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Effects of a Single Histoplasmin Skin Test on the Serological Diagnosis of Histoplasmosis

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Numerous reports have indicated that a single histoplasmin skin test may stimulate humoral antibodies to *Histoplasma capsulatum* antigens in histoplasmin-hypersensitive individuals. Although these investigations concur that antibody elevations are evoked, they vary in the reported degree of incidence and response induced, and they cast doubt on the interpretation of serological tests in the diagnosis of histoplasmosis. Histoplasmin-hypersensitive subjects (114) were bled prior to administration of the skin test, 2 days later, at the time this test was read, and 15 and 30 days after testing. No significant antibody titers were observed at 2 days. At 15- and 30-day intervals, only 17 (15%) of the subjects demonstrated circulating antibodies. All 17 showed agar gel bands; 5 demonstrated no complement-fixation (CF) titers, 10 produced CF antibodies ranging from 1:8 to 1:16, and 2 demonstrated titers of 1:32. The data suggest that skin testing does not interfere significantly with antibody levels in sera drawn approximately 2 days after administration of antigen. However, since titers as high as 1:32 were obtained at later intervals, such reactions should be evaluated cautiously and only after consideration of clinical findings.

The serological procedures most widely used for the presumptive diagnosis of histoplasmosis are the complement-fixation (CF) and agar gel precipitin tests. The soluble mycelial antigen, histoplasmin, is used in all these procedures, but whole yeast-form cells are also used as a second antigen in the CF test. Use of both these antigens in the CF test provides maximal diagnostic coverage, since sera from culturally proven cases of histoplasmosis may react to only one of the antigens (3). A CF titer of 1:8 or greater with either the histoplasmin or yeast-form antigen or the demonstration of one or more precipitin lines is generally considered presumptive evidence of histoplasmosis. Such evidence is often responsible for increased efforts in isolation of the fungus and, especially where serial specimens have been collected, in enabling differentiation of histoplasmosis from other pulmonary diseases or in providing prognostic data.

Since 1959, studies carried out by numerous investigators (4) have demonstrated that CF antibodies or precipitins to *Histoplasma capsulatum* antigens may be induced in histoplasmin-sensitive, but serologically negative, individuals after a single histoplasmin skin test. We do not question the occurrence of these reactions. However, review of the literature indicates that there is considerable variation in results concerning the incidence of reaction to histoplasmin and the level of the histoplasmin antibody titer induced.

The published reports (1, 2, 5-7, 9) reveal that from 3 to 58% of the hypersensitive subjects studied by various investigators responded to a single histoplasmin skin test with antibody titers ranging from 1:8 to as high as 1:256 (Table 1). The titers and frequency of these reactions have led some to believe that the skin test-induced reactions nullify the diagnostic value of CF or other serological tests. The purpose of this study was to evaluate carefully the effects of a single skin test with histoplasmin on the subsequent serology of individuals whose sera were titered by the CF test and were qualitatively analyzed by the agar gel precipitin test. The procedures and reagents used in this study are those used by the majority of public health laboratories.

MATERIALS AND METHODS

Human sera. Sera from 139 patients at the Veterans Administration Hospital, Nashville, Tennessee, were studied. None of these patients suffered from pulmonary disease, and none had knowledge of having been skin-tested with histoplasmin within the last five years. All lived in an area endemic for histoplasmosis.

TABLE 1.
Summary of reports showing the influence of a single positive skin test on serology[a]

Report	No. skin tested	No. positive	CF reactions, no. (%)		Maximal titer	Precipitins, no. (%)		
			Histoplasmin	Yeast		Unidentified	M	M and H or H
Heiner (2)	51	21					19 (90)	2 (10)
McDearman and Young (6)	40	29	1 (3)	4 (14)	1:32			
Nicholas et al. (7)	50	23	4 (17)		1:16			
Sigrest et al. (9)	28	14	1 (7)	0	1:8	4 (29)		
Campbell and Hill (1)	50	12	7 (58)	0	1:256	9 (75)		
Klite (5)	89	32	7 (22)	0	1:16		11 (34)	
Kaufman et al.[b]								
H-42	139	114	10 (9)	2 (2)	1:32		14 (12)	3 (3)[c]
PD	26	19	2 (11)	1 (5)	1:16		4 (21)	3 (15)

[a] All subjects were negative for CF antibodies (<1:8) and precipitins prior to skin testing.
[b] H-42 = National Communicable Disease Center histoplasmin. PD = Parke, Davis & Co. histoplasmin.
[c] One of three showed H band only.

Skin tests. The patients were skin-tested and examined by one examiner (R. T.). A 0.1-ml amount of 1:100 diluted histoplasmin was injected intradermally into the volar surface of the left forearm, 4 to 6 inches (10.2 to 15.2 cm) below the elbow. Disposable needles and syringes were used. The tests were read in 48 hr, and measurement of the largest diameter of induration was recorded. A test was considered positive when the induration measured 5 mm or more. The histoplasmin skin test antigens used were: 1:100 diluted National Communicable Disease Center (NCDC) antigen lot H-42 and 1:100 diluted Parke, Davis & Co. antigen lot Cl 521. The former was used in the major portion of the study, whereas, the latter was applied to a smaller group of subjects.

Bleeding procedure. Each subject was bled on four different occasions. The first bleeding was performed just prior to administration of the skin test (zero-time). The second was taken 2 days later, immediately after reading the results of the skin test. The third and fourth blood samples were taken 15 and 30 days, respectively, after the initial bleeding. Every fifth patient served as a control. He was not skin-tested and had blood drawn only at the aforementioned time intervals. All sera were preserved with Merthiolate (1:10,000) and forwarded to the Fungus Immunology Unit of the NCDC. All sera were stored at −20 C before and after testing. Each serum was tested for CF antibodies, starting with the undiluted serum rather than the usual 1:8 dilution used in other published studies. In addition, each serum was checked for H and M precipitins by the agar gel diffusion test.

CF tests. Sera were titrated by the Laboratory Branch Complement Fixation (LBCF) micro test (10). This test involves five 50% units of complement in 0.05 ml in a total volume of 0.125 ml. The antigen-antibody-complement mixture is incubated for 15 to 18 hr at 4 C. The antigens used were histoplasmin NCDC lots 21, 22, and 23 (optimal dilution 1:8) and intact yeast-like cells, NCDC lots 31 and 32 (optimal dilutions, 1:32 and 1:64, respectively). Sera demonstrating 30% hemolysis or less at a particular dilution were considered positive.

Immunodiffusion tests. Agar gel double diffusion tests were carried out as described by Ouchterlony (8). The test medium consisted of 1.0% Difco Special Agar (Noble), 0.9% sodium chloride, 0.4% sodium citrate, 0.25% phenol, and 7.5% glycine. The final *p*H was 6.7. A 7-ml amount of the above agar was pipetted onto 2 by 3 slides previously coated with 0.2% agar. Patterns consisting of six outer wells, 4 mm from each other and 4 mm from a center well, were cut into the agar. Each well was 3 mm in diameter. Antigens were 5× concentrated histoplasmin NCDC lot H-2, deficient in the M but not the H antigen, and 5× concentrated histoplasmin NCDC lot 2, containing large quantities of both the H and M antigens. The antigenic composition of these mycelial culture filtrates was checked with positive sera from patients with culturally proven histoplasmosis and verified by D. C. Heiner (University of Utah, Salt Lake City). The control histoplasmosis serum was NCDC lot 5736 containing H and M antibodies. The slides with charged wells were incubated at 25 C in a moist chamber and examined daily for 1 week.

RESULTS

Tables 2 and 3 summarize the serological data accumulated on 139 subjects receiving a single skin test with histoplasmin H-42. Of these 139 subjects, 114 were hypersensitive and 25 were skin test negative. Only 17 of the 114 (15%) hypersensitive subjects showed antibody responses after receiving the single skin test.

TABLE 2.

CF titers in sera from 139 individuals prior to and after a single skin test with H-42 histoplasmin

Subject no.	Skin test (mm)	CF titers[a] at indicated no. of days after skin test								Maximal diagnostic CF titer
		0		2		15		30		
		H	Y	H	Y	H	Y	H	Y	
1	28	2	1	2	1	8	1	8	2	8
2	14	0	0	0	0	0	0	0	0	0
3	15	0	0	0	0	2	0	2	0	0
4	20	0	0	0	0	8	4	4	0	8
5	20	0	0	2	2	8	4	8	4	8
6	8	2	4	2	2	2	8	2	4	8Y
7	15	0	0	1	2	8	2	8	0	8
8	15	0	4	0	4	4	8	2	8	8Y
9	17	0	0	0	0	16	0	8	0	16
10	8	0	0	0	0	8	0	8	0	8
11	7	0	0	0	0	2	0	4	0	0
12	15	0	0	0	0	8	0	8	0	8
13	18	0	0	0	0	4	0	4	0	0
14	40	0	0	8	0	32	0	32	0	32
15	12	0	0	0	0	0	0	4	1	0
16	9	4	0	0	0	16	0	16	0	16
17	12	0	0	0	0	32	2	16	2	32
18–114	Positive 5–25	0	0	0	0	0	0	0	0	0
115–139	Negative	0	0	0	0	0	0	0	0	0

[a] Titer expressed as reciprocal of dilution; 1:8 titer or greater considered positive. H = CF titer with histoplasmin, and Y = CF titer with whole yeast antigen.

The distribution of individual antibody responses detected by the CF test is shown in Table 2. Sera from 12 (11%) of the hypersensitive subjects demonstrated CF titers which ranged from 1:8 to 1:32. Of these, 10 (9%) showed CF responses to histoplasmin antigen, and 2 showed 1:8 titers with the yeast-form antigens. The majority of these responses were evident 2 weeks after the skin test. However, in one instance (subject 14), a positive CF reaction was detected as early as 2 days.

Three of the subjects showed low levels of antibodies in their sera prior to skin testing. These levels, however, were below 1:8 and were considered negative. After the skin test, fourfold changes in titer were observed in successive serum specimens from two of these three patients, but none exceeded 1:16.

The sera of the 12 subjects showing CF titers, plus those of 5 additional subjects contained precipitins (Table 3). These precipitins were apparent in the subjects' sera 15 days after the skin test. With the exception of one patient (no. 4), all contained antibody to precipitinogen M in their sera. Two patients demonstrated precipitins to both H and M, and one (no. 4), precipitins to H only.

The data did not reveal any correlation between the size of the area of induration resulting from the skin test and the level of the antibody titer or type of precipitin band demonstrated (Tables 2 and 3). Furthermore, none of the nonsensitive subjects (115 to 139) or the controls showed any humoral antibody response.

To eliminate the possibility that the significantly higher titers observed by Campbell and Hill (1) might be due to the type of skin test antigen used by these workers, we obtained their antigen (Parke, Davis & Co., lot C1-521) and studied the effects of a single skin test with this product on 26 patients. From the data shown in Table 4, it is evident that this antigen did not elicit CF titers significantly higher than those observed after skin testing with the H-42 histoplasmin. Of 19 hypersensitive subjects, 3 (16%) showed antibodies detectable by the CF test. These were noted 2 weeks after the skin test. Of these, two reacted with the histoplasmin antigen with titers of 1:8 and 1:16, and one demonstrated a titer of 1:8 with the yeast-form antigen.

TABLE 3.

Agar gel precipitins in sera from 139 individuals prior to and after a single skin test with histoplasmin H-42

Subject no.	Skin test (mm)	Occurrence of H and/or M precipitins at indicated no. of days after a single skin test								Precipitins detected
		0		2		15		30		
		H	M	H	M	H	M	H	M	
1	28	−	−	−	−	−	+	−	+	M
2	14	−	−	−	−	−	−	−	+	M
3	15	−	−	−	−	−	+	−	+	M
4	20	−	−	−	−	+	−	+	−	H
5	20	−	−	−	−	−	+	−	−	M
6	8	−	−	−	−	−	−	−	+	M
7	15	−	−	−	−	−	+	−	+	M
8	15	−	−	−	−	−	+	−	+	M
9	17	−	−	−	−	+	+	+	+	H, M
10	8	−	−	−	−	−	+	−	+	M
11	7	−	−	−	−	−	−	−	+	M
12	15	−	−	−	−	+	+	+	+	H, M
13	18	−	−	−	−	−	+	−	+	M
14	40	−	−	−	−	−	+	−	+	M
15	12	−	−	−	−	−	+	−	+	M
16	9	−	−	−	−	−	+	−	+	M
17	12	−	−	−	−	−	+	−	+	M
18–114	Positive 5–25	−	−	−	−	−	−	−	−	−
115–139	Negative	−	−	−	−	−	−	−	−	−

TABLE 4.

CF titers in sera from 26 individuals prior to and after a single skin test with PD[a] histoplasmin

Subject no.	Skin test (mm)	CF titers[b] at indicated no. of days after skin test								Maximal diagnostic CF titer
		0		2		15		30		
		H	Y	H	Y	H	Y	H	Y	
1	14	0	0	0	0	0	0	0	8	8Y
2	14	0	0	0	0	16	0	16	0	16
3	25	0	0	0	0	8	0	0	0	8
4–19	7–17	0	0	0	0	0	0	0	0	0
20–26	Negative	0	0	0	0	0	0	0	0	0

[a] Parke, Davis & Co.

[b] Titer expressed as reciprocal of dilution. H = CF titer with histoplasmin, Y = CF titer with whole yeast antigen.

DISCUSSION

It is evident from the results presented here as well as from those presented by others that a single skin test with histoplasmin can induce serum antibody to *H. capsulatum* antigens in hypersensitive individuals (Table 1). Although an earlier report (6) indicated that this induced antibody is principally detected by the yeast CF antigen, it is now generally accepted that this antibody is mainly reactive with the histoplasmin CF antigen. As demonstrated by Sigrest et al. (9), Campbell and Hill (1), Klite (5), and ourselves, the latter response is most frequently detected by the agar gel test rather than by the CF procedure.

Of particular interest were the precipitin reactions elicited by the single skin test with H-42 histoplasmin (Table 3). All of the subjects whose

sera contained CF antibodies (Table 2) also demonstrated precipitins (Table 3). In addition, precipitins were apparent in subjects whose sera were negative for CF antibodies. Of the 17 subjects showing precipitins, 14, as expected, showed only M bands, 2 showed H and M bands, and 1 showed only the H band. Detection of the H band was unexpected since this factor is considered to be diagnostic evidence of active histoplasmosis (11). It is noteworthy that similar H band responses were also observed by us in the several individuals receiving a skin test with the Parke, Davis & Co. histoplasmin. Reactions of this type are rare and have been reported in one instance by Heiner (2), who attributed them to the high antigenicity and concentration of the H antigen in the histoplasmin used in his study. From the data presented, it would appear that the mere presence of an H precipitin in serum is not unequivocal evidence for active histoplasmosis, and that the H precipitin, like the M and CF antibodies can also be elicited by a single skin test.

Contrary to the majority of reports (Table 1), Campbell and Hill (1) reported that 7 of 12 (58%) of the hypersensitive and clinically well subjects studied by them produced CF antibody to histoplasmin with titers as high as 1:256 subsequent to a single histoplasmin skin test. Unfortunately, this report has led many to believe that a high percentage of CF titers on sera from recently skin-tested individuals are induced responses and that CF results are consequently unreliable and no longer of diagnostic value.

Our results (Tables 1 and 2) do not support this contention. Data obtained with the LBCF test indicate a response detectable with the histoplasmin CF antigen in approximately 9% of 114 hypersensitive subjects skin-tested with the H-42 histoplasmin. Based on the 95% confidence interval, 3 to 14% for this sample, it is unlikely that the percentage of reactors in this population exceeds 14%. This percentage of reactors agrees with that noted by Klite (5), Sigrest et al. (9), and Nicholas et al. (7), and is significantly less than the 58% noted by Campbell and Hill (1).

In our study, of the 114 hypersensitive subjects, 8 (7%) converted from negative serology to positive serology with histoplasmin CF titers of 1:8 to 1:16, whereas only 2 (1.7%) showed the maximal titer of 1:32. No titers exceeding 1:32 were observed. These findings are in agreement with those of most other investigators (Table 1). The report of Campbell and Hill (1) describing induced antibody titers as high as 1:256 is exceptional.

The possibility existed that the high titers observed by Campbell and Hill could be attributable to the fact that they used a different skin test antigen than we. To resolve this question, we carried out a limited study with the same lot of antigen (Parke, Davis & Co., lot C1-521) used by them. This study (Tables 1 and 4) revealed that of 19 hypersensitive individuals only 2 (11%) responded to a single skin test with a maximal histoplasmin CF titer of 1:16. Thus, differences observed between our studies and those of Campbell and Hill cannot be attributed solely to the different antigens used.

It appears that within the framework of the serological system used in this study, the administration of a single histoplasmin skin test does not induce changes that materially affect the diagnostic value of the LBCF test. The incidence of reaction to a single skin test with either of the two histoplasmins used in this study was less than 12%. The majority of these responses were in the range of 1:8 to 1:16, with 1.7% of the subjects showing the maximal titer of 1:32. Even before knowledge of the effects of a single skin test was available, the interpretation of titers such as those induced were not considered unequivocal proof of histoplasmosis. For example, although titers of 1:8 to 1:16 are seen in proven cases of histoplasmosis, apparently healthy individuals (7) and individuals suffering from a variety of nonmycotic diseases may show such titers. Even antibody levels of 1:32 cannot be regarded as a sole criterion for diagnosis of histoplasmosis.

It is important to note that the majority of the induced antibody responses were detected only in specimens drawn 15 days after skin testing. In only one case was an antibody response observed at the 48-hr interval, and this was of a low order (1:8). Although it is preferable that blood be drawn for serological studies prior to skin testing, our studies indicate that patients can be bled within 2 or 3 days after being skin-tested, since any antibody induction is usually undetectable at that early date.

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