Lymphocyte response after surgery in the neonate

P. PURI, D. J. REEN, ORLA BROWNE, P. BLAKE, AND E. J. GUINEY

Our Lady's Hospital for Sick Children, Royal College of Surgeons in Ireland, and St Laurence's Hospital, Dublin

SUMMARY Fourteen neonates born with congenital malformations were investigated for lymphocyte function before and after surgery. Total leucocyte and absolute lymphocyte counts were unaltered after surgery. The mean percentage of T-lymphocytes observed either pre- or postoperatively was considerably lower than that reported in older children and adults. While there was an increase in the percentage of B-lymphocytes after operation in the infants, the absolute number of B-cells remained unchanged. The preoperative transformation response of lymphocytes to PHA (mean $12.9 \pm 5.4 \times 10^3$ counts/min) was little different from the postoperative values (mean $12.4 \pm 4.4 \times 10^3$ counts/min). These results suggest that the neonate is immunologically different from older children and adults in its response to anaesthesia and surgery.

It is now widely accepted that anaesthesia and surgical trauma in children and adults results in immunosuppression (Donovan and Soothill, 1973; Cullen and van Belle, 1975). This immunosuppressive effect is apparent within 10-15 min of induction of anaesthesia (Espanol *et al.*, 1974), is greatest in the immediate postoperative period (Park *et al.*, 1971; Slade *et al.*, 1975), and persists for 7 to 10 days (Jubert *et al.*, 1973). There is evidence that anaesthetic drugs depress both specific and nonspecific defences against infection and that this depression influences postoperative morbidity (Moudgil and Wade, 1976).

The lymphatic tissue of the neonate is mature for both cell-mediated and humoral immune response (Miller, 1977). The capacity to mount cellular and humoral immune reactions develops at between 9 and 15 weeks of gestation, but antigenic stimulation is necessary for antibody production on a large scale (Prindull, 1974). The immune system in the newborn in response to a sudden change of environment after birth has not been extensively studied and there are no data on the effect of anaesthesia and surgery on the immune response of the neonate. The purpose of this study was to determine whether an operation

performed under general anaesthesia causes changes in the lymphocyte response of the neonate and, if so, to establish the quality of these changes.

Material and methods

Fourteen infants, 10 boys and 4 girls, born with congenital malformations were studied. Eleven were born at term, two at 38 weeks' gestation, and one at 36 weeks. Pregnancy was complicated by hydramnios in 3 cases. Delivery was normal in 12 babies, breech in one, and forceps-assisted in one. Average birthweight was 3.2 kg, with a range of 2.5 to 4.7. The various congenital anomalies in the 14 neonates were neural tube defects in 7, oesophageal atresias with tracheo-oesophageal fistulas in 3, small bowel atresias in 2, and anorectal anomalies in 2.

Samples of venous blood for various immunological parameters were obtained 2 hours before surgery, usually at the time when the blood sample for crossmatch was being taken, and again 18 hours after the operation. The average age of the infant at the time of the preoperative blood sample was 1.8 days.

The patients were premedicated with atropine 0.02 mg/kg 30 min before operation. Anaesthesia was induced with oxygen, nitrous oxide and 2% halothane, and relaxed for intubation with succinylcholine, 1 mg/kg. For the maintenance of anaesthesia, 50% nitrous oxide in oxygen and, if necessary, 2% halothane were used. Pancuronium bromide (Pavulon) 0.1 mg/kg was used for further

The Children's Research Centre, Our Lady's Hospital for Sick Children

P. PURI, senior research fellow

D. J. REEN, biochemist

E. J. GUINEY, professor of paediatric research

Royal College of Surgeons in Ireland

ORLA BROWNE, lecturer in pathology

St Laurence's Hospital Endocrine/Metabolic Unit

P. BLAKE, biochemist

relaxation in patients undergoing surgery for intestinal obstructions and oesophageal atresias.

The average operating time including anaesthesia was 95 min, with a range of 50 min to 3½ hours. Only one of the 14 infants required blood transfusion during surgery. Two babies were on antibiotics before blood samples were taken.

Blood samples for lymphocyte population studies and mitogen response to PHA were collected in sterile tubes containing preservative-free heparin (20 IU/ml). Blood for total leucocyte and absolute lymphocyte counts was collected in sodium EDTA blood tubes.

Total leucocyte and absolute lymphocyte counts. The leucocyte count was made on an electronic cell counter (Coulter Counter Model ZF), and the differential white cell counts were made on blood smears using Giesma stain. 200 leucocytes were counted to determine absolute cell numbers.

Separation of mononuclear cells. 2 ml heparinised blood was allowed to sediment at room temperature for an hour. The leucocyte-rich supernatant was layered on to a Ficoll-Hypaque gradient of density 1.077 (24 vol 9% Ficoll and 10 vol 33.9% Hypaque) and lymphocytes were separated after centrifugation at 800 *g* for 10 min. The lymphocytes were washed twice in medium 199 and adjusted to a concentration of 10⁶ cells/ml in the same medium. This cell suspension was used for T-cell, B-cell, and null cell counts.

Spontaneous erythrocyte rosettes. 0.25 ml of cell suspension was mixed with an equal volume of a 0.5% suspension of washed sheep red blood cells (SRBC) and incubated at 37°C for 15 min. The cell preparations were then centrifuged at 200 *g* for 5 min at room temperature followed by incubation at 4°C for 18 hours. The cells were resuspended and counted in a Fuchs-Rosenthal haemocytometer. A minimum of 200 lymphocytes were counted and all lymphocytes binding 4 or more SRBC were accepted as positive.

B-lymphocyte identification. 0.1 ml of a working dilution of fluorescein-conjugated antiserum to whole immunoglobulin was added to an equal volume of cell suspension and incubated at 18°C for 30 min. The cells were then washed twice in medium 199, resuspended in one drop of phosphate buffered glycerol, and mounted on conventional microscope slides immediately before ultraviolet microscopical examination, using incident (epi) illumination.

PHA response. Cells collected in Ficoll-Hypaque gradient were resuspended in Dulbeccos Mod. medium (25 mmol/l Hepes buffer with L-glutamine) containing 10% fetal calf serum and 100 units penicillin and streptomycin/ml. Cultures were set up in triplicate in Steralin microplates, containing 10⁵ cells in 200 ml medium and incubated for 3 days in 5% CO₂ and 95% air at 37°C.

PHA-P (Difco) was used as mitogen in doubling dilutions from 1/2 to 16×16⁻³. After 68 hours' incubation, 0.4 μCi ³H-thymidine was added to each culture and the cells were harvested on to glass fibre discs after a further 4 hours' incubation. The discs were washed twice in 10% TCA and twice in methanol, and then dried and counted in a liquid scintillation counter. A dose response curve to PHA was obtained for each infant.

Statistical method. The significance of difference between the mean preoperative and postoperative values was calculated using Student's *t* test for two independent observations.

Results

Total leucocyte and absolute lymphocyte counts. The total leucocyte count (Table 1) showed a wide variation in the numbers of leucocytes both pre- and postoperatively. Even though the mean total leucocyte count showed a drop from 17 264 cells/mm³ (17.3×10⁹/l) preoperatively to 15 407 cells/mm³ (15.4×10⁹/l) postoperatively, half of the patients showed an actual increase in the post-operative total leucocyte count. The preoperative absolute lymphocyte counts were not significantly different from postoperative counts (Table 1).

T- and B-lymphocytes. The percentage of B-lymphocytes increased after surgery in 9 out of 10 infants. While there was an increase in the mean percentage of B-lymphocytes after surgery, the absolute number of B-cells remained unaltered (Table 2). Both the

Table 1 Total leucocyte and absolute lymphocyte counts in neonates pre- and postoperatively

	No. tested	Range	Mean ± 1 SD
<i>Total leucocytes/mm³</i>			
Preoperative	14	8700 – 29 800	17 264 ± 6075
Postoperative	14	10 600 – 22 700	15 407 ± 3328 NS
<i>Absolute lymphocytes/mm³</i>			
Preoperative	14	2262 – 12 336	5350 ± 2953
Postoperative	14	2100 – 7293	4416 ± 1572 NS

NS = Not significant (P>0.05).

Conversion: traditional units to SI—lymphocytes: 1000/mm³ ≈ 1.0 × 10⁹/l.

Table 2 *E*-binding (T), SmIg-positive (B) lymphocytes, and null cells in neonates pre- and postoperatively

	No. tested	Percentage		Absolute no.	
		Range	Mean $\pm$ 1 SD	Range	Mean $\pm$ 1 SD
<i>E-binding lymphocytes (T-cells)</i>					
Preoperative	10	6-65	38.4 $\pm$ 17.3	283-6168	2277 $\pm$ 1659
Postoperative	10	16-58	36.1 $\pm$ 12.4 NS	474-4230	1725 $\pm$ 1207 NS
<i>SmIg-positive lymphocytes (B-cells)</i>					
Preoperative	10	12-31	22.1 $\pm$ 6.7	282-2450	1286 $\pm$ 651
Postoperative	10	11-49	29.2 $\pm$ 10.7 NS	454-1969	1240 $\pm$ 556 NS
<i>Null cells</i>					
Preoperative	10	4-66	39.6 $\pm$ 18.6	108-6236	2448 $\pm$ 1689
Postoperative	10	15-56	34.7 $\pm$ 13.6 NS	504-2724	1446 $\pm$ 665 NS

NS = Not significant ($P > 0.05$).
For conversion see Table 1.

percentage and the absolute number of spontaneous E-rosettes (T-cells) were unaffected by anaesthesia and surgery. The percentage of null cells was noticeably high in the infants and was not appreciably changed after surgery. The mean percentage of null cells was 39.6 ± 18.6 preoperatively and 34.7 ± 13.6 postoperatively (Table 2).

PHA response. The rate of ^3H -thymidine incorporation after PHA stimulation was greatest at a concentration of either 0.06 or 0.1 $\mu\text{l/ml}$ in all infants. The transformation response of lymphocytes to PHA did not change after surgery, the preoperative mean being $12.9 \pm 5.4 \times 10^3$ counts/min and the postoperative being $12.4 \pm 4.4 \times 10^3$ counts/min (Table 3). There was no significant difference at any of the PHA concentrations used in the dose response curve before or after surgery (Figure). Appreciable levels of spontaneous ^3H -

Table 3 *Maximum PHA mitogen response (counts/min $\times 10^3$) in the neonate pre- and postoperatively*

	No. tested	Mean $\pm$ 1 SD
Preoperative	9	12.9 $\pm$ 5.4
Postoperative	9	12.4 $\pm$ 4.4 NS

NS = Not significant ($P > 0.05$).

thymidine incorporation ($1.2 \pm 0.9 \times 10^3$ counts/min) were observed in all patients. At optimum PHA concentrations, a wide variation of maximum incorporation of ^3H -thymidine was observed, especially in patients before operation.

Discussion

While most authors have reported that anaesthesia and surgical trauma in adults and older children produces depression of the cellular immune response

Figure PHA response to increasing mitogen concentrations before and after surgery. Each point represents mean $\pm$ 1 SD for 9 patients.

(Donovan and Soothill, 1973; Cullen and van Belle, 1975), we have been unable to support this finding in the neonate. No overall differences could be demonstrated between the preoperative and postoperative immune response. A number of factors must be considered in relation to this study in the neonate which may explain the differences in our results from those previously reported, and also may help to explain the clearly divergent reported observations on the effect of surgery on the immune response in adults.

When making comparisons of results in relation to the immune status of patients it is essential to select and control as closely as possible a homogeneous group of subjects and to eliminate wide variations in relation to age, degree of immunological sensitisation, clinical status, as well as factors that are known directly to affect immunological competence—such as therapeutic regimens, blood transfusion, and infection.

Age of the patient is of prime importance in relation to immune status, as T-lymphocyte subpopulations and mitogen responsiveness are diminished with increasing age (Augener *et al.*, 1974; Pisciotto *et al.*, 1967). Some patients in our study were relatively homogeneous in regard to age, with a mean age of 1.8 days and a range of 1 to 3.

All patients had a similar brief exposure to their antigenic environment since they were all directly transferred soon after birth from the maternity hospital to the referral centre for urgent surgery.

Several investigators have reported that immunosuppression after surgery is related primarily to the extent of tissue trauma, a more profound decrease in lymphocyte responses occurring after major rather than minor operations (Berenbaum *et al.*, 1973; Salo, 1978). The present study shows that major surgery in the neonate for congenital anomalies is not associated with depression in the immune responsiveness.

Total leucocyte and absolute lymphocyte counts were not significantly altered after surgery. This may be explained by the observations that the infant is undergoing active bone marrow generation with rapid alteration in peripheral blood leucocyte counts, coupled with a developing lymphocytosis relative to later life (Andersen and Andersen, 1974). This normally occurring lymphocytosis in the first few days of life may counteract any specific leucopenic effect of surgery in the neonate.

Similar conditions may explain the unaltered absolute numbers of T- and B-lymphocytes postoperatively. Even though 9 out of 10 infants showed an increase in the percentage of B-lymphocytes after surgery, this increase was not apparent in the absolute numbers owing to the wide range of total

leucocyte counts present in the newborn. The mean percentage of T-cells observed in this study either pre- or postoperatively was considerably lower than that reported for older children and adults (Fleisher *et al.*, 1975). Similarly the percentage and actual numbers of null cells in the infant were higher when compared with the reported levels in adults.

These observations are further supported by our findings on mitogen response to PHA after anaesthesia and surgery. No significant change was found in the transformation response of lymphocytes to PHA after operation. However, an appreciable level of spontaneous ^3H -thymidine incorporation was observed in all patients ($1.2 \pm 0.9 \times 10^3$ counts/min). This may further reflect an active development of lymphocyte production and maturation taking place in the neonate.

These findings suggest that the neonate is immunologically different from older children and adults in its response to known immunosuppressive factors, such as anaesthesia and surgery. Further studies are required on factors that affect the immunological capacity of the neonate in order to understand more fully the complex immunological status of the developing infant in the first few days of life.

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Correspondence to Mr P. Puri, The Children's Research Centre, Our Lady's Hospital for Sick Children, Crumlin, Dublin 12, Eiré.

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