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The effect of soluble β-1,3-glucan and lipopolysaccharide on cytokine production and coagulation activation in whole blood ☆

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Abstract

Soluble β-1,3-glucan has been demonstrated to protect against infection and shock in rats and mice, and clinical studies suggest that administration of soluble glucans to trauma/surgical patients decreases septic complications and improves survival. However, little is known about the precise mechanisms by which glucans influence the state of activation of blood cells, which are responsible for the fulminant cytokine production and the activation of the coagulation system observed in serious gram-negative infection. We studied therefore the effect of an underivatized, soluble yeast β-1,3-glucan and lipopolysaccharide (LPS), either alone or in combination, on tumor necrosis factor-α (TNFα), interleukin-6 (IL-6), IL-8 and IL-10 secretion and monocyte tissue factor (TF) expression in human whole blood. As expected, LPS induced the secretion of substantial amounts of all measured parameters, whereas only minor amounts of TNFα, IL-6, and IL-10 were induced by β-glucan itself. However, β-glucan itself induced the production of significant amounts of IL-8 and TF. Soluble β-1,3-glucan had a strong synergistic effect on the LPS-induced secretion of IL-8, IL-10, and on monocyte TF activity, but not on TNFα and IL-6 production. On the other hand, soluble β-glucan strongly primed LPS stimulation of all parameters, including TNFα and IL-6. β-Glucan also induced detectable neutrophil degranulation within 15 min, whereas a response to LPS was first detected after 90 min. In conclusion, soluble β-1,3-glucan upregulated leukocyte activity, both on its own and in concert with LPS.

Keywords: β-1,3-Glucan; Lipopolysaccharide; Sepsis; Cytokines; Tissue factor; Blood cells

1. Introduction

Glucans are a heterogeneous group of glucose polymers found in the cell walls of plants, bacteria and fungi. The basic structure of branched glucan consists of a linear backbone of β-1,3-linked glucose with β-1,6-linked side branches of β-1,3-glucan. β-1,3-Glucan is the component responsible for the majority of biological activities of zymosan [1], a commonly used leukocyte stimulant derived from the cell wall of Bakers yeast (*Saccharomyces cerevisiae*).

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Glucans are natural biological response modifiers and their effects are many, including anti-tumor response, upregulation of NK cell toxicity and cytokine synthesis, antibacterial and antiviral activity, and stimulation of hematopoiesis and wound healing [2,3]. These effects are thought to be receptor mediated and several different receptors for β-1,3-glucan have been postulated: A phagocytic, non-CR3 receptor on monocytes/macrophages specific for β-1,3-glucans [4,5], a glycosphingolipid β-1,3-glucan receptor on human neutrophils [6], and recently, dectin-1 on macrophages was identified as a potential β-glucan receptor [7]. Furthermore, the toll-like receptors (TLRs) 2 and 6 have been reported to coordinate macrophage activation in response to zymosan particles [8]. Finally, CD11b/CD18 (CR3, Mac-1) has long been recognized as a glucan receptor [9] and has been shown to bind β-1,3-glucan at a cation-independent lectin site located C-terminal to the I-domain of CD11b [10].

Lipopolysaccharides (LPS) are amphipathic glycolipids, constituting the outer leaflet of the outer membrane of gram-negative bacteria. In plasma, LPS is dissociated from LPS aggregates by the acute phase lipopolysaccharide-binding protein (LBP) and transferred to CD14. The LPS receptor CD14 is anchored to the plasma membrane by a glycosylphosphatidylinositol (GPI) anchor and therefore unable to transduce signals to the interior of the cell (for review, see Refs. [11,12]). Two members of the toll-like receptor family, TLR-2 [13,14] and TLR-4 [15,16] in complex with MD-2 [17], have been proposed to act as CD14-associated signal-transducing receptors. LPS binding to its receptor(s) activates several intracellular signaling pathways in the human monocyte, resulting in the activation of a variety of transcription factors including NF-κB (p50/p65) and AP-1 (c-Fos/c-Jun), cellular activation and the production of proinflammatory mediators and cytokines (for review, see Ref. [18]).

LPS is the causal agent of gram-negative infection and of septic shock in particular. High levels of tumor necrosis factor-α (TNFα), interleukin-6 (IL-6), IL-8 and IL-10 are found in the blood of septic patients [19]. TNFα is a central mediator of sepsis, and infusion of TNFα in human volunteers has been shown to mimic the effects of LPS [20]. IL-8 is a potent chemoattractant and stimulator of neutrophil function [21]. IL-10 is regarded as an anti-inflammatory cytokine, and has been claimed to induce hyporeactivity or anergy in circulating leukocytes [22], which is often observed during the course of severe gram-negative sepsis [23]. IL-6 is thought to play a central role in the activation of coagulation activation leading to disseminated intravascular coagulation (DIC) [24], which is initiated by the exposure of tissue factor (TF) on circulating monocytes (for review, see Ref. [25]).

Several studies on the interaction of LPS with different preparations of β-1,3-glucans have been published. Systemic administration of particulate glucan to rats has been shown to increase their sensitivity to endotoxin shock [26], while soluble β-1,3-glucan had no such adverse effects [27]. On the contrary, soluble β-1,3-glucan has been demonstrated to protect against infection and shock in rats and mice [28,29], and clinical studies suggest that administration of soluble glucans to trauma/surgical patients stimulates conversion from leukocyte anergy, decreases septic complications and improves survival [30,31]. However, little is known about the precise mechanisms by which glucans alter the septic state, or how glucans affect the cell biology of leukocytes, which are responsible for the fulminant production of cytokines and the activation of the coagulation system associated with serious gram-negative infection.

The aim of the present investigation was therefore to study the effect of a soluble branched β-1,3-glucan alone, or in combination with LPS, on cytokine production and coagulation activation. All experiments were carried out using human whole blood, which has been shown to be an excellent ex vivo system for studies of LPS-induced blood cell activation and cytokine production associated with sepsis [32,33].

2. Methods

2.1. Reagents

Fragmin was from Pharmacia & Upjohn, Stockholm, Sweden; LPS (strain 026:B6) from Difco, Detroit, MI, USA; Lymphoprep from Nycomed Amersham, Oslo, Norway. An endotoxin-free (<0.05 EU/ml) underivatized aqueous soluble yeast β-glucan with an average numerical molecular weight of approximately 20 kDa was used in the assays (Biotec Pharmacon ASA, Tromsø, Norway). Analysis showed that

the yeast β-glucan had a branched structure with β-1,3-linked side chains anchored through a β-1,6 linkage to the main β-1,3-glucan chain for approximately every 10th–15th glucosyl unit of the main chain [34].

2.2. Whole blood assay system

Whole blood samples anticoagulated with Fragmin (15 IE/ml) were incubated with LPS and β-glucan in a shaker incubator at 37 °C and 180 rpm for different time intervals. The reaction was stopped by adding 100 μl 2% Na_2EDTA/ml whole blood. Mononuclear cells were isolated by density centrifugation on Lymphoprep (Nycomed, Oslo, Norway) followed by a washing step. The cell pellet was resuspended in 0.15 M NaCl, frozen at −20 °C and stored until testing (described in detail previously [35]). Plasma samples were isolated by centrifugation at 1450 × *g* for 10 min and stored at −70 °C until testing.

2.3. Quantitation of TF activity

TF activity was measured in a two-stage amidolytic assay based on the ability of TF to accelerate the activation of factor X by factor VIIa, as previously described [36].

2.4. Quantitation of cytokines and cell activation markers

IL-6, IL-8, IL-10 and TNFα in plasma samples were analyzed using PeliKine Compact ELISA kits from the Central Laboratory of the Netherlands Red Cross, Amsterdam, The Netherlands. PMN elastase in complex with α1 proteinase inhibitor was determined using an immunoassay kit from Merck, Darmstadt, Germany. Platelet factor 4 (PF4) in plasma samples was quantified using ELISA kit from Diagnostica Stago, Asnieres-sur-Seine, France.

2.5. Statistics

All statistical analyses were performed by Statistica 3.0b (Statsoft, Tulsa, OK, USA). Data were analyzed by two- or three-way analysis of variance (ANOVA) followed by Scheffe *F*-test or Student's paired *t*-test, as appropriate. *P*-values <0.05 were considered significant. All results are reported as means with S.E.M.

3. Results

3.1. Effect of increasing LPS and β-glucan concentrations on cytokine secretion and TF activity in whole blood

Whole blood was incubated with increasing concentrations of LPS (0.5, 5, and 50 ng/ml) and β-glucan (2 and 20 μg/ml) for 2 h (Fig. 1a–c, e) or 24 h (Fig. 1d) alone or in combination. Subsequently, cytokine levels in plasma were determined and TF activity in isolated monocytes was measured. As seen in Fig. 1, soluble β-glucan induced the release of small, but statistically significant amounts of TNFα, IL-6 and IL-8 and with respect to the highest dose, also induced TF activity (Fig. 1a–c, e). Nevertheless, the highest dose of β-glucan (20 μg/ml) was still less potent as inducer of all parameters measured even compared to the lowest dose of LPS (0.5 ng/ml). The cytokine levels and TF activity increased dose dependently from 0.5 to 5 ng/ml LPS, and with respect to IL-10 also from 5 to 50 ng/ml LPS (Fig. 1d). The latter in contrast to TNFα and IL-6 secretion where no further increase from 5 to 50 ng/ml LPS were observed (Fig. 1a and b) and with respect to IL-8 secretion and TF activity, a decrease was seen (Fig. 1c and e). When β-glucan and LPS were added together, a strong synergistic effect was observed with respect to TNFα, IL-8, IL-10 secretion and TF activity, but there was seemingly no synergy in IL-6 secretion (Fig. 1b).

3.2. Time course of β-glucan and LPS-induced cytokine production and TF activity in whole blood

To study the effect of β-glucan on the kinetics of cytokine induction and TF expression, 20 μg/ml glucan or saline (as control) was added to whole blood samples. TF activity and cytokine secretion were determined after 2, 4, 8, and 24 h of incubation (Fig. 2). β-Glucan significantly increased TF activity and secretion of all cytokines during the 24-h period (for *p*-values, see legend to Fig. 2), but the kinetics of the individual parameters measured were quite divergent. Glucan-induced TNFα secretion was highest at 2 and 4 h of incubation, declined after 8 h, and was completely abolished after 24 h (Fig. 2a). Glucan-induced IL-6 secretion increased from 2 to 4 h of incubation and then maintained a steady level throughout the incuba-

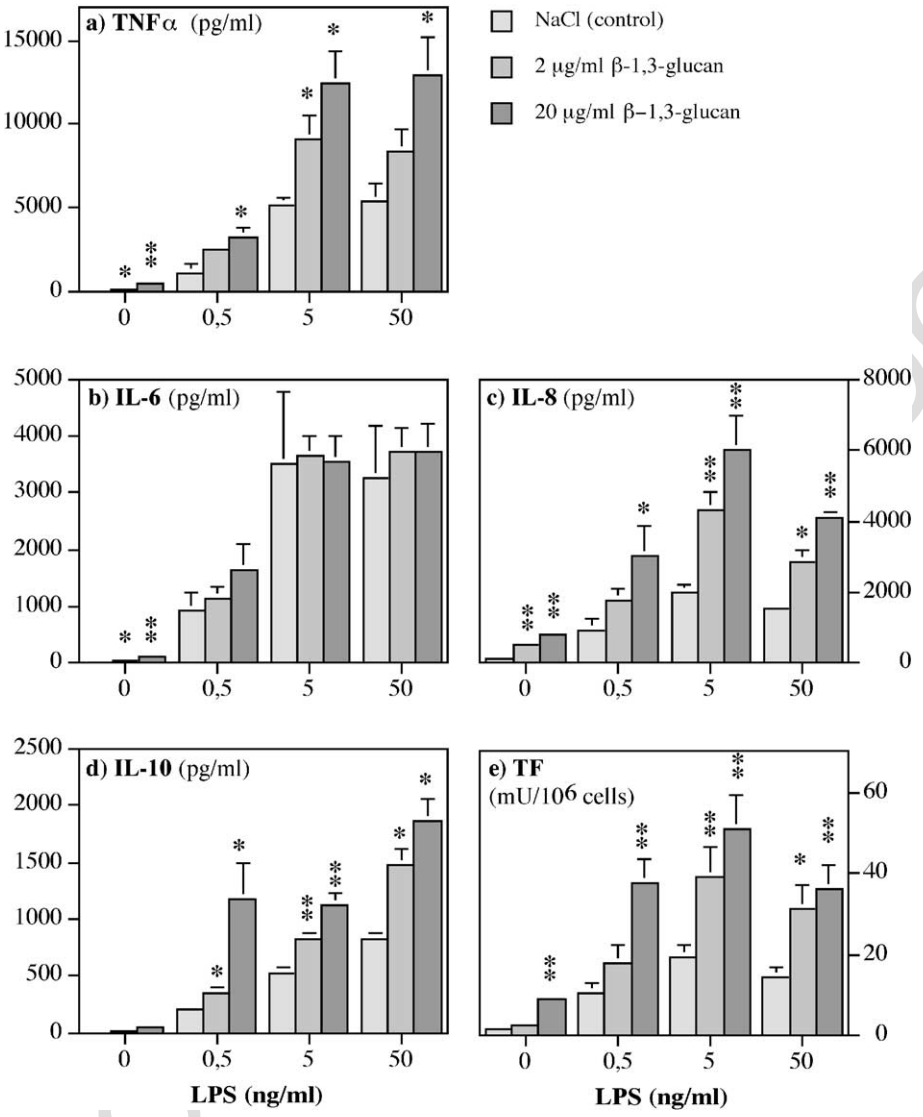

Fig. 1. The effect of β-glucan and LPS on cellular activation parameters in whole blood. Whole blood was incubated with LPS (0.5, 5, and 50 ng/ml) and β-glucan (2 and 20 μg/ml) for 2 (a, b, c, e) or 24 (d) h at 37 °C. TF activity in isolated mononuclear cells and cytokine levels in plasma samples were quantified as described in Methods. Results are expressed as mean with S.E.M., values were considered significantly different in the case of $p < 0.05$ by the Student's *t*-test, $* = p < 0.05$; $** = p < 0.01$. (a) The effect of β-glucan and LPS on TNFα production (pg/ml), $n = 4–5$. (b) The effect of β-glucan and LPS on IL-6 levels (pg/ml), $n = 5$. (c) The effect of β-glucan and LPS on IL-8 production (pg/ml), $n = 5–7$. (d) The effect of β-glucan and LPS on IL-10 secretion (pg/ml), $n = 5$. (e) The effect of β-glucan and LPS on TF activity (mU/10^6 cells) in mononuclear cells, $n = 6–8$.

tion period (Fig. 2b). IL-8 and IL-10 levels continuously increased up to 24 h, but glucan-induced IL-10 secretion was very low in general (Fig. 2c and d). Glucan-stimulated TF activity in monocytes increased from 2 to 4 h, and then steadily decreased (Fig. 2e).

Concurrently, whole blood samples were stimulated with LPS (5 ng/ml) and 20 μg/ml β-glucan or with LPS (5 ng/ml) and NaCl (as control). As shown in Fig. 3, glucan had a significant and enhancing effect on LPS-induced TF activity and TNFα, IL-8, and IL-10

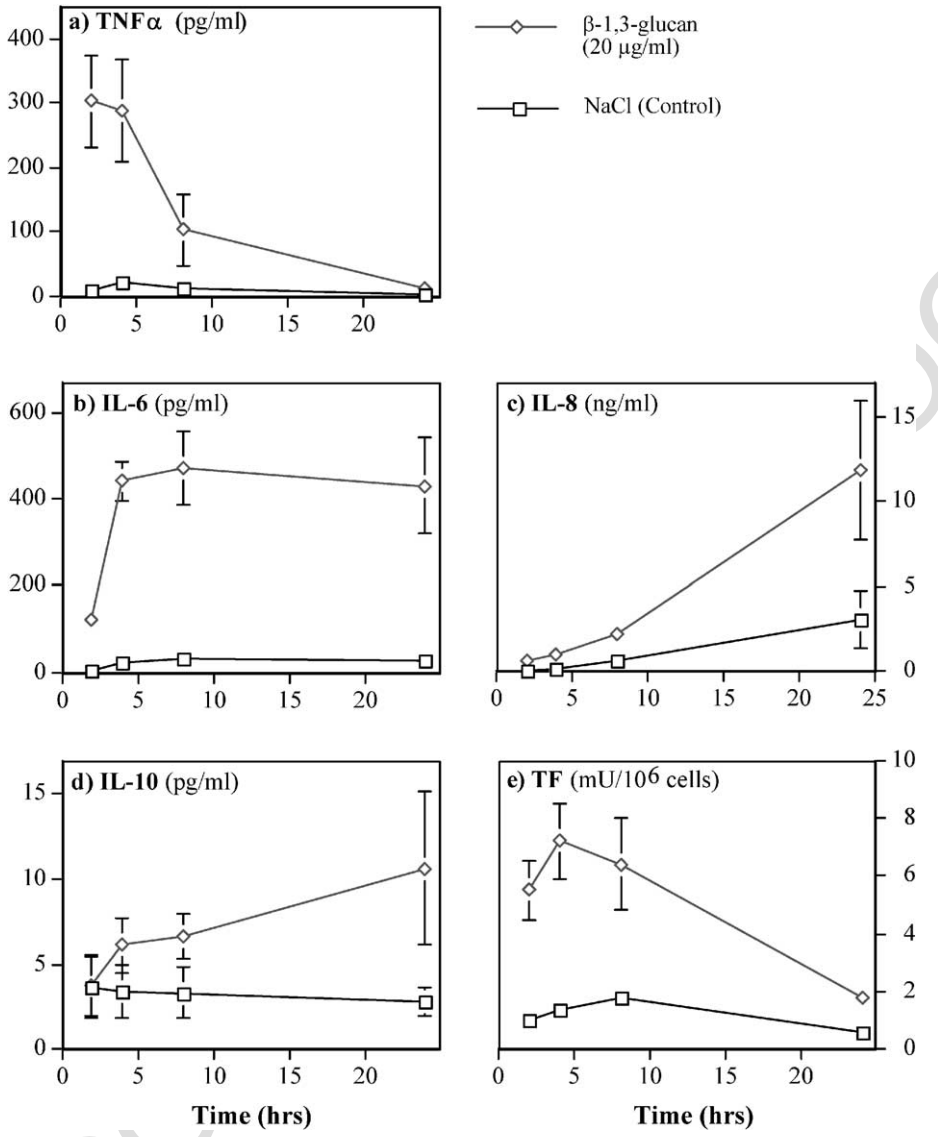

Fig. 2. Time course of β-1,3-induced TF and cytokine production in whole blood. Whole blood was incubated with 20 μg/ml β-glucan or NaCl (control) for 2, 4, 8, and 24 h in a shaker incubator at 37 °C. TF activity in isolated mononuclear cells and cytokine levels in plasma samples were quantified as described in Methods. Results are expressed as mean with S.E.M., values were considered significantly different in the case of $p < 0.05$ by three-way ANOVA. (a) Time course of TNFα secretion (pg/ml) in β-glucan-stimulated whole blood, $n = 5$ and $p = 0.03$. (b) Time course of IL-6 production (pg/ml) in β-glucan-stimulated whole blood, $n = 5$ and $p = 0.02$. (c) Time course of IL-8 secretion (ng/ml) in β-glucan-stimulated whole blood, $n = 4–5$ and $p = 0.0008$. (d) Time course of IL-10 production (pg/ml) in β-glucan-stimulated whole blood, $n = 5$ and $p = 0.03$. (e) Time course of TF activity in monocytes (mU/10^6 cells) from β-glucan-stimulated whole blood, $n = 3–6$ and $p = 0.004$.

secretion, but not on IL-6 secretion (for *p*-values, see legend to Fig. 3). Again, the kinetics of the individual parameters differed a lot. The time courses of LPS-induced TNFα secretion and LPS + β-glucan-induced TNFα secretion paralleled each other. As seen in Fig. 3a, TNFα levels increased up to 8 h and declined afterwards, which was quite different from the time course of TNFα secretion induced by glucan alone (Fig. 2a). LPS alone and LPS + β-glucan-induced IL-6 production became progressively larger up to 8 h and

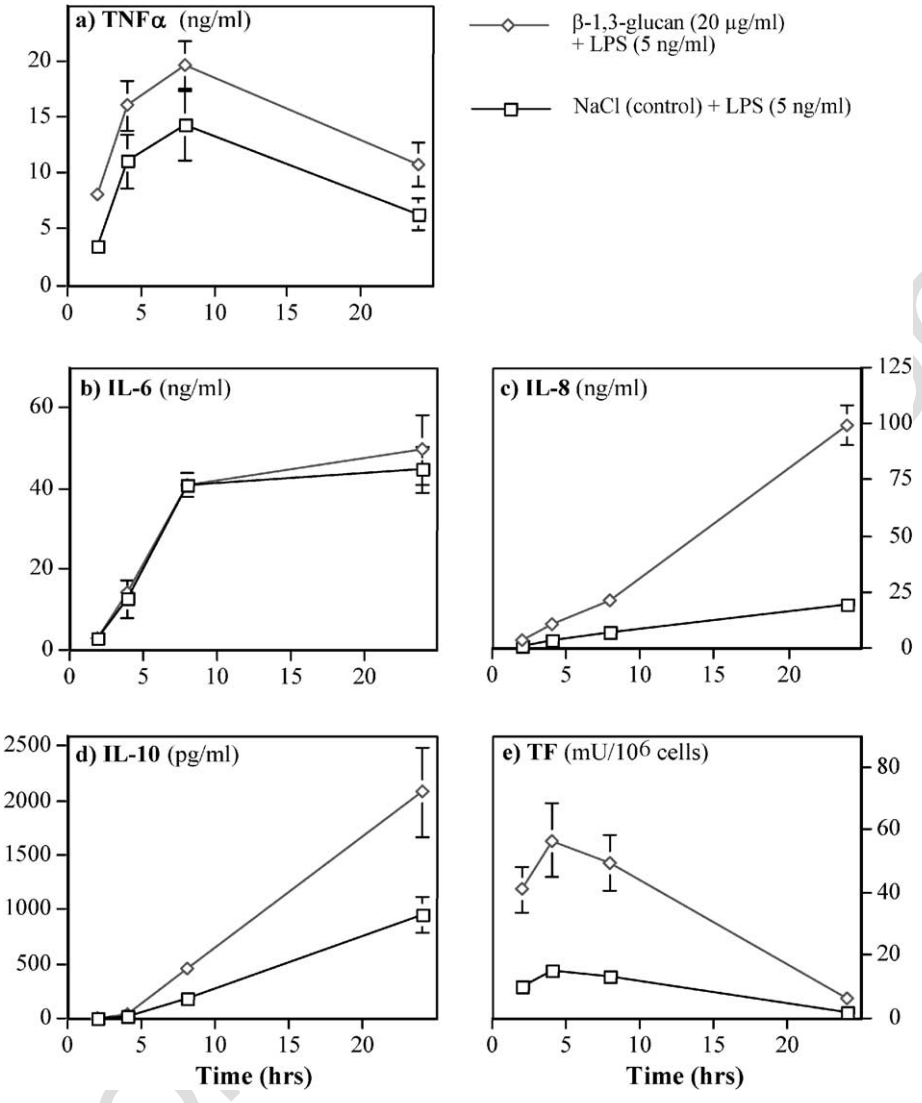

Fig. 3. Time course of LPS and LPS + β-glucan-induced TF and cytokine production in whole blood. Whole blood was incubated with LPS (5 ng/ml) or with LPS and 20 μg/ml β-glucan for 2, 4, 8, and 24 h in a shaker incubator at 37 °C. TF activity in isolated mononuclear cells and cytokine levels in plasma samples were quantified as described in Methods. Results are expressed as mean with S.E.M., values were considered significantly different in the case of $p < 0.05$ by three-way ANOVA. (a) Time course of TNFα secretion (ng/ml) in β-glucan and LPS-stimulated whole blood, $n = 5$ and $p = 0.007$. (b) Time course of IL-6 production (ng/ml) in β-glucan and LPS-stimulated whole blood, $n = 5$. (c) Time course of IL-8 secretion (ng/ml) in β-glucan and LPS-stimulated whole blood, $n = 4–5$ and $p = 0.0002$. (d) Time course of IL-10 production (pg/ml) in β-glucan and LPS-stimulated whole blood, $n = 5$ and $p = 0.0005$. (e) Time course of TF activity in monocytes (mU/10^6 cells) from β-glucan and LPS-stimulated whole blood, $n = 3–6$ and $p = 0.002$.

was then maintained at the same level (Fig. 3b). IL-8 and IL-10 levels increased in a time-dependent manner throughout the whole incubation period both when LPS was given alone or in combination with β-glucan (Fig. 3c and d). The enhancing effect of glucan on LPS-stimulated TF activity peaked at 4 ho (Fig. 3d), was slightly diminished at 8 h and was completely abolished at 24 h.

Table 1 summarizes the results of Figs. 2 and 3. Columns A–D show peak cytokine levels and TF

Table 1
Comparison of the peak values of TF activity and cytokine levels

	A	B	C	D	E	F	G
	Saline	Glucan	LPS	LPS + glucan	(C:D)100	Additive effect (B + C)	Synergism (D:F)
TNFα	0.02 ± 0.005	0.29 ± 0.08	11.0 ± 2.4	16.0 ± 2.3	2.6%	11.29	1.4
IL-6	0.03 ± 0.007	0.47 ± 0.09	40.8 ± 3.1	41.0 ± 2.8	1.2%	41.27	1.0
IL-8	3.0 ± 1.7	11.8 ± 4.1	19.4 ± 1.9	99.2 ± 8.6	60.8%	31.2	3.2
IL-10	2.8 ± 0.8	10.6 ± 4.5	957 ± 166	2184 ± 416	1.1%	967.6	2.3
TF	1.4 ± 0.3	7.2 ± 1.3	14.8 ± 2.9	56.5 ± 118	48.6%	22.0	2.6

Potency of β-1,3-glucan as an inducer compared to LPS and synergism between β-1,3-glucan and LPS.
Columns A–D: Comparison of the peak values of TF activity and cytokine levels (from Figs. 2 and 3): TNFα at 4 h (ng/ml), IL-6 at 8 h (ng/ml), IL-8 at 24 h (ng/ml), IL-10 at 24 h (pg/ml), TF at 4 h (mU/10^6 cells). Whole blood was incubated with saline (control), LPS (5 ng/ml) or with 20 μg/ml β-1,3-glucan for 2, 4, 8, and 24 h in a shaker incubator at 37 °C. TF activity in isolated mononuclear cells and cytokine levels in plasma samples were quantified as described in Methods. Results are expressed as mean with S.E.M. *Column E*: Potency of glucan as an inducer compared to LPS (%). *Column F*: Additive effect of glucan (B) and LPS (C). *Column G*: Degree of synergism between glucan and LPS (experimental value of LPS + glucan (D): theoretically additive effect (F)).

activity in control samples and in samples stimulated with β-glucan, LPS or with β-glucan and LPS in combination. In column E, the potency of LPS and β-glucan as inducers of cytokine secretion and TF activity in monocytes are compared. Twenty micrograms per milliliter β-glucan per se had a strong inducing effect only on TF activity and IL-8 secretion when compared to the effect of 5 ng/ml LPS, since glucan-induced TF activity and IL-8 secretion were 48.6% and 60.8% of LPS-induced TF and IL-8, respectively. However, compared to LPS, glucan itself had almost no effect on TNFα, IL-6, and IL-10 (2.6%, 1.2%, and 1.1% of LPS-induced cytokine secretion). In column F, the additive effect of LPS and β-glucan-induced cytokine secretion and TF activity was calculated, which was used to determine the degree of synergism between LPS and β-glucan (Column G). Column G shows that β-glucan and LPS had a slightly synergistic effect on TNFα production and no effect of IL-6 secretion. However, the effect of β-glucan on LPS-induced IL-8 and IL-10 secretion and TF activity was strongly synergistic (2- to 3-fold increases compared to a strictly additive effect).

3.3. The effect of pretreatment with β-glucan on LPS-induced cytokine secretion and TF activity

Pretreatment with soluble β-glucan has been demonstrated to protect against LPS (endotoxin) shock in a rat model [28], an effect that partly could be explained by an attenuated endotoxin-induced cytokine production as observed in infection models with gram-negative bacteria [37]. In this experiment, we wished to examine the response of blood cells to LPS when the blood was pretreated for various time intervals with β-glucan (Fig. 4). Whole blood samples were incubated with 20 μg/ml β-glucan or saline (as control) for 2, 4, or 8 h, followed by addition of 5 ng/ml LPS and further incubation for 2 h. In line with what was observed in Figs. 1 and 3, preincubation with β-glucan for 2 h significantly increased LPS-induced TF activity, TNFα, and IL-8 secretion, but also IL-6 secretion. A minor stimulatory effect of β-glucan pretreatment on LPS-induced IL-10 secretion could also be observed, though not statistically significant (for *p*-values, see legend to Fig. 4). It should, however, be noted that in comparison to the experiments described in Figs. 1 and 3, the incubation time with LPS in the present experiment was 2 h as compared to 24 h in the former. The effect of preincubation with β-glucan on LPS-induced TNFα secretion tended to decrease as preincubation time increased (Fig. 4a), whereas IL-6 production decreased in both treated and untreated samples with increasing pretreatment time (Fig. 4b). The above in contrast to induction of IL-8, where 8 h pretreatment with β-glucan resulted in the highest LPS-induced activity. The effect of pretreatment with β-glucan on TF activity differed from what was observed with the cytokines, where an increased LPS-induced activity was observed at 2 and 8 h preincubation time, whereas no such effect was observed at 4 h preincubation time (Fig. 4e).

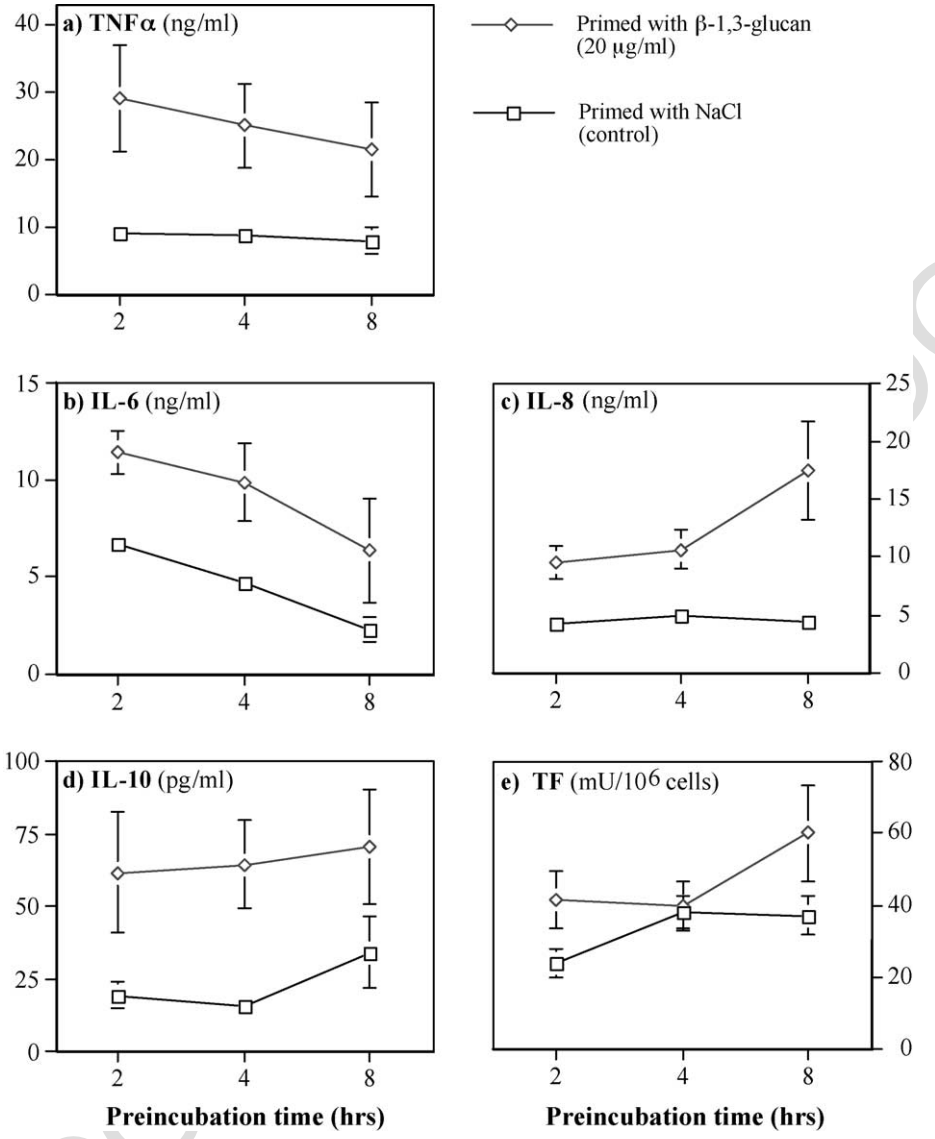

Fig. 4. β-Glucan primes LPS-induced cell activation in whole blood. Whole blood samples were preincubated with NaCl or β-glucan (20 μg/ml) at 37 °C for 2, 4 and 8 h. Then 5 ng/ml LPS was added to all samples and incubation was continued for additional 2 h. TF activity in isolated mononuclear cells and cytokine levels in plasma samples were quantified as described in Methods. Results are expressed as mean with S.E.M., values were considered significantly different in the case of $p < 0.05$ by three-way ANOVA. (a) β-Glucan primes LPS-induced TNFα secretion (ng/ml), $n = 4$ and $p = 0.04$. (b) β-Glucan primes LPS-induced IL-6 production (ng/ml), $n = 5$ and $p = 0.02$. (c) β-Glucan primes LPS-induced IL-8 secretion (ng/ml), $n = 5$ and $p = 0.01$. (d) β-Glucan primes LPS-induced IL-10 secretion (pg/ml), $n = 5$ and $p = 0.06$. (e) β-Glucan primes LPS-induced TF activity (mU/10^6 cells) in mononuclear cells, $n = 3-6$ and $p = 0.04$.

3.4. The effect of β-glucan on PMN and platelet activation in whole blood

The previous experiments showed that β-glucan is capable of specifically activating monocytes, as TF can be regarded as an exclusive parameter of monocyte activation [38]. We wished therefore to determine if soluble β-glucan could also specifically activate PMN and blood platelets. PMN elastase (in complex with α1 proteinase inhibitor) and platelet factor 4 (PF4) were

chosen as markers of PMN and platelet activation, respectively.

Whole blood was incubated with β-glucan, 5 ng/ml LPS or saline (control) for 90 min, blood samples were removed at different time intervals and plasma was tested for activation markers. As shown in Fig. 5a, β-glucan significantly increased PMN elastase levels already after 15 min of incubation as compared to control samples and continued to enhance PMN elastase secretion time dependently throughout the incubation period. This was in contrast to the effect of LPS, where increase in PMN elastase plasma levels first was observed after 90 min (*p*-values, see legend to Fig. 5a). Incubation of blood samples with β-glucan and LPS in combination had no further effect on PMN elastase release (data not shown).

Fig. 5b shows that PF4 secretion increased in a time-dependent way and that β-glucan had no stimulating effect on platelet α-granule release. The effect of LPS on PF4 release was not tested in n this experiment, as we have previously demonstrated that LPS has no effect on PF4 secretion from platelets in whole blood [35].

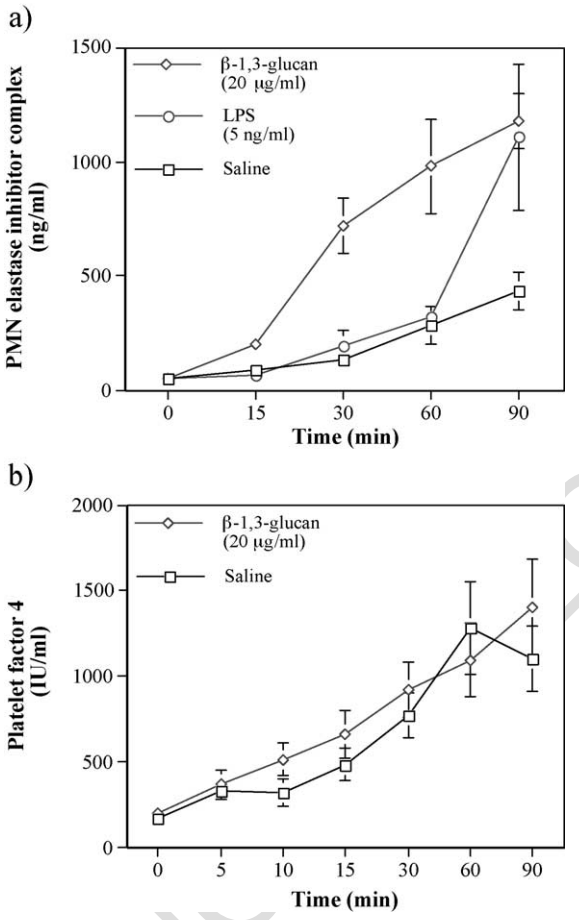

Fig. 5. The effect of β-glucan on PMN and blood platelet activation in whole blood. (a) Whole blood samples were incubated with 20 μg/ml β-glucan, LPS (5 ng/ml) or saline (control) for 0, 15, 30, 60, and 90 min. PMN elastase complexed with α1-proteinase inhibitor in plasma samples was quantified as described in Methods. Results are expressed as mean with S.E.M., values were considered significantly different in the case of $p < 0.05$ by the Scheffe *F*-test, $n = 4–5$; saline versus β-glucan $p < 0.01$, LPS versus β-glucan $p < 0.05$. (b) Whole blood samples were incubated with 20 μg/ml β-glucan or saline (control) for 0, 5, 10, 15, 30, 60, and 90 min. PF4 in plasma was measured as described in Methods, $n = 5$.

4. Discussion

Soluble β-1,3-glucan has been demonstrated to protect against infection and shock in rats and mice [28,29], and clinical studies suggest that administration of soluble glucans to trauma/surgical patients stimulates conversion from leukocyte anergy, decreases septic complications and improves survival [30,31]. However, little is known about the precise mechanisms by which glucans alter the septic state, or how glucans affect the cell biology of leukocytes, which are responsible for the fulminant production of cytokines and the activation of the coagulation system associated with serious gram-negative infection. The present study was therefore initiated to investigate the effect of a soluble β-glucan and LPS, alone or in combination, on leukocyte activation in human whole blood.

In short, we found that β-glucan itself induced significant amounts of IL-8 and TF, but only minor amounts of TNFα, IL-6, and IL-10. As expected, LPS-induced substantial amounts of all measured parameters. β-1,3-Glucan had a strong synergistic effect on the LPS-induced secretion of IL-8, IL-10, and on monocyte TF activity, but not on TNFα and IL-6 production. On the other hand, β-glucan strongly primed LPS stimulation of all parameters, including TNFα and IL-6. β-Glucan also induced detectable neutrophil degranulation within 15 min, whereas a response to LPS was first detected after 90 min.

The time course of LPS-induced TNFα, IL-6 and IL-8 production in whole blood and TF activity in monocytes observed in the present study has also been observed in previous studies [38,39]. β-Glucan, LPS and LPS + β-glucan-induced TF activity was almost completely abolished after 24 h, a pattern also observed with plasma TNFα levels. The decrease in TF activity and TNFα secretion coincided with the increase in IL-10 secretion and could thus, at least in part, be explained by the action of this cytokine known to have anti-inflammatory activity. IL-10 has been shown to inhibit LPS-induced human monocyte tissue factor expression in whole blood [40] and to decrease TNFα production in human monocytes [41].

β-Glucan at a concentration of 2 μg/ml was able to induce the secretion of small amounts of TNFα, IL-6 and IL-8 in whole blood anticoagulated with low molecular weight heparin, and 20 μg/ml β-glucan induced TF activity in monocytes, and production of all cytokines (Figs. 1 and 2). It has been reported that soluble yeast β-glucans activate NF-κB-like and NF-IL-6-like transcription factor complexes in a murine monocytic cell line [42] and in U937 cells [43], and that glucan phosphate increases IKKβ kinase activity in J774a.1 cells [44]. However, 1 μg/ml PGG-glucan did not induce IL-1α, IL-1β, IL-6, IL-8, or TNFα in citrated human whole blood after 3 h of incubation [6]. It seems thus that differences in β-glucan preparation and anticoagulant used to prevent the blood samples from clotting greatly influence the cytokine pattern observed after incubation. Previously we have shown that TF activity and TNFα production in citrated blood was significantly reduced as compared to heparinized blood [35].

Pretreatment of whole blood samples with soluble β-glucan enhanced LPS-induced TF activity and secretion of all cytokines measured independently of the length of preincubation time. Although β-glucan had no effect on LPS-induced IL-6 production when added simultaneously with LPS, preincubation with glucan resulted in increased LPS-induced IL-6 production. These results are in line with two other in vitro studies. Peritoneal macrophages from mice treated with soluble β-glucan showed increased LPS-stimulated NO production and increased PMA-stimulated superoxide production [45], and leukocytes isolated from PGG-glucan-stimulated whole blood displayed enhanced oxidative burst and microbicidal activity when stimulated with fNLP [6]. A paper by Soltys and Quinn [46] shows a differentiated picture of the effect of glucan pretreatment on in vitro cytokine production: When leukocytes were isolated from soluble β-1,6-branched β-glucan-treated mice and subsequently stimulated with LPS, monocytes expressed increased IL-2, decreased TNFα, and no change in IL-6 levels, while lymphocytes expressed increased IL-6, decreased TNFα, and no change in IL-2 and IFNγ levels. Most in vivo studies, however, strongly suggest that pretreatment with soluble glucans reduces cytokine production to subsequent challenge with LPS. Curdlan sulfate and lentinan have been shown to reduce LPS-induced TNFα and IL-1β in BCG-primed mice [47]. The same effect was observed in a model of murine *E. coli* sepsis where TNFα and IL-1 levels in soluble aminated glucan-treated mice were significantly lower than in untreated control animals [48]. Likewise, pretreatment with β-glucan decreased IκBα phosphorylation, NF-κB and NF-IL-6 activation, and cytokine mRNA levels in the lung and liver tissue of mice with surgery-induced polymicrobial sepsis [44,49]. In contrast to the above cited studies, glucan phosphate has been reported to potentiate LPS-induced interferon-γ (IFN-γ) release in mice by increasing production of the IFN-γ inducing cytokines IL-12 and IL-18 [50]. There seems thus to be a very complex picture with respect to how these two compounds interact both in in vitro and in vivo model systems, probably depending on the glucan preparation and the experimental model system used.

In the present study, we observed that β-glucan strongly and synergistically increased LPS-induced TF activity and plasma levels of IL-8 and IL-10, but had only a small synergistic effect of LPS-induced TNFα production and no effect at all on IL-6 production (Table 1, column G). Furthermore, β-glucan per se was able to induce the production of substantial amounts of TF and IL-8, but not of the other cytokines (Table 1, column E). These results might be explained by the ability of glucan to bind and crosslink CD11b/CD18 [9,10]. It has been reported that crosslinking of CD11b/CD18 in PMN induces secretion of IL-8 and IL-1β, but not TNFα and IL-6 [51]. Moreover, aggregation of CD11b/CD18 on the surface of monocytes has been shown to induce procoagulant activity [52]. Signaling pathways activated by clustering and crosslinking of CD11b/CD18 might thus act synergistically

with LPS-induced pathways and lead to upregulation of IL-8 and TF synthesis.

PMN elastase is an important marker of PMN activation [53] and is released upon activation and degranulation. As demonstrated in this paper, β-glucan and LPS behaved differently with respect to the kinetics of PMN activation: β-glucan increased PMN elastase levels already after 15 min of incubation, whereas an effect of LPS first was observed after 90 min. The two compounds did not have any effect on platelet activation. These results show that β-glucan is capable of specifically activating monocytes and neutrophils, but not blood platelets. The results indicate furthermore that β-glucan, in contrast to LPS, can induce PMN degranulation independently of transcription, probably by aggregating CD11b/CD18.

The present study shows that soluble yeast β-glucan is recognized by and interacts with cells of the innate immune system in humans, and that soluble glucan not only modulates, but (in most cases) upregulates leukocyte function, both on its own and in response to LPS. The favorable effect of soluble β-1,3-glucans on patients with septic complications reported in some studies [30,31] might thus at least in part, be explained by a stimulating effect of soluble glucan on leukocyte anergy, which is often observed in serious gram-negative infections (for review, see Ref. [23]). However, this study also shows that LPS and β-glucan exert different effects on human leukocytes, both with respect to the type of responses and also with respect to the kinetics of the induced responses. We have also shown that soluble β-glucan, as does LPS, interacts with the extrinsic pathway of coagulation by inducing TF activity on monocytes. The mechanisms behind this interaction and the cellular receptors involved in this interaction were not addressed in the present study, and need further investigations.

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