Syndecan-4 Deficiency Leads to High Mortality of Lipopolysaccharide-injected Mice*

Kazuhiro Ishiguro‡‡, Kenji Kadomatsu‡, Tetsuhiyo Kojima¶¶, Hisako Muramatsu‡, Mitsunori Iwase¶, Yasunobu Yoshikai*c, Masamitsu Yanada§§, Koji Yamamoto¶, Tadashi Matsuhita§, Masahiko Nishimura‡, Kazuo Kusugami‡, Hidehiko Saito§§, and Takashi Muramatsu†††

From the ‡Department of Biochemistry, §First Department of Internal Medicine, ¶¶Laboratory of Host Defense & Germfree Life, Research Institute for Disease Mechanism and Control, and †††Institute for Laboratory Animal Research, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, Aichi, 466-8550, Japan, the ‡Department of Medical Technology, Nagoya University School of Health Sciences, 1-1-20 Daiko-Minami, Higashi-ku, Nagoya, Aichi, 461-8673, Japan, and the §§Nagoya National Hospital, 4-1-1 Sannomaru, Naka-ku, Nagoya, Aichi, 460-0001, Japan

Syndecan-4 is a transmembrane heparan sulfate proteoglycan belonging to the syndecan family. Following intraperitoneal injection of lipopolysaccharide (LPS), syndecan-4-deficient mice exhibited high mortality compared with wild-type controls. Severe endotoxin shock was observed in the deficient mice: systolic blood pressure and left ventricular fractional shortening were lower in the deficient mice than in the wild-type controls 9 h after LPS injection. Although histological examinations revealed no apparent differences between two groups, the plasma level of interleukin (IL)-1β was higher in the deficient mice than in the wild-type controls 9 h after LPS injection. Consistent with the regulatory roles of syndecan-4, its expression in monocytes and endothelial cells of microvasculature increased in the wild-type mice after LPS administration. Although IL-1β was produced to the same extent by macrophages from syndecan-4-deficient and wild-type mice after LPS stimulation, inhibition of its production by transforming growth factor-β1 was impaired in the syndecan-4-deficient macrophages. These results indicate that syndecan-4 could be involved in prevention of endotoxin shock, at least partly through the inhibitory action of transforming growth factor-β1 on IL-1β production.

Septic shock is defined as sepsis with hypotension or multiple systemic organ failure and is a common cause of death in patients in the intensive care unit. Lipopolysaccharide (LPS) is one of the toxic principles of Gram-negative bacteria (1), and syndecan-4-deficient mice exhibited high mortality compared with wild-type controls 9 h after LPS injection. Although histological examinations revealed no apparent differences between two groups, the plasma level of interleukin (IL)-1β was higher in the deficient mice than in the wild-type controls 9 h after LPS injection. Consistent with the regulatory roles of syndecan-4, its expression in monocytes and endothelial cells of microvasculature increased in the wild-type mice after LPS administration. Although IL-1β was produced to the same extent by macrophages from syndecan-4-deficient and wild-type mice after LPS stimulation, inhibition of its production by transforming growth factor-β1 was impaired in the syndecan-4-deficient macrophages. These results indicate that syndecan-4 could be involved in prevention of endotoxin shock, at least partly through the inhibitory action of transforming growth factor-β1 on IL-1β production.

Cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β, are produced in response to infection or LPS administration. Production of these cytokines has been shown to be important for the cascade leading to endotoxin shock (2–4). Other classes of cytokines such as IL-10 and transforming growth factor (TGF)-β1 play key roles in decreasing susceptibility to endotoxin shock, principally by suppressing the expression of proinflammatory cytokines (5, 6). However, much remains to be clarified concerning the regulatory mechanisms for avoiding endotoxin shock.

Syndecan-4 (also called ryudocan) is a transmembrane heparan sulfate proteoglycan (7, 8) belonging to the syndecan family, which consists of 4 members (9). Heparan sulfate chains of syndecans bind to growth/differentiation factors, anticoagulant factors, and cell adhesion molecules, and have been suggested to participate in various biological phenomena (9, 10). Mice deficient in syndecan genes provide powerful means to analyze the functions of syndecan molecules in vivo (11–15). Thus, syndecan-1 has been shown to participate in Wnt-1 signaling (14) and enhancement of microbial virulence (15), while syndecan-4 in anticoagulation in fetal vessels of placental labyrinth (11) and prevention of k-car rageenan deposition in the kidney (12). Here, we described an unexpected finding that syndecan-4 is involved in a mechanism avoiding endotoxin shock.

**EXPERIMENTAL PROCEDURES**

Generation of Mice Deficient in Syndecan-4 Gene (Synd4)—Synd4(+/−) mice were produced by mating female C57BL/6J mice (Clea, Tokyo, Japan) with the male chimera carrying Synd4(+/−) ES-derived cells, which were generated by homologous recombination as described previously (16). After the Synd4(+/−) mice were backcrossed 6 times to C57BL/6J mice (Clea, Tokyo, Japan), the Synd4(+/−) mice were mated with each other to produce Synd4(+/+) and Synd4(+/−) mice. To expand the size of the colonies, inbreeding of each genotype was performed only for one generation. Sex, age (8–12 weeks old), and weight (20–35 g) of each genotype were matched for the experiments in the present study.

LPS Administration—LPS (Escherichia coli serotype O111:B4) was obtained from Sigma-Aldrich, and dissolved in autoclaved physiological saline at a concentration of 250 or 500 μg/ml. LPS solution was injected intraperitoneally into Synd4(+/+) or Synd4(+/−) or commercially obtained C57BL/6J mice (Clea). Animal experiments in the present study were performed in compliance with the guidelines for the Institute for Laboratory Animal Research, Nagoya University School of Medicine.

Measurement of Systolic Blood Pressure and Left Ventricular Fractional Shortening—Systolic blood pressure in the conscious state was measured using a BP Monitor for Rats and Mice Model MK-2000 (Muromachi Kikai, Tokyo, Japan) according to the manufacturer’s instructions. To assess the left ventricular systolic function, left ventricular fractional shortening was calculated as follows: (diastolic left

---

* This work was supported by Scientific Research Grants-in-aid 10178102, 12670981, and 10CE2006 from the Ministry of Education, Science, Sports and Culture, and for Research on Specific Diseases from 18 U.S.C. Section 1734 solely to indicate this fact.

†† Contributed equally to the results of this work.

‡‡ To whom correspondence should be addressed: Dept. of Biochemistry, Nagoya University School of Medicine, 65 Tsurumai-cho, Showaku, Nagoya, Aichi, 466-8550, Japan. Tel.: 81-52-744-2059; Fax: 81-52-744-2065; E-mail: tmuram@med.nagoya-u.ac.jp.

¶¶ The abbreviations used are: LPS, lipopolysaccharide; BSA, bovine serum albumin; IL, interleukin; LPS, lipopolysaccharide; PBS(−), Dulbecco’s phosphate-buffered saline without calcium and magnesium; Synd4, syndecan-4 gene; TGF-β1, transforming growth factor-β; TNF-α, tumor necrosis factor-α; GST, glutathione S-transferase.
ventricular dimension — systolic left ventricular dimension/diastolic left ventricular dimension) × 100%. These dimensions were determined by left ventricular M-mode echocardiography with an Acuson system equipped with a 1.3-MHz transducer (Sequia Ultrasound System, Mountain View, CA). During the echocardiography, mice were anesthetized (100 mg/kg sodium pentobarbital sodium) and positioned prone on a warmed saline bag, and applied with the transducer from below to avoid preventing respiration.

**Plasma Cytokine Assay**—Blood was taken from the heart into heparinized syringes under mild anesthesia with diethyl ether at the indicated times after LPS injection. Plasma was separated by centrifugation at 5,000 × g for 10 min. Concentrations of TNF-α, IL-1β, and IL-6 in the plasma were determined with immunoassay kits (TFB, Tokyo, Japan).

**Immunohistochemical Staining**—Brains, hearts, lungs, livers, kidneys, and intestines were obtained from 8–12-week-old C57BL/6J mice before, and 1, 3, 9 h after intraperitoneal injection of LPS at a dose of 5 mg/kg. Livers and kidneys of Synd4/H11001 mice were obtained 3 and 9 h after intraperitoneal injection of LPS at a dose of 5 mg/kg. Livers and kidneys of Synd4/H11001 mice before and 3 h after LPS injection were subjected to 10% SDS-polyacrylamide gel electrophoresis and Western blot analysis.

**GST Fusion Proteins**—cDNA fragments corresponding to the ectodomain of mouse syndecan-1 (32–252 amino acids), -2 (27–145 amino acids), -3 (47–384 amino acids), and -4 (24–145 amino acids) were subcloned into pGEX-5X-2 vector (Amersham Pharmacia Biotech). To induce GST fusion proteins, bacteria clones were exposed to 1 mM IPTG (isopropyl β-D-1-thiogalactopyranoside) at 30 °C for 2 h. Bacteria (1 ml) were collected and dissolved with 300 μl of sample buffer (0.125 M Tris-HCl, pH 6.8, 4% SDS, 0.005% bromophenol blue, 20% glycerol, 5% symbol 98-mercaptoethanol), 10 μl of which was subjected to 10% SDS-polyacrylamide gel electrophoresis and Western blot analysis.

**Plasma Cytokine Assay**—Blood was taken from the heart into heparinized syringes under mild anesthesia with diethyl ether at the indicated times after LPS injection. Plasma was separated by centrifugation at 5,000 × g for 10 min. Concentrations of TNF-α, IL-1β, and IL-6 in the plasma were determined with immunoassay kits (TFB, Tokyo, Japan). From the observed OD values, OD values of the controls, which were around 0.070, were subtracted to calculate syndecan-4 immunoreactivity.

**Flow Cytometry**—To isolate peripheral white blood cells, ammonium chloride was used for lysing of red blood cells, which were incubated at 4 °C for 15 min in 2 μg/ml of FITC-conjugated anti-MAC-1 antibody (Pharmingen), at 4 °C for 30 min with anti-syndecan-4 antibody and biotylated anti-Mac-1 antibody or anti-Gr-1 antibody in PBS (−) containing 2% fetal calf serum, and then with anti-rabbit IgG antibody conjugated with fluorescein isothiocyanate and streptavidin-cytochrome 5. A FACS Calibur flow cytometer and Cell Quest software (Becton Dickinson) were used to analyze the stained sample.

**Cell Activation by LPS**—Resident peritoneal exudative cells were collected by peritoneal lavage with cold PBS (−). After washing with RPMI (ICN Biomedicals, Aurora, OH)-HEPES (10 mM, pH 7.4), cells were plated at 2 × 10^5 in RPMI-HEPES containing 1% autologous serum in 96-well plates (Becton Dickinson) and incubated for 2 h at 37 °C in 5% CO₂. Nonadherent cells were removed by washing the cells with RPMI-HEPES twice. Cell activation was performed in 100 μl of RPMI-HEPES containing 1% autologous serum and LPS in the absence or presence of TGF-β1 (R&D System, Minneapolis, MN) and/or IL-10 (Sigma-Aldrich) at the concentrations indicated in the text for 9 h at 37 °C in 5% CO₂. After washing twice with PBS (−), the cells were lysed in 100 μl of 10 mM HEPES (pH 7.4), 0.1% Nonidet P-40, 1.5 mM MgCl₂, 0.5 mM phenylmethylsulfonyl fluoride (19) for 20 min at 4 °C. After centrifugation at 5,000 × g for 10 min, the supernatant was collected and assayed for IL-1β. Inhibition was calculated as follows: (IL-1β production in the cells stimulated with LPS (100 ng/ml) in the presence of TGF-β1 (1 ng/ml)−IL-10 (5 ng/ml) or the combination)/IL-1β production in the cells stimulated with LPS (100 ng/ml) alone) × 100%.

**Resident peritoneal exudative cells were collected by peritoneal lavage with RPMI-HEPES containing 1% fetal calf serum in 10-cm culture dishes (Becton Dickinson) and incubated for 2 h at 37 °C in 5% CO₂. After washing twice with RPMI-HEPES, adherent cells were further cultured for 37 °C for 3 h. Livers were obtained 3 h after LPS injection. After washing with PBS (−), the cells were incubated in 1 ml of PBS (−) containing 0.2% EDTA and 5% fetal calf serum for 10 min at 4 °C and then harvested by mild scraping. After washing the cells with PBS (−) containing 1% BSA, viable cells were counted with the aid of trypan blue staining, adjusted to 1 × 10^6 in 500 μl of PBS (−) containing 1% BSA, and incubated with 125I-labeled TGF-β1 (1 ng/ml, 100,000 cpm) in 4°C for 3 h in the absence or presence of heparin (10 μg/ml). After washing three times with PBS containing 1% BSA, the amount of 125I-labeled TGF-β1 bound to the cells was quantified with an Aloka γ-counter (Aloka, Tokyo, Japan).

**Syndecan-4 inhibition**—Livers were obtained from 8-week-old Synd4/H11001 mice before, 1, 3, and 9 h after intraperitoneal injection of LPS at a dose of 5 mg/kg. Livers were also obtained from 8-week-old Synd4/H11001 mice before and 3 h after LPS injection. Samples were homogenized in Dulbecco’s phosphate-buffered saline without calcium and magnesium (PBS) (−) containing 5 mM EDTA and 1 mM phenylmethylsulfonyl fluoride, and then the homogenate was centrifuged at 100,000 × g for 1 h at 4 °C. The precipitate was homogenized in PBS (−) containing 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 1% Nonidet P-40, and then the supernatant was centrifuged at 100,000 × g for 1 h at 4 °C. Total protein concentration of the supernatant was determined with BCA protein assay kit (Pierce), adjusted to 1 mg/ml in PBS (−) containing 0.5 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, and 1% Nonidet P-40, and then the supernatant was used as the membrane fraction of the liver. One hundred μl of the membrane fraction derived from each sample was applied in a well of a 96-well plate (Becton Dickinson, Lincoln Park, NJ), and the plates were incubated at 4 °C overnight. After washing four times with PBS (−) containing 0.1% Tween 20, the plates were incubated with 4% bovine serum albumin (BSA) in PBS (−) at room temperature for 1 h, blocked with 1% BSA solution, and then the supernatant was diluted with PBS (−) containing 1% BSA (2:200), or with PBS (−) containing 1% BSA alone as a control. After washing, 96-well plates were incubated with anti-rabbit IgG antibody conjugated with horseradish peroxidase. Visualization was performed with tetramethylbenzidine at room temperature for 30 min. The optical density (OD) at 450 nm was determined with ImmunoMINI NJ-2300 (InterMed, Tokyo, Japan).
Roles of Syndecan-4 in the Events after LPS Injection

**RESULTS**

**Syndecan-4 Deficiency Increased Mortality of LPS-injected Mice**—Synd(−/−) mice were found to exhibit significantly (p < 0.01) higher mortality than Synd(+/+) controls after intraperitoneal injection of LPS at a dose of 10 mg/kg (Fig. 1A). We measured systolic blood pressure before, 3, and 9 h after LPS injection, since even Synd(−/−) mice survived until 9 h after LPS injection. Systolic blood pressure was significantly (p < 0.01) lower in Synd(−/−) mice than in Synd(+/+) mice 9 h after LPS injection (Fig. 1B). Left ventricular fractional shortening was also significantly (p < 0.05) lower in Synd(−/−) mice (17.2 ± 2.3%, n = 6) than in Synd(+/+) controls (25.7 ± 2.8%, n = 6) 9 h after LPS injection. Thus, we concluded that Synd(−/−) mice would be more susceptible to endotoxin shock than Synd(+/+) mice. On the other hand, immunohistochemical staining with anti-fibrin(ogen) antibody detected microthrombin in the liver and kidney to the same extent between Synd(+/+) and Synd(−/−) mice, and no severe damage was observed in the liver, kidney, intestine, brain, or heart 3 or 9 h after LPS injection (data not shown).

We investigated possible differences in proinflammatory cytokine levels after LPS administration between Synd(+/+) and Synd(−/−) mice. The profile of TNF-α concentration in plasma was not different between the two genotypes, which reached the maximal level 1 h after, and declined nearly to the basal level 3 h after intraperitoneal injection of LPS at a dose of 10 mg/kg (Fig. 2A). On the other hand, the plasma level of IL-1β was significantly higher in Synd(−/−) mice than in Synd(+/+) mice 9 h after LPS injection, while it was not different between both genotypes until 3 h after LPS injection (Fig. 2B). Syndecan-4 Expression Was Increased 3 and 9 h after LPS Injection—We investigated whether the expression of syndecan-4 was affected by LPS administration. To avoid a loss of mice, the dose of LPS was reduced to 5 mg/kg. Even at the dose of 5 mg/kg, systolic blood pressure 9 h after LPS administration and survival rate were significantly lower in Synd(−/−) mice than in Synd(+/+) controls (data not shown). The specificity of the anti-mouse syndecan-4 antibody was confirmed by Western blot analysis: the antibody reacted only to the GST-syndecan-4 fusion protein among GST fusion proteins carrying the ectodomains of mouse syndecan-1, -2, -3, and -4 (data not shown). Although syndecan-4 expression was not changed 1 h after intraperitoneal injection of LPS (data not shown), it was increased 3 and 9 h after LPS injection in the microvasculature of the lung (Fig. 3B), intestine (data not shown), kidney (data not shown), and liver (Fig. 3, D and E). Strong expression of syndecan-4 was also detected in Mac-1-positive cells 3 and 9 h after LPS injection (Fig. 3, E-G). The assay to quantify syndecan-4 expression in the liver confirmed that its expression was increased 3 and 9 h after LPS injection (Fig. 4). Flow cytometry revealed that syndecan-4 expression was increased in Mac-1-positive cells 3 h (Fig. 5) and 9 h (data not shown) after LPS injection. Syndecan-4 expression in these cells declined 1 day after, and returned to the basal level 4 days after LPS injection (data not shown). Little expression of syndecan-4 was observed in Gr-1-positive cells by immunohistochemical staining and flow cytometry (data not shown). Thus, Mac-1-positive cells expressing syndecan-4 strongly were considered to be monocytes and Kupffer cells in the sinusoids of the liver. When Mac-1-positive cells from Synd(−/−) mice were analyzed, a small number of cells were stained with anti-syndecan-4 antibody and the second antibody, namely anti-rabbit IgG antibody conjugated with fluorescein isothiocyanate (Fig. 5). However, the positive cells were detected similarly even in the absence of

**FIG. 1.** Survival rate and systolic blood pressure after intraperitoneal injection of LPS. A, survival rate after intraperitoneal injection of LPS at a dose of 10 mg/kg (n = 32 per genotype, p < 0.01 (calculated with the Log-rank test). Circles, Synd(+/+) mice; squares, Synd(−/−) mice. B, systolic blood pressure after intraperitoneal injection of LPS at a dose of 10 mg/kg (n = 12 per genotype). Systolic blood pressure of three Synd(+/+) mice 9 h after LPS injection was less than 50 mm Hg, which could not be measured with the apparatus. These values were tentatively considered as 49 mm Hg for statistical calculation. Values represent mean ± S.E. Circles, Synd(+/+) mice; squares, Synd(−/−) mice; *, p < 0.01 (calculated with Student’s t test).

**FIG. 2.** Concentrations of TNF-α and IL-1β in plasma after intraperitoneal injection of LPS. Mice were sacrificed at the indicated times after intraperitoneal injection of LPS at a dose of 10 mg/kg for assay of TNF-α (A, n = 9 per genotype at each time point) and IL-1β (B, n = 6; 18 per genotype at each time point). Values represent mean ± S.E. Circles, Synd(+/+) mice; squares, Synd(−/−) mice; *, p < 0.05 (calculated with Student’s t test).
anti-syndecan-4 antibody (data not shown), indicating that the second antibody reacted nonspecifically with this population of cells. Although a small number of cells stained with the second antibody were also present in Mac-1-positive cells from Synd4(+/+) mice, the number was not elevated by LPS injection (data not shown). Therefore, the increase of Mac-1-positive cells with syndecan-4 expression after LPS injection (Fig. 5) was not due to increase of the nonspecifically stained cells.

**Inhibition of IL-1β Production by TGF-β1 Was Impaired in Synd4(−/−) Macrophages**—Among the cells in which syndecan-4 expression was increased after LPS injection, we used peritoneal macrophages to investigate reasons for the higher levels of IL-1β in plasma of Synd4(−/−) mice after LPS administration. Synd4(−/−) macrophages produced IL-1β in the same amounts as Synd4(+/+) controls when stimulated with LPS at 1, 100, and 10,000 ng/ml for 9 h (data not shown). However, inhibition of IL-1β production by TGF-β1 was impaired in Synd4(−/−) macrophages compared with Synd4(+/+) controls (Fig. 6). Although IL-10 inhibited production of IL-1β at the same level between Synd4(+/+) and Synd4(−/−) macrophages, inhibition by TGF-β1 and IL-10 in combination was also impaired in Synd4(−/−) macrophages (Fig. 6).

Binding of TGF-β1 to Syndecan-4—We investigated whether TGF-β1 binds to syndecan-4 to exert inhibitory effects on production of IL-1β. First, we compared binding of 125I-labeled TGF-β1 to Synd4(−/−) peritoneal macrophages with that to Synd4(+/+) cells. The amount of 125I-labeled TGF-β1 bound to Synd4(−/−) macrophages was significantly (p < 0.01) less than that to Synd4(+/+) controls (Fig. 7). Heparin (10 μg/ml) reduced the binding of 125I-labeled TGF-β1, and abolished the difference between the binding to Synd4(−/−) cells and that to Synd4(+/+) cells (Fig. 7). The results suggest that syndecan-4 is one of the TGF-β1-binding molecules in macrophages. To obtain more data on binding between TGF-β1 and syndecan-4, proteoglycan fraction was isolated from livers obtained 3 h after LPS injection. Syndecan-4 in the proteoglycan fraction was found to bind to TGF-β1, the level of the binding was more than that to BSA, but was less than that to basic fibroblast growth factor, which is known to bind to syndecan-4 (20) (Fig. 8). The binding of syndecan-4 to TGF-β1 was almost completely abolished by heparin or digestion with heparitinase (Fig. 8), indicating that syndecan-4 binds to TGF-β1 via its heparan sulfate chains.

**DISCUSSION**

To evaluate the roles of syndecan-4 in the events after LPS injection, Synd4(−/−) and Synd4(+/+) mice were injected intraperitoneally with LPS. Syndecan-4 deficiency resulted in increased mortality after LPS injection. This finding suggests that syndecan-4 promotes survival of LPS-injected mice. Systolic blood pressure and left ventricular fractional shortening were lower in Synd4(−/−) mice than in Synd4(+/+) controls 9 h after LPS administration, although histological examina-
Roles of Syndecan-4 in the Events after LPS Injection

47487

Fig. 6. Inhibition of IL-1β production by TGF-β1 and/or IL-10. Peritoneal macrophages were incubated for 9 h with LPS (100 ng/ml) alone or in the presence of TGF-β1 (1 ng/ml), IL-10 (5 ng/ml), or both in combination. Vertical range represents relative amount of IL-1β as described under “Experimental Procedures.” Values represent mean ± S.E. * p < 0.05; **, p < 0.01 (calculated with Student’s t test).

Fig. 7. Binding of 125I-TGF-β1 to peritoneal macrophages. Peritoneal macrophages were incubated for 30 min with 125I-TGF-β1 (1 × 10^5 cpm) alone or in the presence of heparin (10 μg/ml) as described under “Experimental Procedures.” Values represent mean ± S.E. * p < 0.01 (calculated with Student’s t test).

Fig. 8. Binding of syndecan-4 to TGF-β1. The proteoglycan fraction was isolated from livers obtained 3 h after LPS injection as described under “Experimental Procedures.” Values represent mean ± S.E. * p < 0.05; **, p < 0.01 (calculated with Student’s t test).

In the present study, syndecan-4 expression in the lung, intestine, kidney, and liver 3 and 9 h after LPS injection. Strong expression of syndecan-4 in Mac-1-positive cells was observed at the same time by immunohistochemical staining and flow cytometry, while Gr-1-positive cells expressed little syndecan-4. Mac-1 is expressed not only in macrophages, including monocytes and Kupffer cells, but also in neutrophils. Gr-1 is expressed in neutrophils, but not in macrophages. Thus, Mac-1-positive cells expressing syndecan-4 strongly were considered to be monocytes and Kupffer cells in the sinusoids of the liver. Zhang et al. (25) have found that TNF-α induces syndecan-4 expression in endothelial cells by both increasing syndecan-4 gene expression in an NF-kB-dependent manner and by extending the half-life of syndecan-4 mRNA. TNF-α concentration in plasma was elevated 1 h after LPS injection, which was prior to syndecan-4 induction. Thus, TNF-α could be one of the molecules involved in induction of syndecan-4 expression after LPS injection.

It is noteworthy that syndecan-4 expression was increased after LPS administration in monocyes/Kupffer cells and endothelial cells, which produce proinflammatory cytokines mediating endotoxin shock (26–28). However, peritoneal macrophages of both genotypes produced IL-1β at the same levels on stimulation with LPS at 1, 100, and 10,000 ng/ml. This finding suggests that syndecan-4 deficiency does not alter the functions of cell-surface receptors involved in production of IL-1β, including LPS receptor, Toll-like receptor-4 (29), and recently identified triggering receptor expressed on myeloid cells-1 (30). Therefore, we examined the hypothesis that increased syndecan-4 expression served to down-regulate the synthesis of IL-1β. Indeed, LPS-induced production of IL-1β was inhibited to a lesser extent by TGF-β1 or TGF-β1 and IL-10 in combination in Synd4(−/−) macrophages than in Synd4(+/+) controls. Previous studies have demonstrated that TGF-β1 binds to heparin/heparan sulfate (31, 32). Our experiments showed that syndecan-4 deficiency reduced the amount of 125I-labeled TGF-β1 bound to macrophages and that syndecan-4 in the proteoglycan fraction from livers obtained 3 h after LPS injection bound to TGF-β1 via its heparan sulfate chains. Heparan sulfate is known to modulate the function of growth factors by promoting their accumulation to specific sites and also serving as their co-receptors (10, 33–35). Thus, the interaction between TGF-β1 and syndecan-4 on macrophages, and probably on en-
Roles of Syndecan-4 in the Events after LPS Injection

Syndecan-4 is a member of the syndecan family, which consists of 4 closely related molecules (10). The plasma membrane also contains the glypican family, which are heparan sulfate proteoglycans linked to the membrane via a glycosylphosphatidylinositol (10). Therefore, it was remarkable that deficiency of only one heparan sulfate proteoglycan significantly alters susceptibility to endotoxin shock. This finding will be important in investigating genetic factors affecting susceptibility to septic shock in patients.

Acknowledgments—We thank Drs. M. Kawabata and T. Imamura for the guidance of 125I labeling of TGF-β1, and K. Sakakura for excellent technical assistance.

REFERENCES
1. Morrison, D. C., and Ryan, J. L. (1987) Annu. Rev. Med. 38, 417–432
2. Li, P., Allen, H., Banerjee, S., Franklin, S., Herzog, L., Johnston, C., McDowell, J., Paskind, M., Rodman, L., and Salfeld, J. (1995) Cell 80, 401–411
3. Wang, S., Miura, M., Jung, Y. K., Zhu, H., Li, E., and Yuan, J. (1998) Cell 92, 501–509
4. Amiel, F., Fitting, C., Tracey, K. J., Cavaillon, J. M., and Dautry, F. (1997) Mol. Med. 3, 864–875
5. Berg, D. J., Kuhn, R., Rajewsky, K., Muller, W., Menon, S., Davidson, N., Grunig, G., and Rennick, D. (1996) J. Clin. Invest. 98, 2339–2347
6. Imai, K., Takeshita, A., and Hanazawa, S. (2000) Infect. Immun. 68, 2418–2423
7. Kojima, T., Shovorak, N. W., and Rosenberg, R. D. (1992) J. Biol. Chem. 267, 4870–4877
8. Kojima, T., Leone, C. W., Marchilhon, G. A., Marcum, J. A., and Rosenberg, R. D. (1992) J. Biol. Chem. 267, 4859–4869
9. Bernfield, M., Kokenyesi, R., Kate, M., Hinkes, M. T., Spring, J., Gallo, R. L., and Luse, E. J. (1992) Annu. Rev. Cell Biol. 8, 365–393
10. Perrimon, N., and Bernfield, M. (2000) Nature 404, 725–728
11. Ishiguro, K., Kadomatsu, K., Kojima, T., Muramatsu, H., Nakamura, E., Ito, M., Nagasaka, T., Kobayashi, H., Kusugami, K., Saito, H., and Muramatsu, T. (2000) Dev. Dyn. 219, 539–544
12. Ishiguro, K., Kadomatsu, K., Kojima, T., Muramatsu, H., Matsuo, S., Kusugami, K., Saito, H., and Muramatsu, T. (2001) Lab. Invest. 81, 509–516
13. Echtermeyer, F., Streit, M., Wilcox-Adelman, S., Saoncella, S., Denher, F., Detmar, M., and Goetinck, P. F. (2001) J. Clin. Invest. 107, R9–R14
14. Alexander, C. M., Reichsman, F., Hinkes, M. T., Lincecum, J., Becker, K. A., Cumberledge, S., and Bernfield, M. (2000) Nat. Genet. 25, 329–332
15. Park, P. W., Pier, G. B., Hinkes, M. T., and Bernfield, M. (2001) Nature 411, 98–102
16. Ishiguro, K., Kadomatsu, K., Kojima, T., Muramatsu, H., Tsuruki, S., Nakamura, E., Kusugami, K., Saito, H., and Muramatsu, T. (2000) J. Biol. Chem. 275, 5249–5252
17. Ishiguro, K., Kojima, T., Taguchi, O., Saito, H., Muramatsu, T., and Kadomatsu, K. (1996) Histochem. Cell Biol. 112, 25–33
18. Yamamoto, K., and Loskutoff, D. J. (1996) J. Clin. Invest. 97, 2440–2451
19. Kitchens, R. L., Ulevitch, R. J., and Munford, R. S. (1992) J. Exp. Med. 176, 485–494
20. Kojima, T., Katsumi, A., Yamazaki, T., Muramatsu, T., Nagasaka, T., Ohsumi, K., and Saito, H. (1996) J. Biol. Chem. 271, 5914–5920
21. Karina, R., Matsumoto, S., Hagashi, H., and Matsushima, K. (1999) Mol. Med. Today 5, 125–132
22. Marsh, C. B., and Wewers, M. D. (1996) Clin. Chest Med. 17, 183–197
23. Dinarello, C. A. (1997) Chest 112, 3218–3295
24. Dinarello, C. A., Gelfand, J. A., and Wolff, S. M. (1993) J. Am. Med. Assoc. 269, 1829–1835
25. Zhang, Y., Pasparakis, M., Kellias, G., and Simons, M. (1999) J. Biol. Chem. 274, 14786–14790
26. Miossec, P., Cavender, D., and Ziff, M. (1986) J. Immunol. 136, 2486–2491
27. Krishnaswamy, G., Kelley, J., Yerra, L., Smith, J. K., and Chi, D. S. (1999) J. Interferon Cytokine Res. 19, 91–104
28. Galanos, C., and Freudenberg, M. A. (1985) Immunobiology 176, 346–356
29. Kaisho, T., and Akira, S. (2000) Crit. Rev. Immunol. 20, 393–405
30. Bouchon, A., Facchetti, F., Weigand, M. A., and Colonna, M. (2001) Nature 410, 1105–1107
31. Lyon, M., Rushton, G., and Gallagher, J. T. (1997) J. Biol. Chem. 272, 18000–18006
32. McCaffrey, T. A., Falcione, D. J., and Du, B. (1992) J. Cell. Physiol. 152, 430–440
33. Tanaka, Y., Kimata, K., Adams, D. H., and Eto, S. (1998) Proc. Assoc. Am. Physicians 110, 118–125
34. Wight, T. N., Kuselis, M. G., and Qvarnstrom, E. E. (1992) Curr. Opin. Cell Biol. 4, 793–801
35. Ruoslahti, E., and Yamaguchi, Y. (1991) Cell 64, 867–869
Syndecan-4 Deficiency Leads to High Mortality of Lipopolysaccharide-injected Mice
Kazuhiro Ishiguro, Kenji Kadomatsu, Tetsuhide Kojima, Hisako Muramatsu, Mitsunori
Iwase, Yasunobu Yoshikai, Masamitsu Yanada, Koji Yamamoto, Tadashi Matsushita,
Masahiko Nishimura, Kazuo Kusugami, Hidehiko Saito and Takashi Muramatsu

J. Biol. Chem. 2001, 276:47483-47488.
doi: 10.1074/jbc.M106268200 originally published online October 3, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M106268200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 35 references, 9 of which can be accessed free at
http://www.jbc.org/content/276/50/47483.full.html#ref-list-1