Efficiency of oral immunization of mice with *Candida albicans* and *Moraxella catarrhalis* heat-killed cells and cross reactivity of induced antibodies

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Abstract

The aim of the study was to evaluate the immune response against Moraxella catarrhalis and Candida albicans antigens in mice (BALB/c) immunized by GALT system. The ELISA and immunofluorescence tests were employed for checking of mice sera activity in the presence of used antigens. Both sources of antigens were efficacious in specific antibody generation by GALT system. Furthermore, obtained results showed cross reactivity between Candida albicans antigens and anti Moraxella catarrhalis sera and also between Moraxella catarrhalis antigens and anti Candida albicans sera.

Key words: *Moraxella catarrhalis, Candida albicans, cross-reactions.*

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Introduction

Moraxella catarrhalis and *Candida albicans* belong to natural microflora of human body. *M. catarrhalis* – gram negative bacterium – is the ubiquitous element of the physiological microflora of the human upper respiratory tract [1-3]. *C. albicans* – yeast-like fungus – is a component of the host residential microbial flora, where – in the majority of humans – it exists as a commensal in gastro-intestinal and genitourinary tracts [4-6]. Cells of *C. albicans* (in contrast to *M. catarrhalis* cells) can exist in open environment [6]. Both microorganisms are usually harmless colonizers of human body. However, in some hosts, they have the capacity to cause a range of diseases. Diseases caused by *C. albicans* are broadly classified into two categories: superficial and invasive candidiasis [4, 5]. The incidence of the last one has increased since the last decades [7]. In recent years *M. catarrhalis* is one of the leading causes of otitis media in young children and lower respiratory tract infections in adults [8]. Existing in the same body both microbes can (directly or/and indirectly) mutually influence each other. Sera and body fluids of the most people have shown the reactivity with *M. catarrhalis* [2] and *C. albicans* [5]. They can also influence on immunological response in general. The extracts of glycoprotein and peptidoglucomannan from *C. albicans* cell wall increase the number of antibody producing cells in mice immunized by other (sheep erythrocytes) antigens [9]. Antigens of *M. catarrhalis* stimulate PBMC to production of IFN [10]. Antigenic structures may be shared among polymers of different microorganisms. Polysaccharides isolated from different species of fungi have shown cross-precipitation in the presence of anti-pneumococcal sera [11]. IgE antibody to *Pityrosporum ovale* showed binding to *C. albicans* cells [12]. It was also shown that *C. albicans* fimbrial adhesin and pilus adhesin of *Pseudomonas aeruginosa* posses a homologous receptor binding domain – identical epitope (adhesintope) [13]. For this report we investigated on animal model the possibility and efficiency of developing the immunlogical response on antigens almost constantly available for animal immunological system and unexpected mutual connections.

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Materials and Methods

Mice

Six – eight week old mice of the BALB/c strain of both sex obtained from Animal Units of this Institute were used.

Microorganisms

Candida albicans 1407 (obtained from Institute of Immunology and Experimental Therapy Microorganism Collection Units) and *Moraxella catarrhalis* (clinical isolate from our Institute collection) were used. Microorganisms were cultured on appropriate media; blood agar (OXOID, 5% sheep blood) and BHI broth for *M. catarrhalis* and Sabouraud solid and fluid medium for *C. albicans.*

Antigens preparation

Thermaly killed cells of *C. albicans* and *M. catarrhalis* were used for mice immunization. Routinely cultured in liquid media bacteria (BHI, 37°C) and yeast (Sabouraud, 37°C) until stationary phase were centrifuged, washed three times in PBS (pH 7.4) and finally suspended in PBS. Tubes with suspended cells were boiled in water bath for 10-15 minutes, cooled and centrifuged. The pallets were suspended in PBS to final concentration of cells 1×10^7 cfu/ml.

Seven formulas of coating antigen preparations for ELISA tests were performed. Cells after washing (as described above) were suspended in borate buffer (pH 8.2; in concentration as above) and finally gave: – 1. viable cells antigens; after boiling, centrifuged and resuspended in borate buffer – 2. killed cells antigens and as the supernatant – 3. extracted antigens. Parallely, the second set of tubes with washed viable and killed cells (suspended in 25 mM tris-HCl-EDTA buffer -pH 6.8) were sonicated (VirSonic 50) 10 × 60 s in 30 s intervals. The crude sonicate extracts were centrifuged (10 000 × g, 15 min, 4°C) and resulting supernatants were ultracentrifuged (100 000 × g, 2 h, 4°C). Pellets and supernatants were suspended in borate buffer (10 ug protein/ml) and gave: – 4. membrane antigens from viable cells; – 5. soluble antigens from viable cells; – 6. membrane and – 7. soluble antigens of killed cells. Respectively for immunofluorescence tests, washed in PBS (three times) cells from stationary phase cultures of *C. albicans* and *M. catarrhalis* were used.

Immunization procedure and sera preparation

Ten groups of mice were used; six of them were given intraperitoneal bacteria or yeast in three doses – 5×10^5 cells/dose at two weeks intervals. The remained four groups were given drinking water with suspensed bacteria or yeast in concentration 2×10^7 killed cells/ml during six weeks. Immunization of mice groups were made in two repetition at one year interval. Before immunization procedure, samples of mice sera were taken and after immunization mice were bled, obtained sera portioned and kept at –70°C.

Antibodies specifity assays

ELISA tests were carried out according Weeman and Schurs [14]. Borate (pH 8.2), PBS (pH 7.4) and citrate (pH 4.9) buffers were employed. Wells of plates (Nunc--Immuno, Maxi Sorp) were coated by all forms of prepared antigens: viable and fixed cells 5×10^5/well; 0.1 ml/well of soluble antigens released into borate buffer during thermal inactivation of cells; membrane and soluble antigens obtained by sonication from viable and fixed cells in concentrations 1 ug/well. Amount of 0,1 ml of tested and conjugated sera diluted in 1% BSA in PBS were added to the wells. Standard of dilution of tested sera was in range1/100 – 1/100 000. Peroxidase conjugated goat anti mouse IgA, IgM, IgG (Jackson Immuno Research) was used in standard dilution 1/5000. Incubations of plates were performed for 2 h at 37°C or overnight at 4°C. All plates were read with Dynatech ELISA spectrofotometric reader at the wavelength 492 nm.

Immunofluorescence tests were performed as follows: 5×10^6 cells of *M. catarrhalis* or *C. albicans* were mixed with tested mice sera (0.15 ml), incubated 1.5 h in room temperature, centrifuged ad washed in PBS (three times), mixed with fluoresceine conjugated goat anti-mouse Ab (0.1 ml), incubated 1.5 h in RT, centrifuged, washed (three times) ad finally suspended in PBS. Fluorescent microscope (Olympus BH-2) were used for visual analysis.

Results

In the preliminary tests the trials were made to select animals by checking their praeimmune sera for presence of specific antibodies against antigens of *C. albicans* and *M. catarrhalis*. Chosen animals – without specific antibodies – were grouped (5 mice in group). Selected groups of mice were immunized with killed cells of *C. albicans* or *M. catarrhalis*. Two routes of immunization were used – oral and peritoneal. The specific activities of challenged animals sera were checked in ELISA (table 1). As seen, used antigens stimulated animals for specific antibody production. Almost all (except of two mice – one for each antigen) animals immunized by Intraperitoneal route produced specific antibodies against used immunogens respectively. The similar results were obtained in case of oral immunization by *Moraxella* immunogen. The oral route of immunization by *Candida* immunogen was less efficient; lower number of immunized animal sera showed positive reaction.

As can be seen, the cross reactions between nonspecific antisera and used immunogens appeared. High number of anti-Moraxella animal sera obtained by both routes of immunizations gave positive reactions with *Candida* antigens. Similarly, high number of anti-Candida sera obtained by peritoneal route recognized *Moraxella* antigens. In contrast – only two of ten anti-Candida sera resulted from the oral route reacted with *Moraxella* antigens. The specificities of highest number of sera recognized within the coating antigens those which contained cell surface constituents of used cells.

Table 1. Reactivity and cross-reactivity of tested mice sera with different forms of *Candida albicans* and *Moraxella catarrhalis* antigens (ELISA)

Antigen used for immunization	Routes of immunization and number of used mice	Number of sera which showed positive reactions with antigens of:													
		Candida albicans (1-7)							*Moraxella catarrhalis* (1-7)						
		A	B	C	D	E	F	G	A	B	C	D	E	F	G
Candida albicans fixed cells	intraperitoneal 15	13	13	14	14	13	13	14	14	–	–	14	10	–	–
	oral 10	2	1	8	2	1	1	5	1	–	–	2	0	–	–
Moraxella catarrhalis fixed cells	intraperitoneal 15	14	–	–	14	11	–	–	14	14	14	14	11	13	14
	oral 10	9	–	–	9	6	–	–	7	5	10	10	7	5	5

A – viable cells; B – soluble antigens; C – membrane antigens; D – fixed (killed) cells; E – extracted antigens; F – soluble antigens from fixed cells; G – membrane antigens from fixed cells.

For some postimmunized sera, titers of specific antibody were determined (table 2). The higher values showed sera specific for *Moraxella* antigens (1/100 000) than those specific for *Candida* (1/5000); obtained by intraperitoneal route than oral one. The highest levels of specific antibodies concentrations obtained by intraperitoneal routes of immunization with *Candida* antigens ranged between 1/1000 (anti-Moraxella specificity) and 1/5000 (anti-Candida specificity); oral routes gave 1/200 (anti-Candida specificity) and 1/1000 (anti-Moraxella).

The further experiments were aimed to visualize effects of specific antibody binding to cell surface (table 3). *Candida* and *Moraxella* cells after reaction with immunized sera and next with fluorescein conjugated goat anti-mouse IgG, A, M antibody were observed under VIS and UV light. Not all of treated cells were fluorescent; fluorescence appeared at about 60% of *Candida* cells and at over 90% of *Moraxella* cells. Most of them showed strong bright. Fluorescence of cells treated by irrelevant serum was very weak, and their number was much lower. Numbers of fluorescent cells were not depending on origins of antisera. Both sources of them gave similar results for *Moraxella* and for *Candida* cells as well.

Discussion

In this study we demonstrated that antigens of *Candida albicans* and *Moraxella catarrhalis* induced, in animal model, immune response by GALT-system (tables 1-3). Oral (mucosal) immunization of BALB/c mice was applied with positive results for administration of: ovoalbumin [15], heat-killed *Shigella flexneri* [16], cholera toxin and *Escherichia coli* enterotoxin [17]. *Helicobacter pylori* urease with cholera toxin as the adiuvant was used for oral immunisation of Swiss Webster mice [18]. Normal human sera (12 from patients with candidiasis and 4 from healthy individuals) when tested against antigens localized to the *C. albicans* cell wall, shared a positive reaction in all samples [19]. Normal human sera (groups of 40 healthy adults and 53 children) tested against *Moraxella* antigens almost always showed positive reaction [20, 21]. Serum samples of patients suspected of bronchopulmonary infection from whom *M. catarrhalis* was (38 individuals) and was not isolated (25 individuals) recognized OMPs of *M. catarrhalis* [3]. All these results support the idea that immune response on antigens (including *C. albicans* and *M. catarrhalis* cells) being in contact with animal/human mucose is a common phenomena.

Next, we demonstrated that antisera challenged by *C. albicans* heat killed cells recognized *M. catarrhalis* cellular antigens and on the other side, antisera challenged by *M. catarrhalis* heat killed cells reacted with *C. albicans* antigens (tables 1-3). Cross-reaction have been demonstrated among antigens of different origins [11-13]. Structures of polymers (polysaccharides, proteins) are the sources of antigenic (epitope) similarities [13, 22]. Possibilities of similarity between antigens of *Moraxella* strains and other bacteria were checked by immunoadsorption experiments [21, 3] and reactions with monoclonal antibodies [3]. Adsorption of *anti-Moraxella* specific sera by antigens of 17 bacteria

Table 2. Levels of specific antibodies against antigens of *Candida albicans* and *Moraxella catarrhalis* in selected mice sera

Immunogens of:	Routes of immunization (number of tested sera)	The range of the specific antibody titers for antigens of:	
		Candida albicans	*Moraxella catarrhalis*
Candida albicans fixed cells	intraperitoneal (4)	1/100-1/5000	1/100-1/1000
	oral (4)	1/100-1/1200	1/100-1/1000
Moraxella catarrhalis fixed cells	intraperitoneal (4)	1/100-1/1000	1/2000-1/100 000
	oral (4)	1/100-1/600	1/100-1/20 000

Table 3. Reactivity and cross-reactivity of selected mice sera with the surface of *Candida albicans* and *Moraxella catarrhalis* cells (immunofluorescence test)

Sera (number) of mice challenged by fixed cells of:	Percent of fluorescent cells of:	
	Candida albicans	***Moraxella catarrhalis***
Candida albicans (2)	60-75	90-95
Moraxella catarrhalis (2)	50-60	90-100
Irrelevant serum	4-10	5-10

species (*Streptococcus*, *Neisseria*, *Haemophilus*) indicated no antigenic cross-reactions [21, 3]. In contrast four *H. influensae* LPS-specific murine mAbs reacted with whole bacterial cells of *M. catarrhalis* [22]. We have not found information about immunological cross-reactiwity between used in our experiments antigens. On the other hand, structural similarities between polysasaccharides of both cell walls exist as they contain the same mono and oligosacchrides(mannans,glucans) [22, 5]. The dynamics of the antigen-antibody reaction process can also strongly influence on final results. This can occur when the antigen (e.g. mannans) has repeatable structure and posses big number of epitopes [23]. Availability of the epitopes can explain differencies in number of fluorescent cells of *C. albicans* and *M. catarrhalis*.

Concluding, *Candida* and *Moraxella* cells influence on human body both as the harmless and the harmfull microrganisms. Their existence elicit antibody production by the host. Specific antibodies and cells, products (OMPs, mannans, mannoproteins) may function as the immunomodulators [9, 10]. Demonstrated in this work cross-reactivity between antigens of tested in experiments microorganisms can be one of the explanation of the existing antibody specificities against antigens not present in host [3]. Finally, its necessary to take this possibility under consideration during analysis of immunodiagnostic tests used for *Candida* and *Moraxella* antibodies detection [5].

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