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THE EFFECT OF ANAESTHESIA ON THE LYMPHOCYTE RESPONSE TO PHYTOHAEMAGGLUTININ

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SUMMARY

The low lymphocyte response to phytohaemagglutinin in patients undergoing tonsillectomy is associated with anaesthesia. There is no serum blocking factor, and the lymphocytes responded normally after washing.

INTRODUCTION

In the course of investigation into possible immunodeficiency in children undergoing tonsillectomy, we found that a proportion of the patients had a very low lymphocyte response to phytohaemagglutinin (PHA) (Donovan & Soothill, 1973). This finding was unexpected and unexplained. There have been other reports of low PHA response after surgery. Riddle & Berenbaum (1967) and Park *et al.* (1971) ascribed this to the effect of surgical stress. However, in our study, the blood was taken after the induction of anaesthesia, but before the start of the operation. The low lymphocyte response could thus either be part of the immunodeficiency underlying the disease, for which there was other evidence, or it could be an effect of the anaesthetic. We have, therefore, investigated this effect in a further series of patients undergoing tonsillectomy.

MATERIALS AND METHODS

Venous blood was taken 10–15 min after the induction of anaesthesia in thirty-six patients undergoing tonsillectomy aged 3 to 13. They were having the operation for the conventional indications, without special selection. Some were also studied before anaesthesia when bleeding was required for clinical purposes. In all patients anaesthesia was maintained by N_2O, O_2 and halothane. Children under 7 years were premedicated with trimeprazine, and induced with cyclopropane and suxamethonium. Those over 7 years had quinalbarbitone premedication and thiopentone and suxamethonium induction.

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PHA response in whole blood

The blood was defibrinated with glass beads. One millilitre was mixed with 9 ml of tissue culture medium RPMI 1964 (Gibco) and 1-ml aliquots were dispensed into ten tubes (Pyrex 1·2 × 7·5 cm). PHA (Purified PHA, Wellcome) was added at a final concentration of 0, 1, 2, 4 and 8 μg in duplicates. One microcurie of [^{3}H]thymidine was added to each culture for the final 4 hr of a 72-hr incubation period. Cells were harvested on glass fibre filter discs as described by Hartzman & Bach (1971) and counted in a liquid scintillation β counter (Philips Liquid Scintillation Analyser).

In some experiments cultures of washed blood cells in foetal calf serum containing medium were performed in parallel with the cultures in autologous serum. One millilitre of defibrinated blood was washed twice in Medium 199 (Wellcome reagents) and resuspended in RPMI 1640 supplemented with 10% heat-inactivated (56°C for 2 hr) foetal calf serum (FCS).

Separation of lymphocytes

Defibrinated blood was centrifuged (5 min at 200 *g*), and the cells were resuspended in Medium 199. Carbonyl iron, 100 mg (Gaf Ltd), was added and incubated for 20 min at 37°C on a Matburn mixer. Iron particles and phagocytes which had engulfed them were removed with a magnet. The blood was then layered on to a Ficoll–Triosil gradient (Harris & Ukaejiofo, 1970) and centrifuged for 20 min at 400 *g*. Cells at the interface were recovered and washed three times.

Spontaneous red cells rosettes

Sheep erythrocytes (Wellcome reagents) less than 10 days old were washed three times in Medium 199 and adjusted to 1% by volume. 0·1 ml of this suspension and 3×10^5 lymphocytes in 0·1 ml of medium were mixed vigorously in 7-mm round-bottomed plastic tubes and centrifuged at room temperature for 7 min at 150 *g*. The tubes were then placed in an ice bath for 2 hr, after which the cells were resuspended by gentle rocking and viewed on a microscope (direct and phase illumination) immediately.

Immunofluorescence staining

A sandwich immunofluorescence technique was used to detect IgG, IgM and IgA on the surface of blood lymphocytes prepared as described above. Rabbit anti-human IgG, IgM and IgA sera (Nordic) and sheep anti-rabbit immunoglobulin (Wellcome) were used. The method and specificity testing with immunoglobulin-coated Sigma cell particles, is described elsewhere (Hayward & Ezer, 1973).

RESULTS

In about half of the samples studied there was no more thymidine uptake by the lymphocytes of the anaesthetized children, when stimulated with PHA at any of the doses used, than in unstimulated cultures. In the rest, thymidine uptake and the ratio of stimulated to unstimulated cultures was less than in the healthy adult controls, and peak activity was often at a relatively high PHA dose, unlike normal cultures. The thymidine uptake by unstimulated cells from the anaesthetized children was also lower than the normal cells (Fig. 1).

The PHA response was measured of lymphocytes washed twice with Medium 199 and resuspended in RPMI + 10% FCS from six anaesthetized children, as well as in their whole

blood. Only one whole blood preparation responded to PHA but all responded after washing (Fig. 2). Since these cultures were in FCS, the values are not comparable with Fig. 1.

Considerable PHA response also occurred when either patients' washed cells or normal cells were cultured in the patients' serum, taken while under anaesthesia (Table 1), so serum blocking factors were not a major factor in the failure of the cells to respond.

In the blood of three children taken for routine purposes shortly before anaesthesia, the

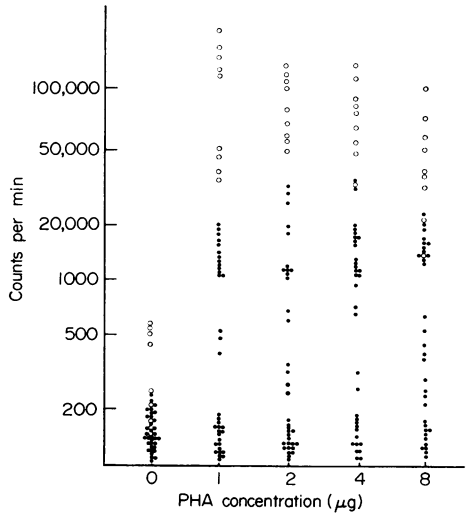

FIG. 1. Thymidine uptake (counts per min) in whole blood cultures without stimulus (zero time) and with various doses of PHA (μg/ml) in thirty-six children anaesthetized for tonsillectomy (●) and in nine healthy adults (○).

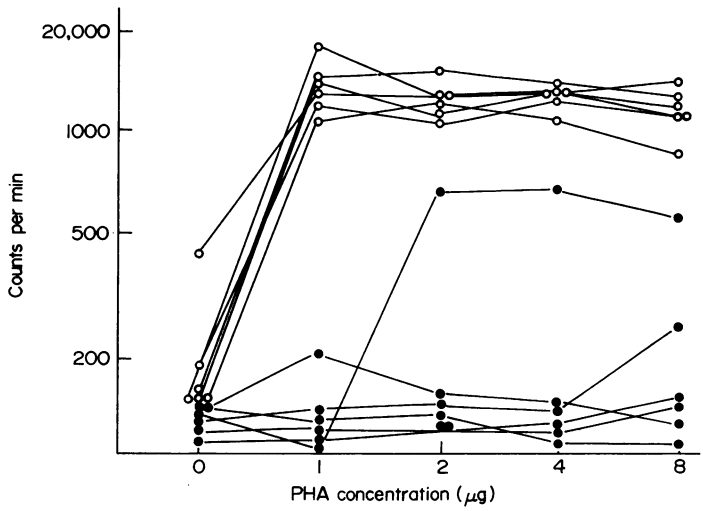

FIG. 2. PHA responses in the whole blood of six children (●) and in their lymphocytes after washing in foetal calf serum (○).

PHA response was similar to that of healthy adults, but the response under anaesthesia was considerably less (Fig. 3). In one, studied 1 month after the operation, the response was again at the pre-operative level.

The proportion of lymphocytes which formed spontaneous rosettes with sheep red cells and which had surface IgG, IgM and IgA detected by immunofluorescence did not differ from that of healthy adult controls ($P > 0{\cdot}10$) (Table 2).

TABLE 1. Peak PHA response (measured in counts per min) of cultures in patients' serum taken under anaesthesia, of patients' cells, collected under anaesthesia but washed after separation, and of normal lymphocytes

Patients' washed lymphocytes	Healthy adult lymphocytes
24,207	30,018
9864	53,923
10,671	22,296

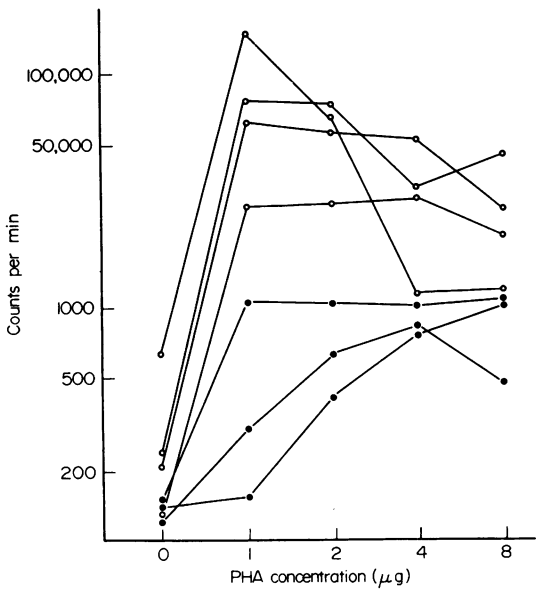

FIG. 3. PHA responses of three children shortly before (○) and while under anaesthesia (●). The two highest peaks are repeat studies on the same child.

The serum levels of IgG, IgM and IgA are shown in Fig. 4. IgM was similar to the control data used by Donovan & Soothill (1973) and Hobbs (1970). Two-thirds of the IgG and IgA values fell below the mean of both control series, but only two children fell below the mean −2 s.d. of the control series, one for IgA and one for IgG.

TABLE 2. Percentage of lymphocytes forming spontaneous red cell rosettes (SRCR) and staining for surface immunofluorescence with anti-IgG, IgM and IgA (SIF). The mean and observed range in the two groups of subjects are given

	Number of patients	SRCR (%)	SIF (%)		
			IgG	IgM	IgA
Tonsillectomy patients	36	55 (43–70)	9·3 (4–17)	9·9 (3–20)	5·2 (2–14)
Adult controls	8	62 (47–82)	11·7 (5–18)	11·5 (6–20)	5 (3–14)

The results are given as the mean percentage followed by the range in parenthesis.

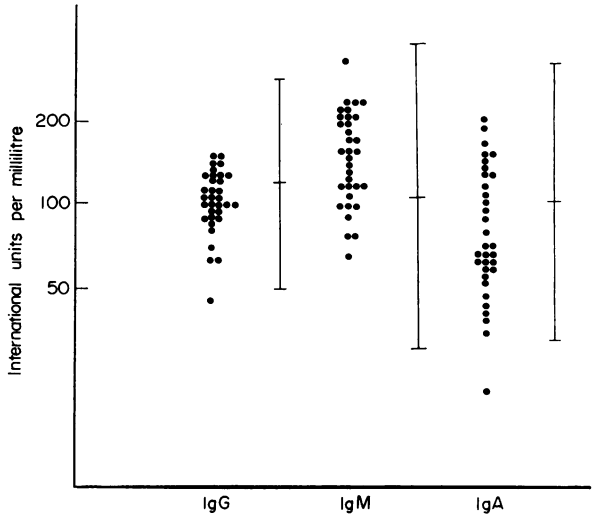

FIG. 4. Serum immunoglobulin concentrations (i.u./ml) in thirty-three children anaesthetized for tonsillectomy. The vertical lines indicate the antilog of the mean ±2 s.d. on logged data for healthy children of similar age.

DISCUSSION

The low PHA response in children anaesthetized for tonsillectomy is corrected by washing, is not associated with a serum suppressive factor, and is not present before the anaesthetic. This suggests that it is an effect of anaesthesia. Most of the reported studies of anaesthetic and analgesic drugs on cell responses are of *in vitro* effects. Salicylates and phenobarbitone suppress the lymphocyte response *in vitro* (Packman, Esterly & Peterson, 1971; Opelltz, Terasaki & Hirata, 1973; Park & Brody, 1971). The latter also report an *in vivo* effect; a few phenobarbitone takers had low PHA responses. Packman *et al.* (1971) reported that the aspirin effect can be removed by washing the cells. Diphenylhydantoin depresses the DNA synthesis in non-stimulated cultures (MacKinney & Booker, 1972). There is also an interest-

ing study of the arrest of mitosis of dividing cells at the root tip of the broad bean after *in vitro* exposure to halothane (Nunn, Lowis & Kimbal, 1972).

In a previous study (Donovan & Soothill, 1973) we studied selected tonsillectomy patients with a defined experience of recurrent sore throats, and noted, besides the defective PHA response, low levels of serum IgA which were significantly related to the infecting organism and to the outcome following tonsillectomy (Donovan, 1973). The IgG values were also rather low. Though the IgG and IgA levels tend to fall below the control mean in this series of unselected tonsillectomy patients (this itself indicates an intrinsic deficiency in a group of patients with recurrent infections), fewer grossly low values were found, suggesting that the selection of our previous series was of greater significance than we had realized. Our present study makes it unlikely that the defective PHA response was related to the disease process, and so its significant relationship to infecting organism must be regarded as coincidence. Sheep red cell rosettes and surface immunofluorescence studied in our present series also failed to detect abnormalities of lymphoid cell populations.

The only factors prior to tonsillectomy which might affect PHA response are anxiety and anaesthesia. It seems likely that admission to hospital is the most stressful experience and the three children with normal response studied before anaesthesia had all just been admitted. Any alteration in PHA response as a result of increased endogenous corticosteroid production also seems excluded by the results of the washing and serum blocking factor studies. Anaesthetic drugs are, therefore, the most probable cause and N_2O, halothane, or suxamethonium are particularly suspected as only a proportion of the poor responders had barbiturates or cyclopropane.

It is common practice to obtain blood for control studies from anaesthetized children. Our results suggest that this cannot be done for lymphocyte studies.

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