

Impaired delayed hypersensitivity in patients with South American blastomycosis

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Fifty-one cases of South American blastomycosis which exhibited a depression of delayed hypersensitivity to infectious antigens and sensitization to dinitrochlorobenzene (DNCB) were investigated. The results showed 54.9 per cent negative intracutaneous reactions to tuberculin, 60.8 per cent to trichophytin, and 64.7 per cent to oidiomycin. These results indicate significantly less reactivity than that in a control group. Sensitization to DNBC was not obtained in 72.6 per cent of the patients, again a significant reduction as compared to that in control subjects. Transfer of tuberculin hypersensitivity by means of lymphocytes from tuberculin-positive individuals was obtained in all 4 South American blastomycosis recipients tested. Based on the above results, some immunological aspects of South American blastomycosis are discussed.

South American blastomycosis is confined almost entirely to South America, with the highest incidence in Brazil. The etiological agent is *Paracoccidioides brasiliensis*, which generally produces granulomatous lesions in the skin and mucosae, lymph nodes, lungs, adrenal glands, spleen, liver, and other organs.

The characteristic chronic nature of the disease, with lymph node enlargement in all cases, and the high percentage of negative intracutaneous tests for several antigens of *Paracoccidioides brasiliensis*¹ suggested some defects in the mechanism of delayed hypersensitivity. For this reason, delayed hypersensitivity was investigated in 51 patients with South American blastomycosis.

MATERIALS AND METHODS

Fifty-one patients, ages 16 to 64, who had had the disease for over a year, were studied, having been classified according to clinical forms and treatment. Twenty two were undergoing daily intravenous treatment with amphotericin B* in doses of 50 to 75 mg. and hydrocortisone succinate in doses of 20 mg. for 15 to 45 days. Fifteen patients had been treated with sulfonamide, and 14 were not treated. Hospitalization was necessary because of the general condition of the patients, which ranged from mildly to severely ill.

The following procedures were used:

1. Intracutaneous tests with antigens which usually produce a high percentage of positive reactions among the normal population of the region studied were performed: (1) standard old tuberculin at 1:1,000 concentration; (2) oidiomycin, prepared according to Lacaz¹; (3) trichophytin at 1:10 concentration prepared by the Instituto Adolfo Lutz.

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Supported in part by the Fundação de Amparo à Pesquisa do Estado de São Paulo, São Paulo, Brazil.

Received for publication March 31, 1970.

*Fungizone, Squibb.

The antigens were injected intracutaneously in 0.10 ml. doses and the reading was made 48 hours later; the diameter of the area of induration was then measured in millimeters.

A control group was used consisting of 51 normal individuals belonging to the hospital staff tested with the same antigens at the same time and under similar experimental conditions.

2. Sensitization with dinitrochlorobenzene (DNCB) was carried out as follows: (1) Sensitizing doses of 2,000 μg of DNCB dissolved in 0.10 ml. of acetone were spread and maintained over a 2 cm.² area of skin on the patient's back for 48 hours, just as in an ordinary patch test. In all 51 patients, erythema and induration with blistering and exudation were observed. All the patients had been tested 48 hours earlier with 100 μg of DNCB in order to eliminate the possibility of previous sensitization. (2) Eighteen to 20 days later, a patch test was made with 100 μg of DNCB on a skin site different from the one used for sensitization. The reactions were read after 48 hours and classified as one to four plus in accordance with the customary procedures. The negative tests were not repeated in the subsequent weeks. (3) Because of ethical problems involved in DNCB sensitization of normal subjects, 29 patients with lepromatous leprosy were used as a control group.

3. Transfer of delayed hypersensitivity to tuberculin was performed. Four tuberculin-negative recipients with South American blastomycosis were investigated. The lymphocytes were obtained from tuberculin-positive donors according to standard techniques.^{2,3} Approximately 15 to 20 million lymphocytes were suspended in 1 ml. of serum from the recipient and injected, the first 0.20 to 0.30 ml. intracutaneously and the remainder subcutaneously in the same place without withdrawing the needle. Three to 5 days later these 4 patients with South American blastomycosis received 0.1 ml. of 1:1,000 tuberculin intracutaneously in areas distant from the sites previously injected with lymphocytes.

RESULTS

The results are summarized in Tables I and II. For statistical analysis, the chi square test was employed. The results are the following:

1. There was a significant depression of delayed hypersensitivity to intracutaneous tests to tuberculin, oidiomycin, and trichophylin (Table I). The chi square test showed the difference with tuberculin to be significant at < 0.02 , oidiomycin significant at $= 0.01$, and trichophylin significant at < 0.05 when compared with the results in the control group of 51 normal individuals. Positive intracutaneous reactions with induration areas of more than 2 cm. in diameter were not observed.

2. A significant decrease in DNCB sensitization (< 0.01 by the chi square test) compared with the control group of patients with lepromatous leprosy was noted (Table II). Only 27.4 per cent of the patients could be sensitized to DNCB.

3. Of the 11 patients with positive intracutaneous tests for infectious antigens, sensitization to DNCB occurred in only 4.

4. In the patients treated with amphotericin B and corticoids, a significant difference (< 0.01) was observed with the tuberculin test, and no significant difference with trichophylin, oidiomycin, and sensitization to DNCB was noted when compared with the other two groups (patients treated with sulfonamide and patients without treatment). There was no important difference between the group of patients treated with sulfonamide and the group not treated.

5. No significant difference in results was observed among the clinical forms of the disease classified as: lymphatic (11 patients), pulmonary (2 patients), tegumental (19 patients), tegumental-lymphatic (10 patients), tegumental-pulmonary (5 patients), and tegumental-lymphatic-pulmonary (4 patients).

TABLE I. Results of intracutaneous tests in 51 patients with South American blastomycosis

Patients	Reactions	Tuberculin	Oidiomycin	Trichophytin
South American blastomycosis (51 patients)	Positive			
	0.5 to 1.0 cm.	7-13.7%	12-23.5%	11-21.6%
	1.0 to 2.0 cm.	16-36.4%	6-11.8%	9-17.6%
	> 2.0 cm.	0	0	0
	Negative	28-54.9%	33-64.7%	31-60.8%
Control group (51 normal individuals)	Positive			
	0.5 to 1.0 cm.	12-23.5%	9-17.6%	11-21.6%
	1.0 to 2.0 cm.	16-31.4%	16-31.4%	16-31.4%
	> 2.0 cm.	7-13.7%	6-11.8%	4- 7.8%
	Negative	16-31.4%	20-39.2%	20-39.2%

TABLE II. Sensitization to DNCB in 51 patients with South American blastomycosis compared with 29 patients with lepromatous leprosy

Reactions	South American blastomycosis	Lepromatous leprosy
Positive		
+	7.8%	17.7%
++	13.7%	24.1%
+++	2.0%	20.7%
++++	3.9%	6.9%
Total	27.4%	68.9%
Negative	72.6%	31.1%

6. Transfer of hypersensitivity to tuberculin by means of lymphocytes was positive in the 4 recipients with South American blastomycosis. The reaction consisted of an induration larger than 1 cm. in diameter in areas injected with 1:1,000 tuberculin. The intracutaneous test for tuberculin was repeated in only one recipient 50 days after the transfer with a positive result.

DISCUSSION

These cases of South American blastomycosis have shown a depression in the delayed hypersensitivity to intracutaneous tests with antigens from infectious agents, as well as a great deficiency in sensitization to DNCB.

As a chemical hapten, DNCB can sensitize over 90 per cent of a group of normal individuals,⁴ but in patients with South American blastomycosis sensitization occurred in only 27.4 per cent. This is a significant deficiency in sensitization to DNCB even when the results were compared with those of a group of patients with lepromatous leprosy in which sensitization to DNCB occurred in 68.9 per cent of the cases. Despite a recognized impairment of delayed hypersensitivity in lepromatous patients,⁵ a significant difference in DNCB sensitization in South American blastomycosis was observed (< 0.01 by the chi square test) when both groups were compared.

Similar behavior has been reported in Hodgkin's disease as well as in other lymphoproliferative diseases, such as, sarcoidosis, ataxia telangiectasia, some viral diseases, uremia, and Di George's syndrome.^{6, 7}

An important theoretical consideration is whether the defect in the mecha-

nism of delayed hypersensitivity precedes the disease or whether it is a consequence of the disease. In South American blastomycosis this problem is not completely understood. In sarcoidosis and Hodgkin's disease there is evidence that the defect occurs as a consequence of the disease.⁸⁻¹² In both sarcoidosis and lepromatous leprosy,¹³ after improvement of the patient's condition following proper treatment there may be a restoration of the lost delayed hypersensitivity.^{14, 15}

Some speculations could be made to explain the defective response to the intracutaneous tests with antigens from infectious agents as well as the impairment of sensitization observed in most of the patients with South American blastomycosis. The fact that some individuals who reacted to tuberculin, oidiomycin, and trichophytin could not be sensitized to DNCB illustrates the distinction between the capacity to react to an antigen to which a subject has been previously sensitized and the ability to develop reactivity upon a primary exposure to a new antigen.¹² Immunologically competent cells having the capacity to respond to tuberculin, oidiomycin, or trichophytin may have persisted in these individuals, while in the course of blastomycosis immunologically uncommitted cells of the same individual may have lost the ability to recognize a new primary antigen. According to Cone and Uhr¹⁶ and Waldorf and associates,⁴ it could be suggested that in these patients the population of cells committed in previous sensitization remains unaffected for many years in contrast to the uncommitted cells which are gradually substituted.

Variable results are obtained from intracutaneous tests of delayed type with blastomycin or paracoccidioidin for diagnostic purposes in North or South American blastomycosis.^{17, 18} Patients in poor general condition with South American blastomycosis usually do not react to intracutaneous tests with various *Paracoccidioides brasiliensis* antigens, while positive tests more frequently are obtained in patients in otherwise good health, with mild localized forms of disease or following recovery after therapy.^{17, 21} Probably results of tests to *Paracoccidioides brasiliensis* antigens depend more upon the general immunological competence of the patient than upon the quality or method of preparation of the antigens.

Severe cases of South American blastomycosis present a high titer of humoral antibodies. In the majority of instances a decrease in the titer of these antibodies is coincident with clinical improvement. Negative or low-titer complement fixation tests for humoral antibodies also are the rule in mild, localized forms of the disease.²¹ Likewise in North American blastomycosis the severe cases usually present negative skin reactions and at the same time positive complement fixation tests.²⁰ Thus, in both diseases there seems to be a dissociation between the presence of circulating antibodies and positive cutaneous reactions. It may be suggested that humoral antibodies block the antigenic sites, preventing the cells responsible for delayed hypersensitivity from reacting with the antigenic sites involved. A similar mechanism is observed in the enhancement phenomenon, as in tolerance to tumor grafts.²²

The beneficial role played by delayed hypersensitivity in resistance to some infections, chiefly those of an intracellular type, is controversial.^{23, 24} South

American blastomycosis develops virtually without a tissue response, with the recipient's macrophages loaded with *Paracoccidioides brasiliensis*, high titers of serum antibodies, and a slight cutaneous reaction to the fungi and their products. This is not a condition peculiar to blastomycosis, as it can also be found in other infections, mainly the intracellular ones, such as, lepromatous leprosy and tuberculosis. In these diseases depressed hypersensitivity has been associated with spreading of the infection and poor prognosis despite high titers of serum antibodies. On the other hand, the recovery of the delayed hypersensitivity is associated with a better prognosis.²⁵

Patients with Hodgkin's disease are usually unable to accept transferred hypersensitivity to tuberculin through lymphocytes, while normal subjects are usually able to do so.^{26, 27} Patients with South American blastomycosis differ in this respect from patients with Hodgkin's disease,²⁸⁻³⁰ for in 4 of our subjects the transfer of hypersensitivity to tuberculin was successful. This means that the cell population normally involved in the acceptance, replication, or transmission of the transfer factor is not affected in South American blastomycosis.

Diseases of a torpid and chronic nature without striking manifestations of defense against the aggressor, such as South American blastomycosis, surely will be better understood with regard to their mechanisms if they are discussed in terms of competence and immunological tolerance.

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