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Release of (1→3)-β-D-Glucan from Depth-type Membrane Filters and Their In Vitro Effects on Proinflammatory Cytokine Production

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Abstract: To clarify the origin of (1→3)-β-D-glucan in blood products and assess the biological activity of filter extracts, we evaluated (1→3)-β-D-glucan extraction from depth filters used to process blood products and their in vitro effects on proinflammatory cytokine production from macrophages. Cellulose or nylon filters were analyzed for (1→3)-β-D-glucan using the Fungitec G test. To evaluate the biological activity of the filter extracts, Mono Mac 6 cells (a human macrophage cell line) were cultured with filter extracts with or without lipopolysaccharide, and tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) concentrations in the culture media were measured. (1→3)-β-D-Glucan was released from seven cellulose filters but the nylon filter level was undetectable. Proinflammatory cytokine production ranged from 74.3% to 119.0% of the control for TNF-α and 81.2% to 115.9% for IL-1β. TNF-α and IL-1β levels were low without lipopolysaccharide. The data indicate that (1→3)-β-D-glucan in blood products is contaminated with the depth filters and that these filter extracts modulate proinflammatory cytokine production from macrophages. **Key Words:** Depth filter—(1→3)-β-D-glucan—Fungitec G test—TNF alpha—IL-1 beta—Blood product.

(1→3)-β-D-Glucan is a polymer of D-glucose that represents a major structural component of the fungal cell wall. Recent studies have indicated that measurement of plasma (1→3)-β-D-glucan levels is a useful screening test for invasive fungal infections (1,2). However, the false positive elevation of plasma (1→3)-β-D-glucan levels, a serodiagnostic test for deep-seated mycoses in patients treated by blood product administration, is suspected. We have shown that the amounts of (1→3)-β-D-glucan in blood products range from 0 to 7510 pg/ml using the Fungitec G test and that the three depth-type filters used to clarify blood products are the source of (1→3)-β-D-glucan in blood products (3).

The Fungitec G test is more sensitive and more specific than the fungal index, an indirect measurement method that was tentatively used for *Limulus* amebocyte lysate-reactive materials (LAL-RM) except endotoxin (3,4). There is much difference between the (1→3)-β-D-glucan levels measured using the Fungitec G test and the fungal index following the report of Miyazaki et al. indicating 60 pg/ml using the Fungitec G test versus 1.2 pg/ml using the fungal index (4). Several papers indicating contamination of glucan-like materials in the area of apheresis have already been reported. Pearson et al. indicate that LAL-RM, except endotoxin, are released from hollow fiber hemodialyzers containing cellulose-based membranes using the LAL test (5). Yamagami et al. (6) and Yoshioka et al. (7) also report that LAL-RM, except endotoxin, are released from membrane dialyzers made of cuprophan. However, neither (1→3)-β-D-glucan nor LAL-RM, except endotoxin, levels have been measured from the depth filters used to process blood products.

In this study, we first used the Fungitec G test to measure the (1→3)-β-D-glucan levels in water extracts from all the depth filters used for blood

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processing by Japanese manufacturers following the results of our questionnaire. Second, the biological activities of the filter extracts on proinflammatory cytokine production from Mono Mac 6 cells (a human macrophage cell line) were evaluated (8).

The biological effects of intravenous injection of (1→3)-β-D-glucan in patients are diverse. The elevation of serum (1→3)-β-D-glucan levels to over 900 pg/ml in cases treated by hemodialysis using a saponified cellulose acetate membrane is reported by Yoshida et al. (9). The level of plasma LAL-RM reaches 150 pg/ml after intravenous injection of 10 g gamma-venin, that is, 450 pg/ml of (1→3)-β-D-glucan following our estimation by the result of (1→3)-β-D-glucan measurement of gamma-venin (3), and this remained even at 30 hr after administration (10). (1→3)-β-D-Glucan remains in the liver and spleen for a long time, more than a month, without major structural changes because of the lack of a specific metabolic pathway for (1→3)-β-D-glucanase degradation in humans (11). However, the inactivation of (1→3)-β-D-glucan by plasma and serum proteins has been reported by Miura et al. (12). In addition, several soluble derivatives of (1→3)-β-D-glucan, especially β-(1→6)-branched (1→3)-β-D-glucans such as lentinan, sizofilan, and grifolan, which show potent immunomodulatory activity, have been developed and used in patients with cancer or sepsis (13). Proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) produced from macrophages, are key humoral mediators of the body's response to infection. Reports by Masihi et al. (14) and Hoffman et al. (15) have indicated that (1→3)-β-D-glucan modulates the release of TNF-α from macrophages in response to lipopolysaccharide (LPS). Pearson et al. have reported that LAL-RM, except endotoxin, do not behave as pyrogens (5), but Yamagami et al. indicated that these induce IL-1 activity and platelet aggregation (6). However, the biological activity of extracts from the depth filters used in the blood manufacturing process in combination with the (1→3)-β-D-glucan levels have not been reported.

MATERIALS AND METHODS

Extraction of (1→3)-β-D-glucan from the depth filters

Seven types of depth filters made of cellulose membrane and one type of depth filter made of nylon membrane were used (Table 1; the product names and materials are indicated in the table footnotes, and they were kind gifts from each company). These filters were used for blood processing by Japanese manufacturers, except for Zeta Plus-SP, which was used in our investigation. (1→3)-β-D-Glucan was extracted by incubating two sheets of 47 Φ filters, shattered into eight pieces, in 50 ml of distilled water (Otsuka distilled water for intravenous injection; Otsuka, Tokyo, Japan) for 72 hr at room temperature using a shaker. The concentration of (1→3)-β-D-glucan in the water extract from each filter was measured using the Fungitec G test (Seikagaku Co., Tokyo, Japan) (4). The Fungitec G test is specific for (1→3)-β-D-glucan, as factors B and C are eliminated in the test. Briefly, each sample, diluted to a measurable range, was added to factor G dissolved in HEPES buffer, and then incubated at 37°C for 30 min. The activated factor G activated the proclotting enzyme, and then the optical density of the

TABLE 1. *Extraction of (1→3)-β-D-glucan from the depth filters*

Product name	Material	(1→3)-β-D-Glucan (pg/ml)
EK[a]	Reclaimed cellulose, diatomaceous earth	43,609
EK1P[a]	Reclaimed cellulose, diatomaceous earth	70.7
EKSP[a]	Reclaimed cellulose, diatomaceous earth	6.3
Milligard CWSS[b]	Cellulose ester	27.3
Zeta Plus-LA[c]	Highly purified cellulose, resin, acid-washed diatomaceous earth	1,521
Zeta Plus-SP[c]	Cellulose, perlite, diatomaceous earth	14,480[e]
Zeta Plus-LP[c]	Cellulose, perlite, resin	2,232
Ultipore N_{66}[d]	Nylon	>1.3

(1→3)-β-D-Glucan levels were measured by the Fungitec G test (pg/ml) after a 72 hr incubation with the filters in distilled water.

[a] U.S. Filter Japan Co., Tokyo, Japan.
[b] Millipore, Tokyo, Japan.
[c] Cuno K.K., Yokohama, Japan.
[d] Nihon Pall, Tokyo, Japan.
[e] Incubation time for water extraction of Zeta Plus-SP was 24 hr in preliminary procedure and processing by Japanese manufacturers.

released p-nitroanilide cleaved from the chromogenic substrate by the clotting enzyme was measured using the kinetic model of a computerized well reader (SK601, Seikagaku Co.).

Scanning electron microscopy

Scanning electron microscopy (SEM) examination was performed to analyze the surface microstructure of the depth filters using a scanning electron microscope (JSM-5500; JEOL, Tokyo, Japan) after sputter coating with carbon (JEC-530; JEOL Ltd.).

Materials

LPS from *Escherichia coli* 0111: B4 and lentinan, as a positive control, were obtained from Sigma-Aldrich Co., Tokyo, Japan and Ajinomoto Pharma Co., Tokyo, Japan, respectively. A stock solution of LPS was dissolved at 2 mg/ml in PBS (GIBCO-BRL, Tokyo, Japan) and lentinan was dissolved at 100 μg/ml in RPMI 1640 medium. The water extracts from the depth filters were further diluted from 0.1% to 1000% with Otsuka distilled water.

Cell culture

The Mono Mac 6 cell line (8) was obtained from DSMZ (Braunschweig, Germany) and maintained in RPMI 1640 medium supplemented with 10% inactivated fetal calf serum (FCS), 50 IU/ml penicillin, 50 μg/ml streptomycin, 1% sodium pyruvate, 0.1% sodium oxaloacetate, 0.05% insulin, and 2% sodium hydrogen carbonate at 37°C in an atmosphere of 5% CO_2 and 100% humidity. All culture media were obtained from Dainippon Pharmaceutical Co., Osaka, Japan. The culture medium was replaced twice a week and cell viability was assessed using 0.4% trypan blue solution. The Mono Mac 6 cells were adjusted to 1.0×10^6 cells/ml in 24-well plates (Corning Costar Co., Tokyo, Japan) and cultured with LPS ranging from 0 to 4000 ng/ml for preliminary experiments to ascertain a suitable concentration. The Mono Mac 6 cells were incubated with 1% of amount of the filter extracts, vehicle (control), or 0–10 μg/ml of lentinan under stimulation by 400 ng/ml of LPS for 24 hr. The culture supernatants were clarified by centrifugation at 17,000 g for 20 min at 4°C and stored at –80°C until the cytokine assays were performed.

Measurement of cytokine levels in the culture supernatant

The concentrations of TNF-α and IL-1β were measured using quantitative enzyme-linked immunosorbent assay (ELISA) kits (Biosource International Inc., Camarillo, CA, U.S.A.) following the manufacturer's manual. Briefly, serial dilutions of the samples and standards were pipetted into the wells and incubated for 2 hr. After washing, a biotinylated monoclonal antibody specific for each cytokine was added and incubated for 1 hr. After removal of the excess biotinylated monoclonal antibody, streptavidin-peroxidase was added and incubated for 30 min. After washing to remove all unbound enzyme, a substrate solution was added and the color intensity of the enzymatic indicator reaction was measured at 450 nm using an ELISA reader (Benchmark Microplate Reader; Bio-Rad, Tokyo, Japan). The cytokine levels were expressed as the percentage ratio of the control.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). The results for all the tests were evaluated by one-way factorial analysis of variance (ANOVA). The correlation between (1→3)-β-D-glucan levels and cytokine levels in the culture media were analyzed. Statistical significance was seen when the P value was less than 0.05.

RESULTS

The (1→3)-β-D-glucan levels from the depth filters are shown in Table 1. (1→3)-β-D-Glucan was released from the seven types of depth filters made of cellulose, but the level from the Ultipore N_{66} filters made of nylon was under the detectable limit. There was a wide range of variation of (1→3)-β-D-glucan levels. The (1→3)-β-D-glucan levels from the EK and Zeta Plus-SP filters, especially, were over 10,000 pg/ml. The (1→3)-β-D-glucan levels from three different products of the EK series filters made from the same materials ranged from 6.3 to 43,609 pg/ml and those from three different products of the Zeta Plus series filters made from different materials ranged from 1,521 to 14,480 pg/ml. The surface microstructure of the depth filters is shown in Figure 1. Cellulose or diatomaceous earth was observed in the three EK series membranes. Cellulose was also observed in the three Zeta Plus series membranes. The surface structures of the Milligard CWSS and Ultipore N_{66} membranes were smoother than the EK series and Zeta Plus series membranes. The (1→3)-β-D-glucan levels in the three EK series filters indicate wide variation despite the same surface microstructure. There was no apparent correlation between the surface microstructure of the depth filters and the (1→3)-β-D-glucan levels.

The proinflammatory cytokine production from the Mono Mac 6 cells is indicated in Tables 2 and 3,

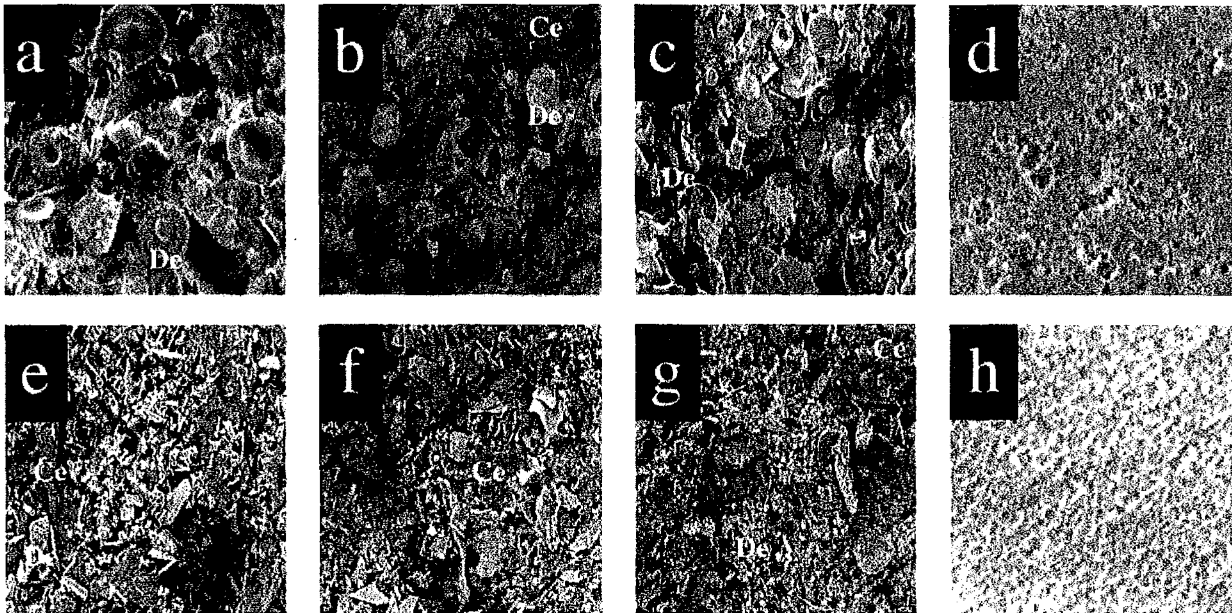

FIG. 1. SEM image shows the surface microstructure of different membranes. EK (a), EK1P (b), EKSP (c), Milligard CWSS (d), Zeta Plus LA (e), Zeta Plus SP (f), Zeta Plus LP (g), Ultipore N_{66} (h). Cellulose (Ce) and diatomaceous earth (De) were observed in the EK series and Zeta Plus series membranes (×1000).

and Figures 2–6. LPS stimulated TNF-α and IL-1β concentrations from Mono Mac 6 cells in a dose-dependent manner, with statistical significance as shown in Table 2. Because unstimulated Mono Mac 6 cells did not produce detectable amounts of these cytokines, we added 400 ng/ml of LPS for the experiments. The control TNF-α and IL-1β concentrations with 400 ng/ml of LPS were 131.4 ± 44.3 pg/ml and 157.4 ± 82.5 pg/ml, respectively. Lentinan, as a positive control for (1→3)-β-D-glucan, significantly decreased TNF-α levels in the presence of LPS, as shown in Table 3. Ten micrograms per milliliter of lentinan reduced the TNF-α production ratio to 93.8 ± 7.8% ($P < 0.01$). The IL-1β production ratio was also decreased to 94.2 ± 13.8% after the addition of 500 ng/ml of lentinan (data not shown).

The TNF-α production ratios with the filter extracts ranged from 74.3 ± 19.8% to 119.0 ± 21.1% (Fig. 2) and the IL-1β production ratios ranged from 81.2 ± 38.8% to 115.9 ± 16.4% (Fig. 3). Milligard CWSS filter extracts significantly decreased the TNF-α production ratio to 80.5 ± 22.1% at the higher (1→3)-β-D-glucan level, 0.273 pg/ml, in a dose-dependent manner ($P < 0.05$). Zeta Plus-LP filter extracts also decreased the TNF-α production ratio to 74.3 ± 19.8% ($P < 0.05$), but the effect was stronger at the lower (1→3)-β-D-glucan level. EKSP filter extracts increased the TNF-α production ratio to 119.0 ± 21.1% at the higher (1→3)-β-D-glucan level, 0.063 pg/ml, ($P < 0.05$). Ultipore N_{66} filter extracts did not change the TNF-α production ratio,

TABLE 2. *Effect of LPS on proinflammatory cytokine production in Mono Mac 6 cells*

LPS (ng/ml)	TNF-α (pg/ml)	IL-1β (pg/ml)
0	3.78 ± 6.34	4.22 ± 2.65
4	106.41 ± 50.48	201.29 ± 79.36
40	136.15 ± 55.69	253.03 ± 120.54
400	181.34 ± 81.25	299.71 ± 146.47
4000	268.24 ± 135.00[a]	362.73 ± 155.92[b]

Values are mean ± SD (n = 3, pg/ml).
[a] $P < 0.01$ with one-way factorial ANOVA.
[b] $P < 0.05$.

TABLE 3. *Effect of lentinan on TNF-α production in Mono Mac 6 cells with LPS*

Lentinan (pg/ml)	TNF-α production (%)
0	100.0 ± 4.093
0.01	98.85 ± 8.32
10	93.81 ± 7.93[b]
10,000	89.83 ± 9.41[a,c]
10,000,000	95.68 ± 5.51

TNF-α levels are normalized to those of the vehicle (control). Values are mean ± SD (n = 6, %).
[a] $P < 0.01$ with one-way factorial ANOVA.
[b] $P < 0.05$.
[c] $P < 0.01$ versus control with Fisher's PLSD.

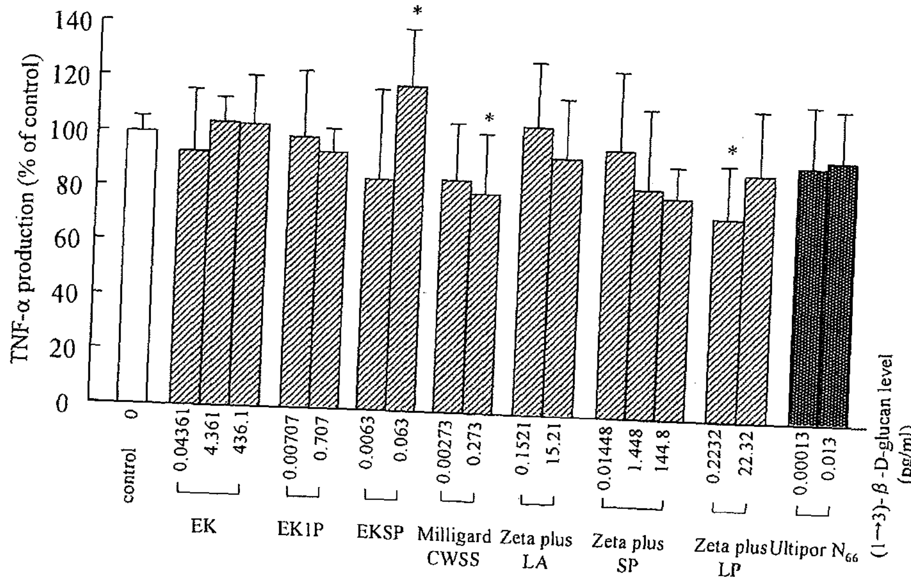

FIG. 2. Effects of the filter extracts and (1→3)-β-D-glucan concentration on TNF-α production in Mono Mac 6 cells with LPS. The filter extracts containing over 10,000 pg/ml of (1→3)-β-D-glucan were further diluted to three concentrations, 0.1%, 10%, and 1000%, with distilled water, and those with less than 10,000 pg/ml were diluted to 10% and 1000%. They were added to a final concentration of 1% of the medium. The values are normalized to those of the vehicle (control). Values are mean ± SD (n = 4). *$P < 0.05$ with one-way factorial ANOVA.

but increased the IL-1β production ratio to 115.93 ± 16.35% at 0.00013 pg/ml of (1→3)-β-D-glucan ($P < 0.05$). There was a positive correlation between the TNF-α and IL-1β production with the filter extracts, as shown in Fig. 4 ($P < 0.01$). There was no statistically significant correlation between the proinflammatory cytokine production and the (1→3)-β-D-glucan levels in the filter extracts, but TNF-α or IL-1β production was lower at the higher (1→3)-β-D-glucan level, as indicated in Figures 5 and 6.

DISCUSSION

Fungitec G test-positive materials, certified as (1→3)-β-D-glucan by the (1→3)-β-D-glucanase digestion experiment (3), are released from the depth filters. With regard to the origin of the (1→3)-β-D-glucan in the filter extracts evaluated, cellulose is the common material used, and other materials, including diatomaceous earth, resin, and perlite, are not commonly used in the filters with the higher (1→3)-β-D-glucan levels. These results indicate that the source of the (1→3)-β-D-glucan is the cellulose in the depth-type filters used for blood product purification (3). Our data are in accordance with the report of Pearson et al. indicating that LAL-RM, except endotoxin, are released from hollow fiber hemodialyzers containing cellulose-based membranes (5). A comparison of the (1→3)-β-D-glucan measured by the Fungitec G test and the LAL-RM, except endotoxin,

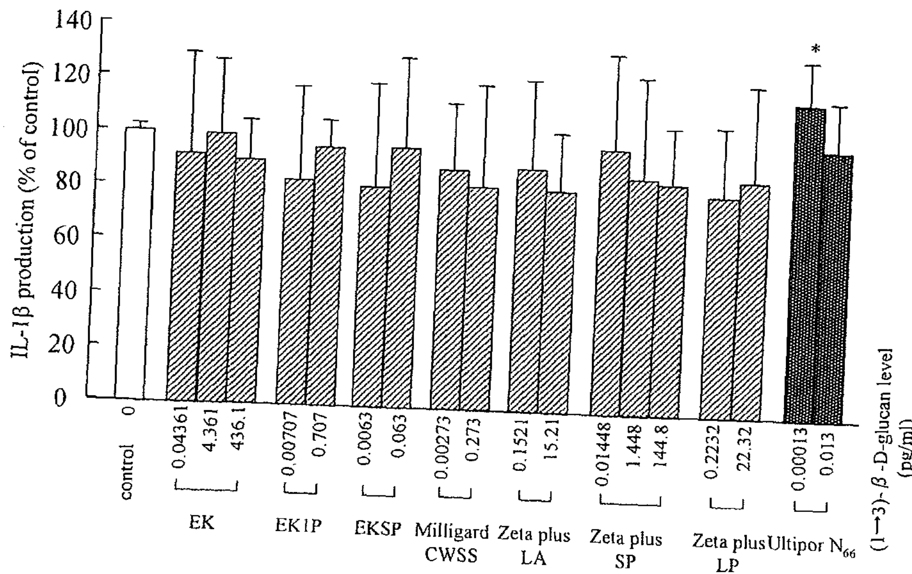

FIG. 3. Effects of the filter extracts and (1→3)-β-D-glucan concentration on IL-1β production in Mono Mac 6 cells with LPS. The filter extracts containing over 10,000 pg/ml of (1→3)-β-D-glucan were further diluted to three concentrations, 0.1%, 10%, and 1000%, with distilled water, and those with less than 10,000 pg/ml were diluted to 10% and 1000%. They were added to a final concentration of 1% of the medium. The values are normalized to those of the vehicle (control). Values are mean ± SD (n = 4). *$P < 0.05$ with one-way factorial ANOVA.

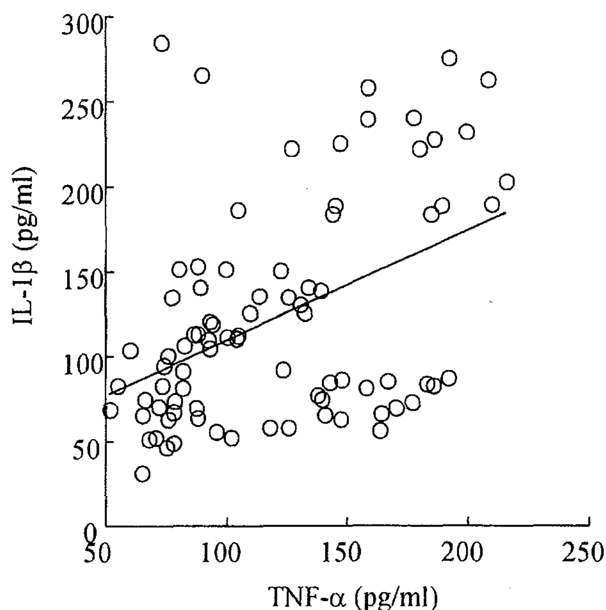

FIG. 4. Correlation between TNF-α and IL-1β concentrations produced with the filter extracts in Mono Mac ells with LPS. Y = 44.834 + 0.648 × X, R = 0.4421, P = 0.00002853; Y, IL-1β concentration (pg/ml); X, TNF-α concentration (pg/ml).

is required, but it is impossible to compare their levels by these two measurement methods due to the lack of precise description of the processed volume during the filter rinse in the Pearson et al. report (5).

The wide range of variations in the (1→3)-β-D-glucan levels observed in the three different products of the EK series filters made from the same materials suggests the importance of a method to process the filter membrane during manufacture. Zeta Plus-LA and LP are higher grades for the reduction of the LAL-RM levels than Zeta Plus-SP according to the Cuno catalog, but the level of (1→3)-β-D-glucan is over 1000 pg/ml in our measurement using the Fungitec G test. Our data indicate that (1→3)-β-D-glucan released from the depth filter is the source of the (1→3)-β-D-glucan in blood products and that it is important to select a suitable depth filter and fractionation method to process blood products in order to avoid high (1→3)-β-D-glucan contamination (3). Minimization of the contamination of (1→3)-β-D-glucan is required to avoid false positive results in serodiagnostic tests for deep-seated mycoses in patients treated by blood product administration. The Japanese Red Cross Plasma Center has recently managed to decrease the (1→3)-β-D-glucan level in blood products by changing the manufacturing process after consideration of a report by us (3).

With regard to biological activity of the depth filter extracts that has not yet been reported, our data indicate that the filter extracts induce significant changes in proinflammatory cytokine production. However, the results are not uniform. Differences in the pro-

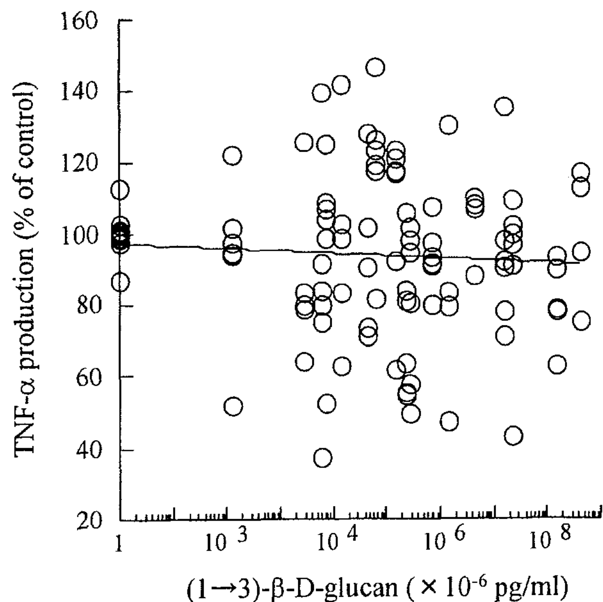

FIG. 5. Correlation between the (1→3)-β-D-glucan levels in the filter extracts and TNF-α production. The values are normalized to those of the vehicle (control). Y = 97.22 − 0.688 × log(X), R = 0.076, P = 0.6162; Y, TNF-α level (% of control); X, (1→3)-β-D-glucan levels (pg/ml).

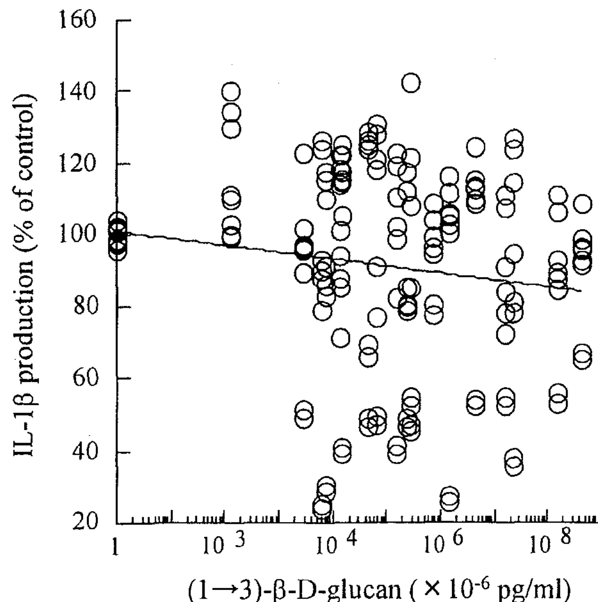

FIG. 6. Correlation between the (1→3)-β-D-glucan levels in the filter extracts and IL-1β production. The values are normalized to those of the vehicle (control). Y = 100.83 − 1.925 × log(X), R = 0.1494, P = 0.6843; Y, IL-1β level (% of control); X, (1→3)-β-D-glucan levels (pg/ml).

inflammatory cytokine production by different filter extracts is noted, that is, TNF-α production was increased by EKSP and decreased by Milligard CWSS and Zeta Plus-LP, and IL-1β production was increased by Ultipore N_{66}. The positive correlation between TNF-α and IL-1β production despite differences in the statistical results for the TNF-α and IL-1β changes suggests that the uniformity in the effects of proinflammatory cytokine production changes with each filter extract. Suppression of TNF-α production by lentinan ranging from 0.01 pg/ml to 10 µg/ml as a positive control for (1→3)-β-D-glucan in our in vitro experiments indicates that TNF-α production is inhibited to 89.8% in accordance with the report of Masihi et al. These authors indicate that TNF-α production was downregulated to 70% by 6.25 µg of lentinan under 25 µg of LPS injected into an in vivo experiment (14). Surprisingly, the levels of suppression of TNF-α production by the extracts of Milligard CWSS, 80.5%, and Zeta Plus-LP, 74.3%, were more prominent than that by lentinan. Our in vitro experimental data indicate that the depth filter extracts contaminating the blood products are active and may influence proinflammatory cytokine production more strongly than lentinan, in accordance with the report of Mashihi et al. indicating that TNF-α and IL-1β are downregulated by glucan under in vivo conditions (14). Pearson et al. have reported that LAL-RM from hollow fiber extracts have biological activity on macrophage functions but they emphasize that the extract is not LPS and do not refer to the activity of the proinflammatory reaction (5). On the contrary, Miura et al. have reported that β-glucan with its protein bound by a substrate, such as serum albumin, is inactive (12). We should be more careful in the use of blood products containing the extracts from depth filters in patients with various immunological disorders.

Filter extracts from hollow fibers for hemodialysis contain three peaks with molecular weights of 23,000–24,000, 3000, and less than 200 in column chromatography, according to the report by Pearson et al. (5). The first peak is considered to be LAL-RM, but the other peaks are not LAL-RM. It appears that materials in the depth filter extracts, other than those from cellulose, have an effect on proinflammatory cytokine production, as the report by Pearson et al. indicated that the filter extracts contain three materials and our data indicate that the difference in cytokine production is unrelated to the (1→3)-β-D-glucan level and the increase of IL-1β production by the nylon filter extracts. Further analysis is required to explain the mechanism for the change in proinflammatory cytokine production by each filter extract, including that from the nylon filter.

We found that the (1→3)-β-D-glucan level in a filtration fluid is decreased by a rinse with distilled water, but increased again up to the level before the water rinsing with 5% albumin solution in an in vitro filtration process (3). The materials extracted by water filtration and those by albumin filtration may be different, and the biological activities of the (1→3)-β-D-glucan extracted in the distilled water and albumin solutions may be different. The biological activity of the albumin solution in combination with the (1→3)-β-D-glucan levels has not been reported. We are currently evaluating of the effect of (1→3)-β-D-glucan using the albumin solution. In addition, in vivo experiments should be performed in the future to determine the effect of these extracts on the immunological condition in different disease conditions.

Recently, the elevation of serum (1→3)-β-D-glucan levels to over 900 pg/ml in cases treated by hemodialysis using a saponified cellulose acetate membrane has been reported by Yoshida et al. (9). However, this can be avoided using a synthetic polymer membrane. Following our results, not only can (1→3)-β-D-glucan contamination make false positive results in serum diagnosis for deep-seated mycoses, but the side effects due to the unexpected biological activity of the filter extracts should also be evaluated.

In summary, we have demonstrated that (1→3)-β-D-glucan is released from depth filters made of cellulose and that the proinflammatory cytokine production from a macrophage cell line is affected by the filter extracts.

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REFERENCES

1. Obayashi T, Yoshida M, Mori T, Goto H, Yasuoka A, Iwasaki H, Teshima H, Kohno S, Horiuchi A, Ito A, Yamaguchi H, Shimada K, Kawai T. 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, Yasuoka A, Miyazaki T, Kaku M, Koga H, Hara K. 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. Usami M, Ohata A, Horiuchi T, Nagasawa K, Wakabayashi T, Tanaka S. Positive (1→3)-β-D-glucan in blood products and release of (1→3)-β-D-glucan from depth type membrane filters for blood processing. *Transfusion* 2002;42:1189–95.
4. Miyazaki T, Kohno S, Koga H, Kaku M, Mitsuoka K, Maesaki S, Yasuoka A, Hara K, Tanaka S, Tamura H. 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.
5. Pearson FC, Bohon J, Lee W, Bruszer G, Sagona M, Dawe R, Jakubowski G, Morrison D, Dinarello C. Comparison of chemical analyses of hollow-fiber dialyzer extracts. *Artif Organs* 1984;8:291–8.
6. Yamagami S, Yoshihara H, Kishimoto T, Sugimura T, Niwa M, Maekawa M. Cuprophan membrane induced interleukin I activity. *Trans Am Soc Artif Intern Organs* 1986;32:98–101.
7. Yoshioka T, Ikegami K, Ikemura K, Shiono S, Uenishi M, Sugimoto H, Sugimoto T. A study on *Limulus* amebocyte lysate (LAL) reactive material derived from dialyzers. *Jpn J Surg* 1989;19:38–41.
8. Ziegler Heitbrock HW, Thiel E, Futterer A, Herzog V, Wirtz A, Riethmuller G. Establishment of a human cell line (Mono Mac 6) with characteristics of mature monocytes. *Int J Cancer* 1988;41:456–61.
9. Yoshida K, Nakajima M, Yamasaki M, Kitano Y, Niki Y, Ohsawa G, Matsushima T. Investigation of influence of hemodialysis membranes and factors associated with hemodialysis on serum (1→3)-β-D-glucan. *Jpn J Infect* 1998;72:245–8.
10. 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.
11. Suda M, Ohno N, Adachi Y, Yadomae T. Tissue distribution of intraperitoneally administered (1→3)-beta-D-glucan (SSG), a highly branched antitumor glucan, in mice. *J Pharmacobiodyn* 1992;15:417–26.
12. 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.
13. Williams DL, Mueller A, Browder W. Glucan-based macrophage stimulators. A review of their anti-infective potential. *Clin Immunother* 1996;5:392–9.
14. Masihi KN, Madaj K, Hintelmann H, Gast G, Kaneko Y. Down-regulation of tumor necrosis factor-alpha, moderate reduction of interleukin-1beta, but not interleukin-6 or interleukin-10, by glucan immunomodulators curdlan sulfate and lentinan. *Int J Immunopharmacol* 1997;19:463–8.
15. Hoffman OA, Olson EJ, Limper AH. Fungal beta-glucans modulate macrophage release of tumor necrosis factor-alpha in response to bacterial lipopolysaccharide. *Immunol Lett* 1993;37:19–25.