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• BRIEF REPORTS •

Intestinal colonization with *Candida albicans* and mucosal immunity

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Abstract

AIM: To observe the relationship between intestinal lumen colonization with *Candida albicans* and mucosal secretory IgA (sIgA).

METHODS: A total of 82 specific-pathogen-free mice were divided randomly into control and colonization groups. After *Candida albicans* were inoculated into specific-pathogen-free mice, the number of *Candida albicans* adhering to cecum and mucosal membrane was counted. The lymphocyte proliferation in Peyer's patch and in lamina propria was shown by BrdU incorporation, while mucosal sIgA (surface membrane) isotype switch in Peyer's patch was investigated. IgA plasma cells in lamina propria were observed by immunohistochemical staining. Specific IgA antibodies to *Candida albicans* were measured with ELISA.

RESULTS: From d 3 to d 14 after *Candida albicans* gavaging to mice, the number of *Candida albicans* colonizing in lumen and adhering to mucosal membrane was sharply reduced. *Candida albicans* translocation to mesenteric lymph nodes occurred at early time points following gavage administration and disappeared at later time points. Meanwhile, the content of specific IgA was increased obviously. Proliferation and differentiation of lymphocytes in lamina propria were also increased.

CONCLUSION: Lymphocytes in lamina propria play an important role in intestinal mucosal immunity of specific-pathogen-free mice when they are first inoculated with *Candida albicans*. The decreasing number of *Candida albicans* in intestine is related to the increased level of specific IgA antibodies in the intestinal mucus.

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INTRODUCTION

Candida albicans are the common opportunistic pathogens[1]; one of their infection routes is overgrowth and translocation in intestinal lumen. So, inhibition of the translocation of *Candida* is an important way to prevent the deadly systemic infection. With the development of the study on mucosal immunity, local antibody production of sIgA has attracted much attention in preventing pathogen[1] and bacterial translocation[2,3]. It has been reported that *Candida albicans* infection of vaginal and oral mucus membrane was specifically inhibited by anti-*Candida albicans* sIgA. But, the mechanism still remains unclear. In the present study, by using *Candidas*' colonization model, we observed lymphocytes proliferation and differentiation in gut-associated lymphatic tissue (GALT) and the relationship between specific IgA and change of *Candida albicans* in the intestine, and further explored the mechanism of host defense against opportunistic pathogen and the effect of specific IgA against *Candida albicans* in intestinal lumen.

MATERIALS AND METHODS

Candida albicans

Candida albican strain cmcc44104 provided by the Burn Institue of Southwest Hospital was amplified in the special selective culture medium. The *Candida albicans* suspension density was modulated to 1.5×10^9 cfu/mL, and stored below 4 ℃.

Grouping of animals

A total of 82 specific-pathogen-free mice (BALB/c) were provided by the Animal Center of Third Military Medical University, and randomly divided into the control and colonization groups. Mice in colonization group were gavaged 0.5 mL *Candida albicans*, and killed on days 3, 7 and 14 after gavage by cervical dislocation. The control animals were treated in a similar way with a vehicle alone, and killed on day 14 after sham-treatment. The mesenteric lymph nodes, cecum and ileum of the mice were taken out.

Candida albicans adhering to ileum

Ilea of 10 cm were rinsed in PBS (0.01 mol/L, pH 7.2) three times until the ilea were translucent, then weighed and homogenized. Homogenized suspension (0.1 mL) was applied on the surface of the selective culture medium at 37 ℃ for 72 h.

Adherence result (cfu/g)=Colony-forming units×dilution/Ileum weight (g).

Quantity of Candida albicans in cecum

Ceca were weighed and homogenized in 5 mL PBS, then the suspension was cultured in the selective medium at 37 ℃ for 72 h. *Candida albicans* quantity (cfu/g)=Colony-forming units×dilution/Cecum weight (g).

Translocation of Candida albicans

Mesenteric lymph nodes (MLN) were taken to be weighed and homogenized and the suspension was applied on the selective medium at 37 ℃ for 72 h.

Lymphocytes proliferation in Peyer's patch and lamina propria

Mice were intraperitoneally injected 5-bromo-2'-deoxyuridine

(BrdU, 10 μg/g bm) at 12 h before cervical dislocation, the intestine and Peyer's patch (PP) were taken for immunohistochemistry staining. BrdU-positive cells in PP and in lamina propria (LP) of intestinal villi were counted.

Number of IgA plasma cell in LP

IgA plasma cells were counted after immunohistochemical stain as 40 villi /per mice and 5 mice/per time-point.

Expression of IgA of Peyer's patch lymphocyte

Peyer's patch lymphocytes were isolated, pooled, washed in RPMI 1640. Then IgA of lymphocytes was measured by flow cytometry.

Specific IgA to Candida albicans in intestinal mucus

Intestinal mucus (0.1 mL) was homogenized in 0.5 mL cold PBS, then centrifuged at 5 000 r/min for 5 min, the supernatant was taken as 1:1 mucus onto 96-well plates and coated by *Candida albicans* as immobilized antigen, which had been fixed in 40 g/L formaldehyde overnight at 4 ℃ for 72 h. Then plates were washed three times with PBS, and blocked by 5 g/L BSA for 0.5 h, the mucus samples were applied to ELISA plates for 1 h below 37 ℃. After that, 96-well plates were washed with PBS, and goat anti-mouse IgA antibodies which coupled with horseradish-peroxidase were added to the wells, 100 μL/well and incubated at 37 ℃ for 1 h. Reaction was stopped by adding one drop of 2 mol/L H_2SO_4 and the result was shown by optical density (OD) at 492 nm.

Relative quantity of specific IgA[4]

The specific IgA positive mucus measured before were serially diluted from 1:1 to 1:16, the content of specific IgA to *Candida albicans* in 1:1 mucus was regarded as 1 U/mL. The mucus was applied to ELISA in order to produce a standard curve. Specific IgA activity to *Candida albicans* was counted as follows:

IgA(U/mg)=IgA relative quantity (U/mL)/Protein content in the mucus (mg/mL).

Statistic analysis

Data were analysed using analysis of variance (ANOVA).

RESULTS

Change of Candidas' adherence and translocation

In the colonization group, the total quantities of *Candida albicans* in intestine were larger on d 3 and 7 after gavage administration, about (34-39)×10^5 cfu/g, declined to 3.2×10^5 cfu/g on d 14. At the early phase after gavaging the mice, *Candida albicans* was found in the MLN, and then disappeared from day 7 to 14. Adherence also showed a declined tendency from the highest on day 3 to the lowest on d 14.

Proliferation of lymphocyte in PP and LP

BrdU incorporation of PP was found in both control and colonization group. BrdU-positive cells were mainly at the verge sites of PP; there were no obvious changes in the colonization group compared with that in the control group. On d 14 after gavaging, LP lymphocytes proliferation in colonization group was significantly higher than that in the control mice (Table 1).

IgA plasma cell in LP

IgA plasma cells increased at all times, the highest was on day 14 (Table 1).

Flow cytometry of mucosal sIgA

There was no difference between the control and the colonization group in the level of mucosal sIgA (Table 1).

Specific IgA to Candida albicans

Specific anti-*Candida albicans* IgA contents on day 14 in colonization group were higher than that in the control group (Table 2).

Table 2 Specific IgA to C*andida albicans* in intestinal mucus membrane

Group	*n*	Positive rate	Content (U/mg)
Colonization			
3 d	20	5/20	33±8
7 d	18	3/18	36±13
14 d	25	10/25	69±25[a]
Control	19	6/19	18.6±6.9

[a]*P*<0.05 *vs* control.

DISCUSSION

Candida albicans is an opportunistic pathogen, which could survive in the intestine and keep the balance of body. sIgA prevents the body from some pathogen infection such as typhoid fever and cholera[5,6], but its role in the balance between opportunistic pathogen and host is not clear yet[7,8]. So we investigated the relationship between *Candida albicans* colonization and the change of local intestinal mucosal immunity by using SPF mice model, in order to understand the mechanism of the balance. We have found that in the early period of *Candida albicans* colonization, *Candida albicans* adherence was serious, while the specific IgA content was lower. Accompanying the increase of specific IgA to *Candida albicans*, quantity of *Candida albicans* adherence and in the cecum decreased, there was negative correlation between specific IgA and quantity of *Candida albicans* adherence, that is to say that specific IgA was the important factor to keep the balance between opportunistic pathogen and host[9]. The colonization of *Candida albicans* could elicit a local mucosal immune reaction and finally limit *Candida albicans*' overgrowth[10,11].

Table 1 Proliferation and differentiation of lymphocytes

Group	Number of BrdU-positive cells sIgA in PP				Number of IgA plasma cells in LP
	n	PP	LP	Positive rates	
Colonization					
3 d	5	75±12	0.30±0.46	7.7±1.2	0.68±0.37
7 d	5	58±20	1.05±1.00	7.7±2.5	0.67±0.54
14 d	5	37±10	3.34±2.35[a]	ND	1.63±0.52[a]
Control	5	70±15	0.78±1.04	10.3±1.8	0.35±0.15

[a]*P*<0.05 *vs* control; PP: Peyer's patch; LP: Lamina propria; IgA plasma cells were counted after immunohistochemical stain as 40 villi/per mice and 5 mice/per time-point.

In the lamina propria, there was B lymphocyte clone that could secret specific IgA against *Candida albicans*[12], but the content was very lower. After *Candida albicans* were gavaged, stronger stimulation of *Candidas*' antigen induced specific B lymphocyte clone proliferation and differentiation, while the number of IgA plasma cell increased. Plenty of specific IgA against *Candida* secreted to the surface of intestinal mucus membrane and formed the antibody barrier. sIgA antibodies were thought to provide mucosal defense by immune exclusion[13]; this refers to their ability to prevent contact between pathogens and epithelial surfaces through agglutination in the intestinal lumen, entrapment of immune complexes in mucus, and clearance by peristalsis. We could draw the conclusion that though *Candida albicans* infection is more common recently, it is possible to prevent *Candida albicans* infection by setting up a specific sIgA antibody barrier in the host.

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