

Some Considerations Regarding the Immunology of Leprosy

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In leprosy, an infection produced by a mycobacterium, *M. leprae*, which is an obligatory intracellular parasite, it is the cell-mediated immunologic phenomena which intervene as defensive mechanisms, and not circulating antibodies.

A high proportion of the general population is immune to the disease. It seems that in each generation there is a small group of persons susceptible to infection who can acquire the lepromatous form. The possibility that the predisposition to acquire the lepromatous form might be a genetic characteristic has been considered (⁴).

There are two different viewpoints in relation with the immunologic disturbance in lepromatous leprosy. One of them considers the lack of immunologic recognition of *M. leprae* as an antigen, capable of eliciting delayed hypersensitivity responses, to be preexisting and specific only for this mycobacterium. The other theory is that the immunologic alteration is produced by the disease itself, and that it includes not only *M. leprae* but other antigens as well.

This last theory compares the immunologic disturbance seen in lepromatous leprosy with that found in granulomatous diseases which compromise lymphoid tissues, such as sarcoidosis and Hodgkin's disease (^{5,6}). It has been shown that in these diseases there is a generalized depression of delayed hypersensitivity reactions which is related to the degree of invasion of lymphoid tissue. In lepromatous leprosy, the depression of cell-mediated immunologic phenomena has been related to the degree of substitution of the lymphocytes of the paracortical area by reticulo-histiocytes. This area is considered as having, in adults, some of the functions of the thymus, such as the control of cellular immunity (⁷).

Several investigators have made studies comparing the response to tuberculin of

leprosy patients and healthy controls; the results published do not agree (⁸).

During the last few years results have been published showing that the percentage of positivity to skin tests with PPD is lower in patients with lepromatous leprosy than in normal controls (^{2,3}). Trials have also been made with fungal antigens, such as trichophyton and oidiomycin, and the reactions of lepromatous patients have been found lower than those of normal controls (^{1,2}). Contact sensitization to dinitro-chlorobenzene and picryl chloride has also given significantly lower responses in lepromatous patients than in controls (^{1,2,9}).

With the purpose of contributing to the investigation of this interesting problem, i.e., the study of whether the alteration of cellular immunologic reactions in lepromatous leprosy is specific or includes other antigens than *M. leprae*, we have carried out the following investigations.

I. Comparison of two diseases considered as due to an immunologic defect of the host: lepromatous leprosy (LL) and diffuse cutaneous leishmaniasis (DCL).

LL and DCL are two diseases with very similar clinical, pathologic, parasitologic and immunologic characteristics (Fig. 1). One of them, LL, is produced by *M. leprae* and the other, DCL, by a leishmanial parasite.

In a previous report, presented at the Nineteenth Convention of the Venezuelan Association for the Advancement of Science, we presented the facts on which we have based our belief that DCL is due to an immunologic defect of the host infected by *L. brasiliensis* and not, as has been proposed, as a disease produced by a different species of *Leishmania*, which has been named *L. pifanoi*.

Table 1 shows the similarities in these two diseases. Clinically, each of them produces generalized diffuse lesions which compromise the skin, mucous tissue and lymph nodes. The only difference is that in LL there are visceral and nervous lesions,

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FIG. 1. DCL patient. Nodules and plaques on the face.

while both these structures remain uncompromised in DCL.

The histologic structure in both diseases is characterized by a granuloma formed solely by macrophages, with a complete lack of lymphoid cells. These granulomas, with their absence of lymphoid cells, are in great contrast with the granulomas found in tuberculoid leprosy (TL) and American cutaneous leishmaniasis (ACL), which are the benign forms of these diseases where there is an active immunologic response from the host.

Skin tests made with the corresponding antigen, lepromin for LL and leishmanin for DCL, show a complete lack of response in both cases.

Another interesting point arises from the great abundance of the infecting agent in host tissue. The parasite-cell relationship is similar in both granulomas; the macrophages show large vacuoles containing a great number of parasites. To these similarities we must add the chronic evolution of both diseases, their resistance to specific therapy and the very frequent relapses

even after the infecting agent has disappeared from the lesions.

The comparison can be closed by pointing out that each of these diseases has a clinical form where the host shows a high level of resistance. This clinical form is, for lepromatous leprosy, tuberculoid leprosy (TL), and for diffuse cutaneous leishmaniasis, American cutaneous leishmaniasis (ACL). TL and ACL are also very similar in their general characteristics.

Also as part of the investigation, we felt it should be determined if the immunologic defect in each of these two diseases was related only to the infecting agent, or if the immunologic anergy might include both parasites.

MATERIALS AND METHODS

Five DCL patients, two females and three males, aged between 24 and 50 years, were tested with standard lepromin containing 160×10^6 acid-fast bacilli per cc. Each patient was injected with 0.1 cc. of the antigen on the volar surface of the forearm.

TABLE 1. Comparison between lepromatous leprosy and diffuse cutaneous leishmaniasis as diseases due to a host-defect.

	Clinical form	Structure	Skin test	Microbiology	Evolution	Therapeutics	Resistant host
Lepromatous leprosy	Diffuse lesions in skin ganglia, mucosa and viscera	Macrophagic granuloma	Mitsuda-negative at 48 hours and 28 days	High number of <i>M. leprae</i> in lesions	Chronic progression during several years	Resistance to treatment. Frequent relapses	Resistant host Tuberculoid Leprosy
Diffuse cutaneous leishmaniasis	Diffuse lesions in skin, mucosa and ganglia	Macrophagic granuloma	Leishmanin-negative at 48 hours	High number of <i>L. brasiliensis</i> in lesions	Chronic progression during several years	Resistance to treatment. Frequent relapses	Resistant host American Cutaneous Leishmaniasis

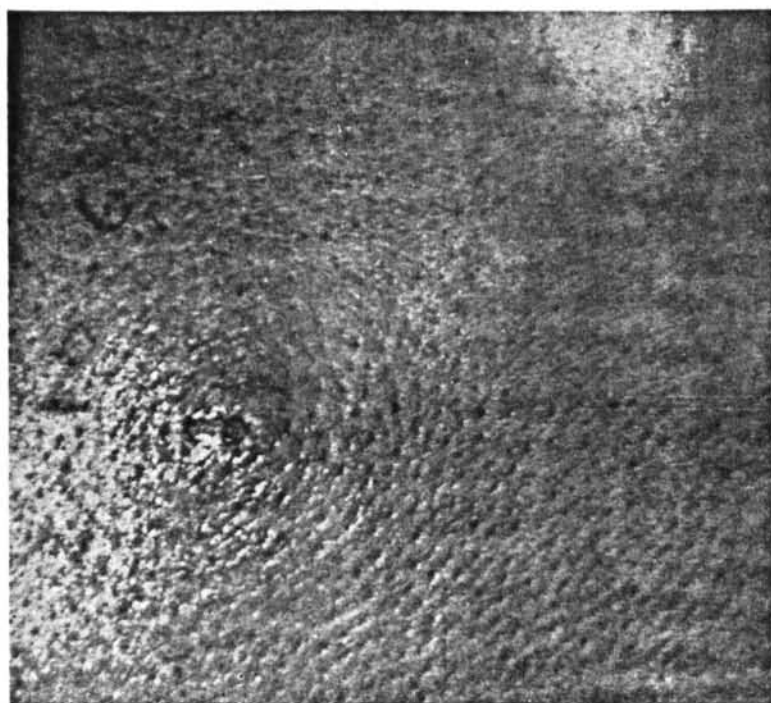


FIG. 2. Inoculation nodule three months old, after injection with material obtained from a DCL in a LL patient.

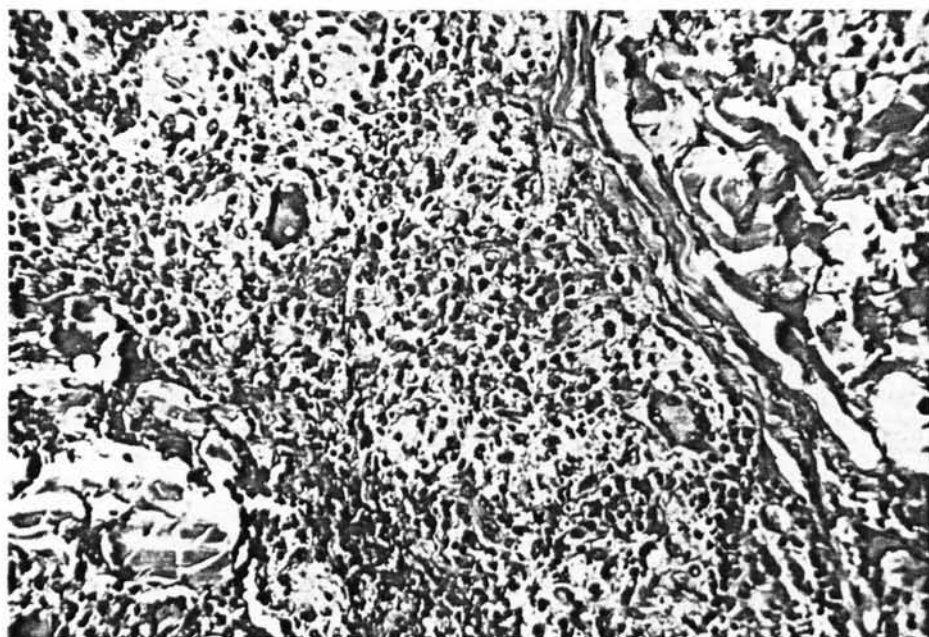


FIG. 3. Tuberculoid granuloma in a nodule resulting from inoculation with DCL material in a LL patient (16 \times).

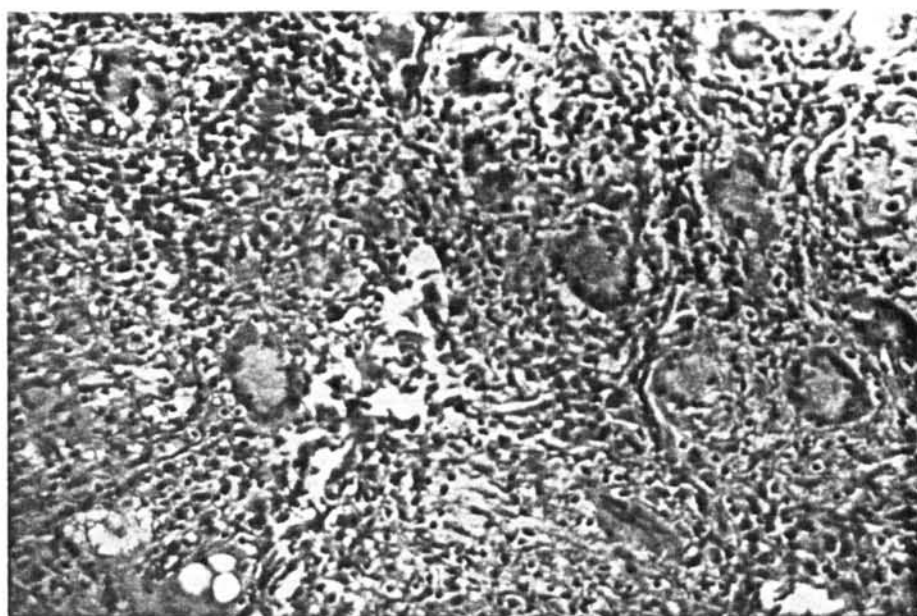


FIG. 4. Tuberculoid granuloma in a nodule resulting from inoculation three months previously of DCL material in a LL patient (45 \times).

Also, five active LL patients, one female and four males, 35 to 65 years old, were injected with parasites obtained from active DCL cases. Nodules from DCL patients were extirpated surgically and macerated in saline with a Ten Broeck grinder. The suspension was diluted to contain 145×10^6 parasites per cc; each patient was injected with 0.1 cc. of the suspension in two different sites on the upper region of the back.

RESULTS

Two weeks after they were injected with lepromin the DCL patients developed an erythematous nodule at the inoculation site. The central diameter of the nodule varied between 7 and 10 mm; two of them had central ulcerations. The nodules were biopsied and the sections stained with hematoxylin-eosin and Fite-Faraco stain. Light microscopy revealed that the structure corresponded to a tuberculoid granuloma.

The LL patients inoculated with parasites from DCL lesions developed, three weeks later at the inoculation site, a nodule (Fig. 2) which at biopsy showed a tuberculoid structure. This nodule became parasitologi-

cally negative four months later on examination by routine procedures. The evolution of these nodules was studied by biopsies one, two and four months after inoculation.

The biopsy at one month showed a large granuloma formed by macrophages, lymphoid cells and some plasmacytes (Fig. 3); inside the granuloma there were numerous epithelioid foci. At this time we found large numbers of leishmanias inside the macrophages. The two-month biopsy showed a granuloma with a tuberculoid organization and few leishmanial parasites. At four months the granuloma was characterized by epithelioid and tuberculoid nodules, abundant lymphocytes and no parasites (Fig. 4).

After the fourth month the lesion regressed completely without treatment. A leishmanin test made on these patients proved positive, with a papule about 20 mm. in diameter. Twelve months later there were only scars at the site of inoculation.

Two of the LL patients were reinoculated with DCL parasites six weeks after the first inoculation. A nodule appeared three weeks later; its structure was formed

by foci of fibrinoid necrosis and epithelioid and tuberculoid nodules. This structure is similar to that of ACL lesions of long duration.

DISCUSSION

DCL patients tested with lepromin showed positive reactions, both clinically and histopathologically. They reacted in the same way as a normal person or a tuberculoid patient. On the other hand, the LL patients inoculated with leishmanial parasites obtained from DCL patients showed self-limiting lesions, identical to those of ACL. As proof of the viability of the DCL parasites injected, we obtained positive cultures in Davis medium, and also experimental lesions in hamsters from the lesions produced in the LL patients.

The LL patients, by producing ACL lesions, reacted to the leishmanial parasite in the same way as the general normal population, indicating a high level of resistance to the disease. This seems further proof that the diffuse form of leishmaniasis is due to an immunologic anergy of the infected host, since the same parasites which produce that type of disease in him produce ACL when inoculated in another host. Sixteen months after inoculation, the experimental lesion produced in the LL patients was completely extinguished.

From these results we reached the conclusion that in each case the immunologic defect producing the disseminated disease was specific for the infecting agent.

From an epidemiologic viewpoint these two diseases show the following. For DCL, few cases in areas where ACL is endemic, favoring the proposition that the disease is due to a pre-existing host defect, since the epidemiologic pattern conditioned by a different species of *Leishmania* would be marked by several cases in a restricted area. For LL, the frequency is much higher, since there are hyperendemic foci where there may be as many as 15 cases per thousand inhabitants.

We believe that the immunologic disturbance giving rise to DCL must be very rare, probably one case in many thousands of individuals living in endemic areas.

II. Investigation comparing groups of lepromatous patients and normal controls on the basis of tuberculin (PPD), trichophytin, oidiomycin and lepromin tests and contact sensitization with dinitro-chlorobenzene.

This investigation was carried out in the two national leprosaria, the Cabo Blanco and Isla de Providencia hospitals. We studied two groups of persons of similar characteristics.

MATERIALS AND METHODS

Two groups of persons were selected. One of them was formed by lepromatous patients with varying degrees of clinical compromise, ranging from patients with no apparent skin lesions, classified as LL₀, to patients with a very advanced form of the disease, LL₃ and also patients with the intermediate stages LL₁ and LL₂. A group of normal controls was formed by the employees of both hospitals, who have lived for long periods (average: 10.7 years) in close contact with the patients under the same environmental conditions and eating the same kind of food. The two groups were equivalent in relation to age and were chosen by pairing and randomization methods. In the LL group there were nine persons who had had tuberculosis, but they had been treated and by the time of the trial had become inactive. In the normal control group there were no cases of tuberculosis.

The antigens used were: PPD, 2 units, from the Statens Serum Institute of Copenhagen; standard lepromin containing 160×10^6 AFB per cc., prepared in the Division of Sanitary Dermatology in Venezuela; trichophytin, diluted 1:50 and oidiomycin diluted 1:100, obtained from the Hollister-Stier Laboratories, Los Angeles, California.

Each person was injected with 0.1 cc. of each antigen on the volar surface of both forearms, using disposable needles. The tests were read at 48 hours for all antigens and at the fourth week again for the lepromin antigen. All injections and readings of tests were made by the authors personally. The criteria for determining positivity were 10 mm. or more of induration for PPD and the 48-hour lepromin test, and 5

TABLE 2. *Healthy controls and patients with lepromatous leprosy according to age.*

Age (years)	Healthy controls		LL ₀		LL ₁	LL ₂	LL ₃
	No.	%	No.	%	No.	% LL ₂ LL ₃	
20-24	4	2.5	3	2.7	4	2.5	
25-29	16	9.8	6	5.5	16	9.8	
30-34	15	9.2	8	7.3	15	9.2	
35-39	28	17.2	21	19.1	28	17.2	
40-44	15	20.5	25	22.7	34	20.9	
45-49	23	14.1	23	20.9	23	14.1	
50-54	17	10.4	10	9.1	17	10.4	
55-59	13	8.0	8	7.3	13	8.0	
60-64	10	6.1	5	4.5	10	6.1	
65-69	3	1.8	1	0.9	3	1.8	
Total	163	100.0	110	100.0	163	100.0	
Arithmetical average	41.6		43.6		41.6		
Standard deviation	10.6		9.4		10.6		
Standard error	0.83		0.89		0.83		

mm. or more of induration for trichophytin and oidiomycin. For the 4-week lepromin reaction, 3 mm. or more of induration was considered positive.

The test for sensitization to dinitrochlorobenzene (DNCB) was carried out in the following manner. A sensitizing dose of 0.1 cc. of a 2 per cent dilution of DNCB in acetone was applied on the lateral surface of the thorax in an area approximately 15 mm. in diameter. Simultaneously, two diagnostic tests were applied to determine previous sensitization. The amounts and the

areas were identical with the sensitizing dose, but the dilutions were 0.2 and 0.05 per cent. The diagnostic tests were read at 48 hours. Fourteen days later the diagnostic tests were repeated, under the same conditions as previously. This part of the trial was made only at Cabo Blanco Hospital, but the groups were kept equivalent by the same methods as used before.

RESULTS

In Table 2 we can see the age distribution of the groups tested. Table 3 shows the

TABLE 3. *Healthy controls and patients LL₀ and LL₁₋₂₋₃ according to their 48 hour reactions and fourth week reaction to standard lepromin.*

Category	48 hour reaction			Fourth week reaction		
	Positives	Negatives	% Pos.	Positives	Negatives	% Pos.
Healthy controls	61	102	37.4	156	7	95.7
Patients LL ₀	9	101	8.2	37	73	33.6
Patients LL ₁₋₂₋₃	6	157	3.7	17	146	10.4

reactions to standard lepromin at 48 hours and at four weeks, in the control group and the groups of LL patients. The difference is statistically significant. The positive lepromin tests in the patient group were, in most cases, just barely in the neighborhood of 10 mm. in diameter for the 48-hour test and 3 mm. for the fourth-week test. Almost all the positive tests were in the LL₀ group. The few positive cases in the LL₁₋₂₋₃ group were patients with very few lesions or patients who had been included in that group only because they were bacteriologically positive.

Table 4 compares the results with PPD in the three groups; the percentage of positive tests is higher in the patient group than in the normal controls, but the difference is not significant.

TABLE 4. Healthy controls and patients LL₀ and LL₁₋₂₋₃ according to their reaction to P.P.D.

Category	Test with PPD		
	Positives	Negatives	% Pos.
Healthy controls	118	45	72.4
Patients LL ₀	94	16	85.4
Patients LL ₁₋₂₋₃	126	37	77.3

Table 5 shows the results of the tests with the fungal antigens trichophytin and oidiomycin. Only the test with oidiomycin shows a statistically significant difference between the control and the LL₁₋₂₋₃ groups.

Table 6 shows the results obtained with DNCB. The difference in sensitization between the patient group and the control group is not statistically significant.

TABLE 6. Percentage of tests positive to DNCB, in two concentrations, among healthy controls and lepromatous leprosy patients.

D.N.C.B.	Groups		
	Controls	LL ₀	LL ₁₋₂₋₃
0.2%			
Positives	44	47	23
Negatives	1	2	2
Total	45	49	25
% positives	97.8	95.9	92.0
0.05%			
Positives	28	36	19
Negatives	17	13	6
Total	45	49	25
% positives	62.2	73.5	76.0

TABLE 5. Healthy controls and patients LL₀ and LL₁₋₂₋₃ according to their skin reactions to trichophytin and oidiomycin.

Category	Test with trichophytin			Test with oidiomycin		
	Positives	Negatives	% Pos.	Positives	Negatives	% Pos.
Healthy controls	61	102	37.4	54	109	33.1
Patients LL ₀	34	76	30.9	26	84	23.6
Patients LL ₁₋₂₋₃	42	121	25.8	21	142	12.9

DISCUSSION

Analysis of the results of the PPD, lepromin, trichophytin, oidiomycin and DNCB tests shows that there were no statistically significant differences in the reactions to the antigens in the groups under study, with one exception; the test with oidiomycin showed a statistically significant difference between the control group and the LL₁₋₂₋₃ group.

The results of this trial seem to show that the immunologic anergy which makes LL patients unable to recognize *M. leprae* as an antigen, is specific for this mycobacterium and does not include other antigens capable of eliciting delayed hypersensitivity reactions.

Our results differ from those of other authors, who find statistically significant differences between lepromatous leprosy patients and normal controls in relation to tests made with PPD, trichophytin, oidiomycin and DNCB.

SUMMARY

We have chosen for presentation in this symposium two aspects of leprosy which seem of great interest in relation to phenomena of delayed hypersensitivity. The first concerns a comparative study between two models of human disease which present very similar characteristics, lepromatous leprosy and diffuse cutaneous leishmaniasis. The second is related to an investigation made in the two leprosaria in Venezuela. This investigation was made by comparing the delayed hypersensitivity reactions to certain antigens in the patients and the personnel working in these leprosaria. The antigens used for these tests were: (a)

lepromin antigen, for which we read the 48 hour or Fernandez reaction, and the 28 day, or Mitsuda reaction, (b) PPD, (c) trichophytin, (d) oidiomycin, and (e) DNCB.

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REFERENCES

1. BUCK, A. A. and HASENCLEVER, H. F. The influence of leprosy on delayed type skin reactions and serum agglutination titers to *Candida Albicans*. *Amer. J. Hyg.* **77** (1963) 305-316.
2. BULLOCK, W. E. Studies of immune mechanisms in leprosy. I. Depression of delayed allergic response to skin test antigens. *New England J. Med.* **278** (1968) 298-304.
3. GUINTO, R. S. and MABALAY, M. C. Note on the tuberculin reaction in leprosy. *Internat J. Leprosy* **30** (1962) 278-283.
4. NEWELL, K. W. *Bull. Wld. Org.* **34** (1966) 827.
5. SONES, M. and ISRAEL, H. L. Altered immunologic reactions in sarcoidosis. *Ann. Intern. Med.* **40** (1954) 260-268.
6. TURK, J. L. *Delayed Hypersensitivity*. Amsterdam, 1967.
7. TURK, J. L. and WATERS, M. F. R. Immunological basis for depression of cellular immunity and the delayed allergic response in patients with lepromatous leprosy. *Lancet*. August 24, 1968, 436-438.
8. WADE, H. W. Reactions to tuberculin in leprosy. *Internat. J. Leprosy* **18** (1950) 373-388.
9. WALDORF, D. S., SHEAGREN, J. N., TRAUTMAN, J. R. and BLOCK, J. B. Impaired delayed hypersensitivity in patients with lepromatous leprosy. *Lancet* **2** (1966) 773-775.