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Obtaining and Experimental Study of Candida Allergens

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Abstract

Due to the need of diagnostics of the widespread allergic diseases connected with infection ofa person by fungi, the question of creation of highly sensitive diagnostic panels of fungal allergens including molds and yeasts is important. Work purpose: creation of experimental technology of preparation of allergens from culture of *C. albicans* and studying of their physical, chemical and immunobiological properties. For receiving allergens we used the inactivated biomass of clinical strains of *Candida albicans*. Cultivation was carried out in the original mineral (without protein) CC1 and ML media in liquid and agarized variants with addition of sugar in various concentrations. In samples of allergens we defined amount of protein, carbohydrates, nucleic acids, specific activity *in vitro* (reaction of degranulation of rat mast cells). It was shown that the content of protein nitrogen varied from 72 to 18900 PNU, carbohydrates from 0,001 to 0,079 mg/ml depending on physiological properties of a population of *C. albicans* and cultivation conditions. For determination of specific activity (reaction of rat mast cells degranulation) of samples used sera from patients with a sensitization to fungi. Allergenic activity of preparations in reaction of degranulation of rat mast cells varied from 3% (spontaneous) to 52%.

The highest specific activity was shown for samples of the allergens obtained from *C. albicans*, grown in the ML medium. The results obtained using of 18 samples of preparations, can be a basis for a choice of an optimum technological mode of allocation of allergens from *C. albicans*.

Keywords: Yeast, *Candida albicans* allergens; Fungal allergens; Mycogenic sensibilization; Allergy

Introduction

Nowadays the number of diseases caused by a sensibilization to different allergens steadily grows all around the world. Mycoallergens are the most widespread allergens in human habitat – according to literary data, frequency of sensibilization to them reaches 60%, depending on fungi species and patients attitude to the risk groups. Yeast allergens, being in the closest contact with human organism, are objects of special interest. High spreading of allergy to *Candida* species is determinated by the colonization frequency of the mucous membrane of gastrointestinal tract and urogenital system by these yeasts. The *Candida* species mainly cause such forms of allergy, as bronchial asthma, an allergic rhinitis and allergic bronchopulmonary mycosis [1]. It is known, that the various allergic conditions, caused by a sensibilization to allergens of *Candida* species, may cause burdening effect on the course of the basic infection -inflammatory disease [2-5]. It shows the importance of fast identification of the allergic fungi sensibilization for therapy optimization [2,6]. At the present time there is no consensus of what components of the *Candida* cell are the main allergens. According to references, such main components of a cell wall of *Candida albicans*, as a polysaccharide mannan, mannoproteins and glucanoproteins, are the most allergenic [6-9], according to other literature sources - such secretory proteins as acidic protease with molecular mass of 31kDa and enolase[10]. It is obvious from different sources that different allergenic components of *Candida* yeast cause lesions of certain human body regions. Identification of the fungi sensibilization is made by detecting the amount of specific IgE in the serum of a patient or by means of scratch skin allergy test with diagnostic preparations of fungal allergens.

Unfortunately, at this moment in Russia industrial production of fungal allergens is stopped, and import diagnostic preparations are too expensive. As different authors consider various parts of *Candida* cell as the most allergenic, there are different methods of getting raw materials for allergen production. Methods of obtaining yeast allergens depend on specific allergen, considering their biochemistry and localization in a fungal cell. For example, mannan-containing allergens are extracted from yeast biomass, which is mechanically destroyed by pressure or autoclaving. After that polysaccharide is sedimented with ethanol multiple times [9,11-14]. Acidic protease of *C. albicans* is obtained by purification of cultural liquid [15] since it doesn't belong to fungi cell components. Yeast enolases are obtained by mechanical destruction of yeast cells and chromatographic refinement of a supernatant on Sephadex™ or sepharose agar [16,17].

Also it is necessary to consider that micromycetes have an essential specific variety, and moreover, the species, causing the greatest number of positive reactions, vary in different regions. Therefore it is necessary to use allergens from species of fungi, dominant in the particular region, for the diagnostic purposes [18]. One of the most important steps of yeast allergens development is standardization of their production [1,2,6,19,20]. Therefore creation of diagnostic panels of allergens from the *Candida* species is very actual.

The purpose of this research is development of technology of receiving allergens from *Candida* species for creation of a native diagnostic panel.

Materials and Methods

As raw materials for preparation of allergens extracts we used thermally inactivated biomass of strains of *Candida albicans* isolated from a clinical material and the reference collection strain. Cultivation

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was carried out in mineral (protein-free) media of CC1 and ML in liquid and agarized variants with various concentrations of glucose, and also in Sabouraud agar. In experimental preparations of allergens we defined protein content, its fractional composition, concentration of carbohydrates and nucleic acids, and also their specific activity.

Amount of protein was determined by Nessler's method and Bradford protein assay [21,22]. Amount of nucleic acids was determined by Spirin's method, concentration of carbohydrates by Dubois method [23,24]. For studying of fractional structure and properties the method of protein separation by means of electrophoresis in polyacrylamide gel (PAAG) and affinity chromatography on Ni - activated sepharose was used [10,17,25].

Specific activity of preparations was determined by rat mast cells degranulation (RMCD) technique [21]. To study specific activity obtained allergen preparations, we used a collection of sera from patients with sensibilization to the fungal allergens, who consulted to Research Advisory Unit of FGBU "Mechnikov Research Institute of Vaccines & Sera". Sera were checked by the RIDA Allergy Screen method, RBiopharm, Germany.

Results

Studying of biochemical properties of the obtained preparations revealed that received allergenic extracts varied by amounts of albuminous nitrogen from 72 to 18900 PNU, carbohydrates from 0,001 to 0,079 mg/ml, nuclear acid from 0,006 to 2,87. Possibly, that could be linked to various content of media and cultivation conditions (Table 1).

To determine the fractional composition we investigated 5 batches of allergenic extracts from *C. albicans* species. Electrophoresis of allergens showed that the protein has a molecular mass of 32 to 97 kDa, which is confirmed the literature data [26]. Using affinity chromatography we obtained two polypeptides with molecular mass of 97, 4 and 35 kDa. Peptide with molecular mass of 35 kDa is 1,3-beta glycosyltransferase Bg12p.Was also did the additional cleaning of *Candida albicans* batches on a column with Ni - Chelating Sepharose sorbent. Affinity chromatography on Ni - activated sepharose of *Candida albicans* allergen showed that in the fractions: wash 60 mm imidazole and wash 0.3 M imidazole contained protein.

Batch	Strain	Protein, PNU	Carbohydrates, mg/ml	Nuclear acids, mg/ml
109	110	8256	0,006	0,039
209	110	2112	0,0245	0,019
309	110	72	0,003	0,021
409	110	312	0,006	0,524
509	110	78	0,002	0,018
609	110	312	0,007	0,498
709	ATCC-885-653	816	0,003	0,008
809	110	768	0,003	0,011
909	110	312	0,001	0,006
1009	110	624	0,002	0,007
1	110	9600	0,017	0,04
2	110	3600	0,02	0,07
3	110	18900	0,068	2,846
4	ATCC-885-653	14100	0,0795	2,412
5	110	14100	0,0595	2,871
6	110	10000	0,051	2,799
14	110	1800	0,033	0,024
15	110	1850	0,02	0,03

Table 1: Studying of biochemical properties of *C. albicans*.

Batch	Strain	Specific activity, %
109	110	45
209	110	52
309	110	10
409	110	8
509	110	20
609	110	6
709	ATCC-885-653	8
809	110	26
909	110	13
1009	110	15
1	110	26
2	110	32
3	110	11
4	ATCC-885-653	4
5	110	4
6	110	3
14	110	28
15	110	29

Table 2: Studying of specific activity of *C. albicans* allergens.

Polyacrylamide gel electrophoresis of *Candida albicans* allergen showed that the protein had molecular mass of about 97 kDa, the concentration of protein in the initial supernatant and wash samples was very low.By means of the RMCD method we examined specific activity of 18 studied preparations of allergens (Table 2). Mast cells of rats were sensibilized by these batches of preparations of allergens and sera of the patients who consulted to Research Advisory Unit of FBGU "Mechnikov Research Institute of Vaccines & Sera", were highly sensibilized to fungal allergens.

Studied preparations of allergens showed different allergenic activity varied from spontaneous (3%) to 52% degranulation. It is necessary to consider that the highest specific activity has No. 109; 209; 809; 1; 2; 14 and 15 batches. The maximum specific activity among them was found for batches No. 209, protein content in this series corresponded to 2112 PNU. Similar high activity was found in batches No. 109, and protein content in this batches corresponded to 8256 PNU.

According to this it is possible to assume that not all proteins which are a part of extract of allergen, possess high allergenic activity. The batches with the greatest specific activity were cultivated on the medium ML with subsequent drying, and extracted with the borate buffer, that will be possible to consider further, for the development of technology of obtaining allergenic preparations from *Candida albicans*. When studying chemical composition and specific activity of batches No. 3,4,5,6 cultivated on media CC1 and Sabouraud agar and extracted with of the Evans Coca buffer, we observed very high protein content from 10000 to 18900 PNU, nucleic acids from 2,4 to 2,87 mg/ml andcarbohydrates from 0,051 to 0,079 mg/ml. At the same time there was no specific activity – (3-15% of degranulation in RMCD methods, respectively), that indicated low allergenic activity of these batches of preparations (Table 2).

Discussion

Research of biochemical composition showed that the ratio of concentrations of proteins, carbohydrates and nucleic acids depends on a method of preparation obtaining of fungi biomass. During studying allergenic activity we found, that the batch cultivated on the medium ML with subsequent drying, and extraction with borate buffer, possessed the highest specific activity. On the basis of the obtained results, for

further researches on creation of diagnostic allergens from *Candida albicans* we selected 8 best batches of preparations. By means of an affinity chromatography on Ni-activated sepharose and polyacrylamide gel electrophoresis it was shown, that proteins with molecular mass of 35 and 97 kDa possessed specific activity.

Based on our results, it is possible to make the conclusion, that our offered experimental technology of obtaining allergenic preparations from *Candida albicans* can be a basis for further development of diagnostic mycoallergen preparations.

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