

Detection of *Candida* Antigen in Sera of Patients with Candidiasis by an Enzyme-Linked Immunosorbent Assay-Inhibition Technique

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A total of 37 serum samples from 27 cancer patients were tested by an enzyme-linked immunosorbent assay-inhibition technique for the detection of *Candida* antigen. In 20 randomly chosen sera from patients without clinical evidence of candidiasis and in 10 sera from patients proven by autopsy not to have candidiasis, the inhibition ranged up to 17%; in contrast, inhibition ranged from 22 to 56% in all seven patients proven by autopsy to have systemic candidiasis, indicating the presence of *Candida* antigen in the sera of these patients. This technique appears promising in diagnosing disseminated candidiasis in cancer patients.

Systemic candidiasis is being encountered with increasing frequency in debilitated individuals such as cancer patients (3). Moreover, the antemortem diagnosis of systemic candidiasis in the immunosuppressed patient is very difficult, often delaying institution of potentially effective therapy. Circulating fungal antigens, presumably of polysaccharidic nature, are believed to be present during *Candida* infections (1, 2, 7). Thus, a technique for detection of such antigens could be a valuable tool permitting the early diagnosis of this fungal infection.

The enzyme-linked immunosorbent assay (ELISA) has been recognized in recent years as a rapid, accurate, and specific method for the detection of antibodies and antigens in various infectious diseases (5). A number of different microplate ELISA systems, including the indirect ELISA, the double-antibody-sandwich ELISA (6), and a blocking assay ELISA (8), have been developed. We report herewith preliminary results from an investigation on an ELISA-inhibition microplate technique for the detection of *Candida* antigen in the sera of cancer patients.

The ELISA-inhibition technique used in the present study is similar in principle to that described by Yolken et al. (8). A reaction mixture of a fixed amount of a rabbit anti-*C. albicans* antibody and the putative antigen-containing sample is transferred to microplates previously coated with *C. albicans* antigen. The unbound rabbit anti-*C. albicans* antibody of the reaction

mixture binds to the antigen on the coated microplates, and this is detected by an enzyme labeled anti-rabbit antibody and assayed spectrophotometrically by color development. Inhibition or blocking of the color development as compared to the control indicates the presence of antigen in the test sample. Since human sera may contain anti-*Candida* antibodies that could result in false negatives, all the sera were pretreated with NaOH (1 volume of 3 N NaOH to 5 volumes of serum; final NaOH concentration, 0.5 N) at 56°C for 2 h, followed by dialysis against phosphate-buffered saline (PBS; pH 7.4) for 24 h. Polyvinyl microplates (Cooke Laboratories, Alexandria, Va.) were coated with *C. albicans* mannan. Mannan was prepared from serotype A *C. albicans*, Hasenclever strain B 311, by the method of Peat (4). On Kjeldahl assay, the mannan contained 0.55% nitrogen by weight. Coating of the microplates was performed by a procedure adapted from Voller et al. (6); namely, wells were filled by using a multichannel pipette (Titertek, Flow Laboratories, Rockville, Md.) with 100 μ l/well of mannan (1,000 ng/ml) made up in a carbonate buffer (pH 9.6) and incubated overnight at 4°C. Before placement in the antigen-coated wells, the NaOH-treated and dialyzed sera were preincubated with whole rabbit anti-*Candida* serum which had been raised by intravenous injection of rabbits with *C. albicans* cells. The preincubation was performed in polystyrene microplates (Linbro Scientific Co., Hamden, Conn.), using PBS-0.05% Tween 20 as diluent (100 μ l of sample and 5 μ l of rabbit antibody in a previously determined concentration per well), and incubated at 4°C overnight.

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The reaction mixture was then transferred to antigen-coated, PBS-Tween-washed plates and incubated for 30 min at 37°C. The plates were shaken out and washed again with PBS-Tween, after which 100 µl/well of 1:1,000 horseradish peroxidase-labeled goat anti-rabbit immunoglobulin G (Miles-Yeda, Rehovot, Israel) was added, and the plates were incubated at 37°C for 60 min. The plates were again washed, and the wells were filled with an enzyme substrate solution (ortho-phenylenediamine and hydrogen peroxide in water). The reaction was terminated with 12 M sulfuric acid, and the color was measured at 488 nm by a spectrophotometer that directly measures the absorbance in the microplates (spectrophotometer designed by the Biomedical Engineering and Engineering Branch, National Institutes of Health). The ELISA sensitivity of each run was checked with mannan (concentration, 1,000 to 1 ng/ml; two-fold dilutions) dissolved both in buffer and in normal human pooled sera (GIBCO, Grand Island, N.Y.) by measuring inhibition of color development of the buffer-mannan and serum-mannan solutions versus the color of nonsupplemented buffer or serum. All samples were run in duplicate. The change in optical density was derived by subtracting the mean optical density of the specimen containing mannan from that of the appropriate control without mannan, calculated as percentage, and expressed as percentage inhibition (Fig. 1). The mannan in various runs

could be detected down to concentrations of 1 to 8 ng/ml when dissolved in buffer and 8 to 31 ng/ml in serum.

This system was used to test 37 stored sera collected from 27 cancer patients from the Pediatric Oncology Branch, National Cancer Institute (Tables 1 and 2). Group I (Table 1) consisted of randomly chosen paired sera (collected at least 2 weeks apart) from 10 patients. None of the patients were considered clinically to have systemic candidiasis. Routine surveillance cultures of nose, throat, and either stool or rectum within 1 week of the serum sample revealed *C. albicans* in 7 of 10 patients, but none of the patients in this group had evidence of systemic candidiasis. The inhibition of the group I sera ranged from no inhibition or even higher optical density values as compared to control, to 12% inhibition (e.g., 12% lower optical density values than the control) (Table 1). A second group of 17 patients (Table 2) was chosen and studied blindly. Seven had autopsy-documented systemic candidiasis, whereas the remaining 10 patients had no evidence of systemic candidiasis demonstrable on postmortem examination. In the group II sera (Table 2), four sera of the seven from patients with proven candidiasis had an inhibition of 40 to 56%, and the three other sera had an inhibition of 20 to 39%. Inhibition of the remaining 10 sera from patients without candidiasis ranged from negative to 17%. The inhibition percentages of the sera from the patients with autopsy-proven candidiasis could corre-

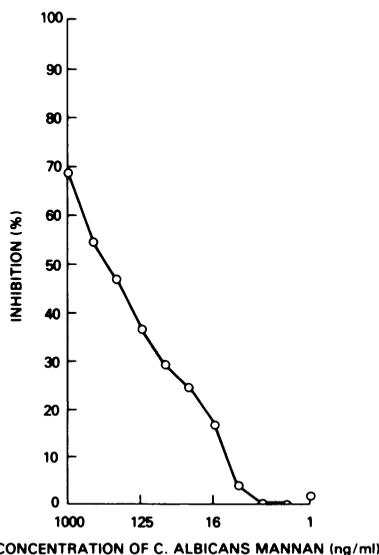


TABLE 2. ELISA-inhibition test in sera of autopsied cancer patients with and without disseminated candidiasis

| Patient no. | Underlying disease | Interval between serum sample and death (days) | Organ distribution of candidiasis at autopsy | ELISA test (% inhibition) |
|-------------|-------------------------------|--|---|---------------------------|
| 11 | Acute lymphocytic leukemia | 9 | Esophagus, ileum, spleen, kidney, lymph nodes, pancreas | 22.5 |
| 12 | Acute myelocytic leukemia | 11 | Lungs, esophagus, ileum, spleen, kidney, lymph nodes, pancreas | 22.2 |
| 13 | Burkitt's lymphoma | 10 | Esophagus, stomach, intestines, spleen, liver, bladder, heart, adrenals, kidneys, brain | 29.1 |
| 14 | Burkitt's lymphoma | 7 | Intestines, liver, spleen, kidney, lungs | 45.7 |
| 15 | Rhabdomyosarcoma | 5 | Stomach, kidney, spleen, liver, lungs, heart | 51.4 |
| 16 | Burkitt's lymphoma | 3 | Esophagus, liver, spleen, kidney, lungs, heart, thyroid | 45.1 |
| 17 | Lymphoma | 2 | Esophagus, stomach, intestines, peritoneum, bladder, heart, pericardium, lungs, pancreas, thyroid, skin | 55.8 |
| 18 | Osteosarcoma | 1 | None | 16 |
| 19 | Acute lymphocytic leukemia | 3 | None | 0 |
| 20 | Lymphoma | 2 | None | 11.8 |
| 21 | Burkitt's lymphoma | 3 | None | 17.3 |
| 22 | Acute lymphocytic leukemia | 1 | None | 0 |
| 23 | Acute myelomonocytic leukemia | 16 | None | 17 |
| 24 | Osteosarcoma | 7 | None | 15.5 |
| 25 | Neuroblastoma | 1 | None | 10.7 |
| 26 | Burkitt's lymphoma | 2 | None | 0 |
| 27 | Osteosarcoma | 4 | None | 0 |

spond to mannan concentrations of about 25 to 540 ng/ml (Fig. 1).

The correlation between the clinical diagnosis of systemic candidiasis and the ELISA-inhibition technique described here suggests that the method may have a diagnostic value. Further studies are necessary to define reliably the range of inhibition which is most indicative of systemic *Candida* infections.

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