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

DANIEL POULAIN,* CHRISTINE FAILLE, CHRISTIAN DELAUNOY, PIERRE MARIE JACQUINOT, PIERRE ANDRÉ TRINEL, AND DANIEL CAMUS

Unité INSERM 42, domaine du CERTIA, 369 rue Jules Guesde, 59650 Villeneuve d'Ascq, France

Received 19 October 1992/Accepted 31 December 1992

Kinetic analysis of candidiasis patients' immunoglobulin G3 response has shown that reactivity towards β(1-2)-linked mannann-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 acetylosis of oligomannoside H-depleted mannan (NGLA) reacted with polydispersed C. albicans high-molecular-weight mannanproteins (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. Candidiasis was documented by (i) isolation of large quantities of C. albicans from normally sterile sites, (ii) clinical parameters, and (iii) availability of 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

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex/age (yr)</th>
<th>Underlying conditions/site of C. albicans isolation</th>
<th>Serum design/day of serum collection*</th>
<th>Serology by IFA/Co-CIE†</th>
<th>ELISA IgG3 reaction* with:</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>F/45</td>
<td>Thoracic traumasisms/blood culture</td>
<td>A1/−09</td>
<td>0.05 ± 0.012</td>
<td>0.059 ± 0.010</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A2/+14</td>
<td>0.317 ± 0.040</td>
<td>0.962 ± 0.050</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A3/+41</td>
<td>0.206 ± 0.016</td>
<td>0.417 ± 0.020</td>
</tr>
<tr>
<td>B</td>
<td>M/70</td>
<td>Gastric perforations/blood culture</td>
<td>B1/0</td>
<td>0.187 ± 0.030</td>
<td>0.73 ± 0.070</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B2/+07</td>
<td>0.450 ± 0.080</td>
<td>1.989 ± 0.100</td>
</tr>
<tr>
<td>C</td>
<td>M/71</td>
<td>Esophagostomy/peritoneal fluid</td>
<td>C1/−16</td>
<td>0.057 ± 0.015</td>
<td>0.087 ± 0.011</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C2/+26</td>
<td>0.065 ± 0.015</td>
<td>0.998 ± 0.080</td>
</tr>
</tbody>
</table>

* M, male; F, female.
† Indicated as days before (negative figures) or after the isolation of C. albicans.
‡ IFA, indirect immunofluorescence assay; reciprocal titer.
§ 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.
∥ Expressed as the optical density ± standard deviation.

* Corresponding author.
The results with NGLA.

(10) 

IgG3.

14-

18-kDa antigen; furthermore, 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 Cl 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 acetylation (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 acetylation, 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
noglobulin 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.

We thank A. Bernigaud and G. Lepage for their valuable technical assistance.

REFERENCES


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 immu-