

Cell-mediated immunity in chyluria

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Cellular immune response to mitogens phytohemagglutinin (PHA) and poke weed mitogen (PWM) was assessed in 13 patients with chyluria and 32 healthy controls. The mean stimulation Index of the patient group was significantly lower than the control group. The degree of depression was neither related to the duration of excretion of chyle nor to the microfilaraemic status.

Transformation of lymphocytes *in vitro* induced by mitogens and antigens is a parameter commonly used to assess cellular immunity in patients with immunodeficiency, auto-immunity and infectious diseases. Host immune response to filarial infection has been under intensive study in the recent past. The varied clinical presentations found among individuals in endemic regions have been related to different types of immunological responses among infected individuals. The common presentation of chronic bancroftian filariasis in Madras includes lymphatic oedema of the limbs, hydrocele and chyluria. Chyluria is the result of obstruction and dilatation of the thoracic duct or its tributaries followed by the rupture of distended lymphatics in the urinary tract¹. To our knowledge, studies in cellular immunity in patients with chyluria are meagre. We report in this communication our observation that peripheral blood lymphocytes from chyluria patients show marked reduction in res-

ponse to phytohaemagglutinin (PHA) and poke weed mitogen (PWM).

Material and Methods

Patients for the study were chosen from those attending the Filariasis Clinic of Government General Hospital, Madras. Thirteen patients with the complaint of passing milky urine were admitted to the study. The clinical history of the patients is summarised in Table I. In addition, 32 healthy subjects were included as controls.

Lymphocyte transformation in vitro : Samples of peripheral blood were drawn from chyluria patients and volunteers, and lymphocytes were isolated by Ficoll-Hypaque density gradient centrifugation. Cells were cultured in quadruplicate in 0.2 ml of RPMI 1640 supplemented with penicillin (100 IU/ml), streptomycin (100 µg/ml), glutamine (300 µg/ml) and 10 per cent autologous plasma in 96-well

Table 1. Transformation of lymphocytes by mitogens in 13 chyluria patients and 32 healthy subjects (control)

Patient no.	Sex/age	Mf in blood	Clinical status	Duration of chyle excretion (months)	Stimulation index*		
					PHA	PWM	
1	M/19		Episodes of fever, rigor and lymphadenitis	4	0	ND	
2	M/22		-do-	6	9	ND	
3	M/47	+ve	-do-	3	7	ND	
4	M/50		-do-	6	2	7	
5	M/20		-do-	3	3	0	
6	F/19		Inguinal lymphadenitis	6	26	ND	
7	M/20		Fever, rigor, lymphadenitis, hydrocele	18	0	0	
8	M/18	-ve	Fever, rigor, lymphadenitis	12	0	0	
9	M/21		Filarial fever	12	33	14	
10	M/28		Previous history of hydrocele	6	1.5	4	
11	M/30		No evidence of filariasis	3	0	0	
12	F/21	-ve	-do-	2	0	15	
13	F/23		-do-	2	3.5	3	
Control subjects (mean of 32)					45	15	

ND, Not done. *For definition, see Material and Methods

(U-bottom) tissue culture plate (Dynatech Laboratories Inc., Alexandria, Va) at a concentration of 0.5×10^6 cells/ml. PHA and PWM were added at a final concentration of 1 μ g/ml and 1/200 dilution, respectively. These concentrations were used, on the basis of our earlier study on human volunteers (unpublished data). Cultures were incubated at 37°C in an atmosphere at 5 per cent CO₂ for 96 h. The proliferative response was measured by adding 1.0 μ Ci of ³H-thymidine (Sp. act. 13000 mCi /mol, Bhaba Atomic Research Centre, Bombay) and incubating for 18 h at 37°C.

Cells were harvested with Mash II (Microbiological Associates, USA) and

deposited on Whatman filter paper. Paper discs were then transferred to biovials containing 2.5 ml of scintillation fluid (4 g of PPO and 0.5 g of POPOP in 1 litre toluene) and counted for 50 sec in a liquid scintillation counter (LSS-20, ECIL, India). Stimulation index (SI) was calculated as follows :

$$\frac{\text{cpm in stimulated cultures}}{\text{cpm in control cultures}}$$

Results and Discussion

The mean stimulation index of 32 healthy subjects was 45 and 15 for PHA and PWM respectively. Eleven out of 13 chyluria patients had a stimulation index of less than 10 for PHA. The remaining

two had moderate stimulation indices of 26 and 33. Considering PWM, 7 out of 9 patients tested had an SI of 7 or less and in the remaining 2 the values were normal. In a variety of parasitic infections such reduction in the cellular immune responses has been observed²⁻⁴. In the present study the degree of depression of mitogen response was neither related to the duration of excretion of chyle nor to microfilaraemic status of the patient. This reduced response was not merely due to a shift in the peak of dose or time kinetic curves, because the reduced response was seen at all time periods of the culture and with different doses of mitogen studied (unpublished data). The reduction in the response may not be due to humoral blocking factors of lymphocyte activation, as the addition of plasma from chyluria patients does not reduce the response of lymphocytes from healthy volunteers to mitogens. Representative data are provided in Table II, similar results have been observed with plasma from a number of chyluria patients. The absolute lymphocyte counts in the peripheral

blood of chyluria patients were considerably reduced. Similar observations were also reported by Date *et al*⁵. The reduction in the mitogen response as such may not be solely due to this reduction in the absolute lymphocyte counts because during *in vitro* lymphocyte culture for both healthy subjects and chyluria patients, the cell suspensions are diluted appropriately in order to have similar number of lymphocytes in each well of the microtitre plate. It is however possible that selective loss of mitogen responsive lymphocytes from the peripheral blood might have occurred. A similar phenomenon has been observed in intestinal lymphangiectasia⁶. Our attempts to collect and characterise the lymphocytes from urine have not been successful, as only 2 out of 13 patients had lymphocytes in their urine.

Our *in vitro* results supplement the observations of Matsumoto *et al*⁷ of marked reduction in skin test reactions to PPD, PHA and DNCB in chyluria patients. The mechanism of this immunosuppression is under further study.

Table II. Blastogenic response of peripheral blood lymphocytes of a normal subject in presence of normal plasma and patient's plasma

Source of plasma	Mean counts/50 sec±SD		
	Control	PHA	PWM
Autologous	552 ± 138	32900 ± 11741	10127 ± 2258
Normal human plasma	1 705 ± 242	12645 ± 4594	6479 ± 1742
	2 550 ± 193	4327 ± 3241	1906 ± 583
Chyluria plasma	1 1052 ± 346	24482 ± 3354	10565 ± 3197
	2 697 ± 546	26606 ± 4858	11090 ± 4295

Lymphocytes were from peripheral blood from a normal subjects

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