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HISTOPLASMIN REACTION. COMPARISON OF A POLYSACCHARIDE ANTIGEN TO THE FILTRATE ANTIGEN

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SUMMARY

This work was planned by taking into account all the knowledge accumulated from the immunological study of paracoccidioidomycosis. It aimed at comparing a polysaccharide antigen from *Histoplasma capsulatum* to a classic histoplasmin with the help of intradermal tests of delayed type of hypersensitivity. Tests were applied to 115 individuals in Santo Amaro, a town in the State of São Paulo. Positive results using classic histoplasmin were obtained in 46.0% cases whereas positive results using the polysaccharide antigen at its highest concentration were obtained in 51.30% cases.

The major conclusion in this investigation is that it is possible to use the polysaccharide antigen as histoplasmin instead of the filtrate antigen.

KEYWORDS: Reaction to histoplasmin; Filtrate antigen; Polysaccharide antigen.

INTRODUCTION

Intradermal tests of histoplasmin are carried out all over the world using the filtrate antigen (classic histoplasmin), which preparation and standardization – time consuming phases – were set up in 1945[5]. The researchers were supplied with such antigen by CDC in USA[28].

Only a few researches were initially carried out basing on the polysaccharide antigen from *Histoplasma capsulatum*[4].

The polysaccharide antigen – obtained from *Paracoccidioides brasiliensis* by a different technique – has been frequently investigated. A lot of scientific information about this antigen has already been compiled – some obtained from serologic reactions[7, 8, 9, 12, 22] and from intradermal tests of delayed type of hypersensitivity[1, 2, 10, 15]. Several epidemiological surveys over paracoccidioidomycosis based on intradermal tests have already been carried out in Brazil[6] and in South America[1, 16]. This antigen has already been compared to other antigens obtained from *Paracoccidioides brasiliensis* in intradermal tests[24] through epidemiological surveys[11] and experimental studies[15, 22]. Studies on this antigen using molecular biology techniques[18, 22] have also been conducted.

This large amount of scientific information about the polysaccharide from *Paracoccidioides brasiliensis* upholds this investigation, which studies the polysaccharide antigen prepared from *Histoplasma capsulatum* by using the very same technique used in the preparation of the polysaccharide antigen from *Paracoccidioides brasiliensis*. This work compares the polysaccharide antigen from *Histoplasma capsulatum* to the classic histoplasmin through epidemiological survey.

MATERIALS AND METHODS

Patients – In the outpatients'Clinic of the Department of Dermatology in the Medical School at the University of Santo Amaro, São Paulo State, Brazil, 115 individuals, 74 female and 41 male, ages varying from 13 to 70 and acting in several different working fields, were submitted to intradermal tests.

This investigation was approved by the Committee of Medical Ethics in the Medical School and all patients were

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accordingly informed about the tests to which they were submitted following their consent.

Antigens

Histoplasmin (filtrate antigen) – A *Histoplasma capsulatum* strain, obtained by the method of FAVA-NETO et al., 1967[13], was isolated from bats'excrement when there was an outbreak of histoplasmosis epidemic infection, affecting the inhabitants of Lagoinha beach, on the north coast of São Paulo State. Such strain is kept under mycelial form in Sabouraud glucose agar, at room temperature, being transferred to another tube containing the same culture medium every three months. When it sown in rich culture medium and incubated at 37° C it easily turns into its yeast form phase.

Method of preparation – According to SMITH et al.[27], a semi-synthetic culture medium was utilized for the preparation of this antigen. In an one litercapacity Erlenmeyer flask containing accordingly sterilized 500 ml culture medium, the *Histoplasma capsulatum* mycelial phase undergoes growth. Various fragments are carefully placed on the culture medium surface so that they remain supernatant. The culture medium is not to be stirred, because the fragments must not sink to the bottom thus obtaining a membrane over the culture medium surface due to the fungus growth. Soon afterwards, the Erlenmeyer flask is placed inside a cabinet, protected against the light and well protected against contamination by air fungi. It is so incubated at room temperature for four months and regularly inspected to check on the fungus growth and absense of contamination. Soon after that, the filtrate is obtained first in a sterilized paper filter and then in a bacteriological filter for a definitive sterilization. After sterility control for fungi and bacteria, merthiolate is added to the concentration at 1:5,000 for preservation. It is generally divided into 5.0 ml volumes and refrigerated at 2-8° C.

Standardization – Standardization is achieved after carrying out various simultaneous intradermal tests with histoplasmin, supplied by CDC in the USA, in selected dilutions. The filtrate histoplasmin here produced was standardized to be used in a 1:500 dilution made in physiological solution containing merthiolate at a:5,000 for preservation.

Distribution and maintenance – The diluted histoplasmin must be divided into low volumes, to be utilized within a month, and refrigerated at 2-8° C.

Histoplasmin (polysaccharide antigen) – The same *Histoplasma capsulatum* strain utilized in the preparation of the histoplasmin (filtrate antigen) was here utilized. The strain was at first turned into its yeast form phase with the help of successive culturing every 5-8 days, in a rich medium, FAVA-NETO[7], FAVA-NETTO et al.[14] and incubated at 37° C. Following that, dense yeast cell suspensions in physiological solution were sown in the same culture medium, divided into Roux bottles. The bottles were then incubated at 37° C for 12 days. Therewith the yeast cells were saved in 10-20 ml physiological solution in each bottle. The suspension was centrifuged at 2,000 rpm for 10 minutes, disregarding the supernatant. The cells were suspended in 10-20 ml acetone and centrifuged, disregarding the supernatant. The cells were rinsed in acetone two more times and later, in a like manner, rinsed three times in 10-20 ml ethylic ether. Cellular sediment was saved and its volume recorded after centrifugation. The tubes used were then refrigerated up to total evaporation of the ether. Having taken into account the recorded volume of the wet ones, the cells were suspended in veronal buffer (v/v) at 20.0%, autoclaving for 20 minutes at 120° C. They were centrifuged, sterilizingly saving the supernatant which is the polysaccharide antigen itself. It was preserved in merthiolate at 1:5,000, divided into low volumes and refrigerated. This antigen cannot be frozen.

Standardization – Standardization is achieved through the quantitative complement fixation reaction, by the method of WADSWORTH, MALTANER & MALTANER'S as employed by FAVA-NETTO[7]. The immunological parameter is represented by an amount of antigen equal to its optimal fixing power for three units of complement (50% hemolysis, 3K). In this investigation the polysaccharide antigen was utilized in two different concentrations 1 × 3K and 2 × 3K, where K is the unit of complement, 50% hemolysis.

Intradermal tests – The tests were carried out through the intradermal injection of 0.10 ml of each antigen on the anterior side of the forearm. Readings were taken after 24, 48 and 72 hours, considering the obtainment of a 0.5 cm or wider mean diameter erythematous papule as a positive result in any of the readings.

RESULTS

The readings of the intradermal tests were taken over three consecutive days – 24, 48 and 72 hours apart from each other – on 89 individuals; over two days on 19 individuals, and in only one day in seven. Table 1 displays the results obtained with the

TABLE 1

Results of histoplasmin tests on 115 individuals with two different types of antigens: filtrate histoplasmin at1:500 and polysaccharide histoplasmin in two different concentrations, 1 × 3K and 2 × 3K.

Individuals	Filtrate histoplasmin		Polysaccharide histoplasmin			
			1 × 3K		2 × 3K	
	Positive	Negative	Positive	Negative	Positive	Negative
115	46	69	55	60	59	56
	40.0%	60.0%	47.8%	52.2%	51.3%	48.7%

two different types of antigen, the polysaccharide antigen having been utilized in two different concentrations.

Similar positive results concerning the two types of antigens were observed in 35 individuals (30.43%) and coinciding negative results concerning the two types of antigens were observed in 42 individuals (36.52%). Positive tests using filtrate histoplasmin were only obtained in 11 individuals (9.57%). Positive ones using the polysaccharide histoplasmin only were obtained in 27 individuals (23.48%).

DISCUSSION

Prepared as herein described and standardized through the completion of several simultaneous intradermal reactions with an already standardized histoplasmin, the classic histoplasmin (filtrate antigen) is a dependable antigen. It does not reveal cross reactions in individuals infected with fungi producing other systematic mycosis. Only its preparation and standardization are time-consuming phases.

Prepared as herein described, the histoplasmin (polysaccharide antigen) can be standardized by using an immunological parameter. The histoplasmin production and standardization can then be proceeded with more easily. The polysaccharide antigen from *Histoplasma capsulatum* has been utilized in serological tests of complement fixation and of precipitation in a liquid medium for a long time (FAVA-NETTO, 1972[9]) and in intradermal tests in some researches (FAVA-NETTO et al., 1967[13]). It has not however been standardized comparatively to the classic histoplasmin (filtrate antigen) through several simultaneous intradermal tests. That is exactly what this work accomplished.

Concerning preparation, standardization and accurate interpretation of the results obtained in intradermal tests (EMMONS et al., 1945[5]; CHRISTIE & PETERSON, 1945[3]; PALMER, 1945, 1946[19, 20]; FURCOLOW, 1958[17]; PRIOR & SASLAW, 1958[21]; SASLAW & CAMPBELL, 1953[25] and SALVIN et al., 1954[23]), classic histoplasmin (filtrate antigen) has been carefully studied.

Eighty-eight epidemiological survey have already been carried out with classic histoplasmin, FAVA, 1996[6], in Brazil. Positivity was quite different on these surveys. It varied from 2.60% to 93.20%.

It is well known that the positivity in intradermal tests depends on the antigen utilized regarding its nature and dose; on the age of the individuals submitted to the tests; on the sampling of the population used; on the geographical area; on the individual's possible contact with *nidi* which might represent any risk factor. Fixing all variables except for one, say geographical area for instance, a comparison of endemicity among two or more geographical areas is feasible. The classic histoplasmin is of the known antigen nature which does not reveal any cross reactions and its dose is standardized in each intradermal test. The antigen validity after dilutions is yet to be known, i.e. for how long it remains active following dilution. In the epidemiological surveys carried out in Brazil, several parameters have not been fixed. As a consequence, the endemicity differences among the several investigated areas cannot be strictly evaluated.

The use of the polysaccharide antigen from *Histoplasma capsulatum* in intradermal tests was suggested by the knowledge gathered from studies, referred to in the introduction to this work, carried out with polysaccharide antigen obtained from *Paracoccidioides brasiliensis* following the very same technique.

The polysaccharide antigen is more easily obtained and standardized and lasts longer. Investigations carried out by SCROFERNEKER & FAVA-NETO, 1988[26], pointed out that the immunological activity of the polysaccharide antigen does not depend on its protein content.

The results achieved in this work attest that it is possible to utilize histoplasmin (polysaccharide antigen) instead of the classic histoplasmin (filtrate antigen). Nevertheless new studies will be carried out in order to find out the dose of polysaccharide antigen to be utilized in intradermal tests, mainly as regards the evaluation of its specificity.

RESUMO

Reação de histoplasmina. Comparação de antígeno polissacarídico com o antígeno filtrado

O estudo envolve a comparação entre o antígeno polissacarídico de *Histoplasma capsulatum* com a histoplasmina clássica em inquérito epidemiológico, através de provas intradérmicas de hipersensibilidade do tipo tardio, realizado em 115 indivíduos da região de Santo Amaro. Os resultados revelaram 46,0% de provas positivas com a histoplasmina clássica e 51,30% de resultados positivos com o antígeno polissacarídico em sua maior concentração.

A principal conclusão da pesquisa: é possível utilizar o antígeno polissacarídico como histoplasmina, em substituição ao antígeno filtrado.

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