Positive (1→3)-β-D-glucan in blood components and release of (1→3)-β-D-glucan from depth-type membrane filters for blood processing

Makoto Usami, Atsushi Ohata, Takeshi Horiuchi, Koichi Nagasawa, Toshio Wakabayashi, and Shigenori Tanaka

BACKGROUND: The false-positive elevation of plasma (1→3)-β-D-glucan level, a serodiagnostic test for deep-seated mycosis, is suspected in patients administered with blood components.

STUDY DESIGN AND METHODS: (1→3)-β-D-Glucan and endotoxin levels in blood components consisting of 12 albumins, 8 immunoglobulins, and 3 blood coagulation factors were measured by fungal infection tests (Fungitec G-test, Seikagaku Co.; the Wako WB003 test, Wako Pure Chemical Industries; and the Endospec ES test, Seikagaku Co.). In vitro release of (1→3)-β-D-glucan from the depth-type filters made by cellulose membrane to process blood components was analyzed through an in vitro filtration process as a source of (1→3)-β-D-glucan in blood components.

RESULTS: The amounts of (1→3)-β-D-glucan in blood components ranged from 0 to 7510 pg per mL in the Fungitec G-test, with wide variations among brands. The positive rates over 20 pg per mL were 75 percent in albumin solutions, 40 percent in blood coagulation factors, and 63 percent in immunoglobulin solutions. (1→3)-β-D-Glucan levels released from the five depth filters ranged from 5 to 2516 pg per mL. The (1→3)-β-D-glucan level in filtration fluid was decreased by rinsing with distilled water, but rebounded again during the albumin filtration process.

CONCLUSION: Depth filters are considered the source of (1→3)-β-D-glucan content in some blood components.

The fungal cell-wall contains the characteristic constituent (1→3)-β-D-glucan and recent studies have revealed that plasma (1→3)-β-D-glucan measurement is useful for screening for invasive fungal infections.[1,2] Accordingly, a fungal infection test (Fungitec G-test, Seikagaku Co., Tokyo, Japan) has been developed to measure plasma concentrations of (1→3)-β-D-glucan. The Fungitec G-test is a highly sensitive and specific test for invasive deep mycoses and fungal febrile episodes in causative fungi including *Candida, Aspergillus, Cryptococcus,* and *Trichosporon.*[2,3] There is one other approved method (Wako WB003, Wako Pure Chemical Industries, Osaka, Japan) to detect plasma (1→3)-β-D-glucan; however, Hossain et al.[3] indicated a lower sensitivity for the Wako WB003 test than the Fungitec G-test in systemic mycosis patients. A deep-seated mycosis is a critical disease condition in immunocompromised patients, including neutropenic patients with hematologic malignancies, patients with solid tumors undergoing chemotherapy or irradiation, and nonneutropenic patients in surgical intensive care units after organ transplantation, with burns, or with intravenous catheters.[4-6] Patients suspected to suffer from a secondary deep mycosis frequently require transfusion of various blood components: IVIG against systemic infectious complication, albumin or plasma protein fractions

From the Division of Surgical Metabolism, Faculty of Health Sciences, Kobe University School of Medicine, Kobe, Japan; Division of Life Science, Graduate School, and Department of Food Science, University of East Asia, Shimonoseki, Japan; SRL Inc., Tokyo, Japan; and Seikagaku Corporation, Tokyo, Japan.

Address reprint requests to: Makoto Usami, MD, PhD, Division of Surgical Metabolism, Faculty of Health Science, Kobe University School of Medicine, 7-10-2 Tomogaoka, Suma-ku, Kobe 654-01, Japan; e-mail: musa@ams.kobe-u.ac.jp.

Received for publication September 13, 2001; revision received March 5, 2002, and accepted April 8, 2002.

TRANSFUSION 2002;42:1189-1195.

against hypovolemia with hypoalbuminemia, and blood coagulation factors against bleeding diathesis.

False-positive elevation of plasma (1→3)-β-D-glucan levels in patients treated by blood components administration is suspected, but the amounts of (1→3)-β-D-glucan present in albumins and blood coagulation factors have not been reported. Ikemura et al.[7] have demonstrated a positive *Limulus* test in immunoglobulin components caused by *Limulus* amebocyte lysate-reactive material; that is, an indirect measurement method to detect (1→3)-β-D-glucan that had been tentatively used as the fungal index before development of the Fungitec G-test. They indicate higher *Limulus* amebocyte lysate-reactive material levels in patient plasma until 1 or 2 days after IVIG injections and suspected cellulose membrane as the source of *Limulus* amebocyte lysate-reactive material in immunoglobulin components. However, the Fungitec G-test is more sensitive than the fungal index, with the correlation between the Fungitec G-test and the fungal index being exponential.[8] It has been reported by Yamagami et al.[9] and Yoshioka et al.[10] that *Limulus* amebocyte lysate-reactive materials are released from a membrane dialyzer made with cuprophan. Recently, the elevation of serum (1→3)-β-D-glucan levels to over 100 pg per mL in cases treated by hemodialysis by use of a saponified cellulose acetate membrane has been reported by Yoshida et al.[11]

In this study, we analyzed (1→3)-β-D-glucan levels in commercially available blood components in Japan by use of the two approved and routinely used methods, the Fungitec G-test and the Wako WB003 test. Endotoxin levels were measured by an endotoxin-specific test (Endospec ES, Seikagaku Co.) in the same blood components to rule out *Limulus*-positive materials. We then hypothesized that depth-type filters made by cellulose membrane used to clarify blood components were the source of (1→3)-β-D-glucan in blood components, and so the release of (1→3)-β-D-glucan from depth-type filters was analyzed by the Fungitec G-test in an in vitro filtration process.

MATERIALS AND METHODS

Blood components

Albumin solutions, plasma protein fractions, blood coagulation factors, and globulin solutions commercially available in Japan were quantitatively analyzed for (1→3)-β-D-glucan levels (Table 1; names of manufacturers are indicated in the table footnotes). Components supplied in lyophilized form were reconstituted with pyrogen-free distilled water attached to the component as described in the package inserts.

Method of (1→3)-β-D-glucan and endotoxin measurement

The *Limulus* amebocyte lysate is composed of two coagulation pathways (Fig. 1). The first pathway contains

TABLE 1. Values of (1→3)-β-D-glucan and endotoxin in blood components*

Product name	Commercial name	Percentage	(1→3)-β-D-Glucan		Endotoxin
			Fungitec G-test	Wako WB003 test	Endospec ES test
Albumin solution	Albumin†	25	4080.0 ± 707.1	245.6 ± 142.5‡	9.6 ± 0.3
	Kenketsu albumin†	25	64.1 ± 0.6	353.8 ± 422.4	20.9 ± 10.8
	Albumin§	25	644.1 ± 772.1	71.5 ± 23.2‡	0.0 ± 0.0
	Albuminar¶	25	406.7 ± 1.0	25.2 ± 26.7‡	0.7 ± 0.9
	Buminate‖	25	65.5 ± 28.9	5.5 ± 0.0‡	0.6 ± 0.9
	Albumin**	25	17.0 ± 3.0	11.8 ± 0.0‡	0.0 ± 0.0
	Albumin††	20	4545.0 ± 49.5	231.8 ± 4.9	0.0 ± 0.0
	Albumin‡‡	20	3780.0 ± 4525.5	173.0 ± 215.5‡	18.3 ± 14.7
	Albumin§§	20	159.7 ± 1.6	44.1 ± 0.0‡	0.0 ± 0.0
	Buminate‖	5	13.0 ± 0.7	1.1 ± 0.0‡	0.0 ± 0.0
Plasma protein fraction	Plasmanate cutter**	4.4	1195 ± 91.9	8.1 ± 1.5	0.0 ± 0.0
	Plasma protein fraction‖	4.4	9.5 ± 2.3	0.5 ± 0.1‡	1.1 ± 1.0
Coagulation factor	Fibrogammin P‡‡		1725.0 ± 63.6	254.4 ± 100.5	5.3 ± 0.2
	Anthrobin P‡‡		1437.5 ± 1704.6	74.7 ± 92.6	0.0 ± 0.0
	Neuart†		0.0 ± 0.0	0.4 ± 0.0	0.0 ± 0.0
Globulin solution	Gamma-Venin‡‡		6930.0 ± 820.2	657.2 ± 47.7	2.0 ± 0.1
	Venilon§		560.0 ± 198.0	62.4 ± 20.0	1.2 ± 1.8
	Sanglopor¶¶		99.3 ± 2.1	40.1 ± 1.8	3.4 ± 0.3
	Kenketsu Venoglobulin-IH†		37.5 ± 6.0	22.0 ± 0.0‡	3.7 ± 1.2
	Venoglobulin-IH†		35.7 ± 24.3	22.0 ± 0.0‡	2.0 ± 2.7
	Gammagard‖		19.4 ± 19.6	22.0 ± 0.0‡	3.4 ± 3.0
	Globenin-I§§		2.4 ± 1.6	22.0 ± 0.0‡	3.1 ± 2.6
	Polyglobin**		1.2 ± 1.0	47.2 ± 0.0	7.1 ± 1.3

* Data are means ± SD (n = 2-4, pg/mL). †Yoshitomi Pharmaceutical Industries Ltd. Osaka (Mitsubishi Welpharma Co., Tokyo, at present), Japan; ‡Measured value less than the value was assigned to the value indicated; §Kaketsuken, Kumamoto, Japan; ¶Centeon, Tokyo, Japan; ‖Baxter Ltd, Tokyo, Japan; **Bayer Yakuhin, Ltd, Osaka, Japan; ††Japanese Red Cross, Tokyo, Japan; ‡‡Hoechst Marion Roussel (Aventis Pharma, at present) Ltd, Tokyo, Japan; §§Nihon Pharmaceutical Co., Ltd, Tokyo, Japan; ¶¶Fujirebio Inc. (UCB Japan Co., Ltd, at present), Tokyo, Japan.

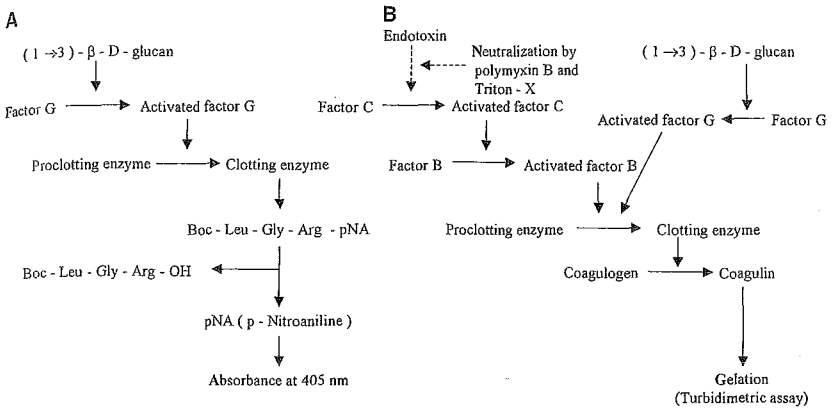

Fig. 1. Schematic diagram of the steps involved in the Fungitec G-test (A) and Wako WB003 test (B). The endotoxin-specific pathway in the Wako WB003 test can be inhibited by the pretreatment solution (polymyxin B and Triton X-100).

factor C, an initiation factor in the *Limulus* clotting system that is mediated by endotoxin, and factor B, a next step serine protease zymogen involved in the *Limulus* clotting system. The second pathway contains factor G, a horseshoe crab coagulation enzyme that is extremely sensitive and specific to (1→3)-β-D-glucan.[3] The Fungitec G-test is specific to (1→3)-β-D-glucan, because factors B and C are eliminated in the test. In brief, each sample (diluted to reach the measurable range) was added to factor G dissolved in HEPES buffer and then incubated at 37°C for 30 minutes. Activated factor G activated a proclotting enzyme, and then the OD of released p-nitroanilide cleaved from the chromogenic substrate by the clotting enzyme was measured by use of the kinetic model of a computerized well-reader (SK601, Seikagaku Co.). The recovery rate of (1→3)-β-D-glucan was calculated in each sample.

The Wako WB003 test is based on the endotoxin-neutralizing effect of the pretreatment solution, which consists of polymyxin B and Triton X, and the turbidimetric measurement of the *Limulus* amebocyte lysate reagent in a gelation reaction of factor G. Increased turbidity was measured by use of a kinetic tube reader (Toxinometer MT 251, Wako Pure Chemical Industries).[3]

The Endospec ES test (Seikagaku Co.) was performed to evaluate activation of factor C by endotoxin without factor G by use of a chromogenic assay the same as in the Fungitec G-test.[12]

Duplicate assays were performed on each sample of blood component, and the mean was used for the analysis.

Extraction of (1→3)-β-D-glucan in an in vitro filtration process of the depth filters

Four types of depth filters were used (Table 2, names of manufacturers are indicated in the table footnotes, gifts from the companies). In vitro filtration by use of 47-mm diameter sterilized depth membranes with filter holders at an ambient temperature and a 2.5 mL per minute flow rate by use of distilled water for intravenous injection (Otsuka distilled water for intravenous injection, Otsuka, Tokyo, Japan) was employed. The concentrations of (1→3)-β-D-glucan in 100 mL volume processed through each filter were measured by the Fungitec G-test and are shown in Table 2. (1→3)-β-D-Glucan levels at the point of each 25 of 100 mL distilled water processed and after 100 mL of 5 percent albumin solution (from Baxter Ltd) were also measured by the Fungitec G-test (Fig. 4).

Digestion with (1→3)-β-D-glucanase

To specify the type of glucans measured by the Fungitec G-test, and to rule out to any other type of glucans like (1→4)-β-D-glucan, in vitro digestion by use of (1→3)-β-D-glucanase as the specific degradation enzyme for (1→3)-β-D-glucan was performed. Five different samples of blood components and two different in vitro extracts from depth filters, both of which indicated high (1→3)-β-D-glucan levels, were prepared. Then (1→3)-β-D-glucan levels were compared before and after the digestion by the endo-(1→3)-β-D-glucanase prepared from *Arthrobacter luteus* by use of the method described by Tanaka et al.[13]

Statistical analysis

Data were expressed as means ± SD, and *t*-test, chi square test, regression analysis, and Sheffe's multiple

TABLE 2. Release of (1→3)-β-D-glucan from the depth filters in an in vitro filtration process*

Product name	(1→3)-β-D-Glucan (pg/mL)	Materials
Zeta Plus-SP†	2515.6 ± 560.2‡	Cellulose, perlite, resin, diatomaceous earth
Zeta Plus-LA†	131.7 ± 80.6	Highly purified cellulose, resin, acid-washed diatomaceous earth
EK1P§	23.1 ± 5.8	Reclaimed cellulose, diatomaceous earth
EKSP§	5.4 ± 1.5	Reclaimed cellulose, diatomaceous earth

* Data are means ± SD (n = 4-5, pg/mL). (1→3)-β-D-Glucan level was measured by the Fungitec G-test.
† K.K. Cuno, Yokohama, Japan.
‡ $p < 0.001$ versus the others (Sheffe's multiple comparison test).
§ U.S. Filter Japan Co., Tokyo, Japan.

comparison test were employed by use of computer software (Statview statistical software package, version 4.51.1; Abacus Concepts, Berkeley, CA). Statistical significance was assured when the p value was less than 0.05.

RESULTS

(1→3)-β-D-Glucan and endotoxin concentrations in blood components

(1→3)-β-D-Glucan and endotoxin concentrations in blood components are summarized as means ± SD in Table 1. (1→3)-β-D-Glucan concentrations measured by the Fungitec G-test ranged from 0 to 7510 pg per mL in one lot of human normal immunoglobulin (Gamma-Venin, Aventis Behring, Tokyo, Japan). The recovery rate of the measurement was 100.2 ± 8.2 percent. There existed a wide range of variation of (1→3)-β-D-glucan levels among components. The difference between lots of the same brand ranged from 0 to 962.5 pg per mL, 29.9 ± 250 pg per mL in all components, except from one albumin component that showed high variations in concentrations ranging from 580 to 6980 pg per mL (mean, 3780 pg/mL). The positive rates of (1→3)-β-D-glucan over 20 pg per mL, the cutoff value of a normal plasma level, were 33 of 50 (66.0%) indicating the numbers of the total components, 18 of 24 (75.0%) in albumin solutions and plasma protein fractions, 4 of 10 (40.0%) in blood coagulation factors, and 10 of 16 (62.5%) in immunoglobulin solutions (Fig. 2). The rates of (1→3)-β-D-glucan over 1000 pg per mL (a very high level; twice the mean plasma level in candidemia patients),[3] were 8 of 24 (33.35) in albumin solutions and plasma protein fractions, 4 of 10 (40.0%) in blood coagulation factors, and 2 of 16 (12.5%) in immunoglobulin solutions. For examples of blood coagulation factors, four Neuart samples and two of four Anthrobin P samples showed 0 pg per mL (1→3)-β-D-glucan, but the residual two of four Anthrobin P and the two Fibrogamin samples indicated over 1000 pg per mL (1→3)-β-D-glucan.

There was no significant difference in the positive rates among the type of blood components. Recently, the Japanese Red Cross Plasma Fraction Center has improved its manufacturing process and decreased the (1→3)-β-D-glucan level to 70 pg per mL, from 4645 pg per mL. This was done immediately after consideration of a report from us.

(1→3)-β-D-Glucan concentrations in blood components measured by the Wako WB003 test ranged from 0.4 to 690.9 pg per mL in one lot of Gamma-Venin (Table 1). There was a clear correlation between the (1→3)-β-D-glucan levels measured by the Fungitec G-test and those by the Wako WB003 test in all samples (Fig. 3): $Y = 33.473 + 0.0629X$, $R = 0.780$, $p = 1.1 \times 10^{-9}$, where Y is the (1→3)-β-D-glucan level measured by the Wako WB003 test (pg/mL) and X is the (1→3)-β-D-glucan level measured by the Fungitec G-test. However, the difference between the two methods in each of the blood components was not consistent. Results for some samples, such as Plasmanate cutter, indicated levels two orders higher when measured by the Fungitec G-test than when measured by the Wako WB003 test. However, results for some samples, such as Gammagard, indicated almost the same level with both tests and results for others, such as Kenketsu Albumin, indicated lower levels by the Fungitec G-test than by the Wako WB003 test.

The endotoxin concentration in blood components

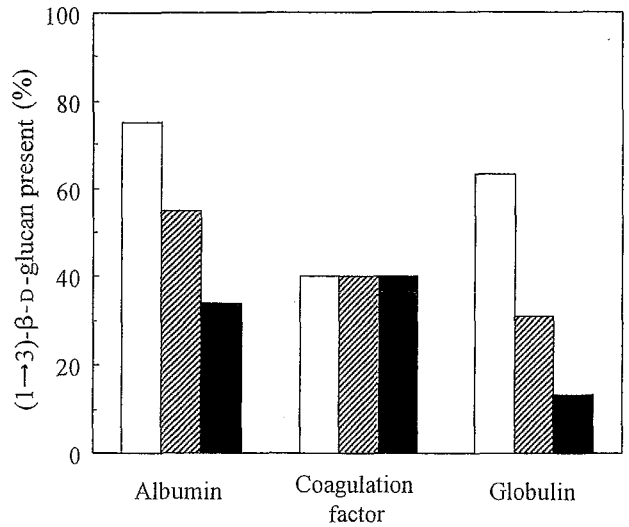

Fig. 2. Positive rates of (1→3)-β-D-glucan in blood components by the different cutoff values. (1→3)-β-D-Glucan level was measured by the Fungitec G-test.

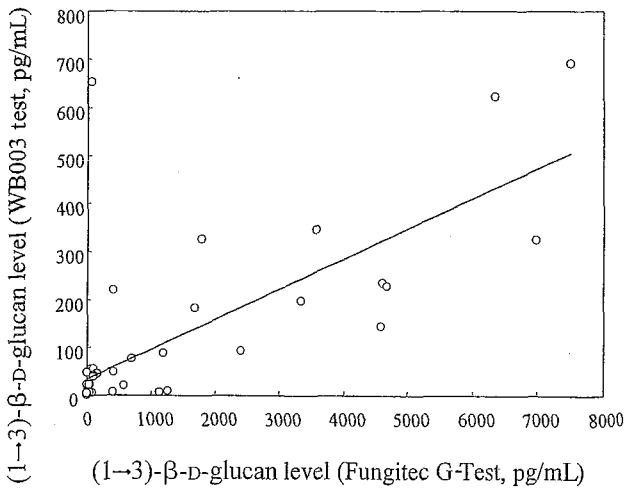

Fig. 3. Relationship between the concentrations of (1→3)-β-D-glucan measured by the Fungitec G-test and the Wako WB003 test in blood components. $Y = 33.473 + 0.0629X$, $R = 0.780$, $p < 0.001$.

ranged from 0 to 29 pg per mL and the positive rate over 10 pg per mL, the cutoff value of a normal plasma level, was 3 of 50 (6%). The recovery rate was 96.7 ± 6.6 percent. The correlation between (1→3)-β-D-glucan and endotoxin levels was low: Y = 1.938 + 0.001X, R = 0.403, p = 0.0037, where Y is the endotoxin level (pg/mL) and X is the (1→3)-β-D-glucan level (pg/mL) measured by the Fungitec G-test (Fig. 4). The endotoxin level was low in blood components and the increased (1→3)-β-D-glucan levels measured by the Fungitec G-test did not correlate with endotoxin levels.

(1→3)-β-D-Glucan levels released from the filters are shown in Table 2. In all filters, release of (1→3)-β-D-glucan was observed ranging from 5.4 to 2515.6 pg per mL during the same in vitro filtration process. (1→3)-β-D-Glucan levels from the Zeta Plus-SP filter were markedly higher than those from the Zeta Plus-LA filter ($p < 1.2 \times 10^{-11}$, Sheffe's multiple comparison). (1→3)-β-D-Glucan levels of each filter indicated differences of materials used, such as perlite and resin, and also indicated differences in the manufacturing process, even with use of the same cellulose and diatomaceous earth. Release of (1→3)-β-D-glucan in a filtration process by use of distilled water and then 5 percent albumin solution is shown in Fig. 5. The (1→3)-β-D-glucan level in the first 25 mL of water processed was 146.9 ± 23.0 pg per mL and decreased gradually to 14.2 ± 14.1 pg per mL in the last 25 mL during processing of 100 mL (p = 0.0070). The mean (1→3)-β-D-glucan level in 100 mL processed by use of distilled water was 37.6 ± 11.2 pg per mL indicated the effectiveness of decreasing (1→3)-β-D-glucan release from the filter by water rinsing. However, the (1→3)-β-D-glucan level rebounded up to 143.1 ± 22.7 pg per mL in the 100 mL processed by use of 5 percent albumin solution (p = 0.031).

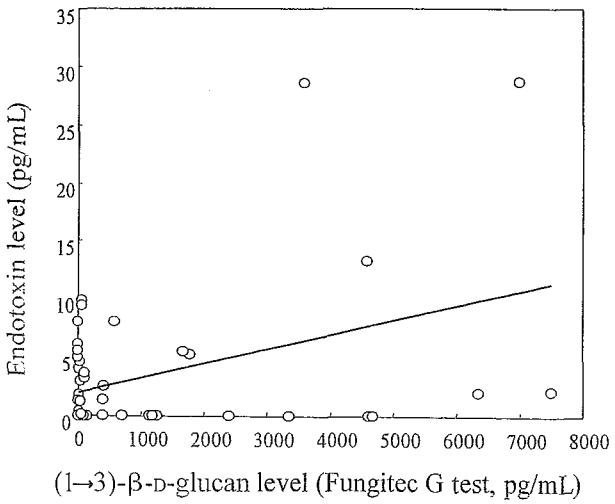

Fig. 4. Relationship between the endotoxin levels measured by the Endospec ES test and the (1→3)-β-D-glucan levels measured by the Fungitec G-test in blood components. Y = 1.938 + 0.001X, R = 0.403, p < 0.005.

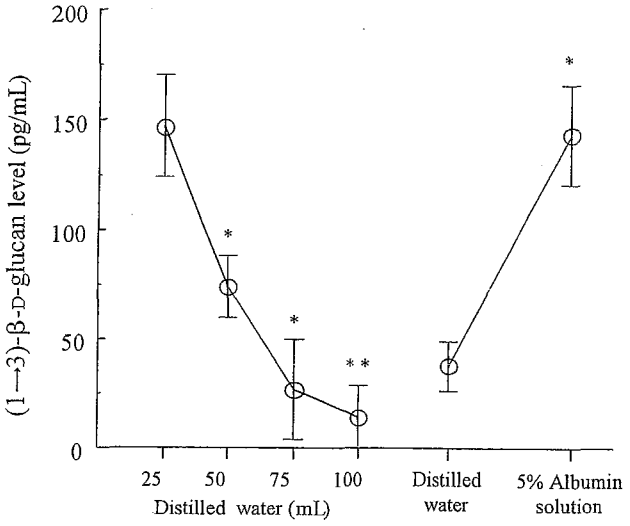

Fig. 5. Changes of (1→3)-β-D-glucan levels during in vitro filtration of the Zeta Plus-LA filter by use of distilled water and 5 percent albumin solution. Each 100 mL of distilled water and 5 percent albumin solution was processed at a 2.5 mL per min flow rate at an ambient temperature. The concentration of (1→3)-β-D-glucan was measured by the Fungitec G-test. *p < 0.05 and **p < 0.01 versus the level in 25 mL processed or the mean level in distilled water statistically analyzed by t-test.

Seven samples indicating high (1→3)-β-D-glucan levels, 4993, 593, 4992, 8536, and 6526 pg per mL of blood components or 37,633 and 4558 pg per mL of filtrates from depth filters, were digested by (1→3)-β-D-glucanase, and thereafter no (1→3)-β-D-glucan activity was observed.

DISCUSSION

The present study clearly demonstrates (1→3)-β-D-glucan content in blood components through the use of depth filters.

Quantitative analysis by specific measurement methods revealed that 66 percent of blood components contain (1→3)-β-D-glucan and that its levels in some blood components are extremely high. The highest (1→3)-β-D-glucan level of 7510 pg per mL is 15 times greater than that of the mean plasma level, 494.7 ± 448.4 pg per mL, as measured by the Fungitec G-test in candidemia patients reported by Hossain et al.[3] Trace levels of endotoxin in the same blood components indicate the specific contamination of (1→3)-β-D-glucan in blood components. The complete reduction of high (1→3)-β-D-glucan levels by endo-(1→3)-β-D-glucanase also certifies that the material in the blood components responsive to the Fungitec G-test is (1→3)-β-D-glucan itself and not any

other material that might increase the number of false-positive results. The wide range of variations in (1→3)-β-D-glucan levels among the different brands of the same blood components and the definitive improvement in decreasing the (1→3)-β-D-glucan level in the albumin solution by changing the manufacturing process achieved in the Japanese Red Cross Plasma Fraction Center clearly indicate that the presence of (1→3)-β-D-glucan in blood components can be avoided. The information we obtained from the Japanese Red Cross Fractionation Center was limited. However, it is suspected that they changed the kind of depth filters in the three steps of the blood purification process, decreasing their (1→3)-β-D-glucan level in albumin solution from 4645 to 70 pg per mL.

The appearance of (1→3)-β-D-glucan in immunoglobulin components shown in this study is comparable to the reports of Ikemura et al.[7] However, there is some difference between the values of (1→3)-β-D-glucan measured with the Fungitec G-test in this study and those of the *Limulus*-reactive material in the reports of Ikemura et al. that were tentatively used as a fungal index before development of the Fungitec G-test. The Fungitec G-test is a direct method that detects (1→3)-β-D-glucan by use of a (1→3)-β-D-glucan-sensitive component, factor G, fractionated from *Limulus* amebocyte lysate. The level of (1→3)-β-D-glucan measured by the Fungitec G-test in our study indicates 307 percent in Gamma-Venin, 2 percent in Venoglobulin, 68 percent in Venilon, 1 percent in Polyglobin, and 13 percent in Glovenin of fungal index levels noted in the report of Ikemura et al. The discrepancy is in accordance with the study of Miyazaki et al., which indicated that the G-test showed a higher sensitivity than the fungal index in a rabbit model of systemic candidiasis.[8]

False-positive elevation of plasma (1→3)-β-D-glucan levels in patients who are intravenously injected with blood components containing (1→3)-β-D-glucan is highly suspected from our results and from the report of Ikemura et al.[7] The plasma (1→3)-β-D-glucan level might reach 300 pg per mL after an intravenous injection of 10 g Gamma-Venin, because the plasma *Limulus*-reactive material level reported is over 100 pg per mL and the ratio of the (1→3)-β-D-glucan level in the Gamma-Venin measured by the Fungitec G-test in this study was three times greater than that of the *Limulus*-reactive material indicated in the previous study. The level would be high enough to lead to a misdiagnosis of deep mycosis.

Comparison between the Wako WB003 test and the Fungitec G-test for the detection of (1→3)-β-D-glucan in blood components indicates that data obtained by the Wako WB003 test are one-eighth of those by the Fungitec G-test, even with a high correlation between the two methods. Hossain et al. reported a one order smaller result by the Wako WB003 test than by the Fungitec G-test in deep candidiasis patients' plasma and the high sensitivity of the Fungitec G-test in detecting plasma (1→3)-β-D-glucan in mycosis patients and rabbits with systemic candidiasis.[3] They suggest that the discrepancy comes from the difference of the sensitivity of the measurements and the standard materials used in the two methods.[3] Also, a higher sensitivity of the Fungitec G-test than the Wako WB003 test from its ability to detect a single helical form of (1→3)-β-D-glucan is reported by Aketagawa et al.[14] The Fungitec G-test has been used in Japan since 1995, and the Wako WB003 test since 1997. A recent result of questionnaire in Japan Adult Leukemia Study Group indicates that over 70 percent of hospitals use the Fungitec G-test as a diagnostic tool for deep-seated fungal infection to measure plasma (1→3)-β-D-glucan level due to its high sensitivity. Both assays are not available in the US yet.

The in vitro filtration experiment reveals that high levels of the Fungitec G-test-positive materials, specified as (1→3)-β-D-glucan by the (1→3)-β-D-glucanase digestion experiment, are released from the depth filters used in blood clarification processes as the origin of (1→3)-β-D-glucan in the blood components. The great differences in (1→3)-β-D-glucan levels in different brands of both the blood components and the depth filters demonstrate the importance of selecting both a suitable depth filter and a suitable fractionation process to manufacture blood components so that (1→3)-β-D-glucan levels are minimized in blood components. More detailed in vitro experiments to trace the filtration conditions, the quantity of filtrate fluids, and/or the rinsing process on the release of (1→3)-β-D-glucan are now being evaluated. The release of *Limulus*-reactive material from a cuprophan membrane for hemodialysis[9,10] and a recent report indicating elevation of serum (1→3)-β-D-glucan level in hemodialysis patients by use of a saponified cellulose acetate membrane[11] suggest the elution of (1→3)-β-D-glucan from cellulose material. The main structure of cellulose is (1→4)-β-D-glucan and the mechanism of (1→3)-β-D-glucan elution from cellulose membrane should be analyzed, together with the development of a membrane filter giving a lesser release of (1→3)-β-D-glucan in future studies.[15] Sources other than filter membranes are not completely excluded because there is no published data to analyze the (1→3)-β-D-glucan level in each process in the manufacture of blood components.

Biologic effects of intravenous injection of (1→3)-β-D-glucan in patients is diverse. Inactivation of (1→3)-β-D-glucan by plasma and serum protein is reported by Miura et al.[16] Moreover, several soluble derivatives of (1→3)-β-D-glucan, especially β-(1→6)-branched (1→3)-β-D-glucans (i.e., lentinan, sizofilan, and grifolan) that show potent immunomodulatory activity, have been developed and are used in patients with cancer or sepsis.[17] However, (1→3)-β-D-glucan remains in the liver and spleen for a long time (more than 1 month) without major structural changes because of the lack of a specific

metabolic pathway of (1→3)-β-D-glucanase in humans.[16] The biologic activity of (1→3)-β-D-glucan released from cellulose membrane has not been evaluated and reported. We are at present evaluating these effects and have found that a filtration fluid indicated some biologic activity of proinflammatory cytokine production from macrophages and lymphocytes in an in vitro condition (manuscript in preparation).

In summary, by use of the specific methods to measure (1→3)-β-D-glucan we demonstrated that some blood components, and filtration fluids from depth filters used for blood processing, contain (1→3)-β-D-glucan. The mechanism of the release of (1→3)-β-D-glucan from depth filters and the biologic activity of these materials needs to be evaluated in future studies.

ACKNOWLEDGMENTS

We are grateful to the companies listed in Tables 1 and 2 for providing gifts of the blood components and the depth filters.

REFERENCES

1. Obayashi T, Yoshida M, Mori T, et al. Plasma (1→3)-β-D-glucan measurement in diagnosis of invasive deep mycosis and fungal febrile episodes. Lancet 1995;345:17-20.
2. Kohno S, Mitsutake K, Maesaki S, et al. An evaluation of serodiagnostic tests in patients with candidemia: (1→3)-β-D-glucan, mannan, candida antigen by Cand-Tec, and D-arabinitol. Microbiol Immunol 1993;37:207-12.
3. Hossain MA, Miyazaki T, Mitsutake K, et al. Comparison between Wako-WB003 and Fungitec G tests for detection of (1→3)-β-D-glucan in systemic mycosis. J Clin Lab Anal 1997;11:73-7.
4. Petri MG, Konig J, Moecke HP, et al. Epidemiology of invasive mycosis in ICU patients: a prospective multicenter study in 435 non-neutropenic patients. Paul-Ehrlich Society for Chemotherapy, Division of Mycology and Pneumonia Research. Intens Care Med 1997;23:317-25.
5. Nassoura Z, Ivatury RR, Simon RJ, Jabbour N, Stahl WM. Candiduria as an early marker of disseminated infection in critically ill surgical patients: the role of fluconazole therapy. J Trauma 1993;35:290-5.
6. Inoue Y, Kohno S, Fujii T, et al. Clinical evaluation of catheter-related fungemia and bacteremia. Intern Med 1995;34:485-90.
7. Ikemura K, Ikegami K, Shimazu T, Yoshioka T, Sugimoto T. False-positive result in *Limulus* test caused by *Limulus* amebocyte lysate-reactive material in immunoglobulin products. J Clin Microbiol 1989;27:1965-8.
8. Miyazaki T, Kohno S, Koga H, et al. G test, a new direct method for diagnosis of Candida infection: comparison with assay for beta-glucan and mannan antigen in a rabbit model of systemic candidiasis. J Clin Lab Anal 1992;6:315-8.
9. Yamagami S, Yoshihara H, Kishimoto T, et al. Cuprophan membrane induces interleukin-1 activity. Trans Am Soc Artif Intern Organs 1986;32:98-101.
10. Yoshioka T, Ikegami K, Ikemura K, et al. A study on *Limulus* amebocyte lysate (LAL) reactive material derived from dialyzers. Jpn J Surg 1989;19:38-41.
11. Yoshida K, Nakajima M, Yamasaki M, et al. Investigation of influence of hemodialysis membranes and factors associated with hemodialysis on serum (1→3)-β-D-glucan [in Japanese]. Jpn J Infect 1998;72:245-8.
12. Obayashi T, Tamura H, Tanaka S, et al. A new chromogenic endotoxin-specific assay using recombined limulus coagulation enzymes and its clinical applications. Clin Chim Acta 1985;149:55-65.
13. Tanaka S, Aketagawa J, Takahashi S, Shibata Y. Activation of a limulus coagulation factor G by (1→3)-β-D-glucans. Carbohydr Res 1991;218:167-74.
14. Aketagawa J, Tanaka S, Tamura H, Shibata Y, Saito H. Activation of limulus coagulation factor G by several (1→3)-β-D-glucans: comparison of the potency of glucans with identical degree of polymerization but different conformations. J Biochem 1993;113:683-6.
15. Stone BA, Clarke AE. Structural characterization and physical chemistry of (1→3)-β-glucans. In: Stone BA, Clarke AE, editors. Chemistry and biology of (1→3)-β-D-glucans. Melbourne, Australia: La Trobe University Press; 1992. p. 1-116.
16. Miura T, Ohno N, Miura NN, Shimada S, Yadomae T. Inactivation of a particle β-glucan by proteins in plasma and serum. Biol Pharm Bull 1997;20:1103-7.
17. Williams DL, Mueller A, Browder W. Glucan-based macrophage stimulators: A review of their anti-infective potential. Clin Immunother 1996;5:392-9.