Radio-isotopic Assessment of Phytohaemagglutinin-Stimulated Lymphocytes from Patients with Protein Calorie Malnutrition

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SUMMARY

Depressed uptakes of ^{3}H-thymidine after phytohaemagglutinin (PHA) stimulation of lymphocytes were found in 37,5% of 24 patients with protein calorie malnutrition (PCM). Folate deficiency accounted for 50% of these depressed uptakes. Iron deficiency was not a significant factor. ^{3}H-uridine uptake (RNA) was reduced in 37,5% of patients, and in the majority of cases when uridine uptake was depressed the same was found for thymidine. An unexpected finding was the lack of correlation between the morphological (100% abnormal) and the radio-isotopic assessment (37,5% abnormal) in PHA-induced lymphocyte transformation.

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The morphological response of peripheral lymphocytes to phytohaemagglutinin (PHA) among children suffering from protein calorie malnutrition (PCM) has been extensively investigated.[1-3] A significant feature of their findings was the wide range of transformation indices within the same series. Geefhuysen *et al.*[2] reported transformation indices varying from as low as 6% to 79%, with a mean of 44%. Grace *et al.*[1] reported a range between 34% and 93% for transformed cells, with a mean of 68%.

In a preliminary study on PCM patients we found that the dominant effect of malnutrition on PHA-stimulated lymphocytes was on untransformed cells, and that these cells showed the most rapid rate of recovery on treatment (Table I). The rise in the number of transformed cells was less affected, while the number of mitotic figures did not show a significant increase during recovery.

A classification of the role of DNA synthesis appears to be called for to explain the observed variations in the number of transformed cells. It is also clear from our preliminary studies that the evaluation of RNA metabolism needs to be fully investigated to determine the significance of the presence of abnormal numbers of untransformed cells.

An investigation of ^{3}H-uridine and ^{3}H-thymidine uptake was undertaken to assess both RNA and DNA metabolism during PHA-induced transformation in malnourished children. In order to correlate the morphological and radio-isotopic assessments of PHA-induced lymphocyte transformation in PCM, both methods were used in some of the patients.

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TABLE I. THE RECOVERY PATTERN OF PHA-STIMULATED LYMPHOCYTES DURING TREATMENT OF PCM

Cell type	Time (days)	Mean ± SD (mean)	Probability of over-all improvement
Untransformed	0	24,1 ± 13,3	
	10	14,1 ± 9,2	0,01 (t_{13} = 2,86)
	20	7,2 ± 2,4	
Transformed	0	77,2 ± 11,2	
	10	82,4 ± 8,5	0,05 (t_{13} = 1,93)
	20	87,0 ± 3,1	
Mitotic	0	3,93 ± 1,8	
	10	4,45 ± 1,9	0,10 (t_{13} = 1,61)
	20	5,84 ± 2,3	

PATIENTS AND METHODS

Patients

Twenty-four Black children with PCM, between the ages of 8 months and 6 years, were divided into two groups so as to minimise the number of tests done on each series.

Group 1 consisted of 16 PCM patients with 11 controls matched for race, age and sex.

Group 2 consisted of 8 PCM patients and 5 controls.

The diagnosis of all patients was established on accepted clinical criteria and a serum albumin level of less than 2,9 g/100 ml.

Bone Marrow

On admission the patients in group 1 had bone marrow smears assessed for the presence of iron stores and megaloblastic changes due to folate deficiency.

PHA Stimulation of Lymphocytes

In group 1 the radio-isotopic determination of DNA, and in group 2 DNA, RNA and morphology of PHA-stimulated lymphocytes, were assessed. Lymphocytes were separated by Boyum's[4] Ficoll Hypaque technique from 5 ml of freshly drawn venous blood collected in 15 ml of saline and 100 units of thromboliquine. The 20 ml of diluted blood was aseptically layered onto 10 ml of Ficoll Hypaque (specific gravity 1,077) in a plastic bottle. The bottles were spun for 40 minutes at 400 *g*. The lymphocytes were collected from the interface and then washed three times using Hanks balanced salt solution. The cell pellets were resuspended in medium 199 supplement with 20% (v/v) AB serum to give a final concentration of 2×10^6 lymphocytes per 3 ml. Three-millilitre amounts of these suspensions were cultured in plastic screw-capped tubes. All the cultures were stimulated with 0,1 ml of PHA (Borroughs Wellcome) as shown in Fig. 1. For the determination of RNA synthesis 0,1 ml containing 20 μCi/ml (specific activity 2,3 Ci/m-mol) of ^{3}H-uridine was added to the cultures, as shown in Fig. 1, while for the DNA determination 0,1 ml of ^{3}H-thymidine containing 20 μCi/ml was used (specific activity 2 μCi/mg).

The cultures were harvested and counted as described previously by Burgess *et al.*[5] The morphological assessment of PHA stimulation was carried out as previously described by Grace *et al.*[1]

Treatment

All patients received a routine high protein diet without vitamin supplements, and antibiotics when indicated, during the period of study.

RESULTS

Group 1

Six of the 16 patients were found to have a reduced ^{3}H-thymidine uptake when compared with the control group (Table II). Of the 5 children who were iron-deficient, 3 had a normal ^{3}H-thymidine uptake, while in the remaining 2 the uptake was reduced. Considering all 16 patients, it was shown that iron was not a significant factor (P = 0,758) in causing depressed uptake levels. Four children were found to be folate-deficient, 3 of whom had a reduced uptake of thymidine, while the remaining child received a blood transfusion before the test. Excluding this patient, the probability of correlation between folate levels and thymidine uptake was significant (P = 0,044).

The 3 folate-deficient patients with reduced thymidine uptake were retested over a period of 40 days while on treatment for PCM. No folic acid supplements were added to their diet during this period. It was found that their uptake levels remained below normal (Fig. 2). Within 20 days of folic acid treatment 2 of the 3 patients showed a marked response, one rising to normal and the other exceeding this level. The third patient showed a good response to treatment, although his thymidine uptake had not reached normal values during the period of our investigation.

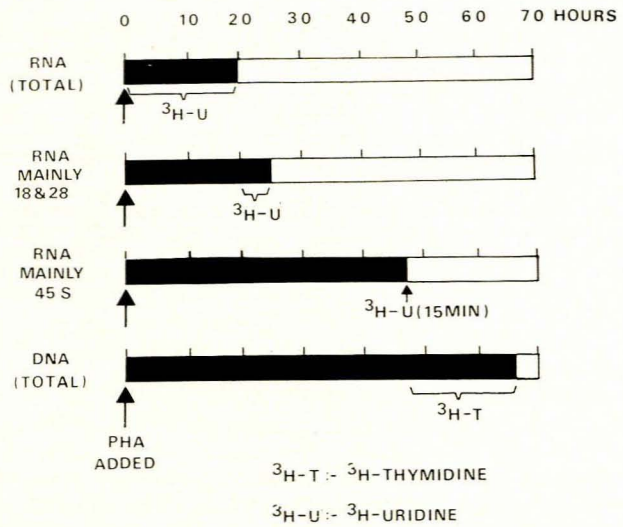

Fig. 1. Standard procedures of culture.

TABLE II. TRITIATED THYMIDINE UPTAKE CORRELATED WITH IRON AND FOLATE DEFICIENCIES AND DEATH IN PCM

PCM patients	^{3}H-thymidine uptake (DNA) Low	Normal	P of correlation using all patients (Fisher's extract method)
Total	**6**	**10**	
Iron-deficient	**2**	**3**	**0,758**
Folate-deficient	**3**	**1***	**0,117 (0,044)†**
Died	**2**	**0**	**0,125**

* Had blood transfusion.
† Recalculated probability excluding patient who had the blood transfusion.

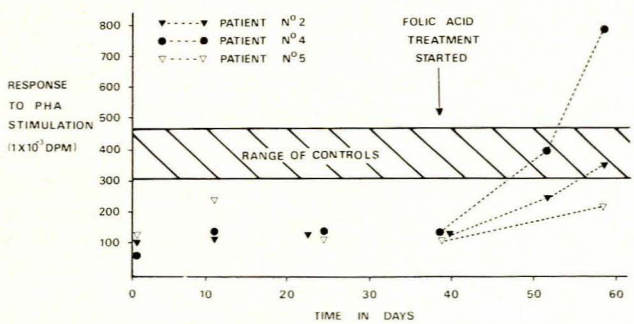

Fig. 2. Response to folic acid treatment of PCM patients with prolonged depression of lymphocyte transformation.

Group 2

The results of both the morphological and radio-isotopic assessments of PHA stimulation are shown in Table III.

TABLE III. COMPARISON BETWEEN THE MORPHOLOGICAL AND RADIO-ISOTOPIC ASSESSMENT OF PHA-STIMULATED LYMPHOCYTES

	Radio-isotopic uptake in DPM x 0^{-3}		Morphological assessment of cell types (%)		
Patient No.	RNA	DNA	Untransformed	Blastoid	Mitotic
1	161	290	24,8	74,2	1,0
2	159	502	20,6	77,4	2,0
3	320	469		Contaminated	
4	120	348	10,2	87,5	2,3
5	248	422	21,5	77,2	1,3
6	106	195	24,3	74,5	1,2
7	47	53	23,0	76,0	1,0
8	151	418	7,2	88,5	4,3
Controls	194	414	4,5	90,9	4,5
(Range)	(139 - 335)	(326 - 491)	(2,3 - 6,8)	(89,1 - 94,1)	(3,3 - 6,8)

Patients 6 and 7 showed considerably reduced levels of ^{3}H-uridine and ^{3}H-thymidine uptake, while patients 1 and 4 showed slightly reduced thymidine and uridine levels, respectively. The morphological assessment of PHA stimulation showed that all of the patients tested had an abnormal pattern of response. No correlation was evident between untransformed cells and ^{3}H-uridine (RNA) uptake ($r_7 = 0{,}030$; $P>0{,}1$) or the blastoid cells and ^{3}H-thymidine (DNA) uptake ($r_7 = 0{,}395$; $P>0{,}1$).

TABLE IV. ASSESSMENT OF DIFFERENT FRACTIONS OF RNA SYNTHESISED DURING PHA STIMULATION OF LYMPHOCYTES

Patient No.	Total	18S and 28S	45S
1	161	125	30
2	159	176	45
3	320	279	38
4	120	87	31
5	248	216	37
6	106	50	12
7	47	48	4
8	151	118	29
Control	194	153	35
(Range)	(139 - 335)	(94 - 302)	(19 - 49)

In a more detailed study of RNA synthesis (Table IV) it was found that patients 6 and 7 had reduced levels of all fractions of RNA. Patient 4, who had normal DNA and reduced RNA synthesis, showed this reduction in the 18S and 28S fractions of RNA, but not in the 45S. The other 5 patients had normal RNA synthesis patterns.

DISCUSSION

The findings show a marked difference between the morphological and radio-isotopic assessments of PHA-induced lymphocyte transformation in PCM. All patients showed an abnormal response when assessed morphologically, whereas only 37,5% were abnormal when using ^{3}H-thymidine.

As no correlation ($r_7 = 0{,}3951$; $P>0{,}1$) was found between transformed cells and DNA synthesis, it was thought that a block at an earlier stage in the cell cycle involving RNA synthesis could explain this finding. However, RNA synthesis was impaired in only 3 of 8 malnourished children, and uridine uptakes did not correlate ($r_7 = 0{,}030$; $P>0{,}1$) with the number of untransformed cells. In the majority of cases when DNA synthesis was depressed, the same was also true for RNA synthesis ($r_s = 0{,}7408$; $P<0{,}05$).

Results of this study, which have correlated folate deficiency with depressed ^{3}H-thymidine uptakes, support the findings of Gross *et al.*,[6] of an impaired lymphocyte response to PHA in patients with folate-deficient megaloblastic anaemia. The role of iron deficiency as a cause of reduced ^{3}H-thymidine uptake[7] is only partly supported by results from the 5 iron-deficient patients in this series. A preterminal state due to septicaemia in 2 PCM patients, was also associated with depressed uptakes of ^{3}H-thymidine.

Although our results show no correlation between the morphological and radio-isotopic evaluation of PHA-induced lymphocyte transformation in PCM, and a derangement of DNA and RNA metabolism in 37,5% of patients, a more detailed study using a greater number of patients is required to interpret the significance of these findings.

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