Gene Expression in Lipopolysaccharide-treated Human Monocytes Following Interaction with Hepatic Cancer Cells

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Abstract. *Background/Aim: Monocytes migrate into the tissue where they differentiate into various types of macrophages with tissue-specific characteristics. When human monocytes are co-cultured with colon cancer cells they exhibit increased mRNA expression of angiogenesis- and signaling pathway-related genes; however, this increase is suppressed by pretreatment with low-dose lipopolysaccharide (LPS). Thus, LPS-treated human monocytes may be useful in suppressing tumor invasion and proliferation in colon cancer. However, it is suggested that the characteristics of tumor-associated macrophages may differ depending on the type of cancer. The function of human tumor-associated macrophages in hepatic cancer remains unclear. In this study, we investigated mRNA expression of various genes in LPS-treated human monocytes following interaction with hepatic cancer cells. Materials and Methods: The human monocyte cell line THP-1 was treated with LPS and subsequently co-cultured with the human hepatic cancer cell line HepG2. mRNA expression of various factors were then analyzed using quantitative real-time polymerase chain reaction (PCR) and DNA microarray. Results: The mRNA expressions of monocyte chemotactic protein-1, vascular endothelial growth factor-A, tumor necrosis factor-α, interleukin (IL)-1β, IL-8, nuclear factor-κB, RelB, signal transducer and activator of transcription 3, IL-10 and transforming growth factor-β in THP-1 cells following interaction with HepG2 cells, were suppressed by pretreatment with LPS. Conclusion: LPS-treated human monocytes may be useful in suppressing tumor invasion and proliferation of hepatic cancer, as well as colon cancer. The co-culture system of monocytes and cancer cells may be beneficial for evaluating antitumor effects in LPS-treated monocytes.*

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Monocytes are released from the bone marrow into the peripheral blood. They then migrate into tissues where they terminally differentiate into various types of macrophages with tissue-specific characteristics. Macrophages have various functions (1-4); although they eliminate cancer cells by inducing inflammatory responses, they are also involved in the development of cancer by inducing chronic inflammation (5). Macrophages that accumulate in tumor tissues are called tumor-associated macrophages. Several reports have described the relationship between prognosis of patients with cancer and the numbers of tumor-associated macrophages. It has been demonstrated that a high number of tumor-associated macrophages is associated with shorter patient survival; however, it is also shown to be correlated with a good prognosis. Such a high number of these macrophages has specifically been shown to be an independent prognostic factor in various cancers (6-9) suggesting that the characteristics of human tumor-associated macrophages may differ depending on the type of cancer (9).

Previously, it was demonstrated that the mRNA expression of chemotaxis- and angiogenesis-related genes in human monocytes increased after interaction with colon cancer cells (10, 11). It was also reported that the increased mRNA expression of those genes in human monocytes following interaction with colon cancer cells were suppressed by pretreatment with low-dose lipopolysaccharide (LPS) (100 pg/ml) (12). Macrophages are known to respond to LPS signaling *via* nuclear factor (NF)-κB (3). It was demonstrated

that unphosphorylated signal transducer and activator of transcription 3 (STAT3) activated transcription by binding to NF-κB and that activated STAT3 contributed to the maintenance of NF-κB activity in cancer cells (13, 14). In addition, macrophages produce interleukin (IL)-10, which acts to inhibit the production of proinflammatory cytokines *via* a Janus-activated kinase (JAK)/STAT3-dependent pathway (15-18). It is, thus, possible that LPS-treated monocytes switch from a proinflammatory to an anti-inflammatory state (18). Against this background, we investigated the mRNA expression of signaling pathway activation- and suppression-related genes in LPS-treated human monocytes following interaction with colon cancer cells. The results demonstrated that increased mRNA expression of these genes were suppressed by pretreatment with low-dose LPS (100 pg/ml) (19), which may suppress tumor progression. Therefore, LPS-treated human monocytes may be useful to suppress tumor invasion and proliferation in colon cancer. Moreover, previous studies demonstrated that the intradermal administration of LPS in mice had a significant suppressive effect on the growth of tumors (20, 21). However, the function of human tumor-associated macrophages in hepatic cancer remains unclear. To clarify this issue, in this study, we investigated the mRNA expressions of chemotaxis-, angiogenesis-, signaling pathway activation- and suppression-related genes in LPS-treated human monocytes following interaction with hepatic cancer cells.

Materials and Methods

Cells. THP-1 cells were obtained from DS Pharma Biomedical (Osaka, Japan) and HepG2 cells from the Japan Health Sciences Foundation (Tokyo, Japan). THP-1 and HepG2 cells were cultured in a 5% CO_2 atmosphere at 37˚C in RPMI 1640 medium and Dulbecco's modified Eagle's medium (Wako Pure Chemical Industries, Ltd., Osaka, Japan) containing 10% fetal calf serum supplemented with 100 units/ml each of penicillin and streptomycin (Wako Pure Chemical Industries, Ltd.), respectively.

Cell co-culture. THP-1 cells were treated with Ultra-pure lipopolysaccharide from *Escherichia coli* (100 pg/ml, 10 ng/ml or 1 μg/ml) (InvivoGen Corporation, San Diego, CA, USA) for 3 h, washed with PBS (–) (Wako Pure Chemical Industries, Ltd.) and re-suspended in RPMI 1640 medium. THP-1 and HepG2 cells were co-cultured using a cell culture insert with a 0.4-μm porous membrane (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) to separate the upper and lower chambers. HepG2 cells were cultured in the upper chamber at 2×10^5 cells/ml and THP-1 cells were cultured in the lower chamber at 2×10^5 cells/ml. THP-1 cells were collected on day 5 after co-culture.

RNA extraction. Total RNA from THP-1 and HepG2 cells was extracted using TRIzol® Reagent (Invitrogen Corporation, Carlsbad, CA, USA), in accordance with the manufacturer's protocol. RNA was quantified by absorbance at 260 nm. cDNA was synthesized using reverse transcriptase with Oligo(dT)20 (Toyobo Co., Ltd., Osaka, Japan).

Quantitative real-time polymerase chain reaction (PCR). Real-time PCR was performed using SsoFast™ EvaGreen® Supermix (Bio-Rad Laboratories, Inc., Hercules, CA, USA) on MiniOpticon (Bio-Rad Laboratories, Inc.). Previously reported primers were used (11, 22). PCR conditions were set at 95˚C for 3 min, followed by 40 cycles of 95˚C for 10 s and 60˚C for 30 s. Relative quantification was performed by normalizing the target expression to that of the housekeeping gene β-actin. Data are expressed as change (n-fold) in mRNA expression compared with that of THP-1 cells incubated without LPS before co-culture.

DNA microarray. Gene expression in THP-1 cells pretreated with 1 μg/ml LPS on day 5 of co-culture with HepG2 cells was analyzed using the fibrous DNA microarray Genopal® (Mitsubishi Rayon, Tokyo, Japan). Data are expressed as change (n-fold) in gene expression compared with that of THP-1 cells incubated without LPS before co-culture.

Results

mRNA expressions of chemotaxis- and angiogenesis-related genes in human monocytes treated with LPS. Previous reports demonstrated that the mRNA expressions of chemotaxis- and angiogenesis-related genes monocyte chemotactic protein-1 (*MCP-1*), vascular endothelial growth factor-A (*VEGF-A*), tumor necrosis factor-α (*TNF-α*), *IL-1β* and *IL-8* were increased in human monocytes following co-culture with colon cancer cells. The mRNA expression of those genes in human monocytes following interaction with colon cancer cells were also suppressed by pretreatment with low-dose LPS (100 pg/ml) (12). Thus, we investigated the mRNA expression of these genes in THP-1 cells pretreated with LPS on day 5 of co-culture with HepG2 cells. The increased mRNA expression of *MCP-1, VEGF-A* and *IL-8* in THP-1 cells was suppressed by pretreatment with LPS (100 pg/ml, 10 ng/ml and 1 μg/ml) on day 5 of co-culture with HepG2 cells. The increased mRNA expression of *TNF-α* and *IL-1β* in THP-1 cells were suppressed by pretreatment with LPS (10 ng/ml and 1 μg/ml) on day 5 of co-culture with HepG2 cells (Figure 1). The results demonstrated that the mRNA expression of chemotaxis- and angiogenesis-related genes in human monocytes following interaction with hepatic cancer cells were suppressed by pretreatment with LPS.

mRNA expression of signaling pathway-related genes in human monocytes treated with LPS. It was reported that the mRNA expressions of signaling pathway-related genes in human monocytes following interaction with colon cancer cells were suppressed by pretreatment with low-dose LPS (100 pg/ml) (19). Thus, we investigated the mRNA expression of *NF-κB, RelB* and *STAT3* in THP-1 cells pretreated with LPS on day 5 of co-culture with HepG2 cells. The increased mRNA expression of *NF-κB, RelB* and *STAT3* in THP-1 cells was suppressed by pretreatment with

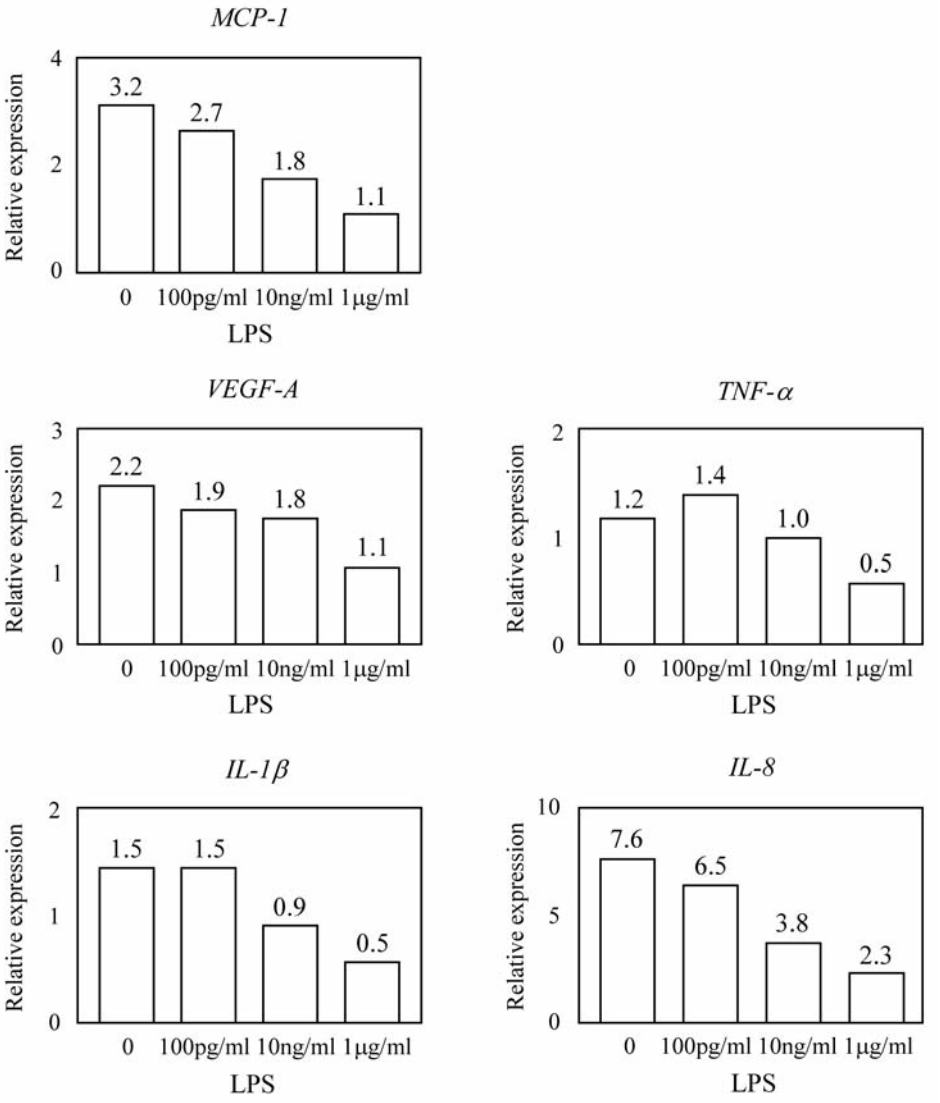

Figure 1. *mRNA expression of chemotaxis- and angiogenesis-related genes in THP-1 cells pretreated with LPS. The mRNA expression of target genes in THP-1 cells were analyzed using quantitative real-time PCR on day 5 of co-culture with HepG2 cells. Relative quantification was performed by normalizing the target expression to that of the housekeeping gene β-actin. Data are expressed as change (n-fold) in mRNA expression compared to that in THP-1 cells incubated without LPS before co-culture.*

LPS (10 ng/ml and 1 μg/ml) on day 5 of co-culture with HepG2 cells (Figure 2). The results demonstrated that the mRNA expression of signaling pathway-related genes in human monocytes following interaction with hepatic cancer cells were suppressed by pretreatment with LPS.

mRNA expression of signaling pathway suppression-related genes in human monocytes treated with LPS. It is known that macrophages produce IL-10, which acts to inhibit proinflammatory cytokine production *via* a JAK/STAT3-dependent pathway (15-18). Thus, we examined the mRNA expression of signaling pathway suppression-related genes in THP-1 cells pretreated with LPS on day 5 of co-culture with HepG2 cells. The increased mRNA expression of *IL-10* in THP-1 cells was suppressed by pretreatment with LPS (100 pg/ml, 10 ng/ml and 1 μg/ml) on day 5 of co-culture with HepG2 cells. The increased mRNA expression of *TGF-β* in THP-1 cells was suppressed by pretreatment with LPS (10 ng/ml and 1 μg/ml) on day 5 of co-culture with HepG2 cells (Figure 3). The results demonstrated that the mRNA expression of signaling pathway suppression-related genes in human monocytes following interaction with hepatic cancer cells were suppressed by pretreatment with LPS.

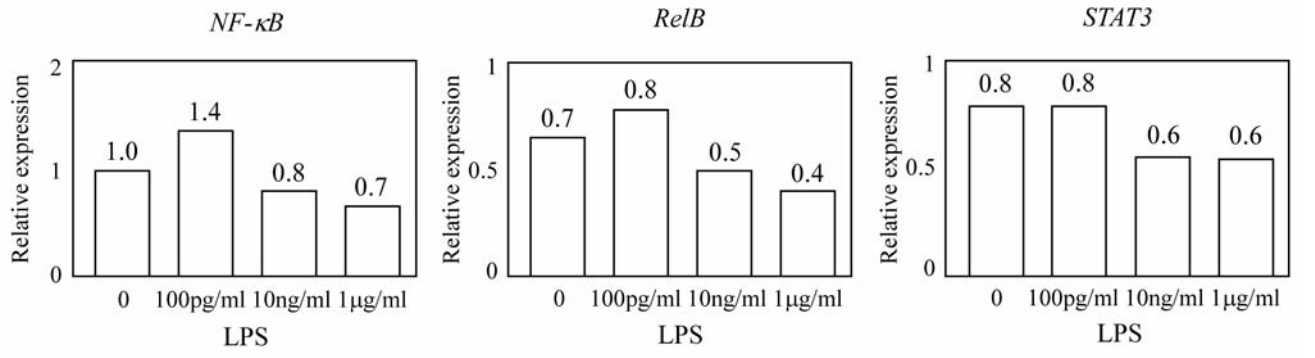

Figure 2. *mRNA expression of signaling pathway activation-related genes in THP-1 cells pretreated with LPS. The mRNA expression of target genes in THP-1 cells were analyzed using quantitative real-time PCR on day 5 of co-culture with HepG2 cells. Relative quantification was performed by normalizing the target expression to that of the housekeeping gene β-actin. Data are expressed as change (n-fold) in mRNA expression compared to that in THP-1 cells incubated without LPS before co-culture.*

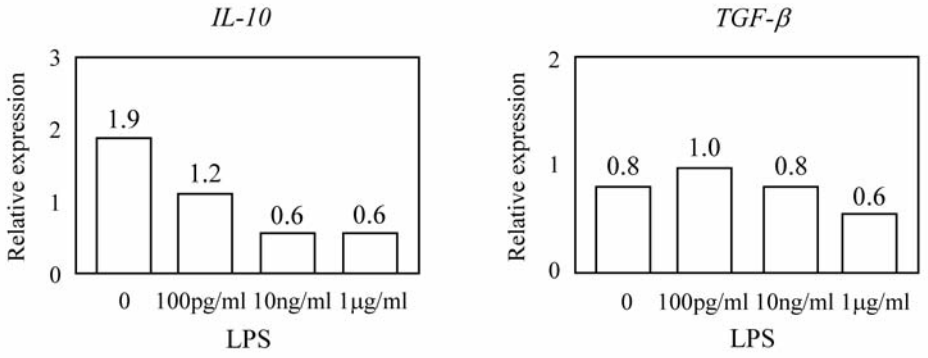

Figure 3. *mRNA expressions of signaling pathway suppression-related genes in THP-1 cells pretreated with LPS. The mRNA expression of target genes in THP-1 cells were analyzed using quantitative real-time PCR on day 5 of co-culture with HepG2 cells. Relative quantification was performed by normalizing the target expression to that of the housekeeping gene β-actin. Data are expressed as change (n-fold) in mRNA expression compared to that in THP-1 cells incubated without LPS before co-culture.*

mRNA expression in human monocytes treated with LPS determined using DNA microarray. We investigated the changes in the expression of 198 genes in THP-1 cells on day 5 of co-culture with HepG2 cells using DNA microarray. It was demonstrated that the increased mRNA expression of *CD86, CXCL6*, interferon *(IFN)α5* and Z-DNA-binding protein 1 (*ZBP1*) in THP-1 cells following interaction with HepG2 cells was suppressed to less than 10-fold by pretreatment with LPS (1 μg/ml) (Figure 4).

Discussion

LPS is a major component of the outer membrane of gram-negative bacteria. It is known that LPS triggers production of proinflammatory cytokines and causes acute shock. Moreover, LPS has a strong antitumor effect on a variety of experimental tumors (20). Previous reports demonstrated that the increased mRNA expression of chemotaxis-, angiogenesis-, signaling pathway activation- and suppression-related genes in human monocytes following interaction with colon cancer cells were suppressed by pretreatment with low-dose LPS (100 pg/ml) (12, 19). LPS-treated monocytes may be able to switch from a proinflammatory to an anti-inflammatory state (18). Thus, we investigated the mRNA expression of those genes in LPS-treated human monocytes following interaction with hepatic cancer cells. The obtained results show that mRNA expression of chemotaxis-, angiogenesis-, signaling pathway activation- and suppression-related genes were suppressed by pretreatment with LPS. The results suggest that LPS-treated human monocytes may be valuable for suppressing tumor invasion and proliferation in hepatic cancer, as well as colon cancer, although the LPS effective concentration to human monocytes is different depending on the type of cancer. Therefore, LPS-treated human monocytes may be useful for hepatic cancer therapy. The co-culture system of

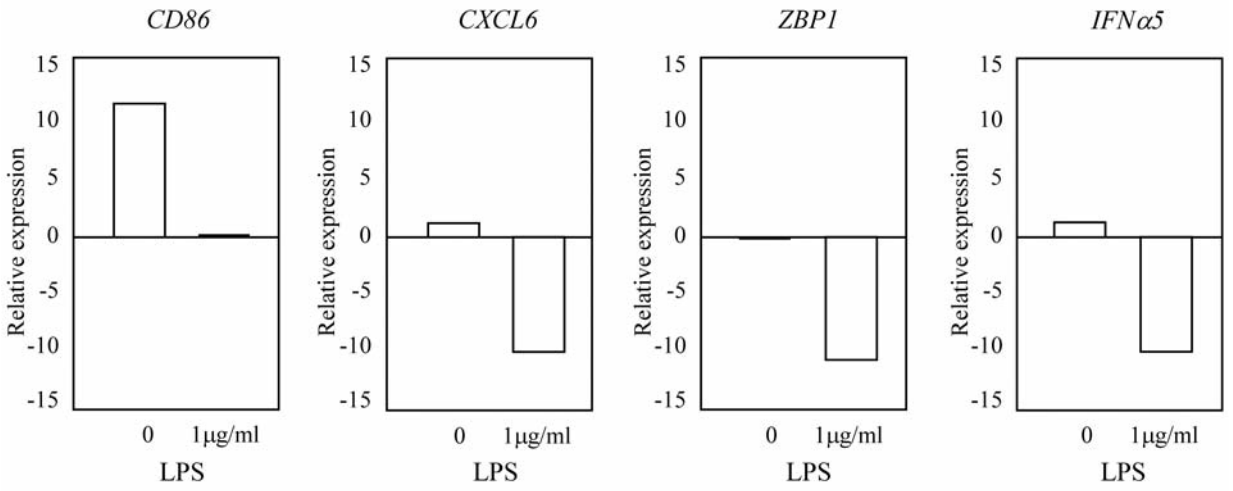

Figure 4. *Gene expression in THP-1 cells pretreated with LPS using DNA microarray. Gene expression in THP-1 cells pretreated with 1 μg/ml LPS was analyzed using DNA microarray on day 5 of co-culture with HepG2 cells. Data are expressed as change (n-fold) in gene expression compared to that in THP-1 cells incubated without LPS before co-culture.*

monocytes and cancer cells may be beneficial to evaluate antitumor effects in LPS-treated monocytes.

DNA microarray analysis demonstrated that the increased mRNA expression of *CD86, CXCL6, IFNα5* and *ZBP1* in human monocytes following interaction with hepatic cancer cells were suppressed to no less than 10-fold by pretreatment with LPS (1 μg/ml). Recently, it was also reported that CD86, a T-cell co-stimulatory molecule, contributes not only to T-cell activation but also to T-cell suppression (23). It is, thus, suggested that LPS-treated monocytes may exert antitumor effects through T-cell regulation. Moreover, it was revealed that up-regulation of CXCL6 through the tetraspanin 12-β-catenin pathway enhanced lung cancer cell invasion and proliferation, although CXCL6 is known to be a chemotaxis-related factor (24). Lastly, two factors identified in the DNA microarray analysis, *IFNα5* and *ZBP1*, are involved in IFN production. It has been demonstrated that IFN has tumor growth suppression and antitumor effects (25, 26). It is, thus, suggested that LPS-treated human monocytes are useful for suppressing tumor invasion and proliferation in many types of cancer. Moreover, the results suggested that LPS can switch from a proinflammatory to an anti-inflammatory role in monocytes.

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