SPHERULIN AND COCCIDIOIDIN: CROSS-REACTIONS IN DERMAL SENSITIVITY TO HISTOPLASMIN AND PARACOCCIDIOIDIN

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Until recently coccidioidin has been the only antigenic preparation available for detecting delayed dermal sensitivity induced by an experience with Coccidioides immitis. It is prepared from autolysates of the mycelial phase (saprophytic) of the fungus. A more sensitive reagent, spherulin, was developed in 1969 from the spherule phase (parasitic) of the organism. Use of spherulin showed that coccidioidin failed to detect approximately 30% of individuals specifically sensitive to C. immitis. However the potential of spherulin to detect cross-sensitivity induced by Histoplasma capsulatum was unknown. This information was considered to be germane because of the capacity of coccidioidin to detect a histoplasmal experience. Accordingly, both reagents as well as paracoccidioidin were compared simultaneously in 365 Colombian soldiers from areas endemic for histoplasmosis but not for coccidioidomycosis. At standard strength both preparations detected non-specific responses in 0.8% to 3% of the histoplasmin negative and positive subgroups, respectively. At 10-times standard strength both preparations cross-detected histoplasmin sensitivity comparably; 5.1% to 7.1% of histoplasmin-positive subjects reacted with the coccidioidal antigens. No pattern of cross-reactivity was observed between paracoccidioidin sensitivity and sensitivity to the coccidioidal antigens.

coccidioidin; histoplasmin; paracoccidioidin; spherulin

INTRODUCTION

The capacity of coccidioidin (1) to detect delayed dermal sensitivity induced by Histoplasma capsulatum is documented clearly. Smith et al. (2) found that up to 9 per cent of 871 individuals sensitized by H. capsulatum reacted to coccidioidin (1:100 dilution). When the strength of the coccidioidin was increased 10-fold, the reactor rate exceeded 50 per cent. And Furcolow and Nelson (communicated to Smith et al. (2) and Smith (3)) reported a 50 per cent reactor rate to 1:10 coccidioidin in a group of children located in Ohio where histoplasmosis is endemic but not coccidioidomycosis (4). Finally, Tucker’s (5) survey of 1000 persons in Panama, also endemic for histoplasmosis but not for coccidioidomycosis, revealed a 1:100 coccidioidin sensitivity rate of 0.8 per cent. Seven of the eight coccidioidin-sensitive individuals were histoplasmin-positive and the eighth reacted...
equivocally to histoplasmin.

This potential for cross-detection of histoplasmin sensitivity by Coccidioides-derived antigens was of concern in evaluations of spherulin, a new and more sensitive skin testing reagent than coccidioidin (6). Although studies in two human populations (7, 8) did not suggest that there was a problem of cross-reaction, it was pointed out (8) that the data on this aspect were not definitive because the possibility of dual fungal experiences could not be excluded. For this reason, reactivity to spherulin was studied in Colombia, in populations from Coccidioides-free areas where histoplasmosis is prevalent. The results are presented in this paper and include data on skin test findings with coccidioidin and paracoccidioidin.

**Materials and Methods**

*Antigens.* Coccidioidin lot 64D2.5, the lot on repository with the Bureau of Biologicals, was used at standard 1:100 dilution (41.6 μg/0.1 ml dose) and at 10-times the standard strength, the 1:10 dilution (416 μg/0.1 ml dose). This lot of coccidioidin was 1.6-fold less dilute than lot 64D4 used earlier (7, 8) but was from the same stock concentrate. At the doses employed in the present study, it produced reactions comparable in size to those elicited by spherulin, as indicated below.

Spherulin (obtained from Berkeley Biologicals, Berkeley, CA) was employed at standard strength (1.4 μg/0.1 ml dose) and at second strength (14 μg/0.1 ml). The term "second strength" will be used hereafter for coccidioidin or spherulin when used at 10-times the standard concentration. Prior to the study, standard strength spherulin and lot 64D2.5 coccidioidin were found to be equipotent in six sensitive volunteers; the mean axes of induration were, respectively, 27.3 and 27.0 mm. Also, this lot of spherulin produced reactions comparable to 2.8 μg of a less potent lot used earlier in epidemiologic surveys (7, 8).

Histoplasmin from Parke Davis and Co. (Detroit, Michigan) was used at the recommended dilution in a total of 254 volunteers. Because of an inadequate supply of this reagent, histoplasmin prepared by the Center for Disease Control (CDC), Atlanta, Georgia, (lot H-42), was also employed. An additional 111 subjects received this material. Both antigens produced nearly identical reactor ratios in men who had come from a single geographic region of the country; 14/44 (31.8 per cent) with Parke Davis's histoplasmin and 34/111 (30.6 per cent) with CDC reagent. For purposes of this study, the antigens were assumed to be bioequivalent.

Paracoccidioidin was used at a standard dose of 40 μg/0.1 ml. This reagent was derived from the mycelial phase of Paracoccidioides brasiliensis and had been partially purified by ethanol precipitation as described elsewhere (9).

*Study population.* Three hundred and sixty-eight Colombian soldiers, ages 18-25, from two regiments stationed in Medellin, comprised the study group. The men of one regiment came from the State of Caldas and those of the second were from the State of Tolima. Both states are endemic for histoplasmosis (10, 11). Their economies are primarily agricultural (coffee and rice) and the men were almost exclusively from farming families. Three individuals who had visited Colombia's one area known to be endemic for coccidioidomycosis (12), Guajira in the northeast of the country, were excluded from the study.

*Design.* All subjects were tested initially with only the standard strengths of the antigens, using both forearms according to the following scheme: Coccidioidin, upper left; spherulin, upper right; histoplasmin, lower right; paracoccidioidin, lower left. Readings were made at 24 and 48 hours. Induration greater than 5 × 5 mm at either reading was scored as positive. At 48 hours, 99 histoplasmin-positive and 107 histoplasmin-negative individuals were given second strength coccidioidin and spherulin. None of these subjects had reacted to the
standard strength coccidioidal reagents. Reactions were recorded as before at 24 and 48 hours. Chi-square analysis (13) was used to determine the significance of difference between reactor ratios.

**RESULTS**

Figure 1 shows, as has already been well-documented (10) for residents of the Colombian Andean valleys, that the rate of histoplasmin sensitivity was high, approximately 27 per cent. But the overall rates obtained with first strength spherulin and coccidioidin were low and ranged between 0.3 and 0.8 per cent. These values translated into rates of 2 and 3 per cent among histoplasmin reactors compared to rates of 0.4 and 1.1 per cent among histoplasmin-negative subjects. Reactor ratios to the two *Coccidioides*-derived reagents were not significantly different.

However, the second strength reagents clearly cross-reacted in some histoplasmin-sensitive individuals (figure 2). Among 99 such subjects, eight reactors were found and four of these reacted to both antigens, three to spherulin only and one to coccidioidin only. The response rates elicited by

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**Figure 1.** Dermal reactions to standard strength coccidioidin, spherulin, histoplasmin and paracoccidioidin. Differences between groups A and A' significant \( (p < .01) \); differences among groups B, C, B' and C' not significant.

**Figure 2.** Dermal reactions to 10-times standard strength coccidioidin and spherulin among histoplasmin sensitive and nonsensitive individuals. Differences between Groups A and A' and between B and B' significant \( (p < .06 \) to \( < .02 ) \). Differences between groups A and B not significant.
the two reagents, 7.1 per cent and 5.1 per cent, were not significantly different. It was significant however that reactors to both coccidioidal antigens came only from the histoplasmin-positive group; none was found among 107 histoplasmin-negative subjects tested (figure 2).

Of 25 paracoccidioidin reactors, 24 occurred in the histoplasmin-positive group (figure 1) compared to one in the histoplasmin-negative group. Cross-reaction by paracoccidioidin in histoplasmin-sensitive individuals is known, as will be discussed further. An analysis of the 25 paracoccidioidin reactors showed that 22 were non-reactive to both spherulin and coccidioidin, two reacted to both antigens and one reacted to spherulin only.

**DISCUSSION**

Spherulin is derived (6) from in vitro grown *Coccidioides immitis* spherules and coccidioidin is a mycelial product (1). Coccidioidal lesions of man and animal characteristically contain spherules (14) although mycelia may be seen occasionally; the spherule structure appears to be the typically parasitic phase of this dimorphic pathogen and the mycelium is its saprophytic form.

Spherules and mycelia differ immunogenically (15) and their lysates also show immunologic differences: Spherulin elicits dermal sensitivity reactions in much lower doses than coccidioidin and detects about one-third more reactors in areas endemic for *C. immitis* (7, 8). A similarly higher reactor ratio for spherulin was found by skin-testing 47 patients with culturally- or serologically-proven coccidioidomycosis (19). Finally, spherulin is more stimulatory than coccidioidin in the lymphocyte transformation test (16) and detects complement-fixing antibodies in certain patients (17) whose sera show no such antibodies with coccidioidin.

Because the coccidioidin skin test is frequently positive in subjects sensitized by *H. capsulatum*, we considered it particularly important to determine if the more sensitive spherulin reagent similarly cross-reacts in such subjects. Figure 1 shows that at standard strength both reagents detected extremely low cross-reactor ratios. These could not be attributed exclusively to sensitization by *H. capsulatum* because some occurred in the histoplasmin-negative group. At 10-times the standard dose, however, cross-detection of histoplasmin sensitivity was apparent; both antigens cross-reacted comparably in 5-7 per cent of histoplasmin-sensitive subjects only.

The nature of the few nonspecific reactions to the standard dose of the coccidioidal antigens (figure 1) remains unknown. Their incidence corresponds very closely with those reported by Tucker (5) in Panama, and by Robledo et al. (12) in Colombia. Possibly they derive from subclinical exposures to unrelated fungi or their products. Goodman et al. (18) have demonstrated complex cross-reaction profiles among a very wide range of fungi; coccidioidin-sensitivity could be induced in guinea pigs by *Histoplasma* and 13 heterologous genera including common fungi such as *Penicillium* and *Aspergillus*.

The possibility that the nonspecific reactions to standard strength coccidioidin and spherulin were etiologically unrelated to fungal sensitivity must also be entertained. Second strength spherulin and coccidioidin elicited no such reactions (figure 2) among nonreactors to the lower doses except when the subjects were histoplasmin-sensitive.

The low cross-reactor ratio by 1:10 coccidioidin in histoplasmin-positive subjects (figure 2) requires further discussion because it differed markedly from the 56 per cent cross-reactor rate reported by Smith et al. (2). The discrepancy may reflect lot differences in coccidioidin. Smith et al. used lot 9 which, at the 1:100 dilution, gave a cross-reactor rate of 9 per cent. Smith's lot 29-31 was used by Tucker (5) who showed a cross-reactor rate of 0.8 per cent.
In the present study Smith's lot 64D2.5 was used and at the 1:100 dilution (figure 1) gave a rate similar to Tucker's. Lot 64 stock concentrate had been diluted 2.5-fold by Smith (20) because it was very potent. After further 1:10 dilution to produce the second strength reagent, it may therefore have contained considerably less total solids than lot 9 which cross-reacted so extensively at the 1:10 dilution.

We were not able to determine if an experience with Paracoccidioides brasiliensis per se might induce cross-reactions with spherulin or coccidioidin. In the study, 22 of 25 paracoccidioidin-positive subjects were negative to the coccidioidal antigens. But the source of their paracoccidioidin sensitivity was not clear; 24 of them were concurrently sensitive to histoplasmin and in some the paracoccidioidin response probably resulted from an experience with H. capsulatum. Two observations support this probability: Among 65 volunteers in the United States (nonendemic for paracoccidioidomycosis), there were 13 reactors to paracoccidioidin (9) among whom 12 were concurrently sensitive to histoplasmin. Secondly, among 3988 subjects studied in Colombia (10) cross-reactivity was evident; patterns of sensitivity and endemicity indicated that there was approximately 6 per cent sensitization by P. brasiliensis and 20 per cent by H. capsulatum.

References
20. Smith CE, personal communication, 1965