INFECTION AND IMMUNITY, Sept. 1979, p. 932-938
0019-9567/79/09-0932/07$02.00/00

Vol. 25, No. 3

Cross-Reactivity Between Antigens of *Coccidioides immitis*, *Histoplasma capsulatum*, and *Blastomyces dermatitidis* in Lymphocyte Transformation Assays

REBECCA A. COX

Department of Research Immunology, San Antonio State Chest Hospital, San Antonio, Texas 78223

Received for publication 9 April 1979

The cross-reactivity of four *Coccidioides* antigens, three *Histoplasma* antigens, and two *Blastomyces* antigens were determined in lymphocyte transformation assays of 11 coccidioidin-reactive, histoplasmin-nonreactive subjects (group I), 13 coccidioidin-nonreactive, histoplasmin-reactive persons (group II), and 13 subjects who were skin test negative to both antigens (group III). Mycelial and yeast (or spherule)-phase antigens of the three fungi were included. Significant cross-reactivity was obtained with both coccidioidins, spherulin, and the alkali-soluble, water-soluble cell wall antigen of *C. immitis*, to the extent that the responses of histoplasmin-reactive persons were not statistically different ($P > 0.05$) from those of coccidioidin-reactive persons. In contrast, optimal dilutions of *Histoplasma* mycelial and yeast-phase lysates effectively distinguished ($P < 0.01$) responses of histoplasmin- and coccidioidin-reactive persons. The alkali-soluble cell wall antigen of *H. capsulatum* showed extensive cross-reactivity at most concentrations and was markedly stimulatory to lymphocytes of skin test-negative persons. Blastomycin elicited significant cross-reactions in histoplasmin-sensitive subjects and to a lesser extent in coccidioidin-sensitive subjects. The alkali-soluble cell wall antigen cross-reacted in cultures of histoplasmin-reactive persons but not in those of coccidioidin-reactive persons. All antigens effectively distinguished ($P < 0.001$) homologous responses of skin test-positive persons (groups I and II) from those of skin test-negative persons (group III). The extensive cross-reactivity in lymphocyte transformation assays in the absence of cross-reactivity in skin tests suggests that these two immune responses may be mediated by different T lymphocyte populations, may be elicited by different antigenic components, or both.

Antigens presently available for immunological studies of coccidioidomycosis, histoplasmosis, and blastomycosis are heterogeneous mixtures containing common antigenic components (10, 13, 18, 21, 22, 30, 31). Accordingly, extensive cross-reactivity occurs between antigens of *Coccidioides immitis*, *Histoplasma capsulatum*, and *Blastomyces dermatitidis* (3-5, 7, 29). Cross-reactivity is most extensive between *Histoplasma* and *Blastomyces*, to a lesser extent between *Histoplasma* and *Coccidioides*, and to an even lesser extent between *Blastomyces* and *Coccidioides*. It is not uncommon that the reaction to the heterologous antigen is stronger than that obtained with the homologous antigen. This is especially true in serological assays (5).

The lymphocyte transformation (LT) assay is commonly used to measure T (thymus derived) lymphocyte function in fungal diseases (1, 6, 11, 16, 20, 26, 27). Briefly, this assay consists of culturing lymphocytes in vitro with antigen for 5 to 7 days during which sensitized T cells recognize and respond to specific antigens by undergoing an increased mitotic division, as measured by radioisotope uptake. Previous reports have suggested that LT assays may prove useful in the diagnosis of fungal diseases (15, 23). The potential value of LT as a diagnostic tool will depend, in part, upon the specificity of responses obtained with fungal antigens. The present study was, therefore, undertaken to assess the extent of cross-reactivity between antigens of *C. immitis*, *H. capsulatum*, and *B. dermatitidis* in LT assays of healthy subjects with known skin test reactivity.

MATERIALS AND METHODS

Study groups. Thirty-seven healthy subjects were divided into three groups on the basis of their skin test reactivity to coccidioidin (1:100, Cutter Labs, Berkeley, Calif.) and histoplasmin (1:100, Parke-Davis, Dallas, Tex.). Group I contained 11 subjects who reacted

(>5 mm of induration) to coccidioidin but not to histoplasmin. Group II contained 13 subjects who were skin test negative to coccidioidin but positive to histoplasmin. Group III contained 13 persons who were nonreactive to both coccidioidin and histoplasmin. The mean skin test responses of the three study groups are summarized in Table 1.

Antigens. Antigens from the saprophytic and parasitic (tissue) phases of *C. immitis*, *H. capsulatum*, and *B. dermatitidis* differ qualitatively and quantitatively (10, 18, 19). For this reason, LT assays were performed with antigens derived from mycelial and yeast (or spherule) phases of the three fungi. Antigens from *Coccidioides* included: coccidioidin TS, a toluene-induced lysate of mycelial-phase cells of *C. immitis* Silveira (28), obtained from Demosthenes Pappagianis, University of California, Davis; coccidioidin XV-77, a mycelial-phase lysate concentrated by ultrafiltration (19), obtained from Milton Huppert, Veterans Administration Hospital, San Antonio, Tex.; spherulin, an aqueous extract of the spherule/endospore phase of strain Silveira obtained from Berkeley Biologicals, Berkeley, Calif. (lot 4A); and C-ASWS, an alkali-soluble, water-soluble cell wall extract of mycelia (32). *Histoplasma* antigens included: histoplasmin H-42, a mycelial-phase autolysate obtained from the Center for Disease Control, Atlanta, Ga.; histoplasmin YP, a yeast-phase lysate obtained from Marilyn Sutcliffe, Veterans Administration Hospital, Nashville, Tenn.; and H-ASWS, the alkali-soluble cell wall antigen obtained from yeast cells. *Blastomyces* antigens included: blastomycin KCB-26, an autolysate of mycelia obtained from Coy Smith, University of Kentucky, Lexington; and B-ASWS, an alkali-soluble, water-soluble extract of yeast-phase cells (9).

Streptokinase-streptodornase (SK-SD, American Cyanamid Co., Lederle Laboratories Div., Pearl River, N.Y.) and *Candida* antigen, obtained from M. Sutcliff, were included in LT assays to compare the responses of the three study groups to antigens unrelated to *Coccidioides*, *Histoplasma*, and *Blastomyces*. Mitogens phytohemagglutinin (PHA, Difco, Detroit, Mich.) and concanavalin A (Con*A*., Sigma Chemical Co., St. Louis, Mo.) were included for the same reason.

Before LT assays, antigens were dialyzed at 4°C against a 40-fold volume of sterile, distilled water to remove preservatives. The nondialyzable antigens were lyophilized and reconstituted back to their original volume in tissue culture medium. Since the availability of the antigens was limited, no attempts were made to determine the amount (as dry weight) of nondialyzable material present. Rather, we chose to refer to the reconstituted antigens as undiluted stocks from which the indicated dilutions were made. The exceptions to this were the ASWS preparations which were quantitated on a dry-weight basis. The protein content of each antigen, determined by the method of Lowry et al. (25), was as follows: coccidioidin TS, 0.64 mg/ml; coccidioidin XV-77, 32.8 mg/ml; spherulin, 0.72 mg/ml; C-ASWS, 0.32 mg/ml; histoplasmin H-42, 1.12 mg/ml; histoplasmin YP, 0.20 mg/ml; H-ASWS, 0.73 mg/ml; blastomycin KCB-26, 0.83 mg/ml; and B-ASWS, 0.71 mg/ml.

TABLE 1. *Skin test responses of groups I, II, and III to Coccidioidin and Histoplasmin*

Group	No. of subjects	Mean skin test responses[a]			
		Coccidioidin		Histoplasmin	
		24 h	48 h	24 h	48 h
I	11	12.0 (0–24)[b]	19.7 (10–45)	0	0
II	13	0	0	19.7 (0–36)	27.4 (13–50)
III	13	0	0	0	0

[a] Induration (millimeters) at 24 and 48 h after intradermal inoculation of coccidioidin 1:100 and histoplasmin 1:100.
[b] Range of induration responses.

LT assays. Sixty milliliters of heparinized blood (20 U/ml) was collected in sterile Vacutainer tubes. The blood was carefully layered over Ficoll-Hypaque and centrifuged at 450 × *g* for 40 min as described by Boyum (2). The mononuclear cells (interface layers) were pooled and washed twice in Hanks balanced salt solution and once in tissue culture medium 199 (TC199, Grand Island Biological Co., Grand Island, N.Y.). Lymphocytes were counted in a Neubauer hemacytometer and suspended to a concentration of 2 × 10^6 per ml of TC199 supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), glutamine (2 mM), and 30% plasma pooled from healthy subjects. One-tenth milliliter of the cell suspensions was dispensed into a series of microtiter wells (Microtest II, Falcon Products, Cockeysville, Md.) containing 0.1 ml of the indicated antigen dilution or, for controls, 0.1 ml of TC199. Antigens and controls were assayed in quadruplicate. The cultures were incubated at 37°C under a 5% CO_2-humidified atmosphere for 5 days. PHA and ConA were added to appropriate wells on day 2 to obtain optimal mitogen responses (72-h cultures). The cultures were pulsed with 0.5 μCi of [^{3}H]thymidine (2 Ci/mmol, Amersham/Searle, Amersham, England) and harvested 5 h later on glass fiber disks using a MASH II harvester (Microbiological Associates, Bethesda, Md.). The disks were transferred to scintillation vials containing 3 ml of a toluene-based scintillation fluid and counted for radioactivity. LT responses were calculated as total counts per minute.

Statistical analysis. Because of the heterogeneity of the variances, the data were analyzed by the Mann-Whitney rank-sums test (17).

RESULTS

LT responses to mitogens, SK-SD, and *Candida*. LT responses of the three study groups were comparable to mitogens PHA and ConA and to antigens SK-SD and *Candida* (Fig. 1) with the exception of PHA responses ($P < 0.05$) in group II (histoplasmin reactive) when compared with group III (skin test negative) subjects.

LT responses to *Coccidioides* antigens. Transformation responses to the four *Coccidioides* antigens are depicted in Fig. 2 to 5. Six to seven dilutions of each antigen were routinely included in each assay to assess the optimal

concentration in terms of sensitivity (responses of coccidioidin-reactive subjects) and specificity (responses of histoplasmin-reactive and skin test-nonreactive subjects). Coccidioidin TS (Fig. 2) and coccidioidin XV-77 (Fig. 3) were similar in their biological activities, i.e., both showed extensive cross-reactivity in histoplasmin-sensitive persons with the exception of coccidioidin TS at a dilution of 1:500. LT responses to spherulin (Fig. 4) indicated this antigen to be more sensitive, in terms of the magnitude of responses in coccidioidin-reactive persons, but without specificity in histoplasmin-reactive persons. A slight stimulatory effect was observed to spherulin 1:10 as evidenced by the response (1,800 cpm) of skin test-negative persons. LT responses to C-ASWS (Fig. 5) were similar to those obtained with spherulin except that a 1-μg level of the cell wall antigen showed some degree ($P < 0.05$) of specificity in assays of histoplasmin-sensitive subjects. The nonspecific stimulatory effect of the ASWS antigen is consistent with previous studies (8, 12).

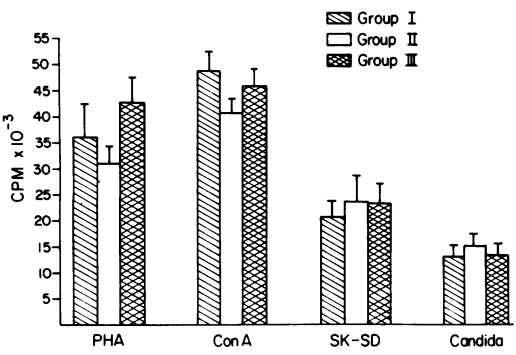

FIG. 1. *LT responses of subjects in group I (coccidioidin reactive), group II (histoplasmin reactive), and group III (skin test negative) to PHA (1:200), ConA (10 μg), SK-SD (50 U), and Candida (1:20).*

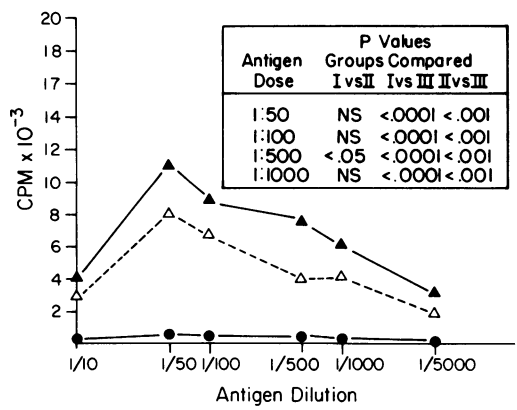

FIG. 2. *LT responses of coccidioidin skin test-positive subjects (▲), histoplasmin skin test-positive subjects (△), and skin test-negative persons (●) to coccidioidin TS. The antigen dilution denotes the dilution added (in 0.1-ml volume) to 2 × 10^5 lymphocytes in 0.1 ml of TC199 with 15% plasma.*

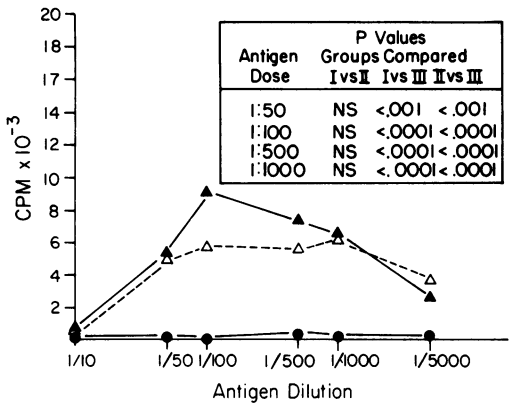

FIG. 3. *LT responses of coccidioidin skin test-positive subjects (▲), histoplasmin skin test-positive subjects (△), and skin test-negative persons (●) to coccidioidin XV-77.*

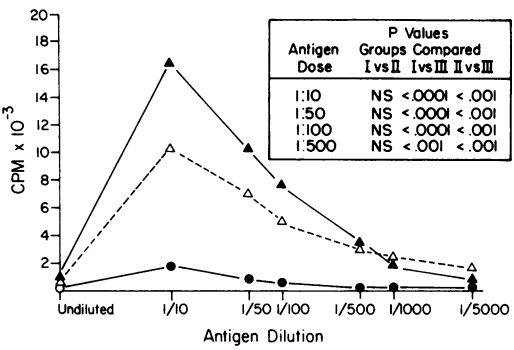

FIG. 4. *LT responses of coccidioidin skin test-positive subjects (▲), histoplasmin skin test-positive subjects (△), and skin test-negative persons (●) to spherulin.*

LT responses to *Histoplasma* antigens. The results obtained with three *Histoplasma* antigens are shown in Fig. 6 to 8. Cross-reactivity with histoplasmin H-42 (Fig. 6) and YP (Fig. 7) occurred in coccidioidin-reactive subjects but was significantly lower than the cross-reactivity observed with *Coccidioides* antigens. Histoplasmin YP appeared to be somewhat less cross-reactive in coccidioidin-sensitive persons since the responses of groups I (coccidioidin reactive) and III (skin test negative) were comparable ($P > 0.05$) at dilutions of 1:10, 1:50, and 1:500. Transformation responses to H-ASWS are

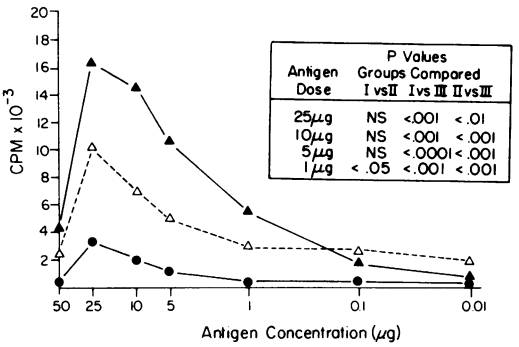

FIG. 5. *LT responses of coccidioidin skin test-positive subjects (▲), histoplasmin skin test-positive subjects (△), and skin test-negative persons (●) to C-ASWS.*

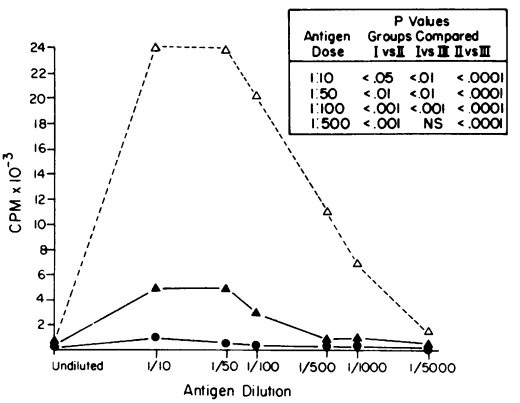

FIG. 6. *LT responses of coccidioidin skin test-positive subjects (▲), histoplasmin skin test-positive subjects (△), and skin test-negative persons (●) to histoplasmin H-42.*

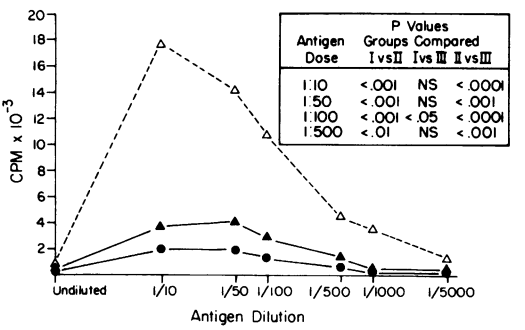

FIG. 7. *LT responses of coccidioidin skin test-positive subjects (▲), histoplasmin skin test-positive subjects (△), and skin test-negative persons (●) to histoplasmin YP.*

shown in Fig. 8. With the exception of the 1-μg dose, responses of histoplasmin-reactive subjects were not significantly different from those of coccidioidin-reactive persons. The nonspecific stimulatory effect of H-ASWS exceeded that of all other antigens studied.

LT responses to *Blastomyces* antigens. Blastomycin KCB-26 (Fig. 9) elicited significant reactions in histoplasmin-sensitive and in coccidioidin-sensitive persons. Essentially no response was obtained in lymphocytes of skin test-negative subjects. Unfortunately, we cannot rule out the possibility of dual sensitivity since an antigen is not currently available for assessing skin test reactivity to *Blastomyces*. However, consistently the responses of histoplasmin-reactive persons were greater, in terms of total counts per minute, to *H. capsulatum* antigen than to *Blastomyces* antigens. The same was true for coccidioidin-reactive persons. It is also pertinent to point out that, without exception, responses of the 13 histoplasmin-reactive subjects were greater to blastomycin KCB-26 than to any of the four *Coccidioides* antigens, a finding that suggests cross-reactivity in LT assays is

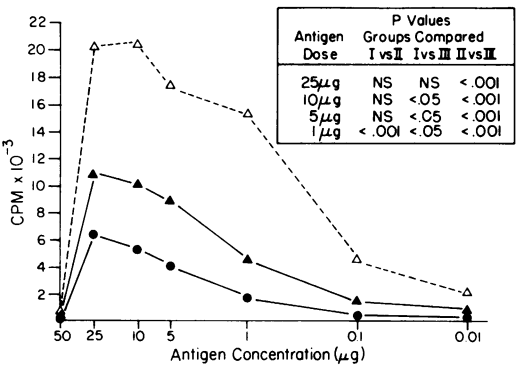

FIG. 8. *LT responses of coccidioidin skin test-positive subjects (▲), histoplasmin skin test-positive subjects (△), and skin test-negative persons (●) to H-ASWS.*

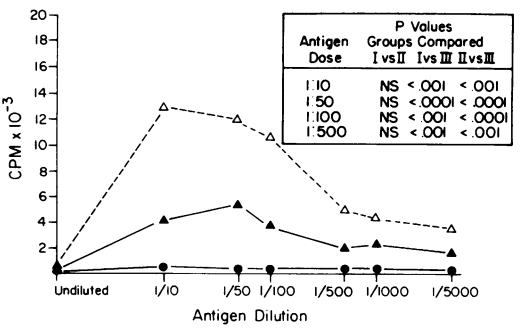

FIG. 9. *LT responses of coccidioidin skin test-positive subjects (▲), histoplasmin skin test-positive subjects (△), and skin test-negative persons (●) to blastomycin KCB-26.*

more pronounced between *Histoplasma* and *Blastomyces* than between *Histoplasma* and *Coccidioides*. In contrast to blastomycin KCB-26, B-ASWS (Fig. 10) did not elicit cross-reactions in coccidioidin-reactive subjects. Significant cross-reactivity was, however, observed in histoplasmin-sensitive persons.

DISCUSSION

Previous studies have documented extensive cross-reactivity between antigens of *C. immitis*, *H. capsulatum*, and *B. dermatitidis* in skin tests and in serological assays (3–5, 7, 29). The results of the present study extend their cross-reactivity to include LT assays. Studies with four *Coccidioides*, three *Histoplasma*, and two *Blastomyces* antigens showed that (i) reactions of healthy, coccidioidin-sensitive persons were significant but not statistically different from the responses of healthy, histoplasmin-sensitive persons with the exception of coccidioidin TS (1:500) and C-ASWS (1 μg); (ii) histoplasmin-reactive subjects responded strongly to histoplasmin H-42 and YP, and their responses were statistically greater than the cross-reactions obtained in coccidioidin-sensitive persons; and (iii) blastomycin KCB-26 elicited significant cross-reactions in histoplasmin-reactive subjects and to a lesser extent in coccidioidin-reactive subjects, whereas B-ASWS showed cross-reactivity with histoplasmin-reactive subjects only. All antigens effectively distinguished responses between skin test-reactive (groups I and II) and nonreactive (group III) subjects. These results are similar to the patterns of cross-reactivity obtained with antigens of *Coccidioides*, *Histoplasma*, and *Blastomyces* in skin tests and in serological assays.

Quantitative and qualitative differences have been reported in studies of antigens from the mycelia and yeast (or spherule) phases of these three dimorphic fungi (10, 18, 19). Recently, Huppert et al. (19) demonstrated 26 protein-containing bands in coccidioidin (XV) using two-dimensional immunoelectrophoresis with anti-coccidioidin. With this reference system, Huppert et al. (18) showed that spherulin contained 10 immune precipitates in common with coccidioidin, C-ASWS contained 8, histoplasmin H-42 and H-ASWS contained 9 and 6, respectively, while blastomycin KCB-26 and B-ASWS contained, respectively, 12 and 5 protein-containing bands that precipitated with anti-coccidioidin. Five immunoprecipitates were common to all antigens, though differences were observed in the relative amounts in each antigen. The presence of common antigenic components among these fungi no doubt contributes to the cross-reactivity observed in immunological assays. Until physiochemical, immunochemical procedures, or both are employed to fractionate antigens of *Coccidioides*, *Histoplasma*, and *Blastomyces*, cross-reactivity will continue to diminish the efficacy of these antigens.

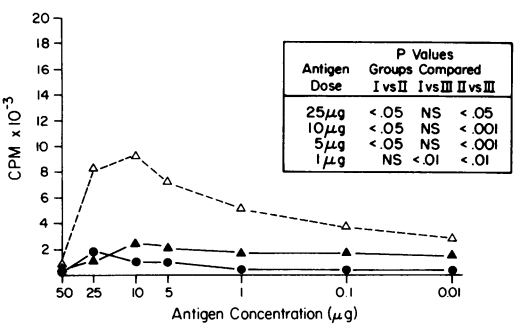

FIG. 10. *LT responses of coccidioidin skin test-positive subjects (▲), histoplasmin skin test-positive subjects (△), and skin test-negative persons (●) to B-ASWS.*

Both yeast-phase and mycelial-phase extracts of the three dimorphic fungi were included in LT assays. Statistically, we were unable to demonstrate any significant differences between the two phases in regard to their sensitivity and specificity. It seems reasonable to assume then that antigens from the mycelial phase and those from the yeast (or spherule) phase both contain the component(s) responsible for LT responses in homologously and heterologously sensitized persons. Whether or not the component(s) that elicits LT responses in homologously sensitized persons is identical to or dissociable from that which elicits cross-reactions is not yet known and awaits fractionation studies.

In previous studies directed towards isolating a more chemically defined antigen from *Blastomyces*, we reported on the efficacy of the ASWS cell wall extract of yeast-phase cells in skin tests, in assays for macrophage inhibitory factor, and in LT assays in the guinea pig model (9, 14). Throughout those studies B-ASWS proved to be more sensitive and specific than blastomycin antigens. In subsequent experiments, the ASWS cell wall fraction of mycelial-phase cells of *C. immitis* was examined for biological activity (8, 11, 12, 32). Comparative studies of C-ASWS with several different coccidioidins and with spherulin showed C-ASWS to be highly sensitive in eliciting delayed-type hypersensitivity responses in sensitized guinea pigs and in infected mice (32). LT and indirect macrophage inhibitory factor assays were limited to healthy, coccidioidin skin test-positive persons,

patients with coccidioidomycosis, and healthy, skin test-negative subjects (8, 11, 12). Responses to C-ASWS were comparable to or better than those obtained with coccidioidin TS and spherulin with the limitation that C-ASWS had a nonspecific stimulatory effect upon LT in healthy, skin test-negative persons. Previous studies with the ASWS cell wall fraction of *H. capsulatum* have not been reported. In the present study, H-ASWS was highly cross-reactive in LT assays of coccidioidin-sensitive persons and was almost mitogenic in lymphocyte cultures of skin test-negative persons, thereby lessening the potential usefulness of this antigen. Cross-reactivity and nonspecific stimulation of lymphocytes also occurred with B-ASWS and C-ASWS, but to a lesser extent. Despite apparent shortcomings of the ASWS cell wall antigens, they do offer the decided advantage of being standardized on a dry weight or chemical basis (9, 32). Mycelial and yeast lysates are rarely reproducible from batch to batch, the exception being spherulin, which can be standardized on a dry-weight basis (24).

In addition to establishing the extent of cross-reactivity of antigens from *Coccidioides*, *Histoplasma*, and *Blastomyces*, the results of the present study suggest that cross-reactivity can occur in LT assays in the absence of cross-reactivity in skin tests. We purposely selected donors who were skin test reactive to coccidioidin but negative to histoplasmin, donors who were reactive to histoplasmin but not to coccidioidin, and donors who were nonreactive to both antigens. The extensive cross-reactivity observed in LT assays in the absence of skin test reactivity to 1:100 dilutions of the heterologous antigens suggests that the T-lymphocyte population that mediates LT responses differs from that which mediates skin test reactivity. Alternatively, it can be argued that the antigen component(s) which elicits LT responses differs from that which elicits skin test responses. It is pertinent to point out that the coccidioidin and histoplasmin antigens used in skin testing differed from those antigens used in LT assays. However, 7 of 13 healthy, histoplasmin skin test-positive subjects were skin tested with and were negative to first strength spherulin (bioequivalent to coccidioidin 1:100), yet these same 7 persons showed significant cross-reactivity to spherulin in LT assays. The question of cross-reactive components versus distinct T-cell subpopulations cannot be resolved until monomolecular antigens are isolated from these dimorphic fungi.

The results of this study diminish the likelihood that LT assays will prove useful in distinguishing clinical infection between these three systemic fungi. Rather, the extensive cross-reactivity between antigens of *Coccidioides*, *Histoplasma*, and *Blastomyces* coupled with the depressed LT responses of most fungal patients to antigens of the infecting fungus (1, 6, 11, 16, 20, 26, 27) severely limits any diagnostic application of this assay.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant AI-13573 from the National Institute of Allergy and Infectious Diseases.

I am indebted to the 37 subjects who volunteered for this study and to Carmen Mead and Evan Pavey for their expert technical assistance.

LITERATURE CITED

1. **Alford, R. H., and R. A. Goodwin.** 1972. Patterns of immune response in chronic pulmonary histoplasmosis. J. Infect. Dis. **125**:269–275.
2. **Boyum, A.** 1968. Isolation of mononuclear cells and granulocytes from human blood. Scand. J. Clin. Lab. Invest. **21**:77–119.
3. **Buechner, H. A., J. H. Seabury, C. C. Campbell, L. K. Georg, L. Kaufman, and W. Kaplan.** 1973. The current status of serologic, immunologic and skin tests in the diagnosis of pulmonary mycoses. Chest **63**:259–270.
4. **Campbell, C. C.** 1960. The accuracy of serologic methods in diagnosis. Ann. N.Y. Acad. Sci. **89**:163–177.
5. **Campbell, C. C., and G. E. Binkley.** 1953. Serologic diagnosis with respect to histoplasmosis, coccidioidomycosis and blastomycosis and the problem of cross reactions. J. Lab. Clin. Med. **42**:896–906.
6. **Catanzaro, A., L. E. Spitler, and K. M. Moser.** 1975. Cellular immune response in coccidioidomycosis. Cell Immunol. **15**:360–371.
7. **Chick, E. W., G. L. Baum, M. L. Furcolow, M. Huppert, L. Kaufman, and D. Pappagianis.** 1973. The use of skin tests and serologic tests in histoplasmosis, coccidioidomycosis, and blastomycosis. Am. Rev. Respir. Dis. **108**:156–159.
8. **Cox, R. A., E. Brummer, and G. Lecara.** 1977. In vitro lymphocyte responses of coccidioidin skin test-positive and -negative persons to coccidioidin, spherulin, and a *Coccidioides* cell wall antigen. Infect. Immun. **15**:751–755.
9. **Cox, R. A., and H. W. Larsh.** 1974. Isolation of skin test-active preparations from yeast-phase cells of *Blastomyces dermatitidis*. Infect. Immun. **10**:42–47.
10. **Cox, R. A., and H. W. Larsh.** 1974. Yeast- and mycelial-phase antigens of *Blastomyces dermatitidis*: comparison using disc gel electrophoresis. Infect. Immun. **10**: 48–53.
11. **Cox, R. A., and J. R. Vivas.** 1977. Spectrum of *in vivo* and *in vitro* cell-mediated immune responses in coccidioidomycosis. Cell Immunol. **31**:130–141.
12. **Cox, R. A., J. R. Vivas, A. Gross, G. Lecara, E. Miller, and E. Brummer.** 1976. *In vivo* and *in vitro* cell-mediated responses in coccidioidomycosis. I. Immunologic responses of persons with primary, asymtomatic infections. Am. Rev. Respir. Dis. **114**:937–943.
13. **Davis, S. K., and H. C. Oels.** 1973. Fractionation of antigenic components of histoplasmin by disc electrophoresis. Am. Rev. Respir. Dis. **108**:708–711.
14. **Deighton, F., R. A. Cox, N. K. Hall, and H. W. Larsh.** 1977. In vivo and in vitro cell-mediated immune responses to a cell wall antigen of *Blastomyces dermatitidis*. Infect. Immun. **15**:429–435.
15. **Deresinski, S. C., H. B. Levine, and D. A. Stevens.**

1974. Soluble antigens of mycelia and spherules in the in vitro detection of immunity to *Coccidioides immitis*. Infect. Immun. **10**:700–704.

16. **Diamond, R. D., and J. E. Bennett.** 1973. Disseminated cryptococcosis in man: depressed lymphocyte transformation in response to *Cryptococcus neoformans*. J. Infect. Dis. **127**:694–697.
17. **Freund, J. E. (ed.).** 1967. Modern elementary statistics, 3rd ed., p. 320–323. Prentice-Hall, Inc., Englewood Cliffs, N.J.
18. **Huppert, M., J. P. Adler, E. H. Rice, and S. H. Sun.** 1979. Common antigens among systemic disease fungi analyzed by two-dimensional immunoelectrophoresis. Infect. Immun. **23**:479–485.
19. **Huppert, M., N. S. Spratt, K. R. Vukovich, S. H. Sun, and E. H. Rice.** 1978. Antigenic analysis of coccidioidin and spherulin determined by two-dimensional immunoelectrophoresis. Infect. Immun. **20**:541–551.
20. **Kirkpatrick, C. H., R. R. Rich, and J. E. Bennett.** 1971. Chronic mucocutaneous candidiasis: model building in cellular immunity. Ann. Intern. Med. **74**:955–978.
21. **Lancaster, M. V., and R. F. Sprouse.** 1976. Preparative isotachophoretic separation of skin test antigens from blastomycin purified derivative. Infect. Immun. **13**:758–762.
22. **Landay, M. E., R. W. Wheat, N. F. Conant, and E. P. Lowe.** 1967. Serological comparison of the three morphological phases of *Coccidioides immitis* by the agar gel diffusion method. J. Bacteriol. **93**:1–13.
23. **Levan, N. E., C. S. Korn, E. G. McNall, and L. Pineda.** 1969. Coccidioidomycosis and lymphocyte transformation, p. 333–339. *In* W. O. Ricke (ed.), Proceedings of the Third Annual Leukocyte Conference, University of Iowa, Iowa City, November, 1967. Appleton-Century-Crofts, New York.
24. **Levine, H. B., and G. M. Scalerone.** 1975. Properties of spherulin, a skin test reagent in coccidioidomycosis. Proceedings of the Third International Symposium on the Mycoses, August, 1974. Pan Am. Health Organ. Sci. Publ. **304**:101–110.
25. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
26. **Musatti, C. C., M. T. Rezkallah, E. Mendes, and N. F. Mendes.** 1976. *In vivo* and *in vitro* evaluation of cell-mediated immunity in patients with paracoccidioidomycosis. Cell. Immunol. **24**:365–378.
27. **Newberry, W. M., Jr., J. W. Chandler, Jr., T. D. Y. Chin, and C. H. Kirkpatrick.** 1968. Immunology of the mycoses. I. Depressed lymphocyte transformation in chronic histoplasmosis. J. Immunol. **100**:436–443.
28. **Pappagianis, D., C. E. Smith, G. S. Kobayashi, and M. T. Saito.** 1961. Studies of antigens from young mycelia of *Coccidioides immitis*. J. Infect. Dis. **108**:35–44.
29. **Smith, C. E., M. T. Saito, R. R. Beard, H. C. Rosenberger, and E. G. Whiting.** 1949. Histoplasmin sensitivity and coccidioidal infection. I. Occurrence of cross reactions. Am. J. Public Health **39**:722–736.
30. **Sprouse, R. F., N. L. Goodman, and H. W. Larsh.** 1969. Fractionation, isolation and chemical characterization of skin test active components of histoplasmin. Sabouraudia **7**:1–11.
31. **Sweet, G. H.** 1971. Antigens of *Histoplasma capsulatum*. II. Separation and characterization of yeast-phase precipitinogens. Am. Rev. Respir. Dis. **104**:401–407.
32. **Ward, E. R., Jr., R. A. Cox, J. A. Schmitt, Jr., M. Huppert, and S. H. Sun.** 1975. Delayed-type hypersensitivity responses to a cell wall fraction of the mycelial phase of *Coccidioides immitis*. Infect. Immun. **12**:1093–1097.