

CANDIDIN: COMPARISON OF TWO ANTIGENS FOR CUTANEOUS DELAYED HYPERSENSITIVITY TESTING

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SUMMARY

A candidin, which is a suspension of killed yeast cells, is commonly used for intradermal tests of delayed hypersensitivity, to evaluate the immunological cellular competence of the patient, when the test is applied along with other similar tests.

When working with a cellular antigen, the histopathology of positive skin tests reveals a cellular infiltrate which not only presents a characteristic hypersensitivity reaction but also a neutrophilic abscess in the central part.

This research presents the results of a comparison between the yeast cell suspension and the polysaccharide antigens, both obtained from the same strains of *Candida albicans*. The results obtained by skin tests in one hundred individuals were 61.0% with the polysaccharide antigen and 69.0% with the yeast cell suspension antigen. Concordant results concerning the two antigens were observed in 82.0% of the individuals. The discussion section presents an assumption to explain the differences of positivity obtained with the two antigens.

We conclude that the polysaccharide antigen can be utilized in the intradermal test of delayed hypersensitivity to *Candida albicans*.

KEYWORDS: Candidin; Delayed hypersensitivity; Polysaccharide antigen.

INTRODUCTION

A polysaccharide antigen from *Paracoccidioides brasiliensis* was described and used for quantitative complement fixation tests (CF) by FAVA-NETTO¹. Its use in the immunological study of 220 paracoccidioidomycosis patients was published in 1961⁵. The standardization of this antigen to be used for intradermal tests of delayed hypersensitivity was then developed⁷.

A total of 30 epidemiological surveys were carried out in Brazil, using the polysaccharide antigen for intradermal tests¹³.

The polysaccharide was compared with other antigens in epidemiological surveys based on intradermal tests^{8,12}. Moreover the histopathology of the skin test was studied^{1,11}. The standardization of the polysaccharide antigen has been developed as an immunological parameter for use on intradermic tests⁶.

This polysaccharide antigen can be stored in a refrigerator for as long as 23 years, showing no loss of immunological activity⁹.

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Taking into account all of these characteristics of the polysaccharide antigen, a similar antigen obtained from *Candida albicans* strains is herein studied.

A comparison to another *C. albicans* antigen, which is commonly used for intradermal tests and which is a suspension of yeast cells, was made and the results are presented.

MATERIAL AND METHODS

Patients – A total of 100 outpatients seen at an Allergy Clinic were submitted to intradermal tests with the two kinds of antigen. The group consisted of 49 males and 51 females. Among them there were cases of candidiasis, dermatomycosis, immediate hypersensitivity such as asthma, urticaria and other diseases.

Yeast cell suspension antigen

The antigen was prepared by the method of CASTRO² who used it for intradermal tests of delayed hypersensitivity in sporotrichosis.

In the present study, the antigen was prepared from four strains of *C. albicans*. After culture for 24 h on three successive days at 37°C on Sabouraud dextrose, the strains were cultured in the same medium for a 48 h period at 37°C. Yeast cell suspension antigens in saline solution containing 1:5,000 merthiolate were obtained. The cell suspensions were heated in a water bath at 56°C for one hour to kill the cells. Following sterility control, the four suspensions were pooled. The pool was standardized by tube 5 on MacFarland scale, aliquoted in 5.0 ml volume and stored at 4-8°C.

The suspension was used diluted 1:2 in saline solution containing 1:5,000 merthiolate.

Polysaccharide antigen

The antigen was obtained from the same four strains of *C. albicans* and prepared by the method of FAVA-NETTO^{4,10}.

The *C. albicans* strains were cultured for 10 days at 37°C on Sabouraud-dextrose⁴, harvested in saline solution and centrifuged at 2,000 rpm for 10 min. The cell pellet was washed in acetone (5-10 volumes) and in ether (5-10 volumes), three times each. After this procedure, the cell volume was recorded. The sediment was stored in the refrigerator until the cells were dried. Then a 20.0% veronal buffer solution suspension (v/v) (volume of cells recorded before drying) was prepared. The veronal buffer suspension was autoclaved at 120°C for 20 min and centrifuged at 2,000 rpm for 20 min. The supernatant containing the antigen was divided into small

volumes and stored. The veronal solution contained merthiolate at 1:5,000 to preserve the antigen.

The polysaccharide antigen was titrated in the complement fixation reaction and used at a concentration which represented 10 times the optimum amount to fix 3 (50%) units of complement.

Intradermal test

Each patient received intradermal injections of 0.10 ml of each antigen into the right and left forearms.

Readings were taken 24, 48 and 72 hours later.

An erythematous papule of 5.0 mm or more in diameter in one reading was considered to be a positive reaction.

RESULTS

The results of the intradermal tests are described in Table 1.

TABLE 1

Candidin test applied to 100 outpatients at an Allergy Clinic.

Number of patients	Antigen			
	Yeast cell suspension		Polysaccharide	
	Positive	Negative	Positive	Negative
100	69 (69.0%)	31 (31.0%)	61 (61.0%)	39 (39.0%)

Similar results concerning the two antigens were observed in 57.0% positive and 25.0% negative tests. Positive tests using cell suspension antigen and negative tests using the polysaccharide antigen were observed in 13.0% of cases. Negative tests using cell suspension antigen and positive ones using the polysaccharide antigen were observed in 5.0% of the cases.

DISCUSSION

When a cellular suspension antigen is used for skin testing, the histopathology of a positive reaction reveals the presence of a neutrophilic abscess in the skin site center. The characteristic cellular infiltration of delayed hypersensitivity can only be seen at the periphery, represented by lymphocytes, monocytes and some eosinophils³. It should be remembered that this neutrophilic infiltration may be responsible for some nonspecific reactivity in the cutaneous delayed hypersensitivity.

The slightly higher positivity observed in this research in the skin test using the yeast cell suspension antigen in contrast with the use of the polysaccharide antigen may be attributed to some nonspecific reaction. However, the advantages of the use of a polysaccharide antigen recommend its employment.

As already emphasized in the Introduction, such advantages are: (a) the antigen is soluble; (b) the histopathological study of a positive reaction reveals an infiltration of cells characteristic of a delayed hypersensitivity reaction; (c) this type of antigen can be stored for a long time with no loss of the immunological activity; and (d) it can be standardized based on an immunological parameter.

RESUMO

Candidina: antígeno para reação de hipersensibilidade de tipo tardio. Comparação de dois antígenos

Candidina, constituída de suspensão de células leveduriformes mortas, é comumente usada em provas intradérmicas de hipersensibilidade retardada, principalmente na avaliação da competência imunológica do paciente, quando usada conjuntamente com outras provas intradérmicas do mesmo tipo. Considerando-se o estudo histopatológico de reação positiva com este tipo de antígeno, é possível obter uma reação positiva não específica na leitura da prova intradérmica.

Esta pesquisa apresenta os resultados obtidos a partir da comparação entre o antígeno de suspensão celular e o antígeno polissacarídico, ambos obtidos a partir das mesmas amostras de *Candida albicans*.

As diferenças observadas no estudo histopatológico de reações positivas com antígeno polissacarídico e com antígeno de suspensão celular podem explicar as diferenças observadas nas porcentagens entre reações intradérmicas positivas com o antígeno de suspensão celular (69,0%) e com o antígeno polissacarídico (61,0%), nas provas intradérmicas realizadas em 100 indivíduos. A coincidência de resultados positivos e negativos nesta pesquisa foi obtida em 82,0% dos indivíduos.

A conclusão da pesquisa: é possível utilizar antígeno polissacarídico como candidina em provas de hipersensibilidade tipo tardio.

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