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INFLUENCE OF A BAGASSE GLUCAN (B_0) ON LEUKOCYTE AGGREGATION AND ACCUMULATION

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ABSTRACT Glucan B_0, isolated from bagasse, activated the classical pathway of complement. B_0-treated-human-serum (B_0TS, 0.1 m*M* B_0, 30 min, 30℃) aggregated human leukocytes dose-dependently. 1 ml B_0TS yielded an aggregative activity of 4.4 ± 1.6 μg hog C5a-desArg equivalents. The leukocyte accumulation in guinea pigs was apparently induced by intrapleural injection of large aggregates of B_0. In cobra venom decomplemented mice, B_0 still caused splenomegaly. Thus the complement activating effect and RES stimulating effect are apparently independent.

KEY WORDS glucans; complement activation; leukocytes; anaphylatoxins (C5a-des-Arg); cobra venoms; splenomegaly

The glucan B_0 from sugar cane had immunopotentiating action by enhancing the functions of host reticuloendothelial system[1]. It activated the classical complement pathway in serum by interacting with immunoglobulins, particularly IgG[2]. Normal human serum contains natural antibodies against B_0 by binding it to their F(ab) regions[3]. The antibodies to glucan could be resulted from previous fungal infections, since the basic β-1, 3 glucan molecular structure is present in all fungal cell wall[4].

Leukocytes change their surface structure and functional characteristics in response to mediators of chemotactic peptides such as C5a which is released after the cleavage of complement components.

The present work reports the influences of B_0 on leukocyte aggregation *in vitro* and accumulation *in vivo*. The relationship of complement activating and spleen enlarging effect of B_0 was also studied on complement depleted mice.

MATERIAL AND METHODS

The purification of B_0 was described previously[1]. For assays, B_0 was dissolved in normal saline or other appropriate buffers.

Preparation of human peripheral leukocytes Human blood was obtained by puncture of the cubital vein; Na_2-EDTA (pH 7.3) was used in a final concentration of 5.4 m*M* as anticoagulant. EDTA-blood 1 vol was added carefully with 2 vol of ice-cold 3% dextran (dissolved in saline, mw 70000, purchased from Pharmacia, Uppsala, Sweden). After 30

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min sedimentation of the erythrocytes, the leukocyte supernatant was collected and centrifuged at 4℃, 150 × *g*, for 15 min. The platelet-containing supernatant was discarded and erythrocytes remaining in the cell pellets were lysed by distilled water for 10–15 s; after reconstitution with 0.45 *M* NaCl to 0.15 *M*, the samples were centrifuged at 4℃, 150 × *g*, for 15 min. The leukocyte pellets were resuspended in Ca^{2+}- and Mg^{2+}-free Gey's solution with 0.5% human serum albumin (HSA; grade "purified", purchased from Boehringwerke, Marburg, Germany) to a final concentration of 1×10^7 cells/ml.

Measurement of leukocyte aggregation Leukocyte suspension 1 ml was incubated at 37℃ under continuous stirring in siliconized glass cuvettes in an "aggregation and shape change monitor" (Born-Michal, MK IV)[6]. After 3 min 0.01 ml 0.1 *M* $CaCl_2$ and $MgCl_2$ solution and 1 min later 0.1 ml B_0treated-human-serum (B_0TS; incubated with 0.1 m*M* B_0 at 30℃ for 30 min) or saline as control were added. Zymosan-treated-human-serum (ZTS; zymosan 2 mg/ml serum incubated at 37℃ for 60 min, centrifuged) was studied simultaneously for comparison. Thereafter, the incubation was continued for further 3 min. Before addition of Ca^{2+} and Mg^{2+} the transmission of the original samples which contained no aggregates was set to zero; 100% transmission was given by the cell-free supernatants obtained after centrifugation. During the incubation the light transmission of the samples was continuously recorded on a XY recorder. Increases in transmission indicated increases in aggregation.

Intrapleural injections Sterile saline as control or B_0-solution in saline were injected (2 ml) into the pleural cavity of guinea pigs weighing 250–350 g. After 180 min the animals were killed. The chest was opened and 2 ml 0.01 *M* EDTA-saline were instilled to prevent aggregation of leukocytes. Then the total pleural fluid was aspirated, its volume measured and the number of leukocytes therein was counted under a phase contrast microscope.

Injections with Evans blue For estimating changes in vascular permeability 5% Evans blue 1 ml/kg was injected into the vena saphena 30 min after intrapleural injections of B_0 solutions or saline. After further 150 min the animals were killed. The concentrations of Evans blue in the supernatants of the pleural fluids were measured photometrically at 623 nm.

RESULTS

Influence of B_0 on leukocyte aggregation B_0 added in a final concentration of 0.1 m*M* to stirred leukocyte suspensions did not induce any aggregation. However, B_0TS aggregated human leukocytes dose-dependently. Dose-response relations obtained with B_0TS and ZTS were compared with those of hog C5a-desArg aggregating potencies. The ED_{50} values of aggregative activity were 4.4± SD 1.6 and 9.3±2.0 μg hog C5a-desArg equivalents for each ml of B_0TS and ZTS, respectively (3 experiments each). Aggregating activities of both activated sera were apparently induced by C5-fragments, most likely by C5a-desArg, as the C5a receptors on leukocytes which had been occupied by pretreatment with hog C5a-desArg in Ca^{2+}-and Mg^{2+}-free medium (to prevent aggregation) were no longer aggregated by B_0TS or ZTS in complete media reconstituted with Ca^{2+} and Mg^{2+}. The difference in potencies was consistent with the consumption of C5: in B_0TS about 30% of C5 had been consumed, in ZTS more than 90% after incubation[2].

Influence of B_0 on leukocyte accumulation and vascular permeability in the pleural cavity of guinea pigs Intrapleural injections of 2 mg B_0, caused a 7-fold increase in leukocyte numbers as compared with injections of saline control (Tab 1). However, after ultracentrifugation under 4℃, 18 h with 100,000 × *g*, B_0(0.1, 0.5 and 2 mg/animal) did not show such a leukocyte-accumulating effect anymore. In contrast to this, the complement-activating effect of B_0 in guinea pig serum was only moderately reduced after ultracentrifugation: CH 50 values after 30 min, 30℃ incubation decreased by 48%

Tab 1. Effect of intrapleural injection of B_0 on leukocytes accumulation and vascular permeability in guinea pigs. $\bar{x} \pm SD$, $^*p>0.05$, $^{**}p<0.05$, $^{***}p<0.01$

	B_0 (mg)	Guinea-pigs	Pleural fluid (ml)	Evans blue (μg)	Total leukocyte ($\times 10^{-6}$)	Serum complement (CH 50)
Control	—	4	0.85±0.06	5.9±1.8	3.0±0.2	1176
B_0	2.0	4	1.00±0.40*	5.6±3.5*	21±9***	606
B_0 after centrifuge 100,000×g	2.0	4	0.75±0.48*	4.6±1.6*	3.5±1.0*	769
	0.5	3	0.30±0.23*	4.0±2.1*	2.9±0.6*	
	0.1	2	0.92±0.36*	6.4±2.8*	4.1±0.1*	

Tab 2. Effect of complement depletion on spleen enlargement of B_0. 3 mice/group, $^*p>0.05$, $^{***}p<0.01$

Group	Treatment	Spleen weight (g/kg)	Total CH 50	Complement (%)	C3 immunodiffusion (%)
1	Control	4.5±0.1	62±15	100	100
2	B_0	7.7±0.4***	13±5***	20	75±2*
3	CVF	4.5±0.5*	5±1***	8	5±1***
4	CVF + B_0	8.2±0.6***	3±2***	4	20±7***

with untreated B_0, but only by 35% with ultracentrifuged B_0 (Tab 1, right column).

Vascular permeability was apparently not affected by either preparation of B_0: Pleural fluid volumes did not change and the amount of Evans blue in the pleural cavity remained on the same level as that of the control animals.

Influence of complement depletion on the gain of spleen weight induced by B_0 The spleen weight of BALB/c mice was markedly increased after a 5-d treatment with ip B_0 (50 mg/kg/d): it increased from 4.5 g/kg in control to 7.7 g/kg in B_0-treated mice (Tab 2). In addition, B_0-treatment led to a decrease of total hemolytic complement activity in serum of mice, namely to 20% of controls; the amount of C3 detectable in radial immunodiffusion was reduced to 75% only. Treatment with cobra venom factor (CVF) ip on d 1,3 and 5 caused almost complete decomplementation[8] (complement fell to <10% in both tests), but did not alter spleen weight (group 3 in Tab 2). In mice treated simultaneously with B_0 and CVF (group 4) the complement level was similarly decreased and the gain of spleen weight was still present (8.2 g/kg; group 4 in Tab 2).

DISCUSSION

B_0 had no direct activity on leukocyte aggregation. The leukocyte stimulating potency induced by B_0TS was via its complement activating action, most likely by B_0 releasing of C 5-cleavage peptide, or its C5a-desArg derivative. This is supported by the finding that B_0TS as well as ZTS caused cross desensitization with purified C5a-desArg.

Intrapleural injection of B_0 in guinea pigs induced significant leukocyte accumulation. After ultracentrifugation of B_0 solution, the effect became less apparent. It seems that leukocyte accumulation is induced mainly by large aggregates of B_0 or its polymerized products. The anticomplement activity remained unchanged after ultracentrifugation, as the authors

have shown previously[2].

CVF caused complete decomplementation in mice. When mice were treated simultaneously with B_0 and CVF, the complement activation was completely blocked, but the spleen still enlarged. It indicates that the complement activating and RES stimulating effects of B_0 are apparently independent.

Glucan is a broad spectrum enhancer of host defense mechanisms[7]. The activation of complement, generating biologically active fragments and inducing the elevation of leukocyte activities by B_0, may well play an important role in modifying a variety of infectious diseases.

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甘蔗渣葡聚糖 B_0 对白细胞凝集和积聚反应的影响

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提要　从甘蔗渣中提取的葡聚糖B_0能激活补体经典通路。B_0处理人血清(B_0TS，0.1 m*M* B_0，30 min，30℃)对人白细胞的凝集作用存在剂量依赖关系。1 ml B_0TS产生的凝集效果相当于 4.4±1.6 μg 猪 C 5a-desArg。豚鼠胸腔内注射 B_0 引起的白细胞积聚作用显然与 B_0 中一些大分子物质有关。在眼镜蛇毒耗竭补体的小鼠，B_0仍引起脾脏增大，说明它激活补体和兴奋 RES 是两个无关的作用。

关键词　葡聚糖；　补体激活；　白细胞；　过敏毒素；　眼镜蛇毒；　脾大

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