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STUDY OF CELL MEDIATED IMMUNE RESPONSIVENESS TO SOLUBLE EGG ANTIGEN AND PHYTOHAEMAGGLUTININ USING THE LYMPHOBLAST TRANSFORMATION TEST IN HUMAN SCHISTOSOMIASIS MANSONI IN EGYPT

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SUMMARY

Soluble egg antigen and phytohaemagglutinin have been used to assess the cell mediated immune responsiveness in patients with schistosomiasis mansoni using the lymphoblast transformation test. Soluble egg antigen gave a higher response in cases with hepatosplenomegaly when compared with cases of simple intestinal bilharziasis, with a peak response at the youngest age groups. Phytohaemagglutinin gave the lowest response in cases with simple intestinal bilharziasis and the higher response was given by the middle age group. This supports the view of the importance of cell mediated immune state of the host in the various manifestations of bilharziasis.

INTRODUCTION

The pathogenesis of schistosomal lesions is now assumed to be partly due to both cell mediated and humoral immunity (WARREN [13]).

The bilharzial granuloma, the main etiological factor in the production of this disease is considered to be a manifestation of delayed hypersensitivity (WARREN et al. [14]).

In a previous study the delayed intradermal test and migration inhibition test showed greater cell mediated immune responsiveness in patients with bilharzial hepatic fibrosis (BHF) than those with simple intestinal bilharziasis suggesting a relationship between the immune state of the patient and the clinicopathological picture of the disease (HELMY KHALIL et al. [6]).

In the present work, the lymphoblast transformation test (LTT), an **in vitro** correlative of cell mediated immunity (CMI), has been used to assess the delayed hypersensitivity in patients with bilharzial hepatosplenomegaly, and simple intestinal bilharziasis using soluble egg antigen (SEA) as the specific antigen, and a universal mitogen phytohaemagglutinin (PHA).

MATERIAL AND METHODS

Sixty male bilharzial patients were subjected to routine laboratory examinations which included complete blood picture, stool examination, plain X-ray chest, liver function tests and percutaneous liver biopsy. Accordingly they were subdivided as follows:

Forty patients with BHF (Group I) as ascertained by liver biopsy which demonstrated the presence of bilharzial ova, their remnants and the bilharzial pigments as well as the associated pathological changes.

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Twenty patients with simple intestinal bilharziasis (Group II), with dysentery as the main complaint. Stool examination showed living bilharzial ova, where liver biopsy did not show hepatic involvement.

The study also included 40 male subjects serving as controls and these were further subdivided as follows:

Fourteen normal healthy controls (Group IIIa).

Six controls with parasitosis other than bilharziasis (Group IIIb) being infected with either ascariasis, ankylostomiasis or enterobiasis.

Twenty controls with liver diseases other than BHF (Group IV).

The latter two groups (III and IV) have been added to assess the specificity of the antigen; and they have been also subjected to the same battery of investigations including liver biopsy.

Antigen Preparation

The SEA was prepared according to the method of BOROS & WARREN [2].

Cell Culture

The method was essentially that of MAINI et al. [8] and WAITHE & HIRSCHHORN [12]. Thirty milliliters of heparinized blood were allowed to sediment at 37°C until the supernatant was substantially clear of RBCs. The supernatant white blood cells rich fraction was removed and centrifuged at 125g for ten minutes and the cell pellet was washed three times and resuspended in Eagle's tissue culture medium suplemented with HEPES buffer (Hopkins & Williams 20 mM/100 ml), penicillin (100 units/ml), streptomycin (100 μg/ml), L-glutamine (Ferak, FRG 30 mg/100 ml) and 10% inactivated human AB serum. The culture tubes were set up in quadruplets with various doses of SEA, without antigen and with the non-specific mitogen PHA (Welcome), at a final dilution of 1%. The cultures were incubated at 37°C in a humid atmosphere. They were the pulsed with one μCi per tube of ^{3}H-thymidine (Amersham 28 Ci/mMO) at the fourth day for 24 hours.

Cell Harvesting and Counting

The cells were collected from the culture wells on the fifth day on-to glass fibres filters, dried at 60°C, washed successively with saline, 5% trichloroacetic acid (Prolabo) then cold methanol (BDH). The samples were counted after addition of 10 ml of scintillation flora (New England Nuclear Boston).

The readings in counts per minute (cpm) have been converted into desintegration per minute (dpm) from an efficiency curve plotted by means of standards (Packard). The degree of response was expressed in net dpm; calculated as dpm of stimulated culture — dpm of unstimulated culture.

RESULTS

Pilot studies on the kinetics of the response to SEA demonstrated that a peak response occurred after about 96 hours of culture and decreased thereafter. Similarly dose response study showed that 10 μg of antigen containing protein per culture resulted in maximum lymphocyte transformation. Hence the peak was read after 96 hours using 10 μg of antigen.

A significant increase of increment blast transformation was noticed in patients of group I when compared with those of group II. Also a significant difference between group II and subgroup IIIa was noted (Tables Ia and b).

TABLE I-A

Mean increment (net desintegration per minute) using SEA, in the different groups studied in the lymphoblast transformation test (LTT)

Group	I	II	III	IIIa	IIIb	IV
Mean	104216.20	70212.55	258.50	259.29	256.67	310.80
S.D.	8912.30	25862.80	102.34	102.16	112.53	251.78

TABLE I-B

Intergroup significance using SEA

Groups compared	t	p
Group I/Subgroup IIIa	43.37	< 0.001
Group II/Subgroup IIIa	10.073	< 0.001
Subgroup IIIb/Subgroup IIIa	0.051	> 0.05
Group IV/Subgroup IIIa	0.6533	> 0.05
Group I/Group II	7.5212	< 0.001

When using 1% PHA, a significant increase of increment blast transformation was found

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in group I as compared to groups II and IIIa. No significant difference was seen between subgroup IIIa, IIIb and group IV (Tables IIa and b).

TABLE II-A

Mean increment (d.p.m.) using 1% PHA in the different groups studied in the LTT

Group	I	II	IIIa	IIIb	IV
Mean	59898.95	47379.10	54327.43	58005.17	46378.00
S.D.	8483.87	5603.08	7550.34	3326.51	10254.74

TABLE II-B

Intergroup significance using 1% PHA

Groups compared	t	p
Group I/Subgroup IIIa	2.172	< 0.05
Group II/Subgroup IIIa	3.084	< 0.005
Subgroup IIIb/Subgroup IIIa	1.1331	> 0.05
Group IV/Subgroup IIIa	1.8457	> 0.05
Group I/Group II	5.9678	< 0.001

When LTT in response to SEA was studied as increment in relation to age, the best response was observed in the youngest age in groups I and II (Tables IIIa and b).

TABLE III-A

Mean increment (d.p.m.) of LTT using SEA in the different age groups

Age groups		< 25 years	25-45 years	> 45 years
Groups:				
I	Mean	110551.00	102993.78	90658.00
	S.D.	6943.63	6917.86	7637.57
II	Mean	94795.00	71155.40	7168.50
	S.D.	5850.40	17667.75	839.34
III	Mean	221.86	269.90	339.33
	S.D.	124.15	85.44	116.24

TABLE III-B

Intergroup significance using SEA

Groups	Age groups	<25 years/25-45 years	25-45-years/>45 years
I	t	3.1441	3.2492
	p	< 0.005	< 0.005
II	t	2.0830	7.0661
	p	> 0.05	< 0.001
III	t	0.9492	1.1487
	p	> 0.05	> 0.05

When LTT response to 1% PHA expressed as increment value was studied in relation to age, the middle age group (25-45 years) showed the highest response in both groups I and II (Tables IVa and b).

TABLE IV-A

Mean increment (d.p.m.) of LTT using 1% PHA in the different age groups

Age groups		< 25 years	25-45 years	> 45 years
Groups:				
I	Mean	60675.54	62497.61	42432.75
	S.D.	8039.10	5347.96	2509.64
II	Mean	46600.63	50495.60	34910.50
	S.D.	3630.22	2755.52	4796.31
III	Mean	56827.57	57124.20	46526,67
	S.D.	4855.00	4342.89	11442.60

TABLE IV-B

Intergroup significance using 1% PHA

Groups	Age groups	<25 years/25-45 years	25-45-years/>45 years
I	t	0.8169	7.2743
	p	> 0.05	< 0.001
II	t	2.5919	6.6573
	p	< 0.02	< 0.001
III	t	0.1321	2.5700
	p	> 0.05	< 0.05

DISCUSSION

The sensitivity of the LTT in relation to SEA is clearly demonstrated by the highly significant blastogenic response in bilharzial patients as compared to healthy controls and the other control groups. A similar conclusion has been be reached by COLLEY [3], WEISS et al. [15] and KY et al. [7], in experimental animals and in human patients. ABOUL-ENEIN et al. [1] and WEISS et al. [15], when comparing the response of LTT to SEA and to worm antigen, obtained significantly higher results with the former antigen.

The antigenic specificity of LTT stimulation was exhibited by the inability of SEA to stimulate lymphocytes of normal control subjects in culture at the same concentration and also by its inability to stimulate culture of limphocytes from patients with other liver diseases or control cases with other parasitosis. The absence of influence by other helminthic infec-

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tions is in accordance with the finding of SHERIF [10] when using extract of miracidia of **Schistosoma mansoni.**

Patients with bilharzial hepatosplenomegaly showed a more significant response to SEA than those with simple intestinal bilharziasis. However, COLLEY [4] stated that chronic infection with **S. mansoni** led to diminished lymphocyte transformation responsiveness to SEA. Apparently this is applicable to advanced cases. ABOUL-ENEIN et al. [1] also found increase in LTT response in cases with hepatomegaly yet, in the more severe cases with ascites, a diminished response to SEA was found. None of our patients had shrunken liver or ascites.

The response to PHA was somewhat higher in cases with hepatosplenomegaly when compared with controls and intestinal cases. On the other hand, ABOUL-ENEIN et al. [1] found no appreciable change in LTT response in bilharzial patients when compared with controls except in advanced cases with ascites when depression was noticed and attributed that to secondary immunodeficiency. Our results may support the view of an innate variation in cell mediated immunity which would be responsible, amongst other factors, for the clinico-pathological outcome of the disease. The present findings are in accordance even with workers using other models than schistosomiasis as WILKINSON & WHITE [16], who suggested a direct correlation between the state of delayed hypersensitivity response of the host and the extent of granuloma formation in tuberculosis.

As regards the age, using SEA, the LTT showed a greater response in those aged less than 25 years. SMITH et al. [11] related the florid reactions observed in young patients infected with **S. haematobium,** and the minimal tissue response in patients around 40 years, to schistosome egg production as well as to host immunological reactivity.

Lymphoblastic transformation in response to PHA gave a different age relation response, which was maximal in the middle age group and agreed with the findings of HAGEN & FRØLUND [5] that a low response is elicited in very young and very old people. Diminished response in older age is due to involution of the thymus, though known to start before puberty, yet the gland's epithelial cells continue to produce thymosin and other hormones necessary for well functioning immune systems till the late twenties when they gradually become deficient (MCBRIDE [9]).

Thus, the higher LTT response observed in relation to SEA in patients with hepatic affection and especially those aged less than twenty five years, in conjunction with the results obtained with PHA, points to a possible relationship between the host cell mediated immunity and the different clinico-pathological manifestations of bilharziasis.

RESUMO

Estudo da resposta imunológica celular a antígeno solúvel de ovo e fitohemaglutinina através do teste de transformação linfoblástica na esquistossomose mansônica humana no Egito

Antígeno solúvel de ovo e fitohemaglutinina foram usados para medir a resposta imunológica celular dos pacientes com esquistossomose mansônica através do teste de transformação linfoblástica. Antígeno solúvel de ovo mostrou resposta maior nos casos com hepatosplenomegalia quando comparados com as formas intestinais simples, as respostas mais altas sendo reencontradas nos grupos etários mais jovens. Fitohemaglutinina mostrou resposta menor nos casos de esquistossomose intestinal simples e resposta maior nos grupos etários de média idade. Esses resultados corroboram a importância da resposta imunológica de tipo celular do hospedeiro nas várias manifestações da esquistossomose.

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