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QUANTITATIVE DINITROCHLOROBENZENE (DNCB) RESPONSIVITY AND PHYTOHEMAGGLUTININ (PHA) INDUCED LYMPHOCYTE TRANSFORMATION IN PATIENTS WITH LEPROMATOUS LEPROSY

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Because we are unable to find a gross generalized impairment of cell-mediated immunity (CMI) in patients with lepromatous leprosy[1], and because such impairment is evidently readily demonstrated by others, we have sought evidence of a subtle deficit in CMI by quantitating in vivo DNCB and in vitro PHA responses, analogous to the studies of Eltringham et al[2] and Levy and Kaplan[3] concerning patients with early Hodgkin's disease.

The subjects of this study are 31 Mexican-born patients, who, by the criteria of Ridley and Waters[4] are clinically classified as LL because of an abundance of bacilli in lesions and the absence of borderline lesions, reversal reactions and nerve trunk neuropathies. Twenty-one of these patients have been classified histologically by the criteria of Ridley[4, 5]: LL, 4; LL or LI, 3; LI, 12; lepromatous but not further classifiable, 2. There were 13 women and 18 men with a median age of 32 years and a median duration of clinical illness of 2 years.

DNCB sensitization was attempted by a sensitizing dose of 2 mg and challenge doses of 0.1, 0.05 and 0.025 mg three weeks later in 25 patients, 20 normal controls and 25 "granulomatous controls" with disseminated coccidioidomycosis (DC). Responses were read as negative if no reaction or erythema (+) was present, or as positive if induration (++) or vesiculation (+++ or ++++) was present.

Lymphocyte transformation by PHA was studied with three day cultures, performed with fetal bovine serum (FBS) in 19 subjects and 29 controls and with autologous serum (AS) in 13 subjects and 17 controls. Lymphocytes, 0.5×10^6 per ml, separated from defibrinated blood by gelatin centrifugation[6], were cultured in RPMI 1640 with 10% serum and phytohemagglutinin-M at dilutions of 1/100, 1/300 and 1/1000. One μ C of tritiated thymidine, 1.9 C/mMole, was added 3 hours before the end of each culture. Tritiated DNA was extracted by the method of Lund and counted for five minutes in a liquid scintillation counter, the final datum expressed as counts per minute (CPM). Final results expressed as CPM were obtained by substracting counts of control cultures from those with PHA. Final results expressed as a stimulation index were obtained by dividing counts from cultures with PHA by those from controls. All cultures were done in triplicate. To seek evidence for variables associated with immuno-deficiency patients with leprosy were subdivided into

three dichotomies using three variables or indices. Patients were separated according to the logarithmic index of bacilli[8] (LIB) by dividing the group at the median. Patients were separated according to the percent of skin surface clinically infiltrated by dividing the group at the median. Patients were separated according to sulfone therapy by its presence or absence. Patients were too few to analyze according to differences in Ridley-histologic category.

The responses to DNCB in normal controls, all DC patients, DC patients with an anti-coccidioidin complement-fixing antibody (CF) titer of 1:32 or less, DC patients with a CF titer of 1:64 or greater and all leprosy patients are shown in Table 1.

TABLE 1. RESPONSES TO DNCB IN PERCENT POSITIVE

	Controls (15)	All DC (25)	DC with CF titer 1/32 or less (10)	DC with CF titer 1/64 or more (15)	Leprosy (25)
Primary Flare	50	28	70	0	32
DNCB Challenge					
0.1 mg	85	46	78	33	80
0.05 mg	60	29	67	7	60
0.025 mg	55	13	33	0	24

DNCB responses in leprosy patients subdivided according to the presence or absence of therapy, and median bisection according to disease extent and LIB are shown in Table 2.

TABLE 2. RESPONSES TO DNCB IN PERCENT POSITIVE

	Treatment		LIB		Extent of involvement	
	(17) none	(8) some	(13) 4.8 or less	(12) 4.9 or more	(13) 20% or less	(12) 21% or more
Primary flare	13	41	46	17	23	42
DNCB challenge						
0.1 mg	63	88	85	75	85	75
0.05 mg	50	65	62	58	54	67
0.025 mg	25	24	38	8	31	17

Responses to DNCB are similar in leprosy patients and in normal controls. Subdivision of the leprosy patients according to differences in disease extent, treatment and LIB does not identify a subgroup consistently different from the normal controls. In contrast, responses to DNCB are less in DC patients than in normal controls and subdivision according to CF titer identifies a group, i.e., those with a titer of 1:64 or greater, which is statistically significantly different from the normal controls at the three challenge doses as well as those with or without a primary flare response, p-value < 0.002.

TABLE 3. RESULTS OF PHA INDUCED LYMPHOCYTE TRANSFORMATION USING AUTOLOGOUS SERUM

	Mean of log of CPM	
	Control (17)	Leprosy (13)
PHA dilution		
1/100	3.98 ± .23	4.02 ± .37
1/300	3.86 ± .25	3.96 ± .27
1/1000	3.41 ± .21	3.45 ± .31
	Mean of Stimulation Index	
PHA dilution		
1/100	65 ± 52	74 ± 77
1/300	48 ± 33	62 ± 54
1/1000	16 ± 11	19 ± 12
	Percent with Stimulation Index < 2.0	
PHA dilution		
1/100	0	0
1/300	0	0
1/1000	0	0

Lymphocyte transformation in autologous serum as judged by means of the log of CPM and by means of the stimulation indices is uniformly greater in leprosy patients than in controls but not statistically significantly so (Table 3). The stimulation index was never less than 2.0.

TABLE 4. RESPONSES TO PHA STIMULATION IN FETAL BOVINE SERUM

	Control (29)	Total LL (19)	Logarithmic Index of Biopsy	
			4.8 or < (9)	>4.8 (10)
PHA dilution	Mean of log of CPM			
1/100	3.96 ± .45	4.02 ± .32	3.93 ± .30	4.10 ± .30
1/300	3.74 ± .55	3.81 ± .34	3.67 ± .35	3.92 ± .26
1/1000	3.39 ± .55	3.22 ± .58	3.08 ± .76	3.33 ± .30
PHA dilution	Mean of Stimulation Index			
1/100	24 ± 15	44 ± 26*	42 ± 28	46 ± 25
1/300	21 ± 12	32 ± 23*	30 ± 23	34 ± 11
1/1000	6 ± 6	13 ± 9	14 ± 11	11 ± 7
PHA dilution	Percent with Stimulation Index < 2.0			
1/100	0	0	0	0
1/300	0	5	11	0
1/1000	14	16	22	10

* p-value < 0.05 by t-test comparing homologous pairs

TABLE 5. RESPONSES TO PHA STIMULATION IN FETAL BOVINE SERUM

	Treatment		Extent of Clinical Illness	
	None (7)	Some (12)	15% or less (9)	20% or more (10)
PHA dilution	Mean of log of CPM			
1/100	4.14 ± .31	3.95 ± .30	4.02 ± .26	4.03 ± .34
1/300	4.08 ± .22	3.67 ± .29*	3.87 ± .40	3.75 ± .23
1/1000	3.15 ± .81	3.25 ± .39	3.48 ± .26	2.98 ± .68
PHA dilution	Mean of Stimulation Index			
1/100	51 ± 30	40 ± 22	54 ± 20	35 ± 28
1/300	38 ± 26	28 ± 20	42 ± 22	23 ± 20
1/1000	10 ± 6	16 ± 10	17 ± 9	8 ± 7*
PHA dilution	Percent with Stimulation Index <2.0			
1/100	0	0	0	0
1/300	14	0	0	10
1/1000	14	17	0	30

* p-value < 0.05 by t-test comparing homologous pairs

PHA induced lymphocyte transformation with FBS is similar in controls and leprosy patients and in subgroups of leprosy patients (Tables 4 & 5). The apparent statistical significance of some data disappears when corrected by multiplying by the number of specificities tested. This study provides no evidence that a subtle, generalized deficit of CMI is present in this group of patients. A subtle generalized defect of CMI cannot be excluded absolutely and we are continuing this investigation utilizing smaller quantities of DNCB and lower dilutions of PHA.

Since generalized deficiencies of CMI are not necessary accompaniments of leprosy, their existence must be attributable to a variable other than lepromatous leprosy itself. We do not know the responsible variable or variables but possibilities to which our present study is directed include disease extent, presence or absence of sulfone therapy, and LIB (antigen load).

Disease Extent. Responses to DNCB and to PHA do not appear to be influenced by disease extent, judging extent by percent of skin surface clinically infiltrated.

Antigen Load and Treatment. A lower antigen load, as judged by LIB, and the presence of sulfone therapy appear to be associated with increased DNCB responses. However, neither a higher antigen load nor the absence of therapy is associated with a consistent, statistically significant reduction in responses to DNCB when compared to normal controls. Differences in antigen load and treatment are not associated with consistent or significant differences in responses of lymphocytes to PHA.

Compared with the use of the CF titer in DC patients, it is possible that the indices employed in this study are not suited for the identification of a variable associated with immunodeficiency. Alternatively, our patients may not have sufficient magnitude of variation to allow identification of a variable that is significantly associated with immunodeficiency.

Variables which might be associated with differences in immunologic responses but which the present study does not cover include Ridley-histologic category, genetic factors, a high prevalence of erythema nodosum leprosum in the absence of chemotherapy and coexistent illnesses particularly malaria and malnutrition.

REFERENCES

1. Rea, T. H., Quismorio, F., Nies, K., Harding, B., DiSaia, P., Levan, N. and Friou, G. Intradermal antigens, epicutaneous haptenes, T cell counts, B cell counts, lymphocyte transformation and autoantibodies in one group of patients with lepromatous leprosy. Int. J. Lepr. 42 (1974) 369-371.

2. Eltringham, J. R. and Kaplan, H. S. Impaired delayed-hypersensitivity responses in 154 patients with untreated Hodgkin's disease. Nat. Cancer Inst. Monogr. 36 (1973) 107-115.

3. Levy, R. and Kaplan, H. S. Impaired lymphocyte function in untreated Hodgkin's disease. N. Engl. J. Med. 290 (1974) 181-186.

4. Ridley, D. S. and Waters, M. F. R. Significance of variations within the lepromatous group. Lepr. Rev. 40 (1969) 143-152.

5. Ridley, D. S. and Jopling, W. H. Classification of leprosy according to immunity: A five group system. Int. J. Lepr. 34 (1966) 255-273.

6. Coulson, A. S. and Chalmers, D. G. Separation of viable lymphocytes from human blood. Lancet 1 (1964) 468-469.

7. Lund, P. A radiochemical assay for glutamine synthetase, and activity of the enzyme in rat tissue. J. Biochem. 118 (1970) 35-39.

8. Ridley, D. S. and Hilson, G. R. F. A logarithmic index of bacilli in biopsies. 1. Method. Int. J. Lepr. 35 (1967) 184-186.