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Probable Presence of β(1-2)-Linked Oligomannosides That Act as Human Immunoglobulin G3 Epitopes and Are Distributed over a *Candida albicans* 14- to 18-Kilodalton Antigen

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Kinetic analysis of candidosis patients' immunoglobulin G3 response has shown that reactivity towards β(1-2)-linked mannan-derived oligomannosides was associated with the recognition through metaperiodate-sensitive epitopes of a 14- to 18-kDa *Candida albicans* antigen unreactive with concanavalin A.

In a recent paper (8), we have provided evidence that reactivity of anti-*Candida albicans* monoclonal antibodies to neoglycolipids (NGL) constructed from *C. albicans* mannan acid-labile oligomannosides (NGLH) and corresponding to homopolymers of β(1-2)-linked mannopyranosyl units (4, 7) was correlated with reactivity against a 14- to 18-kDa *C. albicans* antigen. Conversely, monoclonal antibodies reacting preferentially with NGL constructed with oligomannosides released by acetolysis of oligomannoside H-depleted mannan (NGLA) reacted with polydispersed *C. albicans* high-molecular-weight mannoproteins (HMW MP). Using the methodology of NGL construction, we have also undertaken an analysis of the human antibody response against *C. albicans*-derived oligomannosides to define the molecular basis of the recognition of the mannan molecule by human immunoglobulins (3, 5). The exploration of the human isotypic response against NGLH and NGLA during infectious processes induced by *C. albicans* has recently demonstrated that kinetics of immunoglobulin G3 (IgG3) levels differed greatly, depending on the patients but also on the family of oligomannosides in use (2a). The purpose of this study was therefore to take advantage of this observation by selecting sera with different IgG3 levels against NGLH and NGLA to check the previously established correlated reactivity between NGLH and a 14- to 18-kDa antigen and between NGLA and HMW MP. Table 1 provides information on the sera used. The sera were drawn from patients, hospitalized in intensive care units, retrospectively selected for having presented postsurgery *C. albicans* infection in the absence of other conditions or treatments that would immunocompromise them. Candidosis was documented by (i) isolation of large quantities of *C. albicans* from normally sterile sites, (ii) clinical parameters, and (iii) availability of serial serum samples, near the dates of positive cultures, exhibiting a seroconversion against *C. albicans* antigens as determined by our routine serological diagnostic procedures, i.e., indirect immunofluorescence and co-counterimmunoelectrophoresis (6). Sera from these patients, diluted 1/100, were tested in an enzyme-linked immunosorbent assay (ELISA) against NGLA and NGLH (wells coated with 100 μl of a 2-μg/ml solution of neoantigen) according to a method previously described (8). A peroxidase-labelled monoclonal mouse anti-human IgG3 (Zymed; Biosoft, Paris, France) diluted 1/500 was used to reveal the binding of this IgG subclass to the NGL. This procedure led to the selection of seven serum samples from three patients (out of a group of eight patients) who exhibited strong differences in the kinetics of their IgG3 responses against at least one neoantigen

TABLE 1. Patient and serum information, culture data, and results of routine antibody testing and ELISA

Patient	Sex[a]/age (yr)	Underlying conditions/site of *C. albicans* isolation	Serum design/day of serum collection[b]	Serology by IFA[c]/ Co-CIE[d]	ELISA IgG3 reaction[e] with:	
					NGLH	NGLA
A	F/45	Thoracic traumatisms/blood culture	A1/−09	0/0	0.05 ± 0.012	0.059 ± 0.010
			A2/+14	1,600/1	0.317 ± 0.040	0.962 ± 0.050
			A3/+41	800/4	0.206 ± 0.016	0.417 ± 0.020
B	M/70	Gastric perforations/blood culture	B1/0	400/2	0.187 ± 0.030	0.73 ± 0.070
			B2/+07	800/4	0.450 ± 0.080	1.989 ± 0.100
C	M/71	Esophagostomy/peritoneal fluid	C1/−16	100/0	0.057 ± 0.015	0.087 ± 0.011
			C2/+26	1,600/4	0.065 ± 0.015	0.998 ± 0.080

[a] M, male; F, female.
[b] Indicated as days before (negative figures) or after the isolation of *C. albicans*.
[c] IFA, indirect immunofluorescence assay; reciprocal titer.
[d] Co-CIE, co-counterimmunoelectrophoresis. 0, absence of cospecific precipitin line; 1, faint cospecific precipitin line; 2, cospecific precipitin line; 4, very intense cospecific precipitin line.
[e] Expressed as the optical density ± standard deviation.

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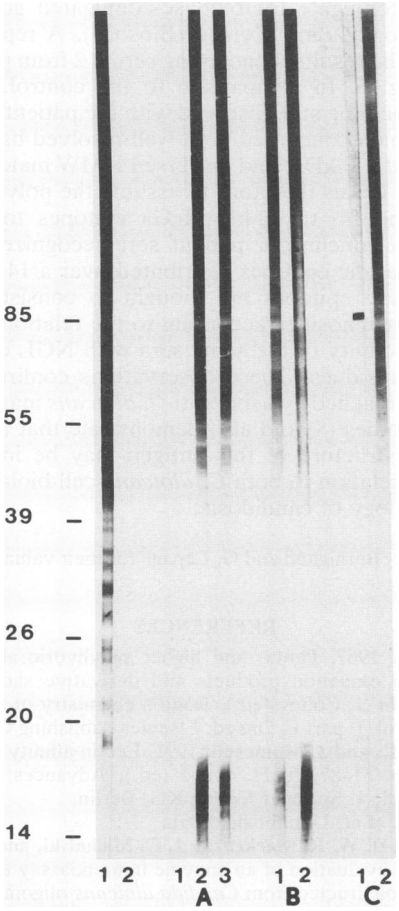

FIG. 1. Western blots of sodium dodecyl sulfate–5 to 15% polyacrylamide gels loaded with an extract of *C. albicans* germ tubes obtained by alkali extraction in reducing conditions. Nitrocellulose strips were probed as follows: lane 1, concanavalin A-horseradish peroxidase; lane 2, anti-human IgG3 (conjugate control); lanes A1 to A3, B1 and B2, and C1 and C2, successive sera from patients A, B, and C, respectively (as identified in Table 1), revealed by anti-human IgG3. Molecular weights (in thousands) are given on the left.

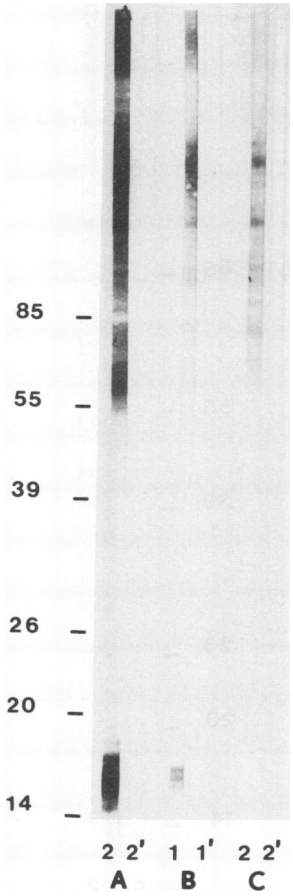

FIG. 2. Same blot as in Fig. 1. The conjugate consisted of anti-human IgG3. Shown are examples of couples of reactions concerning a serum sample from each patient. In comparison to controls, consisting of incubation in buffer at pH 5.5 (lanes A2, B1, and C2), metaperiodate oxidation prior to immunodetection resulted in complete inhibition of IgG3 binding to any antigen (lanes A2′, B1′, and C2′). Molecular weights (in thousands) are given on the left.

(Table 1). All patients (A, B, and C) showed a marked increase of their IgG3 antibody responses against NGLA; patients A and B showed a clear appearance of an IgG3 response against NGLH (followed by a slight decrease for the third serum sample [A3] from patient A), whereas patient C never showed any IgG3 reacting with NGLH during a 42-day period of candidosis serological survey (sera C1 and C2). The same sera, diluted 1/200, were tested in Western blots (immunoblots) against a *C. albicans* germ tube antigen prepared following an alkali extraction in reducing conditions (10) and probed with the same conjugate as used for ELISA (mouse monoclonal anti-human IgG3) diluted 1/200. The results (Fig. 1) showed a complete concordance between the ELISA signal on NGLH and the recognition of the 14- to 18-kDa antigen; furthermore, this concordance also concerned the recognition of HMW MP and ELISA signal on NGLA. Any evolution in the IgG3 level detected by ELISA on NGLH and NGLA for each of the sera correlated respectively with evolution in the recognition of the 14- to 18-kDa antigen and HMW MP in Western blots. These correlations led therefore to the following conclusions: (i) there was a presence of β(1-2)-linked oligomannosides (present in NGLH) acting as IgG3 epitopes and distributed over the 14- to 18-kDa antigen, (ii) there was an absence of detectable oligomannosides released by mannan acetolysis (present in NGLA) acting as IgG3 epitopes within the 14- to 18-kDa antigen, and (iii) there was a presence of oligomannosides released by mannan acetolysis, acting as IgG3 epitopes, distributed over HMW MP. These conclusions, reached by using human IgG3 synthethized during infection induced by *C. albicans*, are therefore in complete agreement with those previously reached by using another type of probe, i.e., a panel of monoclonal antibodies generated against *C. albicans* mannan (8). As most patient sera reacting with NGLA displayed on Western blots patterns similar to that obtained with concanavalin A, this observation also confirmed that the corresponding epitopes were shared by almost all MP present within the blot, at least for those that had relative molecular weights greater than 45,000. The fact that the 14- to 18-kDa antigen is not recognized by concanavalin A is another argument for the absence of accessible α-Man residues at this level (2). In order to nevertheless

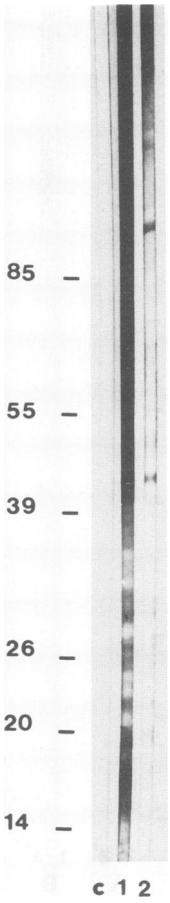

FIG. 3. Same blot as in Fig. 1. The conjugate consisted of total anti-human immunoglobulins. Lane C, conjugate control; lane 2, example of a reaction observed with serum B2. In comparison to the control incubated in buffer at pH 5.5 (lane 1), well-resolved bands and polydispersed HMW components can be still observed after metaperiodate oxidation. Molecular weights (in thousands) are given on the left.

demonstrate the polysaccharidic nature of the epitopes recognized by IgG3 within the 14- to 18-kDa antigen, we have performed Western blotting following treatment of the strips by periodic oxidation (9) (treatment for 20 min at room temperature with 10 mM sodium metaperiodate in 100 mM acetate buffer, pH 5.5). Controls consisted of strips incubated in buffer. This treatment resulted in a complete loss of reactivity against both HMW MP and the 14- to 18-kDa antigen for all patients, whatever the initial serological reactivity against these molecules was (Fig. 2). As β(1-2)-linked mannopyranosyl residues are sensitive to periodic oxidation (1), these results reinforce the hypothesis concerning the fact that these residues correspond to the human IgG3 epitopes distributed over the *C. albicans* 14- to 18-kDa antigen. However, as the treatment of blots by periodic oxidation resulted in a complete disappearance of reactivity for any of the sera tested, we had to check for the absence of artifactual denaturation (in the technical conditions in use) of nonglycosidic epitopes. Controls consisted of the revelation of periodic acid-treated blots with a total anti-human immunoglobulin conjugate (peroxidase-conjugated goat anti-human immunoglobulins [Zymed, Biosoft]). A representative example of the results, concerning serum 2 from patient B, is given in Fig. 3. In comparison to the control, a marked reactivity could be still observed with the patient's immunoglobulins which concerned both well-resolved bands (of 44, 120, 180, and 220 kDa) and dispersed HMW material. These experiments led us therefore to assume the polysaccharidic nature of the 14- to 18-kDa IgG3 epitopes to be highly probable. In conclusion, patient sera recognized, through IgG3, saccharidic epitopes distributed over a 14- to 18-kDa antigen. These epitopes are thought to consist of β(1-2)-linked oligomannosides according to the relations observed with the reactivity of the same sera with NGL constructed from these residues. These observations confirm previous conclusions reached by using anti-*C. albicans* mannan monoclonal antibodies (8) and also demonstrate that the elucidation of the structure of this antigen may be important to consider in relation to both *C. albicans* cell biology and the physiopathology of candidosis.

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