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Characterization of a Rabbit Cationic Protein (CAP18) with Lipopolysaccharide-Inhibitory Activity

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Cationic antibacterial proteins (CAP) were purified from rabbit granulocytes, and the effects of CAP on lipopolysaccharide (LPS)-induced tissue factor generation by murine peritoneal macrophages and human blood monocytes were studied. CAP were purified from rabbit peritoneal leukocytes by using as an assay the agglutination of erythrocytes coated with Re-LPS. Two proteins with CAP activity, CAP18 (18 kDa) and CAP7 (7 kDa), were isolated by acid extraction, ethanol precipitation, affinity chromatography, gel filtration, and reverse-phase high-pressure liquid chromatography. On the basis of protein sequencing, CAP7 was identified as the C-terminal fragment of CAP18, designated $CAP18_{106-142}$. Various forms of LPS (S-LPS, Re-LPS, and lipid A) activate murine macrophages and human blood monocytes to generate tissue factor (tissue thromboplastin). Incubation of LPS for 18 h with partially purified CAP (heparin-Sepharose fraction) inhibited the capacity of LPS to induce tissue factor; however, purified CAP18 inhibited about 75% of the activity of S-LPS after 1 h of incubation. CAP more effectively inhibited S-LPS than Re-LPS or lipid A. Synthetic $CAP18_{106-142}$ inhibited LPS-induced tissue factor generation by murine macrophages. $CAP18_{106-142}$ has greater LPS-binding and LPS-neutralizing activities than CAP18. We hypothesize that CAP18 and the derivative peptide, $CAP18_{106-142}$, bind to LPS and alter the capacity of LPS to initiate disseminated intravascular coagulation. In this regard, CAP may have therapeutic potential for sepsis and endotoxin shock.

Blood coagulation and hemostasis are important components of the host defense system against traumatic injury and at inflammatory sites. Overwhelming infections, particularly those associated with release of endotoxin, can cause excessive activation of the coagulation cascade, a condition termed disseminated intravascular coagulation. The blood coagulation cascade has classically been divided into two parts, i.e., the intrinsic and extrinsic pathways. The intrinsic pathway is initiated through activation of factor XII to XIIa secondary to exposure of collagen from damaged vascular endothelial cells. The extrinsic pathway is initiated by expression of tissue factor (procoagulant, tissue thromboplastin) by activated monocytes and macrophages. Tissue factor converts factor VII to VIIa (15, 18), and the tissue factor-VIIa complex converts factor X to Xa and factor IX to IXa. Both pathways converge at the generation of a prothrombinase composed of a complex of factor Xa, factor V, phospholipid, and calcium ions. Prothrombinase converts prothrombin to thrombin, which cleaves fibrinogen. Because both monocytes and macrophages, as well as endothelial cells, produce tissue factor following exposure to lipopolysaccharide (LPS) (14), the extrinsic pathway is thought to play an important role in LPS-induced disseminated intravascular coagulation (23).

Previously the cationic LPS-binding proteins (CAP) were isolated from mouse bone marrow cells and rabbit granulocytes. These partially purified proteins inhibited LPS-induced tissue factor generation by mouse spleen cells in vivo (2) and LPS-induced lethality to mice (22). Because CAP agglutinate erythrocytes coated with LPS, especially Re-LPS and lipid A (5), a simple assay was developed to purify these molecules. By using this assay, a unique LPS-binding protein composed of two domains was isolated from rabbit granulocytes and sequenced. The amino acid sequence of a 7-kDa protein (CAP7) was used to clone the cDNA and to deduce the nucleotide sequence of the longer 18-kDa protein (CAP18) (9). In the present report, CAP7 and CAP18 are shown to inhibit LPS-induced tissue factor generation by mouse peritoneal macrophages and human blood monocytes.

MATERIALS AND METHODS

LPS preparations. Preparations of *Salmonella minnesota* S-LPS and Re-LPS were purchased from List Biological Laboratories, Inc., Campbell, Calif. Synthetic lipid A (no. 506) was purchased from Daiichi Chemicals, Tokyo, Japan.

Preparation of granulocytes. Peritoneal granulocytes were obtained from rabbits (weighing 2 to 3 kg) that had received 500 ml of saline containing 0.25% sodium caseinate intraperitoneally 16 to 17 h earlier. Cells were washed twice with saline and homogenized in 0.1 M citric acid (20). After centrifugation at 40,000 × *g* for 30 min, cold ethanol was added to the supernatant (80% [vol/vol]) and crude CAP was precipitated.

Purification of CAP. Crude CAP was applied to a heparin-Sepharose CL-6B column (1.0 by 5.0 cm; Pharmacia) equilibrated with 0.05 M Tris-HCl (pH 6.0) containing 0.1 M NaCl. The column was eluted with several concentrations of NaCl. The fractions containing LPS-binding activity were pooled and dialyzed against 0.1% acetic acid and lyophilized. This fraction was dissolved in 0.05 M sodium sulfate–0.02 M sodium phosphate buffer (pH 6.7) containing 1 M NaCl and applied to a TSK 125 (Biogel 125) column equilibrated with the same

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buffer. Molecular weight standards for gel filtration were obtained from BHD Limited, Boom Road, England. These included myoglobin (17.2 kDa), ovalbumin (45 kDa), and lactate dehydrogenase (145 kDa). The fractions containing LPS-binding activity were pooled, lyophilized, and subjected to further purification by reverse-phase (RP) high-pressure liquid chromatography (HPLC) on a Toso-TSK system C8 column (4.6 by 250 mm). Water-acetonitrile gradients containing 0.1% trifluoroacetic acid were used for elution. All glassware and buffer solutions were heated at 250°C for 22 h or autoclaved. A Teflon homogenizer was soaked overnight in 95% ethanol containing 0.2 M NaOH and used for extraction of CAP from granulocytes. Proteins were determined by the method of Lowry et al. (12a) with bovine serum albumin as the standard.

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). Samples were mixed with an equal volume of sample buffer and heated at 100°C for 3 min. Electrophoresis was performed under reducing conditions with 18% SDS gels at a constant current of 30 mA. The running buffer was 0.13 M Tris-HCl (pH 6.8) containing 0.2 M glycine, 0.15% dithiothreitol, and 4% SDS. Gels were stained for protein with 0.025% Coomassie blue R250.

Sequence determination. Purified protein was subjected to gas-phase Edman degradation on an Applied Biosystems (Foster City, Calif.) sequencer with an RP column (2.1 by 250 mm).

Peptide synthesis. Synthetic peptides were prepared by solid-phase Merrifield synthesis and purified by HPLC at the Core Biotechnology Center of the University of California, Davis.

LPS-binding assay. Erythrocyte sensitization with LPS and CAP-mediated hemagglutination were performed exactly as previously described (5). LPS-binding activities of CAP preparations were expressed as minimum agglutinating concentrations (MAC) and as hemagglutination units (HA).

Preparation of peritoneal macrophages. Mouse peritoneal cells were elicited with an intraperitoneal injection of thioglycolate medium (Eiken Chemical Co. Ltd., Tokyo, Japan) and harvested 5 days later by irrigation with pyrogen-free saline. The cells were washed with RPMI 1640 medium, counted, and resuspended at a concentration of 1.5×10^6 to 2.0×10^6/ml in serum-free RPMI medium containing glutamine (0.29 μg/ml), penicillin (50 U/ml), and streptomycin (50 μg/ml). Adherent cells were prepared by incubating 10 ml of a cell suspension in RPMI 1640 medium in a sterile tissue culture flask (50 ml; Nunclon; Inter Med) for 60 min at 37°C in a 5% CO_2 atmosphere. The plastic adherent cells were recovered by scraping the flask with a sterile rubber policeman. The adherent cells were then washed twice with RPMI medium, resuspended in serum-free medium (10^6/ml) in a culture tube (Nunc Cryotube; Inter Med), and stimulated with S-LPS, Re-LPS, or lipid A (0.1 or 1.0 μg/ml) at 37°C in 5% CO_2 for 6 h, which coincided with the peak of tissue factor generation (2). The cell suspension was centrifuged, and the cell pellet was frozen at −80°C until clotting assays were run.

Preparation of human mononuclear cells. Human mononuclear cells were separated from citrated peripheral blood by centrifugation over sodium-metrizoate-Ficoll (Lymphocyte Separation Medium; Japan Antibody Institute, Gumma, Japan). The resulting mixed mononuclear cell suspension was washed three times with RPMI 1640 medium. The cell suspension (10^6/ml) was stimulated with S-LPS (0.1 μg/ml) for 16 to 18 h, which corresponded to the peak time of tissue factor generation (16).

Tissue factor activity assay. The cell pellet was resuspended in Veronal-buffered saline, lysed by three freeze-thaw cycles, and sonicated for 10 s with a hand-held microbe sonicator (Handy Sonic UR-20P; Tomy Seiko Ltd., Tokyo, Japan). Tissue factor activity was measured as modified unactivated partial thromboplastin time (1). One-tenth of a milliliter of a homogeneous suspension of cell lysate (10^6 cells per ml) was preincubated with 0.1 ml of mouse plasma at 37°C for 3 min. Then 0.1 ml of 25 mM $CaCl_2$ that contains phospholipid was added to the mixture, and clotting time was measured with a fibrometer (BioQuest Division, Becton Dickinson, Cockeysville, Md.). A standard curve obtained from serial twofold dilutions of rabbit brain thromboplastin (Simplastin; Ono Pharmaceutical Co.) was used to transpose tissue factor activity into arbitrary tissue factor units (a 1-μg/ml suspension of rabbit brain was assigned a value of 1,000 U).

Statistical analysis. The mean number of tissue factor units ± the standard deviation was determined for three or four samples. Student's *t* test for unpaired data was used to determine statistically significant differences. A two-tailed *P* value of <0.05 was considered significant.

TABLE 1. LPS-binding activity of CAP

Re-LPS concn used for sensitization (μg/ml)	MAC of CAP[a] (μg/ml)		
	Sheep RBC	Human RBC	Mouse RBC
0	500	>100	>100
0.01	—[b]	—	100
0.1	—	—	50
1	—	>100	25
10	—	50	—
100	7.8	12.5	—

[a] Crude CAP (ethanol precipitate) was used. RBC, erythrocytes.
[b] —, not tested.

RESULTS

Purification of CAP. (i) LPS-binding activity: CAP-mediated hemagglutination. The LPS-binding activity of CAP was determined by measuring its ability to agglutinate erythrocytes coated with LPS (Table 1). Crude CAP agglutinated sheep, human, and mouse erythrocytes coated with Re-LPS. The MAC of CAP for sheep erythrocytes coated with 100 μg of LPS per ml were 1.6 to 7.8 μg/ml. The MAC of CAP was inversely related to the amount of LPS used for sensitization, i.e., a small amount of CAP agglutinated erythrocytes coated with a large amount of LPS.

(ii) Purification steps and LPS-binding activity. Figure 1A shows the elution profile of crude CAP on a heparin-Sepharose CL-6B column. LPS-binding activity was found only in fraction III, which eluted with 2.0 M NaCl (MAC, 8.8 μg/ml). The MAC of fractions I and II were >378 and >276 μg/ml, respectively. Fraction III (70 μg/ml; 8 HA) was preincubated with S-LPS, Re-LPS, or lipid A at 37°C for 30 min, and each reaction mixture was added to erythrocytes coated with Re-LPS. All of the preparations inhibited CAP-mediated hemagglutination, and the MICs of S-LPS, Re-LPS, and lipid A were 3.13, 6.25, and 6.25 μg/ml, respectively. Tubes 84 to 88 of fraction III were pooled and subjected to gel filtration on a Biogel 125 column (Fig. 1B). Two fractions exhibiting approximate molecular masses of 44 and 17 kDa had LPS-binding activity. The shaded fraction containing the highest activity with the higher molecular mass was subjected to RP HPLC (Fig. 1C). Two peaks containing LPS-binding activity were identified. The MAC of peaks 1 and 2 were 1.9 and 3.5 μg/ml, respectively.

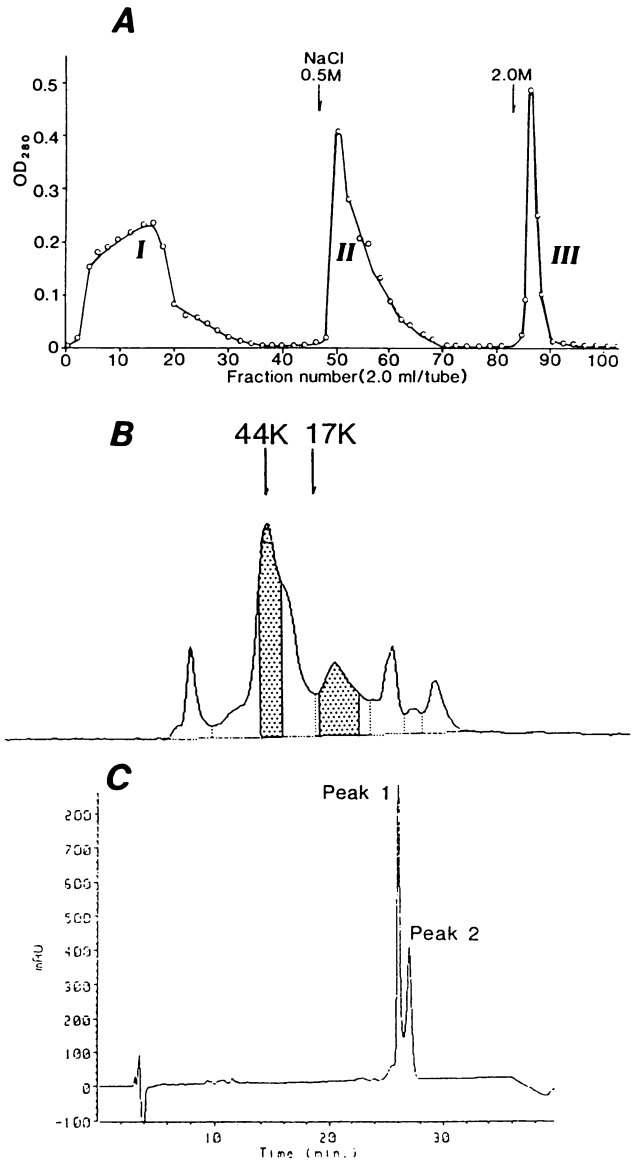

FIG. 1. Chromatography of LPS-binding proteins. (A) Heparin-Sepharose CL-6B column chromatography of crude CAP. Crude CAP (ethanol precipitate; 28 mg) was dissolved in 0.05 M Tris HCl buffer (pH 6.0) containing 0.1 M NaCl and applied to a column equilibrated with the same buffer. Stepwise elution was performed at 4°C. Step I, buffer described above; step II, buffer containing 0.5 M NaCl; step III, buffer containing 2.0 M NaCl. LPS-binding activity was assayed as described in Materials and Methods. OD_{280}, optical density at 280 nm. (B) Gel filtration of fraction III from the heparin-Sepharose CL-6B column. (C) Final purification step of CAP18 on RP HPLC.

(iii) SDS-PAGE. The lyophilized materials of peaks 1 and 2 were analyzed for homogeneity by SDS-PAGE under reducing conditions. The molecular masses of peaks 1 and 2 were estimated with SDS-PAGE to be 18 and 37 kDa, respectively (Fig. 2). A second, large-scale purification was then undertaken to obtain quantities of CAP18 sufficient for primary amino acid sequence analysis. As in experiment 2, fraction III obtained by affinity chromatography was directly applied to RP HPLC, and four peaks (A to D) were obtained (Fig. 3). Each

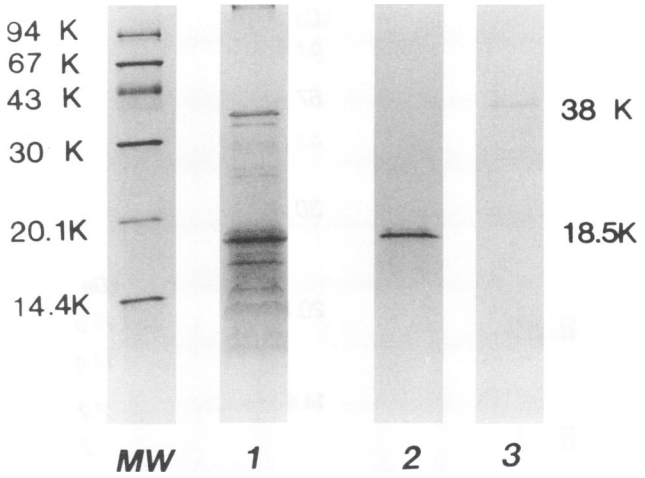

FIG. 2. SDS-PAGE of heparin-Sepharose fraction III and RP HPLC fractions (experiment 1). Lanes: 1, heparin-Sepharose fraction III (7.5 μg of protein); 2, peak 1 of RP HPLC (2.4 μg of protein); 3, peak 2 of RP HPLC (1.1 μg of protein). MW, molecular weight standards. Molecular weights are given in thousands.

fraction was lyophilized and dissolved in 100 μl of 20 mM HCl solution and neutralized with 20 mM NaOH.

All of the peaks had LPS-binding activity. The MAC of peaks A to D were 4.1, 3.0, 1.5, and 9.6 μg/ml, respectively. Peak C was the most active among these proteins. SDS-PAGE of peaks C and D showed bands at 7 and 18 kDa, respectively (Fig. 4). The 18-kDa protein was thought to be identical to CAP18 (lane 2 in Fig. 2).

(iv) Amino acid sequence of CAP18. The four HPLC peaks were subjected to microsequencing. The sequence of CAP7 (Fig. 5A) reveals a high content of cationic residues such as arginine and lysine. Figure 5B shows the location of CAP7 at the 3′ terminus of the CAP18 cDNA (9). The sequences of peaks A and B were identical to those of rabbit defensins (natural peptides 1 and 2).

Activity of CAP. (i) CAP18 inhibits LPS-induced tissue factor generation by murine macrophages. Partially purified CAP (Fig. 1A, fraction III) was tested for inhibition of LPS-induced tissue factor generation by macrophages. Preliminary experiments showed that 45 min of preincubation with 1 μg of CAP per ml was not sufficient to inhibit the tissue factor-inducing activity of S-LPS, Re-LPS, or lipid A (0.1 and 1 μg/ml). By comparison, the activity of 0.1 μg of S-LPS per ml

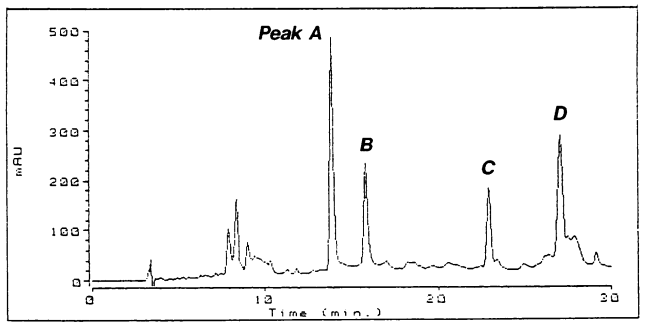

FIG. 3. Elution profile of fraction III material from heparin-Sepharose CL-6B RP HPLC.

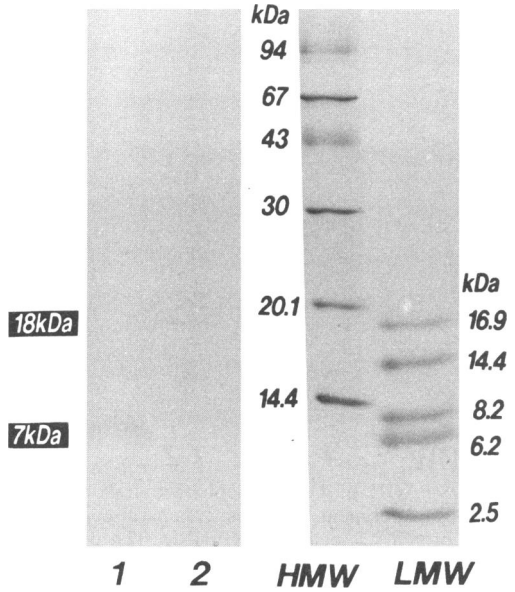

FIG. 4. SDS-PAGE of RP HPLC fractions (experiment 2). Lanes: 1, peak C of RP HPLC (0.4 μg of protein); 2, peak D of RP HPLC (0.6 μg of protein). HMW, high-molecular-weight standards. LMW, low-molecular-weight standards.

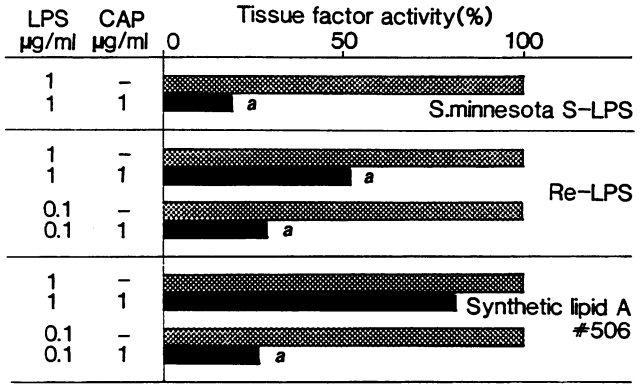

FIG. 6. Inhibition of LPS-induced tissue factor generation by CAP. Each LPS preparation was preincubated with fraction III of heparin-Sepharose chromatography (lot 2) at 25°C for 18 h, and the mixture was added to a macrophage suspension, which was then cultured for 6 h. The mean tissue factor activity of an LPS control (three samples) was taken as 100%. *a*, $P < 0.05$ compared with each LPS control.

was completely inhibited by incubation with LPS-binding antibiotic polymyxin B at 37°C for 45 min. In contrast to these results, the activity of Re-LPS or lipid A was not inhibited, but rather enhanced, by polymyxin B (2). Therefore, each LPS preparation was incubated with CAP for 18 h. In Fig. 6, the tissue factor activity of the LPS control is expressed as 100%. CAP inhibited tissue factor generation induced by all of the preparations. When 1 μg of the heparin-Sepharose preparation per ml was used, 80% of the activity of 1 μg of S-LPS per ml, 50% of the activity of 1 μg of Re-LPS per ml, or 20% of the activity of 1 μg of lipid A per ml was inhibited by CAP. In other experiments, polymyxin B completely inhibited lipid A activity (data not shown).

Subsequent experiments tested purified CAP18 preincubated with S-LPS for 1 to 6 h (Fig. 7). After only 1 h of incubation, 75% of endotoxin activity was inhibited by CAP18. The 37-kDa protein (CAP37 [peak 2 of Fig. 1C]) did not inhibit the LPS activity. It is possible that contaminants present in the semipurified CAP interfered with its interaction with LPS, thus causing more prolonged kinetics in the previous experiments.

(ii) CAP18 and CAP18$_{106-142}$ inhibit LPS-induced tissue factor generation by human blood monocytes. Each of the four purified proteins (Fig. 3, peaks A to D) was tested for the capacity to inhibit LPS-induced tissue factor. Figure 8 demonstrates that 0.1 μg of LPS per ml increased tissue factor activity in human monocytes about 17-fold. After 45 min of incubation of LPS with native HPLC-purified CAP7 (3.0 μg/ml) or CAP18 (9.6 μg/ml), about 50 or 30% of the LPS activity was inhibited, respectively. Both rabbit defensins, natural peptides 1 (16.4 μg/ml) and 2 (6.0 μg/ml), were inactive.

(iii) LPS-neutralizing activity of synthetic CAP18$_{106-142}$. To confirm the LPS-neutralizing activity of CAP18$_{106-142}$, a synthetic peptide consisting of 37 amino acid residues (sequence identical to that of native CAP7) was tested. The LPS-binding activity (MAC) of CAP18$_{106-142}$ was 3.1 μg/ml. All of the LPS preparations from *S. minnesota* Re, *Escherichia coli* J5, *E. coli* O111:B4, *E. coli* O127:B8, *Pseudomonas aeruginosa* F-D type 1, and *Klebsiella pneumoniae* inhibited hemagglutination in-

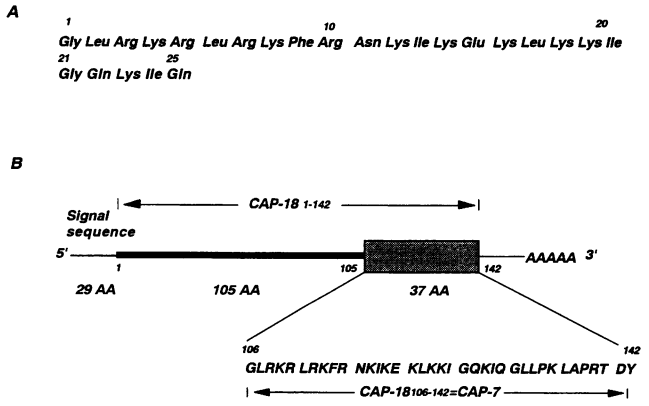

FIG. 5. (A) Amino acid sequence of CAP7. (B) Location of CAP7 on CAP18 cDNA.

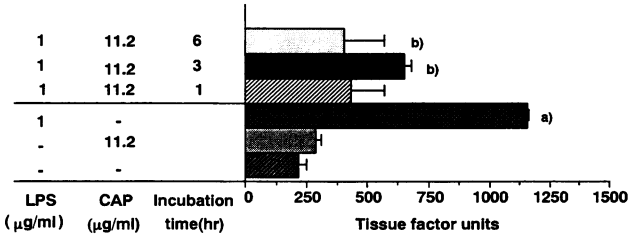

FIG. 7. Inhibition of LPS-induced tissue factor generation by purified CAP18. S-LPS was preincubated with CAP18 at 37°C for 1, 3, or 6 h, and the mixture was added to the macrophage suspension, which was cultured for 6 h. Tissue factor activity was expressed as standard tissue factor units per 10^5 cells. Results were expressed as the mean ± the standard deviation of at least three or four samples. a), $P < 0.05$ compared with medium control. b), $P < 0.05$ compared with LPS control.

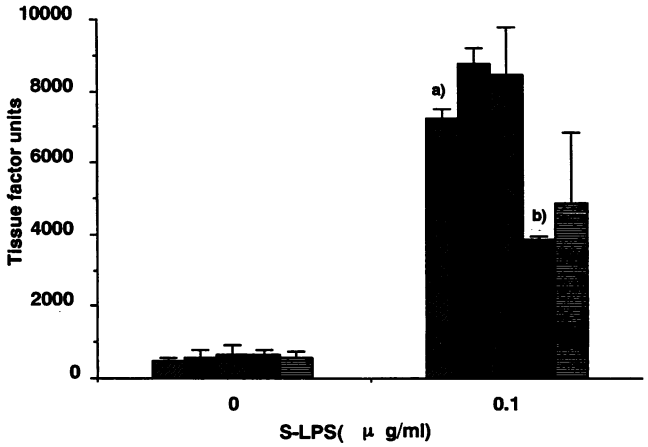

FIG. 8. Effects of LPS-binding proteins on LPS-induced tissue factor generation by human mononuclear cells. S-LPS (2 μg/ml) was preincubated with an equal volume of each LPS-binding protein at 37°C for 45 min, and the mixture was added to the cells, which were cultured for 16 h. Tissue factor activity was expressed as standard tissue factor units per 3.5×10^4 cells. Key: ▨, medium control; ▩, natural peptide 1 (4 HA; 16.4 μg/ml); ▩, natural peptide 2 (2 HA; 6.0 μg/ml); ■, CAP7 (2 HA; 3.0 μg/ml); ▤, CAP18 (1 HA; 9.6 μg/ml). a), $P < 0.01$ compared with medium control. b), $P < 0.05$ compared with LPS control.

duced by 25 μg (8 HA) of synthetic peptide per ml. The MICs of LPS preparations were 15.6 to 62.5 μg/ml. In these experiments, LPS was incubated with peptide at 37°C for 45 min (Fig. 9).

Tissue factor activity of the cells was increased from 50 ± 12 to 480 ± 132 U by stimulation with 1.0 μg of LPS per ml. Synthetic $CAP18_{106-142}$ (1 μg/ml) inhibited the LPS activity significantly (about 91%). LPS stimulatory activity was completely inhibited after only 5 min of preincubation with peptide (data not shown). Peptide alone had no effect on cell viability as determined by the trypan blue exclusion test.

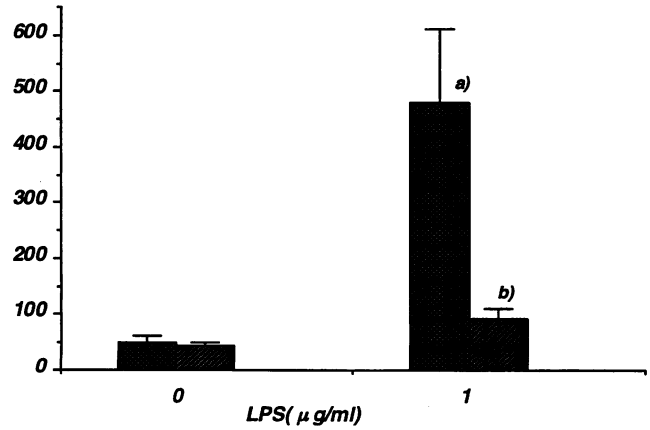

FIG. 9. LPS-neutralizing activity of synthetic peptide. S-LPS (1 μg/ml) was incubated with synthetic peptide at 37°C or 45 min, and then the mixture was added to a cell suspension, which was cultured for 6 h. Tissue factor activity was expressed as units per 1.4×10^5 cells. Key: ▩, medium control; ▨, peptide (1 μg/ml). a), $P < 0.01$ compared with medium control. b), $P < 0.05$ compared with LPS control.

DISCUSSION

LPS activates diverse types of cells to release many cytokines (e.g., interleukins 1, 6, and 8, tumor necrosis factor, etc.) and a plethora of chemical mediators. Overproduction of these soluble mediators in combination with mononuclear cell-generated tissue factor contributes to disseminated intravascular coagulation, shock, and the sepsis syndrome. An important approach to block disseminated intravascular coagulation secondary to endotoxemia is to inhibit directly the LPS released from gram-negative bacteria. Granulocytes contain a number of antimicrobial proteins and peptides important in host defense against bacterial infections. Among the best characterized are bactericidal permeability-increasing protein, CAP37, and the defensins (11, 19, 20). Bactericidal permeability-increasing protein has antibacterial activity against gram-negative bacteria but is not active against gram-positive bacteria (21). Bactericidal permeability-increasing protein is known to block LPS-stimulated complement receptor activation on granulocytes in vitro (13) and LPS lethality to mice in vivo (10). Antibacterial defensins composed of 30 to 40 amino acid residues isolated from rabbit and human granulocytes (11, 19) are homologous with proteins identified in species as distant as insects (6). Defensins show optimal activity against gram-negative and gram-positive bacteria in hypotonic media and relatively neutral pH.

In the present report, we present details of the purification from rabbit granulocytes of two unique LPS-binding proteins, CAP7 and CAP18. For this purification, we used as an assay the agglutination of erythrocytes coated with Re-LPS. CAP-mediated hemagglutination is inhibited by free S-LPS, Re-LPS, and lipid A, indicating that CAP binds to all LPS preparations, including lipid A, as described previously (5). Among the proteins identified in the final HPLC separation were the rabbit defensins (natural peptides 1 and 2). Although these proteins demonstrated LPS-binding activity, they had no associated LPS-inhibitory activity in these experiments.

The sequence of the CAP18-encoding cDNA clone isolated from a rabbit bone marrow cDNA library was recently published (9). The deduced amino acid sequence revealed a putative signal sequence of 29 amino acids and a mature protein of 142 amino acid residues (9). From this cDNA, CAP7 was identified as the C-terminal fragment of CAP18 and consisted of 37 amino acid residues. Therefore, we designated CAP7 as $CAP18_{106-142}$ (Fig. 5B).

Inhibition of LPS induction of tissue factor was used to corroborate the bioactivity of CAP. In the present work, partially purified CAP was shown to require a prolonged (18-h) interaction with LPS to inhibit tissue factor generated from monocytes exposed to S-LPS, Re-LPS, and lipid A. In contrast, purified CAP7 and CAP18 and synthetic $CAP18_{106-142}$ rapidly neutralized the LPS after only 45 min of incubation. Interestingly, polymyxin B inhibited the activity of S-LPS after 1 h of incubation, whereas it enhanced the activity of Re-LPS and lipid A. After 18 h of incubation, polymyxin B completely inhibited the activity of lipid A (2). These results are similar to those of Jacob and Morrison (7) and Rickles and Rick (17), who previously reported inhibiting and enhancing effects of polymyxin B on activities of LPS. Further studies are required to determine the biophysical reasons for these differential effects of LPS-binding peptides on LPS function.

Recent studies have indicated that synthetic $CAP18_{106-142}$ neutralizes several biological effects of LPS both in vitro

(macrophage production of nitric oxide release and tumor necrosis factor) and in vivo (lethality to mice). In addition, this peptide shows antibacterial activity against gram-negative and gram-positive bacteria (3, 8), as well as anticoagulant activity (3, 4). The present studies, demonstrating inhibition of LPS-induced tissue factor release, add an important activity to this list.

CAP18 shows limited homology with other known LPS-binding proteins, such as bactericidal permeability-increasing protein, CAP37, or the defensins (11, 19). Analysis of the deduced primary structures of a pair of 15-kDa antibacterial proteins isolated from rabbit granulocytes revealed homology to CAP18, a cysteine protease inhibitor, and bovine indolicidin (12). With recombinant CAP18, it is possible to characterize the LPS-binding domain and study the LPS-neutralizing activity in various in vitro and in vivo models of endotoxemia. A human homolog of rabbit CAP18 may be useful as a therapy to neutralize LPS in endotoxemia and disseminated intravascular coagulation.

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