

Lymphocyte cultures and skin allograft survival in patients with South American blastomycosis

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Lymphocyte cultures with phytohemagglutinin (PHA), skin allograft survival, intradermal tests with microbial antigens, and sensitization to dinitrochlorobenzene (DNCB) were performed in 10 patients with South American blastomycosis. The lymphocyte stimulation in vitro was depressed in 6 of the patients. Exposure to DNCB resulted in sensitization in 3 of 10 patients. Among the 7 DNCB-negative patients, blast transformation was depressed in 6, and in 2 of these there was prolonged skin allograft survival. The results suggest a severe impairment of delayed hypersensitivity in some patients with South American blastomycosis.

Patients with South American blastomycosis have impaired delayed reactivity to intradermal tests with common antigens such as tuberculin, trichophytin, and oidiomycin, which produce positive reactions in a high percentage of the normal population. Contact sensitization with 2,4 dinitrochlorobenzene (DNCB) is also depressed in these patients.¹

The impaired immune response, chronic nature of the disease, and the lesions in the lymph nodes resemble changes seen in other diseases in which cellular immunity is altered.²⁻¹⁶

The correlation between a depressed in vitro response to phytohemagglutinin (PHA) and the impairment of the delayed response in vivo has been demonstrated in patients with sarcoidosis,^{10, 17} ataxia telangiectasia,^{13, 14} chronic lymphocytic leukemia,^{8, 9, 18} Hodgkin's disease,¹⁹⁻²³ Sjögren's syndrome,²³ and DiGeorge's syndrome.^{24, 25}

The purpose of this investigation was to compare in patients with South American blastomycosis the response of peripheral lymphocytes in vitro to PHA with in vivo tests of delayed hypersensitivity, such as skin allograft survival, intradermal reactions with common antigens, and sensitization to DNCB.

MATERIALS AND METHODS

Ten patients with a clinical and laboratory diagnosis of South American blastomycosis were studied (Table I). None of them was receiving corticosteroids at the time of the investigation.

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TABLE I. Distribution of patients according to sex, age, and duration and clinical form of the disease

Patient	Sex	Age (years)	Duration (years)	Clinical form
L. M.	M	44	3	Cutaneous-pulmonary
A. P.	M	14	5	Lymphatic-visceral
M. A.	F	18	$\frac{1}{4}$	Lymphatic-visceral
A. M.	M	59	$\frac{1}{5}$	Pulmonary
S. F. S.	M	35	$\frac{1}{4}$	Lymphatic
B. S. O.	M	48	10	Adrenal-pulmonary
E. K.	M	24	5	Lymphatic
J. B. R.	M	15	4	Lymphatic
M. P. M.	F	26	1	Lymphatic
A. B.	M	20	14	Cutaneous

Lymphocyte cultures

Peripheral heparinized blood was obtained from the 10 patients and from 10 normal control subjects. After separation of the leukocyte-rich plasma, the cells were counted, centrifuged, and resuspended in Eagle's tissue culture medium (Grand Island Biological Co., Chagrin Falls, Ohio) containing 20 per cent autologous plasma. The lymphocyte concentration was adjusted to 0.3×10^6 per milliliter, and 2.5 ml. of this suspension was added to each culture tube. PHA-M (Difco Laboratories, Detroit, Mich.) (0.15 ml. of a 1:10 dilution) was added to triplicate tubes at the beginning of the culture. Control tubes did not receive PHA. The same procedure was repeated substituting autologous plasma for normal homologous plasma. Tritiated thymidine, 2 μ c (Schwarz Bio Research, New York, N. Y.), was added to each tube 5 hours prior to termination of culture at 72 hours. The tubes were centrifuged at 150 g for 15 minutes, the supernatants removed, and the cell buttons resuspended in 5 ml. of a 5 per cent solution of trichloroacetic acid. The tubes were re-centrifuged and the cells again washed with the trichloroacetic acid. 2N Methanolic potassium hydroxide, $\frac{1}{2}$ ml., was then added to each tube and the mixture incubated for 24 hours at 70° C. Bray's scintillation solution,²⁰ 10 ml., was added to each tube. The contents of the tubes were then transferred to scintillation counting vials, and the vials were analyzed for their radioactivity in a Beckman scintillation counter. Each sample was counted for 10 minutes and the results expressed as counts per minute.

Skin-grafting technique

A 4 mm. trephine was used to obtain grafts from normal unrelated ABO compatible donors. All grafts were of full thickness and held in place with sterile semipressure dressings without sutures. The grafts were examined macroscopically, daily.

Cutaneous tests

Tests for intradermal delayed reactions to tuberculin, trichophytin, and oidiomycin, as well as DNCB sensitization, were performed as previously described.¹

RESULTS

Lymphocyte transformation stimulated by PHA in cells from blastomycosis patients was reduced as compared to that of the normal control subjects (Table II). Cells from 6 patients showed a depressed response, and 4 responded in a manner similar to that of the control subjects.

As shown in Table III, there was no difference in the degree of transformation when the cells obtained from the blastomycosis patients were cultured in the presence of homologous normal plasma instead of autologous plasma.

TABLE II. Response of peripheral lymphocytes from patients with South American blastomycosis and normal control subjects to PHA

Subjects	Uptake of tritiated thymidine ($\times 10^3$)*	
	Cultures without PHA	Cultures with PHA
Blastomycosis patients		
L. M.	0.7	85
A. P.	0.7	0.8
M. A.	0.5	120
A. M.	0.4	68
S. F. S.	0.8	1.2
B. S. O.	1.1	1.4
E. K.	0.6	6
J. B. R.	0.9	10
M. P. M.	0.9	0.9
A. B.	0.5	64
Normal controls		
C1	0.7	90
C2	0.5	66
C3	0.9	117
C4	0.7	60
C5	0.5	83
C6	0.9	72
C7	0.6	57
C8	0.5	123
C9	1.0	94
C10	0.7	81

*Average of triplicate determinations given as counts per minute.

As can be seen in Table IV, there was generally a good correlation between the in vitro response to PHA, intradermal testing, and DNCB sensitivity. Prolonged allograft survival was observed in only 2 patients. However, there were certain differences in the response of some of the patients when studied by the various techniques. Patient L. M. with a cutaneous-pulmonary form, Patient A. M. with a pulmonary form, and Patient A. B. with a cutaneous form responded normally to all the stimuli employed. Patient M. A. with a lymphatic-visceral form responded normally to PHA, rejected a skin allograft in a normal time, was positive to one of the intradermal tests, but could not be sensitized to DNCB. Patients E. K. and J. B. R. with lymphatic forms showed low levels of lymphocyte stimulation, rejected the skin allograft normally, and failed to respond to the intradermal tests and DNCB sensitization. Patients S. F. S., with a lymphatic form, and B. S. O., with an adrenal pulmonary form of the infection, did not respond to DNCB and gave positive skin tests only to trichophytin. Skin allograft survival was not studied in these two patients, since they declined to submit to these tests. Finally, Patients A. P. and M. P. M. with lymphatic forms showed impaired responses to all the tests employed.

DISCUSSION

The in vitro blastogenic response of human peripheral lymphocytes to PHA stimulation has been studied by several authors^{19, 27} as a possible measure of the capacity to manifest delayed hypersensitivity reactions in vivo. The correlation between the response to PHA and that to other tests of immunological

TABLE III. Response to PHA of peripheral lymphocytes from patients with South American blastomycosis cultured in the presence of autologous or normal homologous plasma

Blastomycosis patients	Uptake of tritiated thymidine ($\times 10^3$)*	
	Medium with autologous plasma	Medium with homologous plasma
M. A.	120	105
A. B.	64	62
J. B. R.	10	18
E. K.	6	6.3
M. P. M.	0.9	1.5
A. P.	0.8	0.8

*Average of triplicate determinations given as counts per minute.

TABLE IV. Comparison between the in vitro response to PHA, intradermal antigen tests, sensitivity to DNCB, and allograft survival

Blastomycosis patients	Response to PHA	Response to intradermal antigens			Sensitivity to DNCB	Allograft survival (days)
		Tuberculin	Trichophytin	Oidiomycin		
L. M.	P	P	P	N	P	8
A. P.	N	N	N	N	N	17
M. A.	P	N	P	N	N	7
A. M.	P	N	P	N	P	7
S. F. S.	N	N	P	N	N	Not done
B. S. O.	N	N	N	N	N	Not done
E. K.	D	N	N	N	N	8
J. B. R.	D	N	N	N	N	7
M. P. M.	N	N	N	N	N	+ 60
A. B.	P	N	P	P	P	Not done

P = positive; N = negative; D = depressed.

function has been variable in the diseases studied.^{18, 21} However, it seems that PHA is a more powerful stimulus than the antigens used for delayed intradermal reactions and DNCB sensitization.^{18, 20}

The 10 patients with South American blastomycosis reported here presented varying degrees of impairment of delayed hypersensitivity. Three patients reacted normally to all the tests employed, 2 presented impaired responses to all test stimuli, and the others showed intermediate behavior. In South American blastomycosis, as in Hodgkin's disease,^{5, 19} the ability to respond to various stimuli appears to be quantitative, so that a powerful stimulus could evoke a response in patients unreactive to weaker stimuli.

In the blastomycosis patients studied, a rank of reactivity can be established. The first response to be lost is probably the delayed cutaneous reaction to intradermal tests and the ability to become sensitized to DNCB. Patients showing this kind of impairment may still manifest a normal response to PHA and can reject skin allografts normally. Patients with depressed but not abolished responses to PHA may still reject skin allografts normally. These observations suggest that the failure of peripheral lymphocytes to respond to PHA in vitro is followed by a decreased capacity to reject skin allografts. Rejection of skin allografts is probably the most difficult manifestation of delayed hypersensitivity to suppress. Indeed, in the 2 patients in whom an increased survival time of

skin allografts was observed, there was no response to the skin tests and a complete absence of stimulation of the peripheral lymphocytes by PHA in vitro. In similar studies on patients with Hodgkin's disease, the failure to obtain positive delayed skin test reactions is not always accompanied by prolonged survival of skin allografts.⁴

An important theoretic concept is whether the involvement of the delayed hypersensitivity mechanisms precedes the disease or whether it is a consequence of it. In sarcoidosis and Hodgkin's disease there are some evidences that the immunologic defect occurs as a consequence of the disease.^{2, 4, 6, 28-30} In these patients there is an oscillation of immunologic reactions according to the clinical stage of the disease, i.e., patients in remission respond to antigenic stimuli in a normal way.

In South American blastomycosis, this question is not yet resolved. The humoral response against the etiologic agent seems to be normal. Circulating antibodies can be detected by complement fixation and precipitation,³¹ but they do not seem to provide protection. On the contrary, it has been observed that a decrease in antibody titers may coincide with clinical improvement.³¹

An attempt to establish a relationship between the clinical form of the disease and the impairment of the cellular immunity showed that the patients affected with the lymphatic form of the disease had a more severe involvement of cellular immunity. However, larger numbers of patients must be studied before reliable generalizations can be made.

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