Lepr. Rev. (1976) **47**, 193-199

DNCB—Reactivity in Patients with Leprosy In Kenya

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Sensitization followed by graded challenges of dinitrochlorobenzene (DNCB) were performed in 105 leprosy patients (Bantu) in Kenya (22 tuberculoid, 53 borderline and 30 lepromatous leprosy). The results were compared with those obtained in a group of 38 relatives (index cases 5 lepromatous leprosy patients) and in a group of healthy controls (no known household contact with leprosy). All patients showed a diminshed DNCB reactivity as compared to healthy controls. In the group of relatives of lepromatous leprosy patients no decrease of DNCB reactivity (as compared to local controls) was observed.

The percentage of DNCB reactors in healthy controls in Africa proved to be significantly lower than the percentage of DNCB reactors in healthy controls of Caucasion and Negro ancestry in Holland. The factors possibly influencing these results are discussed.

Introduction

In several studies evidence is given that cell-mediated immune reactivity is depressed in patients with lepromatous leprosy. Impaired responses were noted to intracutaneous test antigens (Waldorf *et al.*, 1966; Bullock, 1968), and to contact sensitization with 2,4-dinitrochlorobenzene (DNCB) or picrychloride (Waldorf *et al.*, 1966; Bullock, 1968, Turk and Waters, 1969). Besides prolonged skin allograft survival has been reported in lepromatous patients (Job *et al.*, 1967) and in both tuberculoid and lepromatous patients (Han *et al.*, 1971). Reports on *in vitro* tests with leucocytes of leprosy patients have been contradictory (Dierks and Shepard, 1968; Sheagren *et al.*, 1969; Nelson *et al.*, 1971; Wong *et al.*, 1971) and consequently there is some doubt whether in lepromatous leprosy a generalized depression of CMI reactivity actually occurs.

Moreover, some findings point to an impairment of CMI reactivity in patients with tuberculoid leprosy (Bullock, 1968). Recently Mendes *et al.* (1974) showed significantly impaired responses to DNCB in tuberculoid leprosy patients with normal responses to intracutaneous test antigens (tuberculin, oidiomycin, trichophytin and lepromin).

Because of this controversy of opinion we decided to start a systematic study on the immune reactivity of leprosy patients. In this paper we report on the results of DNCB sensitization testing in leprosy patients in Kenya. Patients representing the whole spectrum of leprosy from polar tuberculoid to polar lepromatous were included in these tests. A comparison was made with similar

Received for publication 11 February, 1976.

tests carried out in relatives of lepromatous patients while results were matched with results obtained in two groups of healthy controls with residence in Kenya and in the Netherlands, respectively.

Methods

SENSITIZATION PROCEDURE

Sensitization tests with D CBNwere performed according to the methods described by Bleumink *et al.* (1974) with minor modifications.

1. The solutions used for sensitization and challenge were prepared at the Biochemical Laboratory (Head: E. Bleumink, Ph.D.), Department of Dermatology, Groningen, The Netherlands, and issued in polythene vials which were sealed after the removal of acetone by evaporation. The vials were stored at 4° C and prior to use in the field were reconstituted with a standard volume of acetone.
2. The sensitization dose of 2 mg DNCB in acetone as a rule was applied to the volar aspect of the forearm. Moslems, however, because of ritually prescribed daily ablutions, were sensitized at the volar aspect of their *upperarm.*
3. Challenges were performed 14 days late; by patchtesting with 3 and 10 ug of DNCB, omitting tests with 30 ug in order to prevent erroneous readings (Hartman, 1976).
4. The reactions were examined 48 h later and graded as 2, 3 or 4 plus reactions omitting 1 plus reactions (erythema only) because of their inconspicuousness on African skin. The sum of the plus reactions is designated as DNCB-score. A score of 2 or more is taken as positive evidence for cell-mediated immune reactivity to this particular component.

PATIENTS AND CONTROLS STUDIED

A. In Kenya three groups of persons were studied:

1. 105 leprosy patients; 75 persons from Western Kenya admitted to the Leprosy Hospital at Alupe and 30 in-patients of the Tumbe Leprosy Centre (Coast Province). Of these 47 were in a reactional state or had recently suffered from a reactional state (reversal reaction or ENL). All of them had received treatment with diaminodiphenylsulphone (DDS) or Lamprene. Some patients were treated with corticosteroids.
2. 38 relatives (first and second degree) of 5 patients with (polar) lepromatous leprosy living in the Coast Province. Physical examination revealed that 3 of them had untreated tuberculoid leprosy.
3. 27 healthy villagers near Tumbe without a family history of leprosy.

B. In the Netherlands 2 groups of controls were studied:

1. A group of 105 Dutch out-patients of the Department of Dermatology at Groningen, presenting with minor skin disease and obviously free of leprosy.
2. A group of 7 healthy negroes of S. American ancestry born in Holland.

The classification of leprosy patients was based on clinical history, physical examination, determination of skin smears and skin biopsies. All patients were (re)classified according to the Ridley-Jopling scale (1966).

Details like age, sex, tribe, reactional state, reason of admission, period of treatment, BCG vaccination and other factors of possible influence on the DNCB reactivity were recorded.

Some biopsies were taken for histological evaluation of our test results.

Finally all volunteers were clinically examined and the clinical history and diseases were noted.

GENERAL HEALTH SCREENING

Physical examination revealed that a great many people in the 3 groups investigated had a palpable spleen. Anaemia was commonly encountered (18%). All of them had a history of repeated attacks of malaria and a number of them (58%) had a history of schistosomiasis. Kwashiorkor and marasmus are well known diseases in the areas under survey.

Results

Leprosy patients both in Alupe as well as in Tumbe Leprosy Centre, were classified by clinical examination in five groups (Table 1). Since DNCB reactivity partly depends upon age, data are given for 4 separate age groups (Table 2). Only 4 out of 105 Patients (4%) with leprosy were found reactive to DNCB as compared to 11 out of 38 in the group of relatives (29%) and 7 out of 27 (26%) healthy controls in Kenya. The difference between the leprosy patients and the latter two groups is statistically significant ($P > 0.01$). The differences are even

TABLE 1

Classification of leprosy patients, reactional state and DNCB reactivity

Type	Admitted at Tumbe	Reactional state	DNCB-test	Admitted at Alupe	Reactional state	DNCB-test
1. TT	3	0	0	–	–	–
2. TB	8	5	0	32	12	1
3. BB	7	3	0	7	3	1
4. BL	6	3	0	28	13	1
5. LL	6	2	0	8	6	1
	30	13	0	75	34	4

TABLE 2

Results of patch tests with DNCB in leprosy patients, relatives of LL patients and controls in Kenya (1) and in Holland (2)

Age years	Leprosy patients		Relatives of LL* patients		Controls in Kenya		Controls in Holland (Dutch)		Controls in Holland (negroes)	
	No	*Pos*	*No*	*Pos*	*No*	*Pos*	*No*	*Pos*	*No*	*Pos*
0-30	37	2	22	7	14	3	48	48	6	6
31-50	54	1	13	3	10	3	35	35	1	1
51-70	12	1	2	1	12	1	10	8	0	0
70	2	1	1	0	1	0	10	2	0	0
	105	4	38	11	27	7	103	92	7	7

No = Number.
Pos = Positive.

* Lepromatous leprosy (polar form).

TABLE 3

Summary of DNCB-tests in leprosy patients and controls in Kenya

		Number Challenged (read)	Positive	%
Group A	Leprosy patients			
	Alupe (Western Kenya)	75	4	5
	Tumbe (Coast Province)	30	0	0
	Total	105	4	5
Group B	Family members of 5 lepromatous patients (1st and 2nd degree)			
	Coast Province	38	11	29
Group C	Control Group	27		
	Coast Province		7	26

Differences between A and B, A and C statistically significant ($P < 0.01$)

more striking (Table 3) if only those persons living in the Coast Province are taken into account. None out of 30 leprosy patients and 7 out of 27 healthy controls could be sensitized to the contact allergen. Also 3 children with high resistant tuberculoid leprosy (TT) found among the relatives of patients were negative to DNCB.

TABLE 4

Data of persons in Kenya with positive reactions to DNCB

Age	Sex	Leprosy class	BCG vaccinated persons	DNCB-score	Palpable spleen*
Group A		Leprosy patients (4)			
40	f	BL	+	8	++
35	f	TB	–	2	–
31	m	BB	–	2	+
33	m	LL	–	2	–
Group B		Family members (11)			
16	f	–	+	5	++
9	f	–	+	2	–
32	f	–	–	4	+
4	f	–	+	8	+
20	f	–	–	2	+++
45	f	–	–	2	–
40	m	–	–	5	–
70	f	–	–	2	++
8	m	–	+	2	–
17	f	–	–	3	–
22	f	–	–	4	–
Group C		Controls (7)	–		
14	f	–	+	5	–
15	m	–	+	5	++
15	f	–	+	2	+
21					
19	f	–	–	4	+++
24	m	–	+	3	–
20	m	–	–	3	+

* Spleen size measured in fingers: + = one finger below the costal arch,
2+ = two fingers great and so on.

The 4 positive reactors among leprosy patients from Western Kenya were classified as tuberculoid-borderline (1), borderline (1), lepromatous-borderline (1) and polar lepromatous (1), (Table 4). In the DNCB positive reactors we found 2 female and 2 male patients. Three of them were in a reactional state either with neuritis or erythema nodosum leprosum. There was no relation to duration of treatment or type of treatment (DDS or Lamprene). All patients receiving corticosteroids showed negative responses. Even very recent polar tuberculoid cases proved to be completely negative to DNCB, even by histological examination of skin biopsies taken from challenge sites in 4 patients.

Prior BCG vaccination did not influence DNCB reactivity.

In persons with a palpable spleen in the 3 groups investigated, no correlation was found between the spleen size and DNCB reactivity. In healthy controls in Holland all individuals under 50 years of age were responding to DNCB sensitization; older individuals were less sensitive (Table 2). All negroes born in Holland could be sensitized. These results sharply contrast with those found in healthy controls in Kenya, of whom only 26% showed reactivity to DNCB upon sensitization. These differences were found highly significant ($P < 0.001$).

Discussion

In leprosy patients of all types a low percentage of reactions to DNCB was found. The fact that only 1 out of 11 tuberculoid patients responded to DNCB challenge does not corroborate the assumption of Turk and Bryceson (1971) that cellular immune reactivity is intact in tuberculoid leprosy. The data presented tend to confirm the observation of Lim *et al.* (1972) stating that difference in immune reactivity in the various types of leprosy might be more a difference of degree than of kind.

The observation of marked impairment of immune reactivity to DNCB both in leprosy patients and also in healthy controls in Kenya contrasts with other investigations, first of all with our own normal results obtained by examining DNCB reactivity in a control group consisting of Negroes and Caucasians born in Holland. Similarly in Africa (Uganda) Ziegler *et al.* (1969, 1970) observed a high rate of DNCB reactivity in small groups of patients with malignant melanoma and Burkitt's lymphoma.

Different explanations can be given for the low percentage of skin reactors to DNCB in leprosy patients and healthy controls in Kenya. Weigand and Gaylor (1974) testing different concentrations of DNCB upon stripped and normal skin of Negroes and Caucasians in USA concluded that the stratum corneum of Negroes provided a more effective barrier to chemical substances like DNCB. This observation is at variance with the finding of Verhagen (1974) that contact dermatitis is not rarely encountered in Kenya, though the number of sophisticated allergens obviously is smaller than in industrialized countries. The fact that Hartman (1976) in Kenya found a lower percentage of contact dermatitis as compared to figures reported from Sweden (Hellgren, 1967) may largely be explained by the above mentioned relative lack of potential allergens in Africa.

Walker *et al.* (1967) in investigating genetic factors operative in contact dermatitis found normal skin reactivity to DNCB, even at low challenge dosage, in Caucasians as well as in Negroes and Asians. The well known fact of the declining rate of positive skin reactors in older groups (e.g. Waldorf *et al,* 1968) cannot explain the low percentage of DNCB reactors neither in our study with few

patients and controls in age groups beyond 50 years nor in Africa as a whole due to the relative preponderance of young age groups in tropical countries.

Impaired immune reactivity to DNCB in bacilliferous infections may be explained by massive infiltration of paracortical zones of lymph nodes by histiocytes containing ingested *M. leprae*, but in paucibacilliferous types of leprosy this explanation cannot be put forward. The common depression of cellular immune reactivity due to malnutrition and to various endemic infections of viral, bacterial or protozoal origin is well known.

Both in India (Chandra, 1972) and in Thailand (Edelman *et al.*, 1973) a significantly lower frequency of sensitization to DNCB (resp. DNFB) has been reported in malnourished children. Similarly in South Africa Smythe *et al.* (1971) demonstrated profound depletion of the thymolymphatic system and severe depression of cellular immune responses in patients with malnutrition as compared to healthy controls.

The synergistic interaction of malnutrition and infection is well recognised on the basis of clinical observations and epidemiological data (Chandra, 1972).

A recent report of a WHO scientific group (1973) sums up a number of viral and bacterial infections, many of them present in tropical countries, primarily causing non-specific loss of cell mediated immunity in man and test animals.

In the Coast province of Kenya schistosomiasis, helminthic infections, tuberculosis, leprosy and filariasis are endemic (Hartman, 1976). The area is known for the hyperendemicity of malaria (Kortman, 1972). Malnutrition in younger age groups is frequently encountered. Therefore the explanation most likely to apply to the reported low percentage of skin reactors to DNCB both in leprosy patients and in healthy controls in Kenya is to be found in environmental factors in which an interplay between malnutrition and parasitic endemic infections is operative.

Future immunological studies of leprosy patients preferably should include investigations to be carried out in an environment in which concurrent endemic disease cannot possibly interfere with the collection of data needed to elucidate the patho-immunological mechanisms involved in leprosy patients.

Acknowledgements

Permission and help was given by the Kenyan Government both in Alupe leprosy hospital, Western Province (Head: A. de Rijk M.D.B.S.) and Tumbe leprosy centre, Coast Province (Head: K. Waaldijk M.D.B.S.). Thanks are due to the Dutch Leprosy Foundation at Amsterdam, for financially supporting this investigation and to the East African Leprosy Research Centre, Alupe (Head: Dr Y. Otsyula) for assistance and transportation facilities.

Furthermore, I thank the Staff of the Department of Dermatology for technical support and helpful discussion. Finally I am most grateful for the cooperation of all volunteers both in Kenya as well as in Holland.

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