

# The response to phytohaemagglutinin (PHA) of lymphocytes from cancer patients

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**SYNOPSIS** The uptake of tritiated thymidine by lymphocytes from 100 cancer patients and 31 normal subjects was tested in the presence and absence of phytohaemagglutinin (PHA) in a medium containing autologous or homologous serum. Significant differences were found between normal subjects and cancer patients in both the time course and degree of uptake. Serum from normal individuals restored the response of lymphocytes from cancer patients towards normality whilst serum from other cancer patients had no effect. Serum from cancer patients impaired the response of normal lymphocytes.

It has been suggested that cellular immune potential in man and animals may be assessed by measuring the capability of small lymphocytes to transform in the presence of PHA (Bloom, 1971). There are several reports that this is impaired in certain diseases including cancer (Gatti, 1971). The reason for the impairment of transformation in cancer patients has been attributed either to abnormality of the lymphocytes or to the presence of inhibitors in sera. The transformation of lymphocytes from healthy individuals has been shown to be reduced by incubation with sera from cancer patients (Silk, 1967; Whittaker, Rees, and Clark, 1971). Other workers have failed to detect inhibitors and some have even described stimulatory factors in the sera of patients with cancer (Kasakura and Lowenstein, 1967; Golob, Israsena, Quatrone, and Becker, 1969; Al-Sarraf and Vaitkevicius, 1971).

Recently there has been evidence that PHA-induced transformation of lymphocytes may be influenced by a number of factors apart from the presence or absence of disease, eg, general anaesthetics (Park, Brody, Wallace, and Blake, 1971), day-to-day spontaneous variation (McIntyre and Cole, 1969), and differences in technique such as varying periods of incubation (Bredt and Mardiney, 1969; McIntyre and Cole, 1969; Nelson, 1969; Park *et al*, 1971) and concentrations of PHA (Ducos, Miguères, Colombies, Kessous, Poujoulet, 1970; Carr and Stites, 1972). Any investigation involving so many variables requires a large series of cases. However, only small numbers of cancer patients have

been studied in each of the reported series. In the present investigation, lymphocytes from 100 cancer patients and 31 healthy individuals were cultured for up to 168 hours in a medium containing either autologous or homologous serum, with or without PHA. The cancer patients were divided into three groups depending on whether the lymphocytes were obtained (a) before operation, (b) within one year after operation, (c) more than one year after operation.

## Materials and Methods

Blood was obtained by venepuncture from 31 healthy individuals and from 100 cancer patients. None of the patients had received radiotherapy. None had received chemotherapy within two weeks of taking the blood sample. The patients included both children and adults suffering from a wide variety of tumours as shown in table I. Although the control group was smaller than the cancer patient group an attempt was made to make the age distribution of the two groups comparable. The normal adults in the group were

Tumour	No. of Patients
Leukaemia	5
Lymphoreticular neoplasms	11
Melanoma	6
Nervous tissue neoplasms	26
Sarcomas	16
Breast carcinoma	9
Genito-urinary tract neoplasms	23
Other neoplasms	4

Table I *Diagnosis in the 100 patients studied*

hospital workers and the children inpatients suffering from a variety of simple non-neoplastic conditions.

Lymphocytes were separated by the method of Coulson and Chalmers (1967) using a sterile 3% solution of pigskin gelatin and carbonyl iron powder. The serum was decplemented by heating for 30 minutes at 56°C. Cells, after being twice washed, with medium 199, were resuspended to contain  $1 \times 10^6$  cells/tube in 1 ml of medium 199 containing 30% of either autologous or homologous serum-gelatin mixture. A vial containing freeze-dried reagent grade PHA (Burroughs Wellcome) was reconstituted in 5 ml of physiological saline, 5  $\mu$ l of this solution was added to each of the culture tubes, and they were incubated at 37°C in an atmosphere of 5% CO<sub>2</sub> in air. Whenever possible, cultures were done in quadruplicate. Three hours before termination, 2  $\mu$ Ci of <sup>3</sup>H Td R (5 ci/m Mo1) was added to all cultures. Radioactive thymidine incorporation into DNA was measured by a slightly modified method of Hartog, Cline, and Grodsky (1967). Cells were successively washed with 1% acetic acid, twice with 5% trichloroacetic acid, and twice with methanol. The precipitate formed was dissolved in 0.2 ml of hyamine hydroxide at 56°C for 30 minutes, after which scintillation fluid was added. The results obtained by scintillation counting are expressed as log<sub>10</sub> CPM. The statistical test employed to analyse the results was determined in the main by the number of observations. For a small number of observations from two related series Wilcoxin's signed rank sum test was used, and the Mann-Whitney u-test for two unrelated series. Otherwise, Student's t test was used.

## Results

A pilot study of the lymphocytes of 10 cancer patients and 10 healthy individuals cultured in autologous serum in the presence of varying concentrations of PHA was carried out. The maximum response with least scatter and a minimum variation in the values of replicates was obtained by using 5  $\mu$ l of PHA per culture.

Table II shows the uptake of <sup>3</sup>HTdR by cultured lymphocytes from healthy donors and cancer patients, with and without phytohaemagglutinin. Statistical analysis of the responses obtained at 72, 120, and 168 hr in healthy donors and cancer patients within one year of operation shows that peak transformation occurred at 72 hr (table III). However, samples obtained from cancer patients, either preoperatively or more than one year after operation gave results at 72 hr which were not significantly different from those at 120 hours. It is apparent from tables II and III that in healthy donors and cancer patients as a whole (table III, group D), 72-hour responses were greater than at 120 hr and at 120 hr greater than at 168 hr, in the presence of PHA.

Responses of healthy lymphocytes cultured for 72 hr, with or without PHA, were significantly greater than those of cancer patients (table IV). From the same table it can be seen that comparing lymphocytes from healthy donors and cancer patients, no statistical difference between the responses was obtained at 120 hr and 168 hr either with or without phytohaemagglutinin.

The results of the incubation of lymphocytes

Source of Lymphocytes	Period of Incubation		
	72 Hours	120 Hours	168 Hours
<i>Healthy Individuals</i>			
With PHA	4.95 ± 0.22 (31) <sup>1</sup>	4.44 ± 0.63 (16)	4.22 ± 0.51 (16)
Without PHA	2.92 ± 0.55 (31)	2.47 ± 0.47 (16)	2.29 ± 0.47 (16)
<i>Cancer Patients</i>			
<i>Before operation</i>			
With PHA	4.59 ± 0.58 (19)	4.65 ± 0.22 (17)	4.21 ± 0.49 (13)
Without PHA	2.61 ± 0.29 (19)	2.70 ± 0.49 (16)	2.39 ± 0.21 (13)
<i>&lt; One Year after Operation</i>			
With PHA	4.53 ± 0.53 (42)	4.29 ± 0.46 (32)	3.91 ± 0.65 (30)
Without PHA	2.63 ± 0.51 (42)	2.72 ± 0.79 (32)	2.55 ± 0.59 (30)
<i>&gt; One Year after Operation</i>			
With PHA	4.83 ± 0.26 (39)	4.66 ± 0.28 (19)	4.09 ± 0.53 (22)
Without PHA	2.71 ± 0.45 (39)	2.65 ± 0.47 (17)	2.29 ± 0.26 (22)
<i>All Cancer Patients</i>			
With PHA	4.66 ± 0.47 (100)	4.51 ± 0.39 (68)	4.03 ± 0.58 (65)
Without PHA	2.66 ± 0.45 (100)	2.70 ± 0.63 (68)	2.44 ± 0.47 (65)

Table II Mean and standard deviations of <sup>3</sup>HTdR uptake log<sub>10</sub> of CPM by lymphocytes from normal subjects and cancer patients in the presence and absence of phytohaemagglutinin

<sup>1</sup>All tests carried out in autologous serum.

<sup>2</sup>Number of individuals

Source of Lymphocytes	Treatment	Statistical Significance of Differences in Response at Different Times			
		72 Hours vs 120 Hours	72 Hours vs 168 Hours	120 Hours vs 168 Hours	
Healthy donors	With PHA	72 > 120 (P < 0.01)	72 > 168 (P < 0.001)	NS <sup>1</sup>	
	Without PHA	72 > 120 (P < 0.01)	72 > 168 (P < 0.001)	120 > 168 (P < 0.01)	
Cancer patients Before operation	With PHA	NS	72 > 168 (P < 0.01)	NS	
	Without PHA	NS	NS	NS	
	< 1 year after operation	With PHA	72 > 120 (P < 0.05)	72 > 168 (P < 0.001)	NS
		Without PHA	NS	NS	NS
	> 1 year after operation	With PHA	NS	72 > 168 (P < 0.001)	120 > 168 (P < 0.05)
		Without PHA	NS	72 > 168 (P < 0.001)	NS
Total before and after operation	With PHA	72 > 120 (P < 0.02)	72 > 168 (P < 0.001)	120 > 168 (P < 0.02)	
	Without PHA	NS	NS	NS	

**Table III** Statistical analysis to compare time of peak transformation in normal individuals and cancer patients

<sup>1</sup>NS = statistically not significant.

Groups Compared	Treatment	Statistical Significance of Differences in Response at Different Times (Student's t test)		
		72 Hours	120 Hours	168 Hours
1 Healthy donors (A) Vs cancer patients before operation (B)	With PHA	A > B (P < 0.02)	NS <sup>1</sup>	NS
	Without PHA	A > B (P < 0.02)	NS	NS
2 Healthy (A) vs cancer patient < 1 year after operation (B)	With PHA	A > B (P < 0.001)	NS	NS
	Without PHA	A > B (P < 0.05)	NS	NS
3 Healthy (A) vs cancer patient > 1 year after operation (B)	With PHA	A > B (P < 0.05)	NS	NS
	Without PHA	NS	NS	NS
4 Healthy (A) vs total cancer patients before and after (B)	With PHA	A > B (P < 0.001)	NS	NS
	Without PHA	A > B (P < 0.01)	NS	NS

**Table IV** Statistical analysis to compare differences in height of peak transformation in healthy subjects and cancer patients

<sup>1</sup>Statistically not significant.

Source of Lymphocytes	Source of Serum	Statistical Analysis		
		Autologous (A)	Homologous	
			Healthy Donors (B)	Cancer Patients (C)
Cancer patients	4.80 ± 0.40 (21) <sup>1</sup>	4.94 ± 0.49 (23)	4.81 ± 0.33 (19)	A vs B (P < 0.05) A vs C (NS) <sup>2</sup> A vs B (NS) A vs C (P < 0.01)
Healthy donors	4.96 ± 0.36 (22)	5.01 ± 0.19 (17)	4.65 ± 0.74 (32)	

**Table V** Phytohaemagglutinin-induced transformation at 72 hours of lymphocytes from cancer patients and healthy donors in the presence of autologous or homologous serum (mean standard deviation of log<sub>10</sub> CPM)

<sup>1</sup>The number in parentheses is the number of individuals tested.

<sup>2</sup>Statistically not significant.

obtained from healthy donors and cancer patients with autologous and homologous serum are presented in table V. The transformation of cancer patients' lymphocytes is significantly increased when incubated with sera from cancer patients. No significant difference was found when normal homologous serum was substituted for autologous serum in the transformation of lymphocytes from normal subjects. Similarly, the substitution of autologous serum from other cancer patients did not alter the

depressed transformation of lymphocytes from cancer patients.

## Discussion

The present study shows that the peak uptake of tritiated thymidine by lymphocytes from healthy individuals, in the presence and in the absence of PHA, occurs at 72 hr; the peak transformation in cancer patients was often later, both with and

without phytohaemagglutinin. Lymphocytes obtained from cancer patients within one year of operation behaved differently and 72-hr responses were greater than those at 120 hr, ie, a tendency to show a more normal response.

Stimulation of lymphocytes of cancer patients who had been clinically free of disease for more than one year showed a slightly but not statistically significantly greater response than occurred in lymphocytes taken from patients either before operation or within one year of operation. The low response in cancer patients compared with healthy individuals at 72 hr confirms some earlier reports. However, the differences between the responses of cancer patients' lymphocytes were not significantly different from those of healthy individuals at 120 and 168 hours. This might be one of the reasons for conflicting reports.

The responses of cancer patients' lymphocytes cultured without PHA follow a similar, though much lower, pattern when compared with the corresponding PHA-stimulated group. It is possible that the transformation seen in the absence of PHA in our study might have been due to the presence of antibiotics and the serum-gelatin mixture in the culture media.

In the present study incubation of lymphocytes derived from healthy donors and cancer patients in autologous and homologous sera supports the contention that the sera from cancer patients significantly depresses the ability of healthy lymphocytes to transform and the addition of sera from healthy individuals improves the response of cancer patients' lymphocytes. The addition of homologous healthy serum to the medium failed to alter the responses of healthy lymphocytes. Similarly, the addition of homologous cancer serum to cultures of cancer patients' lymphocytes had no effect. The nature of the serum inhibitors is not known; an  $\alpha_2$ -globulin (Cooperband, Bondevik, Schmid, and Mannick, 1968), polypeptide (Scheurlen, Schneider, and Pappas), and IgG (Barnes, Bishun, and Holliday, 1970) have been suggested. It is also possible that there is a lack of growth-stimulating factor in the sera of cancer patients.

The relevance of the depressed lymphocyte transformation and the inhibitory action of cancer patients serum on autologous and homologous lymphocytes is problematical. Whether it represents partial failure of immunological surveillance and hence might be important aetiological or is a result of the disease state could only be satisfactorily solved

by a long-term prospective study. That the abnormality demonstrated preoperatively tends towards normality in lymphocytes from patients some time postoperatively suggests a tumour-dependent effect rather than an aetiological factor.

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