated IgA and IgM were detectable in all 24 samples. Total concentrations of IgA and IgM correlated significantly with the concentrations of total secretory component associated IgA and IgM, respectively, at each of the sampling times (each $r_s \geq 0.64$, each $p < 0.01$).

Concentrations of secretory component associated anti-*E coli* antibody were measured in

aspirates collected at birth and at four or five weeks after birth from nine patients who had detectable IgA and IgM anti-*E coli* antibody at four or five weeks. At birth none of these nine patients had detectable secretory component associated antibody despite the presence of IgM anti-*E coli* antibody in two of the nine samples tested. Three of the nine samples collected at four or five weeks had detectable amounts of secretory component associated anti-*E coli* antibody.

Discussion

Immunoglobulin in secretions may be acquired from secretion by lymphoid cells resident in mucosa, by transport of systemically produced or acquired immunoglobulin, or a combination of these mechanisms. The results of the longitudinal study of very premature neonates reported here do not determine definitively whether the immunoglobulin or antibody detected in the secretions in prematurity was produced locally by mucosal tissue, or whether it was produced systemically. The decrease in median IgG concentrations in the tracheobronchial aspirates with increasing birth age is consistent with a half life of about four weeks, and thus the IgG may have been from the mother. Concentrations of IgG anti-*E coli* antibody also decreased with increasing postnatal age. There was, however, no correlation between concentrations of IgG and of IgG anti-*E coli* antibody in tracheobronchial aspirates after 2 weeks postnatal age. Thus some IgG antibody may have been produced locally in response to mucosal antigenic stimulation after a postnatal age of 2 weeks.

Support for the concept of local production of immunoglobulin in the mucosa is seen in the rise in concentrations of IgA and IgM with increasing postnatal age, and the increase in the proportion of samples containing IgA and IgM anti-*E coli* antibody with increasing postnatal age. The presence of secretory component associated IgA and IgM in samples collected at birth also suggests maturation of specific mechanisms for acquisition of antibody in secretions. These findings could be the result of the induction of local antibody production at mucosal sites, or of maturation of specific mucosal immunoglobulin transport mechanisms. These mechanisms seem to become active for IgM more rapidly than for IgA, but concentrations of IgA became greater than those for IgM in tracheobronchial secretions by the end of the fifth week of life. IgG concentrations, however, remained four times greater than those of IgA at 5.5 weeks postnatal age.

There were no significant correlations between the concentrations of each immunoglobulin isotype in cord blood samples and in the tracheobronchial aspirates. Similarly the values for tracheobronchial aspirates did not correlate with gestational age. These results suggest that immunoglobulin and antibody present in tracheobronchial secretions is not acquired by passive leakage alone but rather by active mechanisms, which may be dependent on postnatal age rather than gestational age in neonates

of less than 30 weeks' gestation. In some neonates, this active process may have begun prenatally, in that tracheobronchial IgG concentrations at birth were greater than concentrations of IgG in cord serum in 10 of 23 neonates studied (43%). Concentrations of IgA in tracheobronchial secretions at birth were also greater than in cord serum in three of 23 neonates (13%). Concentrations of IgM in cord blood were, however, higher than in tracheobronchial fluid in all neonates. Interestingly, concentrations of IgM in tracheobronchial fluid were significantly higher at birth in the seven neonates born after prolonged (>24 hours) rupture of the membranes than in those for whom the duration of rupture of membranes was less than 24 hours before delivery ($p=0.03$, Mann-Whitney U test). In contrast, the median concentration of IgM anti-*E coli* antibody in tracheobronchial fluid from these seven neonates did not differ from the remaining neonates ($p=0.78$). Similarly, there were no significant differences between these two groups in the median concentrations in tracheobronchial fluid of total IgG, total IgA, or IgG, IgA, and IgM anti-*E coli* antibodies. Prolonged rupture of the membranes may therefore act as a stimulus to the appearance of IgM in the respiratory tract, but does not alter concentrations of IgG and IgA and does not lead to production of anti-*E coli* antibody.

IgA antibody to *E coli* in tracheobronchial secretions could originate from maternal breast milk if aspiration of breast milk being given by nasogastric tube feeding occurred. IgA anti-*E coli* antibody was, however, present in samples from seven neonates who had not received breast milk at any time before sample collection. IgA anti-*E coli* antibody was also detectable in three neonates at intubation before any feeds, consistent with the findings of Mellander *et al*, who suggested that IgA antibody in mucosal secretions at birth and in amniotic fluid during pregnancy may be induced by transplacental transfer of maternal IgG anti-idiotypic.¹⁹ The three neonates who had secretory component associated anti-*E coli* antibody at four to five weeks postnatally had each received some breast milk, and thus it is not known whether this antibody was produced locally or came from the milk.

The findings of immunoglobulin concentrations in tracheobronchial fluid from very premature neonates in this study are similar to those of Forsyth *et al*, in that there was a slow rise in concentrations of IgA and IgM with increasing postnatal age.¹¹ In contrast, the rate of rise of concentrations of IgA and IgM was much more rapid in tracheal aspirates from neonates of more than 32 weeks' gestation.¹¹ In previous studies of neonatal breast milk, we have shown that IgA concentrations rise rapidly in other secretions from neonates born at full term during postnatal life.¹² There was also a rapid rise in concentrations of IgA antibody and secretory component associated antibody in neonatal breast milk to another enteric antigen, β lactoglobulin, during the first two to three weeks of life in neonates born at full term.¹² The results of our study suggest that very pre-

mature neonates, although apparently able to acquire mucosal IgA immunoglobulin and specific antibody, do so at a slower rate than gestationally older infants, which suggests slower postnatal maturation of mucosal antibody transport and production than in those born at full term. The presence of secretory component associated immunoglobulin provides presumptive evidence that some of this antibody is produced locally, consistent with the findings of secretory component containing epithelial cells in the respiratory tract during early gestation.^{10, 20} Paradoxically, however, Takemura and Eishi were not able to find any cells containing immunoglobulin in segmental bronchi in neonates of up to 37 weeks' gestation¹⁰; thus the origin of the immunoglobulin and antibody reported in this study and in that of Forsyth *et al* remains to be determined.¹¹

The production of specific antibody at mucosal surfaces may arise as a result of local antigenic stimulation, or may be a consequence of homing of mucosally associated lymphocytes after exposure to antigens at a distant mucosal site.²¹ Only five neonates in this study had clinical and radiological evidence of pulmonary infection at any time during the study period, and *E coli* was not isolated from the bronchial secretions of any infant. These five infants did not have higher concentrations of immunoglobulin or antibody in aspirates than the non-infected neonates.

Previous studies of the microbiological flora in premature neonates (mean gestational age 30 weeks) from this hospital showed that throat swabs from 45% of infants at 17–20 days of age grew *E coli*.²² *E coli* was also detectable in the faeces of 60% of infants studied at 17–20 days of age.²² In the study reported here we did not perform routine cultures of tracheobronchial aspirates or faeces at the time of collection of samples for immunoglobulin and antibody estimations. We are therefore not able to say whether the appearance of specific antibody to *E coli* in respiratory tract secretions, if of mucosal origin, is the result of homing of committed lymphocytes from the gastrointestinal tract or is the result of local antigenic stimulation after colonisation of the upper or lower respiratory tract.

The findings of this study suggest that the very premature neonate is able to acquire specific antibody at mucosal surfaces within the first few weeks of postnatal life. The maturation of this process may, however, be slower than that seen in the infant born at full term. It will be necessary to carry out cultures of respiratory tract associated lymphoid cells to determine whether local mucosal production of antibody is possible at this stage of gestation. Further studies will be necessary to see if the therapeutic administration of intravenous immunoglobulin to very premature neonates will affect concentrations of antibodies in the respiratory tracts in these neonates.

We thank the nursing staff of the neonatal intensive care units, Royal Children's Hospital, Royal Women's Hospital, and Monash Medical Centre, for collecting the samples, and Mr O Wenger for collecting the data. We thank Dr C Murray, Institute of Medical and Veterinary Science, Adelaide, for the

E. coli standard strains and the staff of the department of microbiology, Royal Children's Hospital, for the preparation of *E. coli* antigens. Dr FH Sennhauser was supported by grants from the Swiss National Research Foundations (No 83.337.06) and the Swiss Academy of National Sciences, and Mrs A Balloch received grants from the University of Melbourne.

- 1 Giacola GP, Neter E, Ogra P. Respiratory infections in infants on mechanical ventilation: the immune response as a diagnostic aid. *J Pediatr* 1981;**98**:691-5.
- 2 Harris H, Wirtschafter D, Cassady G. Endotracheal intubation and its relationship to bacterial colonisation and systemic infection of newborn infants. *Pediatrics* 1976;**58**:816-23.
- 3 Remington JS, Klein JO. *Infectious diseases of the fetus and newborn infant*. Philadelphia: WB Saunders, 1983: 679-735.
- 4 Ballou M, Cates KL, Rowe JC, Goetz C, Desbonnet C. Development of the immune system in very low birth weight (less than 1500 g) premature infants: concentrations of plasma immunoglobulins and patterns of infections. *Pediatr Res* 1986;**20**:899-904.
- 5 Kilian M. Perturbation of mucosal immune defence mechanisms by bacterial IgA protease. *Bull Europ Physiopathol Respir* 1983;**19**:99-104.
- 6 Reynolds HY. Lung immunology and its contribution to the immunopathogenesis of certain respiratory diseases. *J Allergy Clin Immunol* 1986;**78**:833-47.
- 7 Burnett D. Immunoglobulins in the lung. *Thorax* 1986;**41**:337-44.
- 8 Quie PG. Lung defense against infection. *J Pediatr* 1986;**108**:813-6.
- 9 Merrill WW, Naegel GP, Olchowski JJ, Reynolds HY. Immunoglobulin G subclass proteins in serum and lavage fluid of normal subjects. *Am Rev Respir Dis* 1985;**131**:584-7.
- 10 Takemura T, Eishi Y. Distribution of secretory component and immunoglobulins in the developing lung. *Am Rev Respir Dis* 1985;**131**:125-30.
- 11 Forsyth K, Koh L, Lawrence A, Bradley J. Immunoglobulin profile of tracheal aspirate fluid in intubated children. *Clin Exp Immunol* 1988;**71**:357-61.
- 12 Robertson DM, Forrest PJ, Frangoulis E, Jones CL, Mermelstein N. Early induction of secretory immunity in infancy: specific antibody in neonatal breast milk. *Arch Dis Child* 1986;**61**:489-94.
- 13 Turner KJ, Carter PM, Hosking CS, Finger WK. The Australian reference preparation of human serum immunoglobulins. *Australian Journal of Experimental Biology and Medical Science* 1980;**58**:351-6.
- 14 Turck M, Petersdorf RG, Fournier MR. The epidemiology of non-enteric *Escherichia coli* infections: prevalence of serological groups. *J Clin Invest* 1962;**41**:1760-5.
- 15 Lidin-Janson G, Hanson LA, Kaijser B, et al. Comparison of *Escherichia coli* from bacteria patients with those from feces of healthy school children. *J Infect Dis* 1977;**136**:346-53.
- 16 Vogt RF, Phillips DL, Henderson LO, Whitfield W, Spierto FW. Quantitative differences among various proteins as blocking agents for ELISA microtitre plates. *J Immunol Methods* 1987;**101**:43-50.
- 17 Jackson S, Montgomery RI, Mestecky J, Czerkinsky C. Normal human sera contain antibodies directed at Fab of IgA. *J Immunol* 1987;**138**:2244-8.
- 18 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;**153**:265-75.
- 19 Mellander L, Carlsson B, Hanson LA. Secretory IgA and IgM antibodies to *E. coli* O and poliovirus type 1 antigens occur in amniotic fluid, meconium and saliva from newborns. *Clin Exp Immunol* 1986;**63**:555-61.
- 20 Sturgess JM. Ontogeny of the bronchial mucosa. *Am Rev Respir Dis* 1985;**131**(suppl):S4-7.
- 21 Mestecky J. The common mucosal immune system and current strategies for induction of immune responses in external secretions. *J Clin Immunol* 1987;**7**:265-76.
- 22 Blakey JL, Lubitz L, Barnes GL, Bishop RF, Campbell NT, Gillam GL. Development of gut colonisation in pre-term neonates. *J Med Microbiol* 1982;**15**:519-29.