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Anti-grifolan antibody reacts with the cell wall β-glucan and the extracellular mannoprotein-β-glucan complex of *C. albicans*

Michiharu Uchiyama, Naohito Ohno*, Noriko N. Miura, Yoshiyuki Adachi, Toshiro Yadomae

Laboratory for Immunopharmacology of Microbial Products, School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

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Abstract

We have recently prepared a rabbit antibody (Ab) against a fungal branched β-(1 → 3)-D-glucan, grifolan (GRN) obtained from *Grifola frondosa*. In this study, we examined the reactivity of *anti*-GRN Ab against a pathogenic fungus, *Candida albicans*. *Anti*-GRN Ab was strongly reacted with acetone dried, autoclaved, NaOH treated, as well as NaClO treated *C. albicans*, assessed by FACS. The binding was inhibited by GRN, a solubilized *Candida* spp. β(1 → 3)-D-glucan (CSBG), and a extracellular mannoprotein-β-glucan complex (CAWS). By ELISA analysis, binding affinity of *anti*-GRN Ab to GRN and CSBG was different. These facts strongly suggested that *anti*-GRN Ab reacted with the cell wall β-glucan in several ways. The Ab would be useful for the immunochemical diagnostic test of the deep-seated mycosis.

Keywords: β-Glucan; *Candida albicans*; Cell wall; Grifolan; *Anti*-β-glucan antibody; Diagnostic test

1. Introduction

Antitumor 6-branched β-(1 → 3)-D-glucan has been clinically used for cancer patients as a biological response modifier (BRM) (Hamuro, Takatsuki, Suga, Kikuchi & Suzuki, 1994; Hamuro, Kikuchi, Takatsuki & Suzuki, 1996; Kimura, Tojima, Fukase & Takeda, 1994; Matsuoka, Seo, Wakasugi, Saito & Tomoda, 1997). Optimization of the administration protocol of BRM is still needed to enhance therapeutic outcome. Monitoring concentration of the β-glucan is an important parameter for the above purpose. Grifolan (GRN) is an antitumor 6-branched β-(1 → 3)-D-glucan isolated from *Grifola frondosa* (Adachi, Suzuki, Ohno & Yadomae, 1998; Ohno, Suzuki, Oikawa, Sato, Miyazaki & Yadomae, 1984). We have recently prepared antibody (Ab) to GRN by immunizing albumin conjugated GRN to rabbits (*anti*-GRN Ab) (Adachi, Ohno & Yadomae, 1994; Adachi, Miura, Ohno, Tamura, Tanaka & Yadomae, 1999). The *anti*-GRN Ab reacted with 6-branched β-(1 → 3)-D-glucans but not with α-glucans and β-(1 → 6)-D-glucans. By using solubilized derivatives of linear β-(1 → 3)-D-glucan, such as hydroxyethylated- or carboxymethylated-curdlan, the *anti*-GRN Ab did not react with these glucans, suggesting the importance of branching point on the reactivity. By using this Ab, we have established a sandwich ELISA system to detect 6-branched β-(1 → 3)-D-glucans in various biological fluids. The Ab was also applicable to the immunohistochemical detection of 6-branched β-(1 → 3)-D-glucans.

Candida spp. is a medically important fungi, to induce a disseminated candidiasis and candidemia in hospitalized immuno-compromised patients. Cell wall of *Candida* is mainly composed of two polysaccharides, mannan and β-glucan. At least a part of β-glucan was basically insoluble in H_2O or NaOH. In contrast, the appearance of β-glucan in the blood is an indication of deep-seated fungal infections (Kawagoe, Nakao, Kanbe, Tamura, Tanaka & Takagi, 1998; Miyazaki, Kohno, Mitsutake, Maesaki, Tanaka, Ishikawa et al., 1995; Nakao, Kato, Kanbe, Tanaka, Tamura, Tanaka et al., 1994; Obayashi, Yoshida, Tamura, Aketagawa, Tanaka & Kawai, 1992; Obayashi, Yoshida, Mori, Goto, Yasuoka, Iwasaki et al., 1995; Yoshida, Roth, Grunfeld, Feingold & Levin, 1996). Limulus test is a well-established method for the diagnosis of both Gram (−) sepsis and invasive fungal infection (Nagi, Ohno, Adachi, Aketagawa, Tamura, Shibata et al., 1993). To diagnose deep-seated fungal infections, (1 → 3)-β-D-glucan-specific chromogenic kit (Fungitec G test MK) has been developed and applied clinically (Aketagawa, Tanaka, Tamura, Shibata & Saito, 1993; Mitsutake et al., 1996; Saito, Yoshioka, Uehara, Aketagawa, Tanaka & Shibata, 1991; Tamura, Arimoto,

* Corresponding author. Tel.: +81-426-76-5561; fax: +81-426-76-5561.
E-mail address: ohnonao@ps.toyaku.ac.jp (N. Ohno).

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Tanaka, Yoshida, Obayashi & Kawai, 1994; Tanaka, Aketagawa, Takahashi, Shibata, Tsumuraya & Hashimoto, 1993). We have prepared a water-soluble limulus factor G reactive substance, CAWS, from the cultured supernatant of *Candida* spp (Uchiyama, Ohno, Miura, Adachi, Aizawa, Tamura et al., 1999). By chemical, biochemical and immunochemical methodologies, CAWS was mainly composed of a mannoprotein-β-glucan complex, which resembles part of the yeast cell wall. We have also developed a simple protocol to obtain a solubilized *Candida* spp. β-glucan (CSBG) by sodium hypochlorite (NaClO) oxidation and subsequent dimethylsulfoxide (Me_2SO) extraction of acetone-dried whole cell preparations (Miura, Ohno, Adachi & Yadomae, 1996; Miura, Miura, Ohno, Adachi, Watanabe, Tamura et al., 1998; Ohno, Uchiyama, Tsuzuki, Tokuhaka, Miura & Adachi, 1999). CSBG was free from the cell-wall mannan, gave a symmetrical peak by gel filtration, and was soluble in dilute NaOH. By physical and chemical methodologies CSBG was found to be mainly composed of β-(1 → 3) and β-(1 → 6)-glucosidic linkages with small branches.

It is generally accepted that isolation and identification of a pathogenic fungi from the patient is important to the diagnosis of mycosis, however, cultivation of fungi is a time consuming method and has often failed to isolate the candidate. Molecular biological tests using the polymerase chain reaction was applied very recently, but still need modifications. Thus, there is still required to develop additional rapid and simple methodologies to detect fungal infections. During the immunochemical characterization of *C. albicans* we have found that *anti*-GRN Ab reacted with the cells of *C. albicans*. In this study, the epitope structures of the cell wall β-glucan responsible to the *anti*-GRN Ab were examined.

2. Materials and methods

2.1. Materials

C. albicans IFO1385 was purchased from the Institute for Fermentation, Osaka, maintained on Sabouraud agar (Difco, USA) at 25°C and transferred every 3 months. *C. albicans* (CA) was cultured by the synthetic medium as described previously (Uchiyama et al., 1999). Zymolyase 100 T was from Seikagaku Corp. (Tokyo). *Candida* water soluble polysaccharide fraction (CAWS) was prepared from *C. albicans* IFO1385 by a previously published procedure. (Uchiyama et al., 1999). Curdlan (CRD) was from Wako Pure Chemical Industries, LTD, Osaka. Grifolan (GRN) was prepared by fermentation of the mycelium of *G. frondosa* as described previously (Ohno, Adachi, Suzuki, Oikawa, Sato, Ohsawa et al., 1986b). Laminaran (LAM) was from Tokyo Kasei Kogyo CO, LTD, Tokyo. Small GRN (sGRN) and small curdlan (sCRD) were prepared by formolysis of the parent glucans. 3,3′,5,5′-Tetramethylbenzidine (TMB) microwell peroxidase substrate was from Kirkegaard and Perry Laboratories Inc, USA. The visking tube (MW cutoff; 3500) was from Spectrum Medical Industries, Inc.

2.2. Preparation of anti-GRN Ab

GRN was conjugated with bovine serum albumin (BSA) and the resulting complex was immunized into rabbits as described previously (Adachi et al., 1994, 1999). Briefly, GRN-BSA dissolved in phosphate buffered saline was emulsified with an equal volume of Freund's complete adjuvant and injected subcutaneously into Japanese white rabbits (500 μg/injection) every other week. Serum was collected at 42nd day after the first immunization. The GRN-specific Ab was purified by passing from a column of GRN-conjugated aminocellulofine affinity gel (Seikagaku Corp. Tokyo). The biotinylation of *anti*-GRN Ab was performed by mixing the Ab with NHS-LC-biotin (Pierce, Rockford, IL) in bicarbonate buffer (pH 8.4).

2.3. Preparation of autoclaved, NaOH treated, and NaClO treated Candida and CSBG

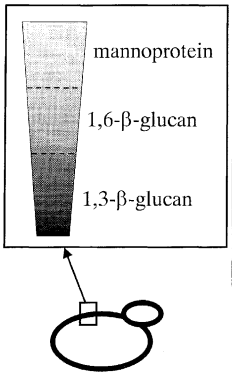
Preparation of the fractions of *C. albicans* was followed by the procedure used previously (Miura et al., 1996; Miura, Ohno, Miura, Adachi, Shimada & Yadomae, 1999; Ohno et al., 1999). Briefly, acetone-dried cells of *C. albicans* IFO1385 (2 g) were suspended in distilled water (1 l) and autoclaved for 1 h. After centrifugation, the precipitate was dried with ethanol and acetone (autoclaved cell). The acetone-dried cells of *C. albicans* (2 g) was suspended in 200 ml of 0.1 M NaOH and incubated for 1 day. After centrifugation, the precipitate was washed extensively with distilled water and then dried with ethanol and acetone (NaOH treated cell). The acetone-dried cells of *C. albicans* (2 g) was suspended in 200 ml of 0.1 M NaOH with 50 ml of NaClO solution for 1 day at 4°C. After the reaction was completed, the reaction mixture was centrifuged to collect the insoluble fraction. Insoluble fractions were dried by washing with ethanol and acetone (NaClO treated *Candida*, OX-CA). OX-CA suspended in dimethylsulfoxide was ultrasonically disrupted and the resulting supernatant was dried with ethanol and acetone (CSBG).

2.4. Zymolyase digestion of CSBG (OX-CAZNDF)

CSBG (20 mg) suspended in 10 ml of acetate buffer (50 mM, pH 6.0) was mixed with 1 mg of zymolyase 100T. After overnight incubation at 45° the reaction mixture was boiled 3 min to inactivate the enzyme. The reaction product was dialyzed (MW cutoff: 3500) with distilled water (3 $l \times 5$ times, 4°C, 2 days), and the reaction mixture was evaporated, then lyophilized.

2.5. ELISA for anti-GRN antibody

Immune plate (Nunc 442404, F96 Maxisorp) was used for all ELISA experiments in this study. Phosphate buffered



Outline of polysaccharide fractions used in this study

name	property	1)	2)	3)	4)
GRN		+			
sGRN	partially hydrolyzed GRN	+			
CSBG			+	+	
OX-CAZNDF	Zymolyase digested CSBG			+	
laminanan			+		
CRD			+		
sCRD	partially hydrolyzed CRD		+		
Lam7	laminaraheptaose		+		
CAWS			+/-	+	+

1) 6-branched (1→3)-β-glucan (monoglucosyl branch)
2) linear (or low branch) (1→3)-β-glucan
3) 3-branched (1→6)-β-glucan
4) mannoprotein

Fig. 1. Schematic representation of the structure of β-glucans used in this study.

saline containing 0.05% tween 20 (Wako Pure Chemical Co) (PBST) was used to wash plate (150 μl/each well). Carbonate buffer (pH 9.6, 0.1 M) was used to bind the antigen or antibody. The plate was blocked by 0.5% bovine serum albumin containing PBST (150 μl/each well). Soluble antigen was added with mouse sera.

2.6. Flow cytometory

C. albicans ($\sim 10^5$) was suspended in a staining buffer composed of 3% fetal bovine serum in PBS. Biotinylated *anti*-GRN Ab was added in the presence or absence of polysaccharide and incubated at room temperature for 30 min. After extensive washing with the staining buffer, streptoavidine-FITC (PharMingen, Becton Dickinson, Mountain View, CA) was added and incubated for additional 30 min. The resulting cell was suspended in 0.5 ml of the staining buffer and analyzed on a FACS Calibur cytofluorometer (Becton Dickinson).

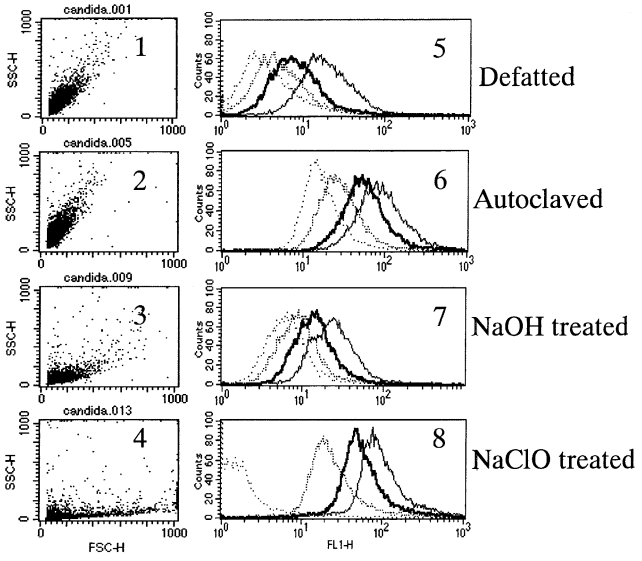


Fig. 2. Reactivity of *anti*-GRN Ab with *C. albicans* and derivatives assessed by flow cytometory.. A suspension of acetone dried cell (1, 5), autoclaved cell (2, 6), NaOH treated cell (3, 7), or NaClO treated cell (4, 8) were mixed with various concentration of biotinylated *anti*-GRN Ab (1, 1/2, 1/4 μl/assay). After 30min incubation at room temperature, the reaction mixture was washed and then reacted 30 min with streptoavidine-FITC. The resulting products were analyzed by FACS Calibur. 1 ~ 4, scattergram, 5 ~ 8, histogram of fluorescence.

3. Results

3.1. Anti-GRN Ab reacted with cells of C. albicans assessed by FACS

As mentioned in the introduction, we have found, during preliminary investigation, that *anti*-GRN Ab reacted with acetone-dried cells of *C. albicans*. To confirm the observation, reactivity of the *anti*-GRN Ab with the cells of *C. albicans* and its cell wall modified derivatives were examined by FACS. Acetone-dried cells, autoclave extracted residue, sodium hydroxide extracted residue, and sodium hypochlorite extracted residue were used. The ratio of cell wall mannan to β-glucan was decreased in this order, and the sodium hydroxide extracted residue contained mainly β-(1 → 6)-glucan and β-(1 → 3)-glucan moieties (Fig. 1). Sodium hypochlorite extracted residue contained mainly β-(1 → 3)-glucan with almost no nitrogen content (data not shown). A two-fold dilution of *anti*-GRN Ab was reacted with these cells and the bound Ab was detected by streptoavidine-FITC. The result was shown by the scattergram and the histogram of fluorescence intensity (Fig. 2). Fluorescence of the cells was increased dose dependently in all of the cell preparations. Reactivity of the Ab was rather stronger in the sodium hypochlorite extracted residue, suggesting the reactive sites were on the cell wall β-glucan of *C. albicans*. In addition, the Ab binding to the cell was competed by GRN and several polysaccharides, such as laminaran, and cell wall polysaccharides prepared from *C. albicans* (Fig. 3). Inhibitory activity of these polysaccharides were quantitatively compared using NaOH extracted residue and NaClO-treated residue (Fig. 4). Comparing relative binding, OXCAZNDF and CSBG strongly inhibited the Ab binding to the NaOH-residue. These polysaccharides containing 3-branched (1 → 6)-β-linked glucan segment. NMR analyses suggested removal of these segment during NaClO treatment of *C. albicans*. It is evident that after removal of these segments, epitopes, at least in part, still remained on the cell of *C. albicans*. These facts strongly suggested that the *anti*-GRN-Ab was specifically bound to the several regions of the cell wall of *C. albicans.*

3.2. Specificity of anti-GRN Ab assessed by ELISA

To clarify the specificity of *anti*-GRN Ab, reactivity of the Ab to various polysaccharides, especially prepared from *C. albicans*, was screened by ELISA. The structural feature of the polysaccharides used in this study are listed in Fig. 1. Fig. 5 shows the representative result of ELISA using the polysaccharide-coated plate. The strongest reaction was

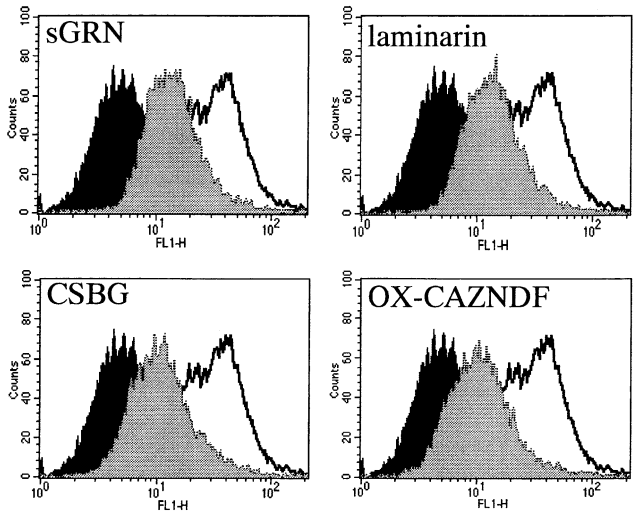


Fig. 3. Inhibition of *anti*-GRN Ab-*C. albicans* interaction by various polysacchriades. *Anti*-GRN Ab (1 μl/assay) was reacted with *C. albicans* (10 μg/assay) in the presence or absence of various polysaccharides (sGRN, laminaran, CSBG, and OX-CAZNDF, 8 μg/assay). The experiment was performed as described in legend of Fig. 1.

shown by the GRN-coated plate, but CSBG- or LAM-coated plates also showed significant reactivity. To further characterize the reactivity, soluble polysaccharides were added to the ELISA system. As shown in Fig. 6, the reactivity to GRN and CSBG were significantly inhibited by GRN and by CSBG, respectively. However, interestingly, the reactivity to GRN was only weakly inhibited by CSBG. Similarly, GRN weakly inhibited the reactivity to CSBG. These facts strongly suggested that *anti*-GRN Ab contains Ab showing reactivity to several different epitopes.

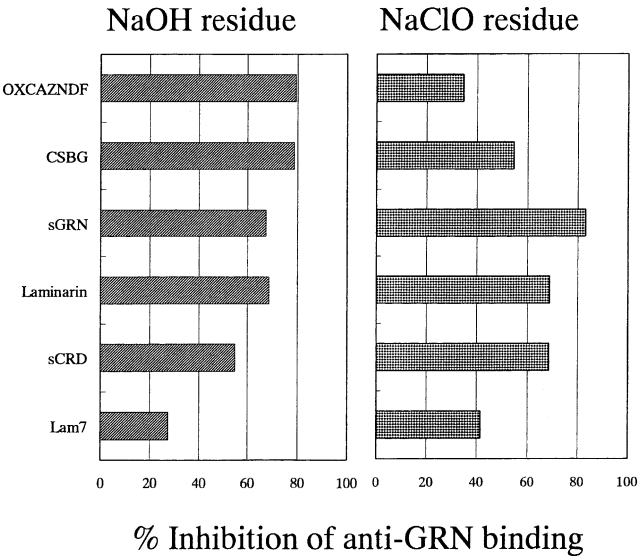


Fig. 4. Relative binding capacity of various polysaccharides to *anti*-GRN Ab assessed by NaOH treated and NaClO treated *C. albicans* using flow cytometory. *Anti*-GRN Ab (1 μl/assay) was reacted with *C. albicans* (10 μg/assay) in the presence or absence of various polysaccharides (8 μg/assay). The experiment was performed as described in legend of Fig. 2. % Inhibition was calculated by comparing geometoric mean value in the absence of soluble polysaccharide.

3.3. Extracellular polysaccharide of C. albicans, CAWS, reacts with anti-GRN-Ab

We have previously established a method for preparing the extracellular polysaccharide fraction, CAWS, of *C. albicans* by culturing it in a synthetic medium and subsequent ethanol precipitation (Uchiyama et al., 1999). CAWS was mainly composed of mannoprotein-β-glucan complex, and showing significant activity on the limulus G-test, specific for β-(1 → 3)-D-glucan. As shown in Fig. 5 CAWS did not strongly reacted with *anti*-GRN Ab. However, there is no doubt that the reactivity was strongly related to the concentration of plate-bound antigen. As shown above, the content of the β-glucan was relatively low, and this might be related to the lower reactivity shown in Fig. 5. To confirm the reactivity of CAWS to the *anti*-GRN-Ab, FACS analysis (Fig. 7) and indirect ELISA method (Fig. 8) were applied.

Fig. 7 shows inhibitory activity of CAWS on *anti*-GRN Ab to *C. albicans*. CAWS inhibited the Ab binding (Fig. 7, left), and the inhibition was dose dependent (Fig. 7, right). To know the binding specificity more precisely, the binding was compared by indirect ELISA using the polysaccharide bound plate. Inhibitory activity of CAWS to each bound antigen was compared with those of GRN. Significantly, CAWS strongly inhibited the binding of the Ab to CSBG and OX-CAZNDF. Both of the polysaccharides contained 3-branched β-1,6-glucan segment, suggesting the β-glucan segment in CAWS has binding capacity to the *anti*-GRN.

4. Discussion

β-Glucan preparations, especially the water-soluble form, are now being used for antitumor immunotherapy. We have long been working on analyzing the relationship between the structure and activity of β-(1 → 3)-glucans (Ohno, Miura, Adachi & Yadomae, 1997; Yadomae & Ohno, 1996) We started the projects using fungal β-(1 → 3)-glucans, such as GRN, SSG from *Sclerotinia sclerotiorum*, OL-2 from *Omphalia lapidescens*, and PVG from *Peziza vesiculosa* (Hashimoto, Suzuki & Yadomae, 1991; Hashimoto, Ohno, Adachi & Yadomae, 1997; Nemoto, Ohno, Saito, Adachi & Yadomae, 1993; Nemoto, Ohno, Saito, Adachi & Yadomae, 1994; Ohno, Suzuki & Yadomae, 1986a; Ohno, Saito, Nemoto, kaneko, Adachi, Nishijima et al., 1993; Ohno, miura, Chiba, Adachi & Yadomae, 1995; Ohno, Hashimoto, Adachi & Yadomae, 1996; Sakurai, Hashimoto, Suzuki, Ohno, Oikawa, Masuda et al., 1992; Suzuki, Ohno, Chiba, Miura, Adachi & Yadomae, 1996; Tsuzuki, Tateishi, Ohno, Adachi & Yadomae, 1999). We prepared and purified these glucans mainly from mushroom and their principal structure is 6-branched β-(1 → 3)-glucan, in which the branch is β-monoglucoside. To extend the project we attempted to establish monoclonal antibodies

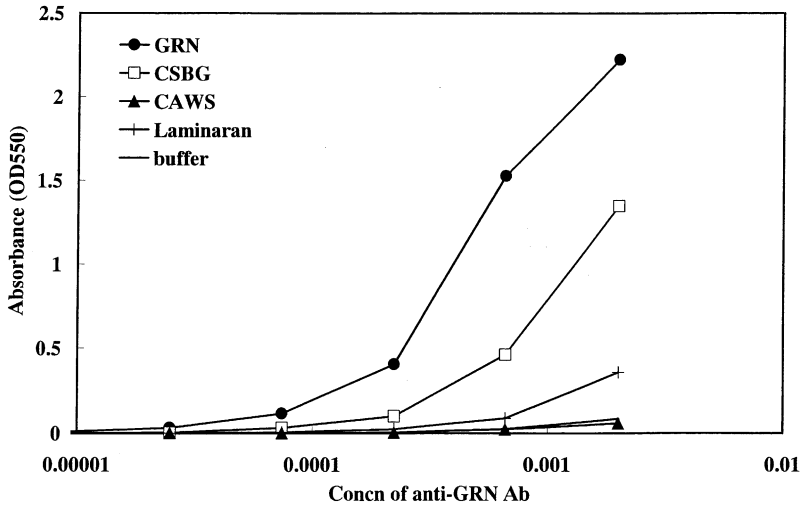


Fig. 5. Relative binding capacity of various polysaccharides to *anti*-GRN Ab assessed by polysaccharides coated ELISA plate. A ELISA plate was coated with indicated polysaccharides (25 μg/ml in carbonate buffer). After blocking and washing, *anti*-GRN Ab was serially diluted and added to the plate. Concentration of the plate bound Ab was determined by reacting with the peroxidase conjugated Streptoavidine. Color development was performed by the TMB substrate system.

to these polysaccharide antigens, however, none of the clones could be established either in mice or rats, even using adjuvants. On the contrary, highly immune sera were obtained in rabbits by albumin-conjugated GRN (Adachi et al., 1994). We also established a solubilization procedure for the yeast cell wall β-glucan (CSBG), and found that it contained a significant proportion of linear β-(1 → 6)-linked glucan segments in addition to a β-(1 → 3)-linked segment (Fig. 1), and that the primary structures of GRN and CSBG were significantly different (Ohno et al., 1999). Recently, we analyzed the Ab response of *C. albicans* immune mice to CSBG and found that a significant number of mice produced *anti*-CSBG Ab (Uchiyama, Ohno, Miura, Adachi, Tamura, Tanaka et al., 2000). In addition, these murine Abs scarcely reacted with GRN, suggesting the immunochemical independence between GRN and CSBG. However, the data shown in this study indicated that rabbit *anti*-GRN Ab strongly reacted with CSBG on the cell wall of *C. albicans*. The difference might be due to the immunological difference of animal species and the *anti*-GRN showed broader specificity.

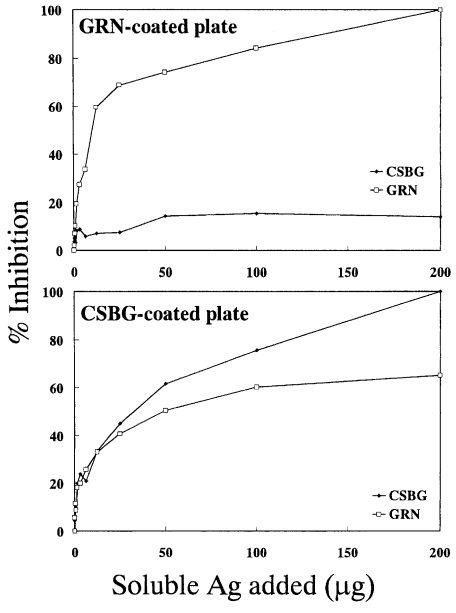


Fig. 6. Comparison of *anti*-GRN Ab to GRN-coated and CSBG-coated plates by competitive ELISA. Various concentration of GRN and CSBG were added to each well and performed ELISA as shown in Fig. 4.

Precise characterization of the microbial cell surface, including the bacterial peptidoglycan, viral envelope, and fungal cell wall, is significantly important from various point of view, such as the target for developing new medicine, analyzing immune reactivity and escape mechanisms, and obtaining basic mechanisms for the organization of the organelle. Immunochemical detection of the cell wall components is one general approach together with biochemical technique and microscopy. Comparing the *anti*-GRN Ab specificity between GRN and CSBG, the relative binding was significantly different, i.e. CSBG weakly inhibited binding of the Ab to GRN-coated plate. In addition, effective polysaccharides in inhibiting the Ab to *C. albicans* assessed by FACS were different, i.e. CSBG was a good inhibitor in sodium hydroxide treated cells but not in sodium hypochlorite treated cells. These facts strongly suggested that the Ab used several epitopes on the cell wall β-glucan of *C. albicans*. It is a quite surprising observation for us, because the *anti*-GRN Ab contained reactivity to structurally very different components, 6-branched β-(1 → 3)-glucan and 3-branched β-(1 → 6)-glucan segments. The physicochemical property of these glucans were generally different: the former one is insoluble in water or produced gel depending on the molecular weight and degree of branching (DB), and the latter is highly soluble in water.

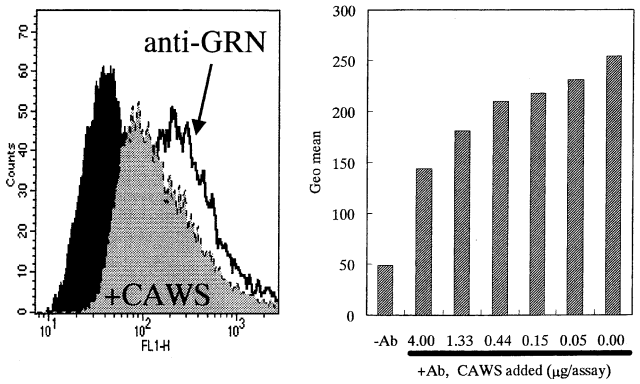


Fig. 7. Reactivity of CAWS to *anti*-GRN Ab assessed by flow cytometory (left). *Anti*-GRN Ab (1 μl/assay) was reacted with the NaClO oxidized *C. albicans* (10 μg/assay) in the presence or absence of CAWS (8 μg/assay). (right) Geometric mean channel of *C. albicans* reacted with *anti*-GRN Ab in the presence of various concentration of CAWS.

A common feature of these glucans are the presence of 3- and 6-linked branching point. In *C. albicans*, both of the segments are known to be present, but DB of the former one is lower than GRN. Considering these facts, the 3-branched β-(1 → 6)-glucan segment would largely contribute to the reactivity of *C. albicans* to the *anti*-GRN Ab.

The major cell wall polysaccharide of *C. albicans* are the mannan and the β-glucan. The mannan is linked to the β-glucan via the protein portion, localized in the outer part of the cell wall, and we have shown that at least a part of the mannoprotein-β-glucan complex was released into the culture medium during growth (CAWS). Patients with deep-seated mycosis often showed high concentration of β-glucan in the plasma. It can be detected by the limulus factor G test. The method has been applied and widely used for diagnosis, however, we think that the additional diagnostic tests are still needed to increase the accuracy. In the

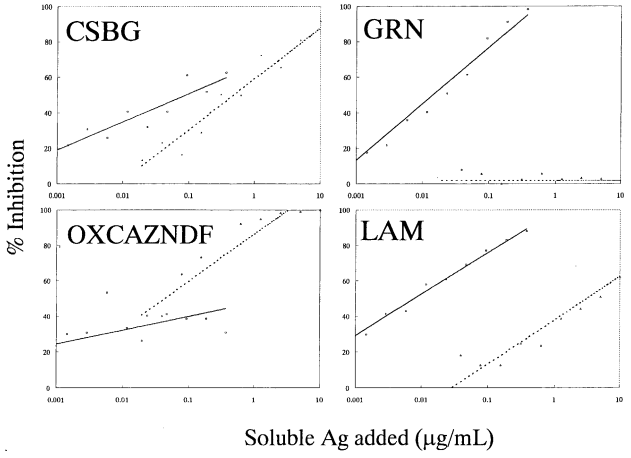


Fig. 8. Reactivity of CAWS to *anti*-GRN Ab assessed by competitive ELISA. ELISA plates were coated with various polysaccharides and then blocked. Various concentration of GRN (solid line) or CAWS (dotted line) were mixed with *anti*-GRN Ab (500 fold dilution) and then added on the ELISA plate. After incubation, bound Ab was determined by peroxidase conjugated Streptoavidine, and TMB substrate system.

glucan segment of CAWS, the ratio of the β-(1 → 6)-linked segment was significantly higher than the β-(1 → 3)-segment. The *anti*-GRN Ab might be reacted strongly with β-(1 → 6)-segment of CAWS. The clinically applied diagnostic test, limulus G-test, is known to react only with the β-(1 → 3)-linked glucan segment. These facts strongly suggested that the reactive site of *anti*-GRN Ab and limulus factor-G were diferent and thus Ab would be useful for the diagnostic test of deep-seated mycosis.

We tested a limited number of fungi in this study. It was surprising for us that the antibody established against β-(1 → 3)-glucan from mushroom strongly reacted with yeast. It is true that both mushroom and yeast contained β-(1 → 3)-glucan in the cell wall but single beta-linked glucose as the sidechain was only detected in mushroom. In contrast, yeast contained very long β-(1 → 6)-linked glucan as the sidechain. From this study, we discovered the complexity of the immuno-cross reactivity. The data indicated the specificity of the *anti*-GRN Ab is wider and usable as not for specific species but for group specific detection. Precise characterization of the Ab, especially the spectrum of the reactivity to various fungi, is under way.

β-Glucan is widely used as the biological response modifier and applied clinically for cancer patients. In contrast, some β-glucans are thought to be responsible for inflammatory respiratory diseases, such as farmer's lung and sick building syndrome (Fogelmark, Goto, Yuasa, Marchat & Rylander, 1992; Fogelmark, Sjostrand & Rylander, 1994; Rylander, Fogelmark, Mc William & Currie, 1999; Thorn, Beijer & Rylander, 1998). Standardization of β-glucan concentration in the environment might be important to reduce β-glucan mediated diseases. The *anti*-GRN Ab could also be used for this purpose.

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