CELL MEDIATED IMMUNITY IN PATIENTS WITH VIRCHOWIAN HANSENIASIS BEFORE AND AFTER TREATMENT WITH TRANSFER FACTOR

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ABSTRACT — Cell mediated immunity (CMI), bacterial index (BI), morphological index (MI), skin and lymph nodes biopsies were evaluated in 15 patients with virchowian hanseniasis before and after treatment with transfer factor (TF) obtained from human spleens. The patients were divided in 3 groups: group I (control) received only sulforiti, group II received sulfone plus TF and group III received only TF.

There was no difference in the numbers of peripherical T and B lymphocytes of patients and normal controls. Before the treatment with TF, there was an impaired response of the patient's peripheral lymphocytes to PHA stimulus, in the presence of autologous or homologous plasma. This depressed response was corrected after treatment With TF in the patients of group III. In none of the patients a positive Mitsuda reaction was observed before and after treatment with TF.

The improvement of the MI observed in group IH, treated only with TF was remarkably similar to the patients treated only with sulfone.

This work points out that TF has a role in the treatment of patients with virchowian hanseniasis, based on the improvement of CMI, MI, on histopathology of skin biopsies and clinical conditions.

Key words: Hanseniasis. Cell mediated immunity. Transfer factor.

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1 INTRODUCTION

It has been shown that patients with Virchowian hanseniasis (COCHRANE & SMYLY, 1964; RABELLO JR., 1938; RABELLO JR. & AZULAY, 1975; ROTBERG, 1937) present a deficit of. cell mediated immunity (CMI) (BLADEN, 1974; BULLOCK JR., 1968, 1978; CONVIT *et* a/., 1971; GODAL, 1978; HAN *at al.,* 1971; MACKANESS & BLADEN, 1967; MILLER & OSOBA, 1967; NELSON, 1974; NELSON *et al.,* 1971; NORTH, 1973; SER. INF. TEC. OMS, 1973; REA *St* LEVAN, 1977; TURK, 1970; TURK & BRYCESQN, 1971; WAL, DORF *at al.,* 1966; WHO, 1973).

In vivo as well as in *vitro* tests (BLOOM, 1971; DAGUILLARD, 1972; FUNDENBERG *et al.,* 1971; MILLS, 1966; ROCKLIN, 1974) there is no response of the T lymphocyte to the antigens of *Mycobacterium leprae* (BULLOCK & FASAL, 1971; GODAL *at al.,* 1971; GODAL *at al.,* 1972; HAN *at al.,* 1971; MYRVANG *at* a/., 1973). For other antigens this lack of response is more frequent when the disease duration and the bacilli load are more intense and when the virchowian polar characteristics are well characterized (GODAL *at* a/., 1972; HAN *at al.,* 1971; MYRVANG *at al.,* 1973; SAHA & MITTAL, 1971; TURK & BRYCESON, 1971; WALDORF *et al.,* 1966; WHO, 1973).

Several authors have used, with discordant results, transfer factor (TF) (BULLOCK *et al.,* 1972; CANDIDO SILVA *at* a/., 1973; FABER *at al.,* 1979; GODAL, 1974; HASTINGS *at al.,* 1976; LAWRENCE, 1968; SAHA *et al.,* 1975) or total blood transfusion (ALMEIDA GONÇALVES & CUSTÓDIO, 1975; LIM *et* a/., 1972) or viable leukocytes (ANTIA & KHANOLKAR, 1974; BULLOCK *at al.,* 1972; PARADISI *et al.,* 1969; SAHA *et al.,* 1975) in patients with the Virchow cells type (BRIEGER & ALLEN, 1964; KHANOLKAR, 1964) in an attempt to obtain the transfer of skin reactivity or to reactivate the immunodeficient mechanisms. The TF, as a terapeutic weapon, had its efficiency demonstrated in several diseases with deficiency of CMI (BASTEN *et al.,* 1975; CATANZARO & SPITLER, 1976; GRAYBILL *et al.,* 1973; GRISCELLI *et al.,* 1973; HITZIG & GROB, 1974; KIRKPATRICK & GALLIN, 1974; LAWRENCE, 1969, 1974; LEVIN *et* a/., 1970, 1973, 1975; MENDES & MENDES, 1976; PABST & SWANSON, 1972; ROCKLIN, 1975; ROCKLIN *et al.,* 1970; SCHULKIND & AYOUB, 1975; SILVA *et* a/., 1976; SPITLER *et al.,* 1975; VETTO *at* a/., 1976).

Our aim was to compare, in a doubly blind study, the CMI of virchowian and dimorphous patients, treated with TF, TF plus sulfone or only sulfone. These patients did not receive any previous treatment.

2. MATERIALS AND METHODS

Fifteen male patients with clinical illness, duration up to 5 years (this generally corresponds to a 10 years period of disease development), were selected for this study. They did not receive any previous treatment and the age range was between 18 and 40 years.

The patients, all of them Mitsuda negative, were classified according to the criteria adopted in Madrid (COCHRANE & SMILY, 1964) based on dermatological, histological and bacteriological findings. Eleven of them were classified as virchowian patients and four as dimorphous ones. The patients remained in the hospital for 4 months, which was the time necessary to complete the study. To avoid any bias on the distribution of the patients in groups, a previous assortment was done, following the patients' admission order to the hospital.

2.1 Group definition and patients'allotment

Group one: Patients treated with 100 mg of sulfone (DDS) everyday, orally, and 1 ml

of sterile saline, subcutaneously, as placebo, twice a week, during 8 weeks (control group). Group two: Patients treated with 100 mg of DDS everyday, orally, and 1 ml of TF, subcutaneously, twice a week, during 8 weeks. Group three: Patients treated with a placebo tablet everyday, orally, and 1 ml of TF, subcutaneously, twice a week, during 8 weeks.

The different placebos were necessary since there were no elements permitting exclusion of possible effects related to subjectivity. A control group receiving only the two placebos was not included on the research plan for ethical reasons. All the tests were done before and after treatment, without knowing to which group the patients belonged.

2.2 Bacteriological examination: bacterial index (BI) and morphological index (MI).

Material was obtained by scarification of the left and right nostrils and also of six symmetrical areas, generally of auricular lobes, elbows and knees or other more evident lesion sites. The slides were sent to the laboratory (Laboratório da Divisão de Hansenologia e Dermatologia Sanitária do Instituto de Saúde da Secretaria de Saúde de Sao Paulo) under a code number. The BI has followed the Ridley's criteria (RIDLEY, 1975) and the MI was done according to World Health Organization's (WHO, 1966) criteria.

2.3 Histopathology of skin and inguinal lymph nodes.

Skin was obtained by biopsies of every evident skin lesions. The lymph nodes were obtained by surgery, the right ones before treatment and the left ones after it. For histological analysis, the material was stained with hematoxylin-eosin and by the Fite-Faraco method. The determination of BI and MI on the skin biopsies and on the lymph nodes were done according to the classification proposed by WHO (CENTRO ...1974). After treatment, bacteriological examination and skin biopsies were done, whenever possible, near the original site.

2.4 Evaluation of cellular immunity "in vivo"

2.4.1 Skin tests

Fernandez and Mitsuda types of reaction (CONSIGLI, 1958; GUINTO, 1968; KUPER, 1964; MITSUDA, 1924; REES, 1964; ROTBERG, 1944). Antigens prepared and supplied by Secretaria da Saúde do Estado de Sao Paulo containing 40x10' bacilli/ml. The skin tests results were expressed according to WHO's criteria (WHO, 1970). PPD-RT 23, containing 2 UT/0,1 ml (Divisão Nacional de Tuberculose, Brasil). SK-SD (Varidase), Lederle Laboratories, Pearl River, N.Y., USA, containing 40 SK units and 10 SD units per 0,1 ml. PHAc — Purified phytohemagglutinin for clinical use, Wellcome Reagents, England, containing 20 lig/ml.

The reading of PHAc reaction (BLAESE et al., 1973; BONFORTE *et al.,* 1972; LAWLOR *et al.,* 1973; MOTA, 1973) were done after 24 hours and of PPD and SK-SD reactions, after 48 hours. The induration area was expressed in millimeters as the average of the two largest diameters. As recommended by MOTA (1973), the reactions were considered positive: for PPD, whenever there was a measurable induration; for SK-SD and PHAc an induration of 5 mm or greater.

2.4.2 Sensitization with DNCB (2,4 Dinitrochlorobenzene) CATALONA *et al.,* 1972.

Sensitization was done with a solution of 2.000 p,g of DNCB (Carlo Erba, Milan, Italy) in 0,1 ml acetone. After two weeks, a solution of 100 d,g in 0,1 ml acetone, was applied as a challenging dose: the result was expressed as described by DUPIJY & PREUD'HOMME (1968).

2.5 Evaluation of cellular immunity "in *vitro*"

2.5.1 Rosette formation with sheep erythrocytes (E).

T lymphocytes were detected by rosette formation with E as described elsewhere (LAY *et al.,* 1971; MENDES, 1975; MENDES *et al.,* 1973, 1974). Five milliliters of heparinized blood were mixed with 1 g of iron powder (Carlo Erba, Milan, Italy), incubated at 37°C for 10 min, and then separated in a Ficoll-Hypaque gradient. (Pharmacia Fine Chemicals, Uppsala, Sweden; Winthrop Products Inc., New York, USA) (THORSBY & BRATLIE, 1970).

The lymphocytes removed from the interphase (97% pure), were washed three times in a Hanks' balanced salt solution (HBSS, Grand Island Biological Co., Gibco, USA) at pH 7,2 and adjusted to 3x10 in HBSS at pH 7,2. For rosette formation 0,1 ml of E was mixed in 6x50 mm glass tubes with 60 ;J.1 of lymphocyte suspension (3x105 cells/ml) and

with 40 ti,1 of normal inactivated AB serum absorbed with E. Triplicate tubes were incubated at 37°C for 5 min, centrifuged at 200 *g* for 5 min, and finally, incubated at 4°C for 1 h. After this, one drop of methylene blue (0,33% in HESS) was added to each tube and the cell bottom gently resuspended with a Pasteur pipette. The percentage of rosette forming cells was determined microscopically in a hemocytometer. At least 300 lymphocytes were counted and only cells possessing at least three adhering erythrocytes were scored as rosette forming cells.

2.5.2 Rosette formation with human erythrocytes sensitized with antibody and complement (HEAC).

The method has been previously described (MENDES, 1975; MENDES *et* a/., 1974, 1973) to evaluate the percentage and total number of B lymphocytes. A suspension of washed human erythrocytes (HE) was adjusted to 2,5% in HESS and incubated for 30 min with equal volumes of a subagglutinating dilution of rabbit antiserum (A) to HE stroma. To 2 ml of HEA suspension, 0,1 ml of mouse complement was added and the mixture was incubated for 30 min at 37°C. The resulting HEAC suspension was washed and adjusted to 0,5% in HESS. For rosette formation, 0,1 ml of HEAC at the concentration of 0,5% was mixed at room temperature with 0,1 ml of lymphocyte suspension (2x106 cels/ml) in 6x50 mm *glass* tubes. The mixture was immediately centrifuged at 200 g for 5 min at room temperature. One drop of methylene blue (0,33% in HBSS) was added to each tube and the percentage of rosette forming cells was determined as described for E.

2.5.3 Lymphocyte culture

Lymphocyte culture was performed by methods employed in previous studies (LESER et al., 1977). Peripheral heparinized blood was obtained from patients and normal control subjects. After separation of the leucocyte-rich plasma, the lymphocyte concentration was adjusted to 0,4x106/m1 of Eagle's minimal essential medium (MEM — Grand Island Biological Company, USA) containing 20% autologous or homologous plasma (pool prepared previously from normal people). Each culture tube received 2,5 ml of this suspension and triplicates were prepared receiving 0,1 ml of phytohemagglutinin (PHA-P, Difco Laboratories, USA) diluted to 1:50. Control cultures were incubated without mitogen. The cultures were incubated by 3 days at 37°C in 5% Co:) atmosphere concentration; 2,5 p,Ci of Tritium-labelled thymidine (New England Nuclear, USA) was added in each tube for 6 hours before harvest of the cultures. Isotope incorporation by harvested cell cultures was counted in a Beckmann scintillation counter. The results were expressed as lymphoblastic-transformation index (LTI) which is the ratio: mean cpm of triplicate stimulated tubes/mean cpm triplicate control tubes.

2.6 Transfer Factor.

Transfer factor (ARALA-CHAVES *et al.*, 1976; BALLOW *et al.,* 1975; BLOOM, 1973a, 1973b; BURNET, 1974; GOTTLIEB *et al.*, 1973; GROB *et al.*, 1976; KROHN *et al.*, 1976; LAWRENCE, 1949, 1955, 1969; LAWRENCE & AL-ASKARI, 1971; LAWRENCE *et al.*, 1960; ZUCKERMAN *et .al.,* 1974) was prepared from human spleens as described previously (MUSATTI *et al.*, 1976). Normal human spleens were obtained from four cadaveric donors of kidney grafts at the time of necropsy. The capsules were removed and the organs minced. For each 100 g of spleen fragments, 100 ml of sterile phosphate-buffered saline at pH 7,2 were added and then blended in a Virtis homogenizer at 15,000 rpm for 5 min in an ice bath. For a complete disruption of the cells, the homogenized mass was frozen and thawed 10 times. Ten mg of DNase (Worthington, xl crystalized) and 500 mg of MgC12 were added and the material was then dialysed against 10 times its volume of distilled water at 4°C for 48h. Dialysed material of the 4 spleens were pooled and lyophilized in samples of 40 ml each. Prior to its use, the lyophilized material from 40 ml (corresponding approximately to lx10° lymphocyte) was reconstituted with 1,0 ml of sterile saline and filtered through GS 0,22 Millipore filter (Millipore Co., Bedford, USA). After filtration, sterility tests and HbsAg tests were done; both were negative in all samples.

2.7 Statistical methods.

In view of the nature of variables the following non-parametric methods were used: Mann-Whitney, Wilcoxon and Kruskal-Wallis. The two tailed tests was adopted whenever it could not be foreseen, through the available knowledge, the direction of the difference indicated on the alternative hypothesis. When the elements permitted such prevision, the one-tailed test was adopted (SIEGEL, 19(5; SOKAL & ROHLF, 1969).

3. RESULTS

3.1. Evaluation of cellular immunity *in vitro*

3.1.1 Determination of T and B lymphocytes

Table I points out the values obtained for each patient, before and after the treatment. Table II contains the results obtained from 20 normal persons. Comparing all the variables the analysis shows that statistically there is no significant differences among: the three groups of patients, before and after the treatment (Kruskal-Wallis' test: $p > 0,102$ in every case) ; the examined results in a group of 15 patients, before and after the treatment (Wilcoxon test: $p > 0,094$ in every case) ; the results obtained with a group of 15 patients, before the treatment, and the normal persons (Mann-Whitney, with $P > 0,010$ in all the cases).

3. 1 . 2 Lymphocyte cultures

Table III points out the lymphoblastic transformation indexes noted in patients before and after the treatment and also in 20 normal persons. The results referring to these normal persons, associated to a hundred cultures from other normal people during the same period, have led us to consider as normal figures, LTI over twenty. Adopting this criterion, the results of the cultures with autologous plasma, before the treatment, have shown that 60% (9/15) of the patients had depressed response. When homologous plasma was used, 47% (7/15) of the patients were immunodeficient under such conditilons.

The results of the lymphocyte cultures, with both types of plasma, obtained from the patients, before the treatment, were compared to those of the normal persons and the observed differences were statistically significant, which pointed out a CMI deficiency evaluated by this method (Mann-Whitney tests: $p < 0,001$ in both cases). Figure I shows these differences.

The fact that statistical analysis does not point out a significant difference among the median indexes obtained with homologous plasma and autologous plasma, would indicate that there are no blastogenic inhibitory substances in the autologous plasma (Wilcoxon test: ($p > 0,05$ in normal persons and in patients before the treatment).

The statistical analysis concerning the LTI differences among the three groups of patients, before the treatment, considering the results of the homologous and autologous plasma cultures separately, has shown that these differences are not significant (Kruskal-Wallis test: $p > 0,102$ in both cases).

After the treatment, the three groups did not show significant LTI differences in cultures with homologous plasma (Test of Kruskal-Wallis: $0,051 < p < 0,10$). On the other hand, in cultures with autologous plasma, the differences among the groups were significant (Test of Kruskal-Wallis: $0,01 < p < 0,05$). The groups I and II did not differ from each other (Mann-Whitney test: $0,075 < p < 0,111$), but together, they differed from group Ill (Mann-Whitney test: $0,01 < p < 0,02$).

To analyse the median situation of each group, with the same kind of culture before and after the treatment, the adequate statistics test is the Wilcoxon's ; one-tailed test using pairs of values. In this, with 5 pairs of numbers the null hypothesis rejection, according to the adopted criteria, can only occur with $p = 0,0312$, when all the differences have the same signal The simple inspection of the table III permits a verification that only in Group III, with the use of autologous serum, the LTI were always higher

TABLE 2 Number of lymphocytes, percentual and absolute number of T and B lymphocytes in the peripheral blood of patients, before and after treatment with transfer factor.

GROUP	PATIENT	LYMPHOCYTES									
		TOTAL		T %		T/mm³		B %		B/mm³	
		before	after	before	after	before	after	before	after	before	after
I	J.P.	1411	3037	62	62	903	1883	27	20	381	607
	S.A.R.	1949	2486	58	61	1130	1516	22	24	429	597
	R.Z.	2880	2850	59	66	1345	1881	20	25	456	712
	O.F.M.	2946	3608	53	66	1561	2381	19	25	560	902
	M.C.V.B.	3880	3802	71	55	2755	2091	26	27	1009	1026
X		2613	3157	61	62	1539	1950	22	24	567	769
Mi		2880	3037	59	62	1345	1883	22	25	456	712
II	J.R.	1352	2038	71	63	960	1284	22	24	297	489
	G.A.	1672	2548	57	61	953	1554	27	28	451	713
	A.G.O.	2402	2964	66	57	1585	1689	29	26	697	770
	J.P.D.	2700	1915	48	59	1296	1053	12	20	324	383
	J.M.F.	4120	4557	67	66	2760	3008	23	19	948	866
X		2445	2804	62	61	1511	1717	23	23	543	644
Mi		2402	2548	66	61	1256	1554	23	24	451	713
III	B.S.N.	1927	2561	70	66	1349	1690	18	10	347	256
	I.S.	1997	2331	58	68	1158	1585	23	25	459	583
	N.P.S.	3238	4259	64	57	2072	2428	20	19	648	809
	W.S.L.	3266	1462	53	65	1731	950	22	26	718	380
	W.C.	4337	2440	73	68	3166	1659	18	26	781	634
X		2953	2611	64	65	1895	1662	20	21	591	532
Mi		3238	2440	64	66	1731	1659	20	25	648	583
X		2672	2857	62	63	1648	1777	22	23	567	648
Mi		2700	2561	64	63	1349	1689	22	25	459	634

TABLE 2 Number of lymphocytes, percentual and absolute number of T and B lymphocytes in the peripheral blood of normal controls.

TOTAL OF LYMPHOCYTES	T %	T/mm³	B %	B/mm³
1196	58	694	16	191
1376	58	798	24	330
1577	53	835	29	457
1708	62	1059	18	307
1736	62	1076	20	347
1839	60	1103	25	450
1885	69	1301	21	396
1932	61	1178	20	386
2046	72	1473	22	450
2054	70	1437	12	246
2102	67	1408	22	462
2408	60	1444	15	361
2560	74	1894	12	307
2580	57	1471	26	671
2580	76	1960	24	619
2968	59	1751	29	860
3168	68	2154	21	665
3279	54	1770	14	459
3370	60	2022	27	909
3706	69	2557	19	704
X 2304	63	1469	21	479
Mi 2078	62	1440	21	450

after the treatment, i.e., only in this case the null hypothesis could be rejected. Figure II points it out.

3.2 Evaluation of cellular immunity through skin tests

Table IV presents the results of the late intradermic reactions and sensitization to DNCB in the three groups, before and after treatment. As it can be seen in none of the patients a positive reaction to Mitsuda antigen was observed, both in early and late readings, nor there were significant modifications concerning the responses to other antigens.

3.3 Bacterial and morphological indexes of skin smears and nasal MUCUS

Considering the results of BI and MI obtained through the examination of skin lesions and nasal mucus (Table V) we have to point out the fact that patients of Group III, who have only received TF, presented important modifications concerning both indexes, mainly MI, that shows a reduction of acid-fast bacilli. It was relevant the MI reduction of patients IS, NPS, WSL and specially WC, whose MI was 6,5 before the treatment, coming down to zero after it.

TABLES 3 Cultures of peripheral lymphocytes of patients and normal controls, with PHA, in the presence of autologous and homologous plasma. Lymphoblastic transformation indexes before and after treatment with transfer factor.

		PATIENTS				NORMAL CONTROLS		
GROUP	PATIENTS	BEFORE		AFTER		N.º	autologous	homologous
		autologous	homologous	autologous	homologous			
	J.P.	26	23	24	19	1	81	59
	S.A.R.	11	9	22	35	2	51	54
I	R.Z.	27	41	22	28	3	21	33
	O.F.M.	21	33	19	16	4	27	32
	M.C.V.B.	10	24	32	63	5	37	52
						6	27	62
X		19	26	23.8	32.2	7	27	16
Mi		21	24	22	28	8	29	29
						9	20	146
	J.R.	17	17	19	21	10	29	46
	G.A.	22	41	18	19	11	49	98
II	A.G.O.	19	18	15	11	12	21	50
	J.P.D.	50	40	51	31	13	48	64
	J.M.F.	4	3	4	4	14	208	193
X		22.4	23.8	21.4	17.2	15	35	33
Mi		19	18	18	19	16	91	76
						17	98	21
	B.S.N.	24	19	36	37	18	62	61
	I.S.	19	36	57	61	19	32	27
III	N.P.S.	8	3	29	20	20	27	79
	W.S.L.	18	15	29	56			
	W.C.	4	45	44	39			
X		14.6	23.6	35	42.6			
Mi		18	19	36	35			
X		18.7	24.5	28.1	30.7		51	61.6
Mi		19	23	24	28		33.5	53

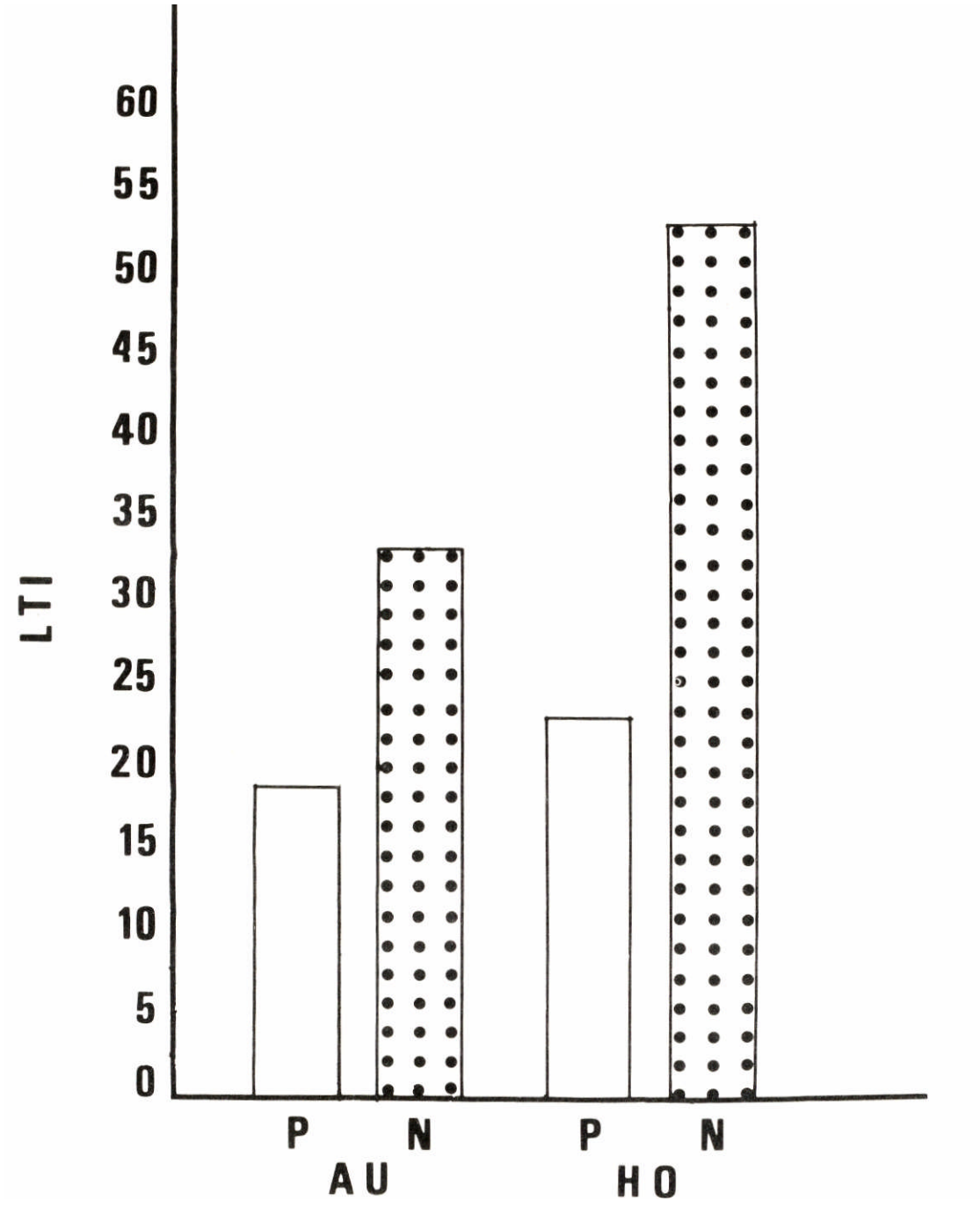

FIGURE 1 Cultures of peripheral lymphocytes of 15 patients (P) and 20 normal controls (N), with PHA, in the presence of autologous (AU) and homologous (HO) plasma. Median of lymphocytes transformation indexes (LIT).

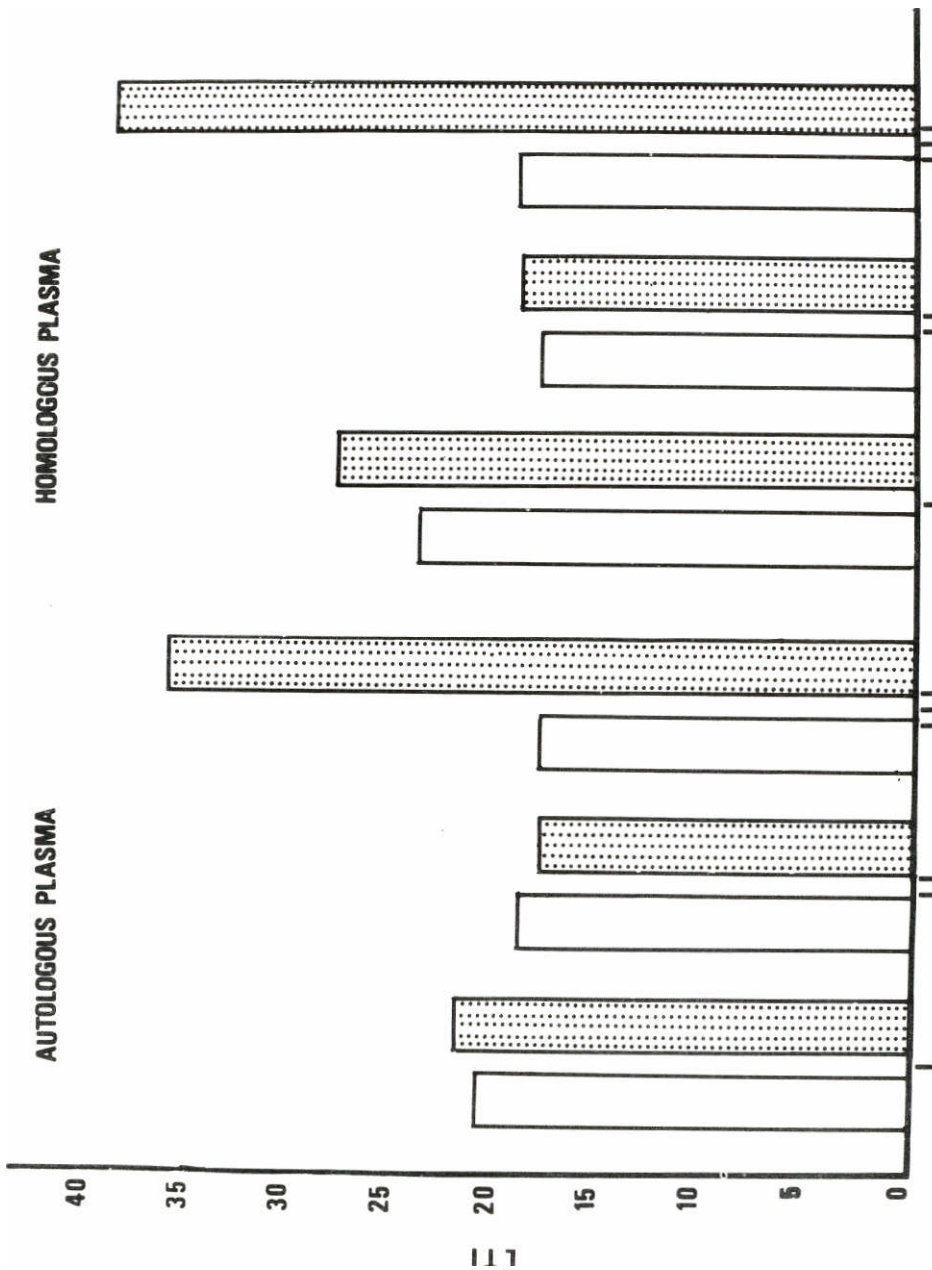

FIGURE 2 Cultures of peripheral lymphocytes of patients (3 groups), with PHA, in the presence of autologous and homologous plasma, before and after treatment with transfer factor. Median of lymphoblastic transformation indexes (LIT).

TABLE 4 Delayed skin reactions to Mitsuda antigen, PPD, SK-SD, PHA$_e$ and DNCB in patients before and after treatment with transfer factor.

GROUP	PATIENTS	MITSUDA ANTIGEN		PPD		SK-SD		PHA$_e$		DNCB	
		before	after	before	after	before	after	before	after	before	after
	J.P.	N	N	N	N	N	8	N	10	N	++
	S.A.R.	N	N	N	N	8	6	N	N	N	N
I	R.Z.	N	N	N	N	6	12	N	5	++	++
	O.F.M.	N	N	N	N	N	N	N	N	++	+++
	M.C.V.B.	N	N	N	N	N	N	N	N	++	+++
	J.R.	N	N	N	N	N	N	N	N	N	N
	G.H.	N	N	16	23	N	N	N	N	+++	++
II	A.G.O.	N	N	28	16	N	12	N	6	N	N
	J.P.D.	N	N	10	22	6	5	5	6	N	N
	J.F.M.	N	N	16	N	21	N	7	N	N	N
	B.S.N.	N	N	N	N	11	20	N	14	++	++
	I.S.	N	N	N	N	6	8	N	5	++	+++
III	N.P.S.	N	N	28	9	N	N	8	6	+++	+++
	W.S.L.	N	N	5	3	N	N	N	N	N	N
	W.C.	N	N	30	1(	N	N	5	5	++	+

TABLE 5 Bacterial and morphological indexes in material obtained from nasal mucosa and skin lesions of patients, before and after treatment with transfer factor.

GROUP	PATIENTS	BI		M	
		before	after	before	after
I	J.P.	3.13	2.00	2.00	0.00
	S.A.R.	3.75	0.63	0.63	0.15
	R.Z.	4.38	3.75	2.88	0.00
	O.F.M.	5.38	5.00	12.50	7.35
	M.C.V.B.	5.13	2.00	4.38	0.63
II	J.R.	0.25	0.00	0.00	0.00
	G.A.	0.25	0.00	0.00	0.00
	A.G.O.	1.25	0.25	0.00	0.00
	J.P.D.	4.80	4.30	1.00	0.50
	J.M.F.	3.88	2.88	4.38	0.00
III	B.S.N.	4.50	4.50	3.13	2.00
	LS.	4.63	4.38	14.75	1.75
	N.P.S.	5.00	4.38	5.88	0.25
	W.S.L.	5.00	3.63	7.50	0.25
	W.C.	4.63	2.00	6.50	0.00

3.4 Histopathology of skin and lymph nodes

The reports sent by pathologists concerning the histopathology of skin and lymph nodes, before and after the treatment, took into consideration: the composition of the infiltrated cells, bacillary and morphological indexes and disease type classification. While considering the improvement of each case, a higher importance was given to MI, because of the short interval between the two exams. Comparing group I and group III reports, it was observed that TF can cause a histophatological evolution compared to those of patients which received DDS. The interpretation of the histophatological skin results and those of the lymph nodes of group II patients compared to group I and III, was hindered by the casual allotment of 3 dimorphous patients.

TABLE 6 Dermatological evaluation of patients after treatment in 3 groups.

GROUP	PATIENTS	EVALUATION
I	J.P.	moderate improvement
	S.A.R.	moderate improvement
	R.Z.	great improvement
	O.F.M.	no change
	M.C.V.B.	moderate improvement
II	J.R.	great improvement
	G.A.	great improvement
	A.G.O.	great improvement
	J.P.D.	moderate improvement
	J.M.F.	moderate improvement
III	B.S.N.	moderate improvement
	I.S.	no change
	N.P.S.	great improvement
	W.S.L.	moderate improvement
	W.C.	great improvement

3.5 Clinical dermatological evolution

Table VI only shows the clinical improvement of each patient, according to a dermatologist who did not know to which group the patient belonged. It was possible to observe that the clinical improvement of group III patients is, at least, comparable to those of group I. The best results were obtained with patients who received a combined treatment (group II) ; however, the inclusion of the dimorphous patients hindered the comparison of group III, formed by virchowian patients only.

4. DISCUSSION

The evaluation of cellular immunity can be done by in vivo or in *vitro* tests. The calculation of the T lymphocytes in the peripheral blood is one of the parameters to be used. Our results have shown that the number, in percentages and absolute values, of the T lymphocytes causing rosette formation, in 15 patients before the treatment, did not differ from the number observed on normal persons.

Identical results were obtained by REA *et al.* (1976), while DWYER *et al.* (1973), MENDES *et* a/. (1974) and UM *et al.* (1974) have observed percentages and absolute numbers of T lymphocytes significantly lower in virchowian patients. The inguinal lymph nodes of the patients presented the paracortical areas replaced by cells of the reticulo-histiocyte system, with a large number of bacilli, as already observed by TURK & WATERS (1968, 1971) and PTAK *et al.* (1970). For some authors (HAN *et al.*, 1971; LIM *et al.*, 1974) these morphological alterations would explain and imply on a reduction in the number of T lymphocytes in the peripheral blood (NATH *et al.*, 1974. PTAK *et* at., 1970; SAHA & MITTAL, 1971; TURK & WATERS, 1968, 1971).

We have to remember that not all lymphatic system presents the same performance, since the paravertebral and mesenteric lymph nodes of the virchowian patients can present a normal histology (Fleury, R. Personnal communication).

For better characterization of the lymphocyte populations, we also determine the percentage and absolute number of B lymphocytes. In accordance with the results obtained by REA *et al.* (1976) and using the same method, we did not notice any difference between patients and normal persons. But MENDES *et al.* (1974), obtained, in virchowian patients, B values which were inferior to those normal persons, while NATH *et al.*, (1974) came to an exactly opposite conclusion, using the same method. High levels were observed by GAJL-PECZALSKA *et al.* (1973) and DWYER *et al.* (1973), using other method. It is not easy to explain these differences, considering only the diversity of methods adopted by different researches. Variations related with the clinical type, previous treatment and duration of the disease might be involved.

We do not believe there is any incompatibility between the normal results obtained from the T lymphocytes counting and the depressed response to PHA stimulation. The T cell determination has proved to be efficient as a quantitative measure of thymus-dependent population, but is does not necessarily seem to express its funcional capacity. The absence of correlation between the normal T lymphocytes numbers and the depressed CMI was also observed, in neoplastic diseases and paracoccidioidomycosis (KOPERSZTYCH *et al.*, 1976; MUSATTI *et al.*, 1976).

The results of the stimulation of peripheral lymphocytes by PHA (FUN-

DENBERG *et. al.,* 1971; GRAYBILL & ALFORD, 1976; JANOSSY & GREAVES, 1971, 1972; NASPITZ & RICHTER, 1968; NOWELL, 1960; OPPENHEIM & SCHECTER, 1976; WYBRAN *at al.,* 1973) in the presence of autologous and homologous plasma, have shown that some patients, virehowian and dimorphous, presented a deficit of CMI calculated by this parameter, while the others presented indexes which could be compared to those of normal persons. On the other hand, we did not find a significant statistical difference when comparing the **LTI** in cultures with homologous plasma to those with autologous plasma, i.e., in our patients the low LTI could not be atributed to the presence of plasmatic inhibitory factors (BULLOCK & FASAL, 1971; COOPERBAND *et. al.,* 1972; HOKAMA *et al.,* 1974). The depressed response to PHA according to our experience conditions, would depend on some characteristics of the lymphocyte itself, during the development of the disease (HAN *et al.,* 1971; UM *et al.,* 1975; SAHA & MITTAL, 1971; TALWAR *et. al.,* 1977; TURK & WATERS, 1968).

Similar results were related by BULLOCK & FASAL (1968), MEHRA *et al.* (1972), in the presence of homologous and autologous plasma; PARADISI *et al.* (1968), DIERKS & SHEPARD (1968), HOKAMA *et al.* (1947), LIM *et al.* (1975) and JOB *at al.* **(1970** using only autologous plasma; by HAN *et. al.* (1971) and TALWAR *at* a/. (1972) using only homologous plasma and by WONG *et al.* (1971) using heterologous plasma.

Different results were reported by SHEAGREN *et al.* (1969), PAGNANO (1974) and JOHN *et al.* (1974) in the presence of homologous plasma; by ULRICH *et* a/. (1972) in cultures with autologous plasma and by REA *et al.* (1976) using autologous and heterologous sera. They did not find any difference between virchowian patients and normal persons.

After the treatment, the comparative analysis of the LTI in the three groups has supplied enough elements to set up the hypothesis that the administration of TF, twice a week each dose corresponding to 1x109 lymphocytes, during 8 weeks, can increase the peripheral lymphocyte response to the PHA stimulation.

The mechanisms of the TF action that would cause this response improvement are still unknown. Several authors (BALLOW *et* a/., 1975; LEVIN *et* a/., 1973; ROCKLIN, 1975), who have used the TF as a treatment for various human diseases, suggest different answers to this question. All of them agree that a sufficient number of lymphocytes is required for this action. The **TF would alter the proportion of several T lymphocytes sub-populations (supressors, helpers and effectors) and induce their maturation, release of lymphokines (BLOOM** *at al.,* **1974; DIJMONDE** *at al.,* 1969; PICK & TURK, 1972), which would draft new T lymphocytes and act directly on the macrophages (FOWLES *et al.,* 1973; GODAL *et al.,* 1971; KRAHENBUHL *et al.,* **1973, 1976;** MC GREGOR *at al.,* 1971; MELTZER & OPPENHEIM, 1977; SIMON & SHEAGREN, 1971).

In none of 10 patients (group II and III) receiving the TF a positive reaction to Mitsuda antigen was observed, on both early and late readings, as reported by others (ALMEIDA GONÇALVES & CUSTÓDIO, 1975; ANTIA & KHANOLKAR, 1974; SAHA *et al.,* 1975). However, positive Fernandez reactions and, rarely, a positive Mitsuda reaction were obtained from Mitsuda positive donors in patients treated with TF (BULLOCK *et al.,*

1972; MENDES et a/., 1974; PARADISI *et* a/., 1969; SAHA et a/., 1975). The use of cadaveric spleens for the TF preparation did not permit the knowledge of their delayed hypersensitivity to several antigens. Comparing the results from groups II and III, we observed that the convertion of negative reactions to the antigens used was small (4 times in 30 possibilities).

TF had an important effect on the clearance of the bacilli estimated by BI and MI. UM *et al.* (1972), SAHA *et al.* (1975) and ALMEIDA GONÇALVES & CUSTÓDIO (1975) obtained results similar to ours, although with several transfusions of viable leukocytes, obtained from Mitsuda positive donors. This clearence mechanisms could be explained by an allogenic effect (GODAL *et al.*, 1971; LIM *et al.*, 1972).

For SAHA *et* a/. (1975) the bacili elimination would only occur after an interaction of the lymphocytes of the positive Mitsuda donors with macrophages of the virchowian patient. This was their explanation for the inefficiency of the TF obtained from the same donors and injected in virchowian patients during 8 months.

More recently, FABER *et* a/. (1979) studied the effect of TF combined with clofazimine on 7 virchowian patients. During a period of 20 weeks, each patient received a total of 9 TF units (unit = 5x108 lymphocytes). The TF was obtained from lymphocytes of the peripheral blood of Mitsuda positive donors. A result different from ours was observed : the group which received TF and clofazimine and a control group which received only clofazimine presented no differences regarding the clinical course of the disease, the evolution of the skin biopsies and the changes in skin test reactivity to various antigens as well as the lymphocyte transformation in vitro to various mitogens and antigens.

On the other hand, HASTINGS *et al.* (1976) obtained a result similar to ours, treating 4 virchowian patients with 36 doses of TF prepared from peripheral blood of donors which reacted to Mitsuda antigen (7x10'' lymphocytes per patient) during 12 weeks. In this work, however no coniparative study of the cellular immunity was done, before or after treatment.

The acquired resistence to intracellular microorganisms infections, as *Mycobacterium tuberculosis* and *Listeria monocytogenes* (BLADEN & LANGMAN, 1972; LANE & UNANUE, 1972), follows the cellular immunity induced by the cooperation between lymphocytes and macrophages. The parasite would induce a blastogenic response of the commited T lymphocytes; during this response lymphokines would be released and recruit other cells, amplifying the inflamatory reaction (DAVID & DAVID, 1972; MACKANESS, 1969, 1971; NATHAN *et al.*, 1971) Recent in *vitro* tests suggest that one of these lymphokines, the macrophages migration inhibition factor (MIF), in addition to its specific role, can also activate the macrophages for the killing of intracellular bacteria (NATHAN *et al.*. 1973; SIMON & SHEAGREN, 1971).

HAN *et* a/. 1974, using the migration inhibition test, with lymphocytes and macrophages of virchowian and tuberculoid patients, in the presence of *M. leprae* antigens, showed that the virchowian patients' lymphocytes were unable to produce MIF, while the tuberculoid patients' lymphocytes can produce MIF and inhibit both virchowian and tuberculoid macrophages. Furthermore, Convit et al., 1974, observed that the injection of a solution containing 40x106 *M. leprae* and 0,1 mg

of BCG in the arms of virchowian patients who were bacteriologically negative after several years of treatment, induced the following modifications: the biopsy on the site where the antigen was injected with BCG produced, a granuloma formed by vacuolated macrophages with abundant giant cells, epithelioid nodules, large number of lymphocytes and complete absence of bacilli. In the biopsy on the injection site which contained the same bacteria only, there was a macrophagic granuloma with many viable bacteria inside the macrophages without lymphocyte infiltration. The results of this study led the authors to suggest that virchowian patients' macrophages have the necessary enzymes for the elimination of *M. leprae,* when adequately stimulated, and this stimulus is provided by the injection of an antigen to which the host does respond, such as BCG.

All the above evidence could suggest that TF would act upon the T lymphocytes, releasing lymphokines stimulating the phagocytic cells which would reduce the bacillary load (GERY *ct al.,* 1972; GERY & WAKSMAN, 1972; KRAHENBUHL *et al.,* 1973, 1976; MC GREGOR & KOSTER, 1971; MELTZER & OPPENHEIM ; 1977; NATHAN *et al.,* 1971, 1973; SIMON & CHEAGREN, 1971). This, in fact, was observed in 4 of the 5 patients of group III. The interpretation of the results of group II is impaired by the absence of acid fast bacilli in three dimorphous patients, before the treatment. In the remaining two patients, one showed a moderate reduction and the other a strong one.

After the casual distribution of the patients in three groups, it was observed that group III included more patients with higher morphological indexes. Even considering that the interpretation of MI should be cautious, it is worth pointing out that in group III the index can be compared to that of group I, which received a treatment with sulfone.

Histological alterations, as the presence of epithelioid cells or the reappearance of lymphocytes in the paracortical areas which would indicate an improvement of the immunocellular system (OORT & TURK, 1965; TURK & WATERS, 1971) were observed in one patient of the third group (WC) and in one of group II (AGO). WC's skin biopsy after the treatment showed few epithelioid an giant cells. This histological change, characteristic of a dimorphous patient, could be attributed to the action of TF.

Analysing the dermatologic lesions, it is important to emphasize the regression of the lesions observed in the patient WC, treated only with TF. Such improvement coincided with the observation of epithelioid cells and the elimination of acid fast bacilli. In the other four patients of the group III the bacillar reduction did not show a close correlation with the clinical conditions and histopathological findings, although three of them presented clinical improvement.

HASTINGS (1976) obtained results similar to ours, using TF. SAHA *et al.* (1975), using TF in virchowian patients, did not notice clinical improvement associated to the absence of histological and MI modification.

Using viable cells transfusions ALMEIDA GONÇALVES & CUSTODIO (1975) and LIM *et al.* (1972), observed clinical improvement.

The proposal of using human spleen as a TF source was reinforced by the results obtained. This method is of particular importance, because it permits the supply of large quantities of TF, which is relevant for intensive treatment.

These results support the hypothesis that TF is important in the treatment of hanseniasis, specially for virchowian cases and particularly for sulfone resistant patients (BROWNE, 1974) or for those presenting an intense reaction during the sulfone treatment (RIDLEY, 1969). Reactions such as a fever, local pain and erythema nodosum, observed on patients which received TF, were never serious enough to justify interruption of the treatment.

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RESUMO — A proposição do trabalho foi a de estudar as manifestações da imunidade celular medida por células (CMI), in vivo e in vitro, em hansenianos Mitsuda- -negativos antes e após o tratamento. Foi constituído um grupo homogêneo de 15 pacientes obedecendo aos seguintes critérios: pacientes Mitsuda-negativos, do sexo masculino, de 18 a 40 anos, virgens de qualquer tipo de tratamento anterior e com tempo de doença relatado em torno de 5 anos. Foram formados 3 grupos experimentais definidos como segue: Grupo I — pacientes que receberiam 100 mg de sulfona diariamente e 1 ml de salina, por via subcutânea, como placebo, duas vezes por semana; Grupo H — pacientes que receberiam 100 mg de sulfona diariamente e 1 ml de fator de transferencia (FT), preparado a partir de limfócitos obtidos de bago humano, por via subcuthnea, duas vezes por semana; Grupo III — pacientes que receberiam um comprimido como placebo diariamente e 1 **nil** de FT, por via subcutânea, duas vezes por semana.

Os pacientes foram submetidos aos seguintes exames: anamnese e exame clinico dermatológico, índice baciloscópico (IB) e morfológico **(IM),** histopatologia de pele e de linfonodos ingiiinais, determinação de linfócitos T e B no sangue periférico e cultura de linfócitos com estimulo pela fitohemaglutinina (PHA) no 3.° e 14.° dias. Após a coleta de todo o material, o esquema terapêutico foi iniciado e mantido por 8 semanas consecutivas. Findo este período de tratamento, os pacierites foram reavaliados considerando-se todos os exames citados acima mais a reação de Mitsuda.

A hipótese de que o Ft tenha papel significativo a desempenhar na terapêutica *da* hanseniase foi corroborada pelos seguintes resultados: a) em 5 pacientes virchowianos que receberam apenas o FT, houve nitida melhora da resposta A estimulação pela PHA **In vitro,** normalizando-se os valores daqueles que, antes do tratamento, mostravam depressão dessa resposta; b) nesses mesmos pacientes, a evolução do IM foi comparável â observada em 4 virchowianos e 1 dimorfo que receberam sulfona; também foram comparáveis os resultados globais de evolução histopatológicos em biopsias de linfonodos e, principalmente, de pele, nos dois grupos de pacientes; é especialmente digna de nota a mudança da forma virchowiana para a dimorfa, em um dos pacientes tratados apenas com FT; c) na avaliação dermatológica das lesões cutâneas, **o** tratamento pelo FT proporcionou resultados semelhantes aos observados com a terapêutica sulfiinica; d) o tratamento com **FT,** isolado ou associado A sulfona, não promoveu conversão da reação ao antígeno de Mitsuda.

Os pacientes do Grupo **H** que receberam o FT e sulfona mostraram evolução dermatológica e dos quadros histopatológicos de biopsias de linfonodos e de pele semelhante A. observada nos outros dois grupos. Entretanto, não houve modificação da resposta h estimulação pelo PHA em cultura de 3 dias.

Em relação à transformação blistica dos linfócitos antes do tratamento, observamos: **a)** *as* medianas dos indices de transformação linfoblústica, com estimulação pela fitohemaglutinina em meios de cultura contendo soro autólogo ou soro homólogo, foram nos pacientes observados antes do tratamento, significantemente menores do que nos controles "normais". Alguns pacientes, todavia, apresentaram valores dentro

da normalidade. **b)** nas mesmas cultures, não foram encontradas diferenças significantes entre as medianas dos indices obtidos em meios com soro aut6logo e com soro nomólogo, tento em pacientes antes do tratamento, quanto os controles "normais".

As medianas do número absoluto e da percentagem de linf6citos T e de linf6citos 8, em pacientes, antes do tratamento, não diferiram significantemente das observadas em controles "normais". Também não diferiram essas medianas, quando comparadas aos resultados dos pacientes antes e depois dos tratamentos.

A proposição de se utilizer bago humano como fonte de FT, encontrou apoio nos resultados obtidos. Este método assume particular importância por permitir a obtenção de grandes volumes do referido produto, o que será relevante para a aplicação extensiva dessa terapêutica.

Palavras chave: Ha.nseniase. Imunidade celular. Fator de transferência.

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