Detection of antibodies against *Candida albicans* ribosomes by the enzyme linked immunosorbent assay

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Abstract

Enzyme linked immunosorbent assay was found to be a convenient method for the investigation of antibodies in mice immunized with *Candida albicans* ribosomes. Antibodies against the ribosomal antigen were detected in all the sera of mice (ICR and BALB/c) immunized with ribosomes and incomplete Freund’s adjuvant and in some of the sera of mice immunized with ribosomes only; the titer of antibodies varied from 1:320 to 1:10 240. Vaccination of mice with ribosomal protein and IFA resulted in a high titer of antiribosomal antibodies. Treatment of ribosomes with pronase abrogated the capacity of the ribosomes to elicit anti ribosomal humoral responses, suggesting that the antibodies detected were directed against the protein moiety of the ribosomes. The presence of antibodies in sera of immunized mice could not be correlated with the protection afforded by the ribosomal vaccination.

Introduction

Host defense against candidiasis involves probably various innate and acquired mechanisms. The role of cell mediated immunity (CMI) is well established especially in chronic mucocutaneous candidiasis (4). The importance of antibodies is debatable (2, 10) but they may play some role in the resistance to systemic candidiasis.

We previously reported that vaccination of mice with *Candida albicans* ribosomes induced protective immunity against experimental systemic candidiasis (7). CMI responses were induced in animals immunized with ribosomes only or with ribosomes with incomplete Freund’s adjuvant (IFA) as detected by *in vitro* and *in vivo* assays (8).

Humoral immune responses of the immunized mice were investigated by the gel-immunodiffusion and immune adherence tests. Antibodies against *C. albicans* were detected only in sera of mice immunized with ribosomes and complete Freund’s adjuvant. No such antibodies could be found in sera of mice inoculated with ribosomes alone or with ribosomes emulsified with IFA (11). These findings pointed to the possibility that the tests employed for detection of antibodies were not sensitive enough.

The purpose of the present study was thus to use a sensitive technique such as the enzyme linked immunosorbent assay (ELISA) in order to determine whether anti ribosomal antibodies are produced by the immunized animals and whether the presence of such antibodies can be correlated with protection induced by the ribosomal vaccination.

Materials and methods

*Preparation of antigens and immunization of animals*

Preparation of ribosomes and their physical and chemical characteristics were previously described in detail (7). Briefly, *C. albicans* cells grown in liquid medium at 28 °C were disrupted mechanically using a Braun MSK Homogenizer and ribosomes were obtained by differential centrifugation. Ribo-
somes were quantitated by their protein content as determined by the Lowry reaction (9). Ribosomal protein was extracted from the ribosomes by a modified procedure of Fogel & Sypherd (3). ICR or BALB/c female mice were immunized subcutaneously by two inoculations (14 days apart) either of 100 µg ribosomes (with or without IFA) or with 100 µg of ribosomal protein emulsified with IFA. Additional groups of mice were inoculated with live (two inoculations of $6 \times 10^4$ live organisms/mouse) or killed (two inoculations of $5 \times 10^7$/mouse) *C. albicans* organisms.

In some experiments, mice were immunized with enzyme treated ribosomes, too. The ribosomes were incubated at 37 °C for 1 h with each enzyme (RNase, pronase or trypsin) in the ratio of 50 µg enzyme/mg of ribosomes (wet weight).

Mice were bled from the retro-orbital venous plexus 14 days after the booster inoculation; sera of all the mice in each group were pooled and stored in small aliquots at -70 °C until used. After the bleeding, mice were challenged intraperitoneally or intravenously with live *C. albicans* organisms and surveilled for 30 days to assess resistance against systemic experimental candidiasis.

**Enzyme linked immunosorbent assay (ELISA)**

The indirect ELISA used for determination of anti ribosomal antibodies was based on the method of Voller *et al.* (12).

The antigen – namely, *C. albicans* ribosomes – was diluted to the desired concentration in 0.1 M carbonate buffer (pH = 9.6) and adsorbed to disposable microtiter plates (Dynatech) – a volume of 100 µl was put into each well. Following an overnight incubation at room temperature and washing with saline containing 0.05% Tween 20, the plates were dried and stored at -70 °C until needed. Two-fold dilutions (starting at 1:40) of the sera, in 0.05 M phosphate buffered saline (pH =7.3) containing 0.05% Tween 20 and 0.1% BSA (PBS-T), were made and each well was filled with 100 µl of the diluted sera. Each test was carried out in duplicate. The plates were incubated overnight at 4 °C, washed and dried and then the wells were refilled with 100 µl of Alkaline Phosphatase anti-Mouse IgG (Miles, Yeda, Rechovot) diluted 1:100 with PBS-T. Following an incubation of 3 h at 37 °C, washing and drying, 100 µl of the phosphatase substrate (p-nitrophenyl phosphate disodium, Sigma,

![Fig. 1. ELISA test performed with sera of mice immunized with ribosomes as reflected by absorbance at 405 nm: (●—●) Sera of BALB/c mice immunized with ribosomes + IFA, (●—■) Sera of BALB/c mice injected with buffer + IFA, (■—■) Sera of ICR mice immunized with ribosomes + IFA, (○—□) Sera of ICR mice injected with buffer + IFA.](image-url)