# Expression of Chemotaxis- and Angiogenesis-related Factors in Human Monocytes Following Interaction with Colon Cancer Cells Is Suppressed by Low-dose Lipopolysaccharide

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**Abstract.** *Background: We have previously reported that mRNA expression of chemotaxis- and angiogenesis-related factors in human monocytes increased following interaction with colon cancer cells. Recently, it was also reported that mRNA expression of the chemotaxis-related factor, monocyte chemotactic protein (MCP)-1, in mouse macrophages following treatment with low-dose lipopolysaccharide (LPS) was significantly lower compared to that following treatment with high-dose LPS, and that low-dose LPS failed to activate the classical nuclear factor (NF)-κB pathway. In the present study, we examined changes in mRNA expression of chemotaxis- and angiogenesis-related factors in human monocytes following low-dose LPS treatment and subsequent interaction with colon cancer cells. Materials and Methods: The human monocyte cell line THP-1 was treated with LPS and subsequently co-cultured with the human colon cancer cell line DLD-1. mRNA expression was analyzed by quantitative real-time PCR. Results: mRNA expression of MCP-1, vascular endothelial growth factor (VEGF)-A, tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-8 in THP-1 cells treated with low-dose LPS (100 pg/ml) decreased compared to untreated THP-1 cells after five days of co-culture with DLD-1 cells. Conclusion: mRNA expression of chemotaxis- and angiogenesis-related factors in human monocytes following interaction with colon cancer cells is suppressed by prior treatment with low-dose LPS. Thus, low-dose LPS treatment of human monocytes may be useful for prevention and therapy of colon cancer.*

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Monocytes are known to differentiate from multipotential myeloid stem cells in the bone marrow. From there they are released into the peripheral circulation where they circulate for several days before migrating into various tissues. There they differentiate into tissue-specific macrophages according to each particular microenvironment (1-3). Macrophages play an important role in innate immunity, such as that in the defense against foreign substances. Macrophages accumulated in tumor tissues are termed tumor-associated macrophages, and they are considered to promote the progression of malignancies (1, 4-11). We previously reported that mRNA expression of chemotaxis- and angiogenesis-related factors in human monocytes increased following interaction with colon cancer cells (12, 13). It is possible that human monocytes may migrate and accumulate in tumor tissues in response to macrophage-derived chemotaxis-related factors, and that tumor-associated macrophages may promote tumor progression and metastasis by releasing macrophage-derived angiogenesis-related factors. Therefore, inhibition of the production of chemotaxis- and angiogenesis-related factors in tumor-associated macrophages may provide a useful form of anticancer therapy.

Lipopolysaccharide (LPS) is an extracellular membrane component of Gram-negative bacteria. LPS circulates in the plasma of healthy humans at a low concentration (14-16). A high level of LPS is known to cause severe systemic inflammation and acute septic shock (16). In addition, it has been shown that high-dose LPS causes a robust induction of various pro-inflammatory mediators in macrophages (17, 18), but it has also been suggested that LPS helps in the treatment of various diseases, such as cancer and allergies, by controlling innate immunity involving macrophages (19-21).

Recent studies have revealed that mRNA expression of the chemotaxis-related factor, monocyte chemotactic protein (MCP)-1, in mouse macrophages was significantly reduced by low-dose LPS treatment compared to those that treated with high-dose LPS, and that low-dose LPS failed to activate the classical nuclear factor (NF)-κB pathway (22-24).

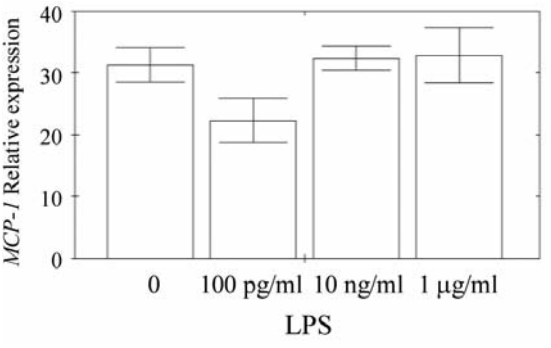


Figure 1. *MCP-1 mRNA expression in THP-1 cells treated with LPS. mRNA expression after five days of co-culture was analyzed by quantitative real-time PCR. Relative quantification was performed by normalization to the value of the housekeeping gene β-actin. Data are expressed as fold change in mRNA expression compared to that in THP-1 cells incubated without LPS before co-culture.*

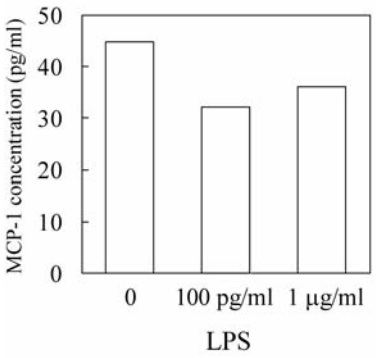


Figure 2. *MCP-1 protein concentration in the co-culture supernatants of THP-1 cells treated with LPS. The cell culture supernatants were collected after five days of co-culture. The MCP-1 protein concentration in the co-culture supernatants with and without LPS treatment was measured by ELISA.*

Therefore, unlike high-dose LPS, it is possible that low-dose LPS may aid in the treatment of various diseases involving macrophages. However, the effects of low-dose LPS on mRNA expression of chemotaxis- and angiogenesis-related factors in human monocytes remain unknown. In the present study, we examined changes in mRNA expression of chemotaxis- and angiogenesis-related factors in human monocytes following interaction with colon cancer cells after exposure to low-dose LPS.

## Materials and Methods

*Cells*. DLD-1 cells obtained from the Japan Health Sciences Foundation and THP-1 cells obtained from DS Pharma Biomedical were cultured in a 5% $CO_2$ atmosphere at 37˚C in RPMI-1640 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.).

*Cell co-culture*. THP-1 cells were treated with ultra-pure *Escherichia coli* LPS (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 resuspended in RPMI-1640 medium. THP-1 and DLD-1 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. DLD-1 cells were cultured in the upper chamber at $2\times10^5$ cells/ml, and THP-1 cells were cultured in the lower chamber at $2\times10^5$ cells/ml. THP-1 cells were collected at day 5 after the start of co-culture.

*RNA extraction*. Extraction of RNA from THP-1 cells was performed using TRIzol® Reagent (Invitrogen Corporation, Carlsbad, CA, USA) according to 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 PCR*. mRNA expression was analyzed by quantitative real-time PCR (Model MiniOpticon; Bio-Rad Laboratories, Inc., Hercules, CA, USA) using SsoFast™ EvaGreen® Supermix (Bio-Rad Laboratories, Inc.). Previously reported primers were used (12, 13). After initial heat denaturation at 95˚C for 3 min, PCR conditions were set at 95˚C for 10 s and 60˚C for 30 s for 40 cycles. Relative quantifications were performed by normalization to the value of the housekeeping gene β-actin. Data were expressed as fold change in mRNA expression compared with those in THP-1 cells incubated without LPS before co-culture.

*Enzyme-linked immunosorbent assay (ELISA)*. The cell culture supernatants were collected at day 5 after the start of co-culture and stored at −20˚C until assay. The MCP-1 protein concentration in the co-culture supernatants was measured using an ELISA kit (Funakoshi Co, Ltd., Tokyo, Japan) according to the manufacturer's protocol.

## Results

*MCP-1 mRNA expression in human monocytes following LPS treatment*. We have previously reported that *MCP-1* mRNA expression increased in THP-1 cells in a time-dependent manner from day 3 after the start of co-culture with DLD-1 cells (12). In the present study, we examined the effects of low-dose LPS on *MCP-1* mRNA expression in THP-1 cells at day 5 after the start of co-culture with DLD-1 cells. *MCP-1* mRNA expression in THP-1 cells increased 31.3-fold without LPS, 22.3 fold with 100 pg/ml LPS, 32.4-fold with 10 ng/ml LPS, and 32.9-fold with 1 μg/ml LPS after five days of co-culture with DLD-1 cells, compared to that in THP-1 cells incubated without LPS before co-culture. Thus, *MCP-1* mRNA expression in THP-1 cells treated with low-dose LPS (100 pg/ml) decreased by 28.8% compared to that in untreated THP-1

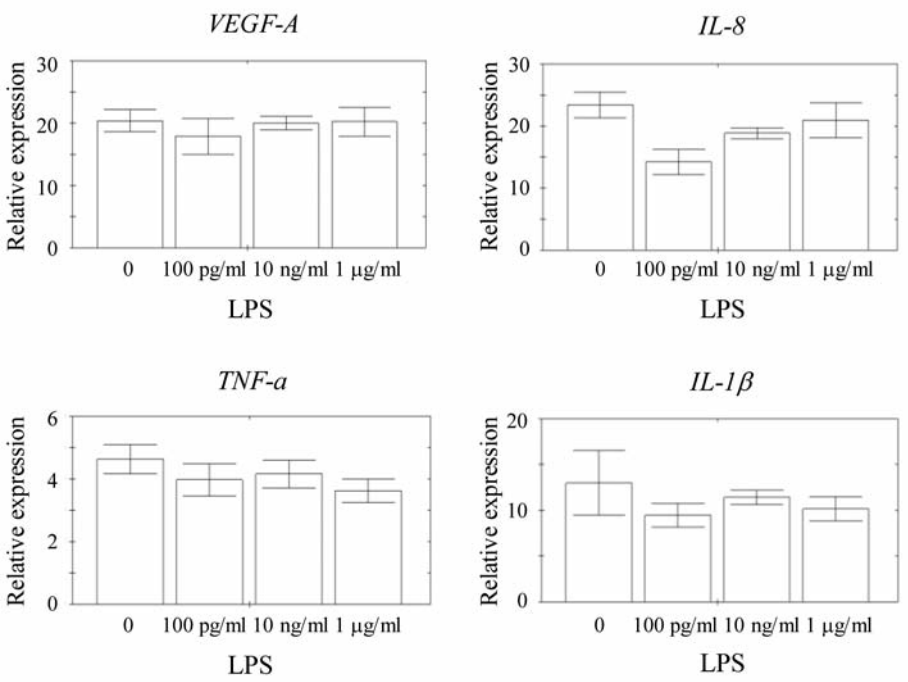


Figure 3. *mRNA expression of angiogenesis-related factors in THP-1 cells treated with LPS. mRNA expression after five days of co-culture was analyzed by quantitative real-time PCR. Relative quantifications were performed by normalization to the value of the housekeeping gene β-actin. Data are expressed as fold change in mRNA expression compared to those in THP-1 cells incubated without LPS before co-culture.*

cells after five days of co-culture with DLD-1 cells. Conversely, *MCP-1* mRNA expression in THP-1 cells treated with high-dose LPS (10 ng/ml or 1 μg/ml) was no different to that in untreated THP-1 cells after five days of co-culture with DLD-1 cells (Figure 1). These results suggest that the increase in *MCP-1* mRNA expression in human monocytes following interaction with colon cancer cells is suppressed by low-dose LPS.

*MCP-1 protein expression in the co-culture supernatants of human monocytes treated with LPS*. The MCP-1 protein concentration in supernatants from co-cultures with and without LPS treatment was measured in order to confirm protein expression. Thus, MCP-1 protein concentration in the co-culture supernatants of THP-1 cells treated with low-dose LPS (100 pg/ml) decreased by 28.1% compared with those without LPS after five days of co-culture (Figure 2). According to our previous report, *MCP-1* mRNA expression in THP-1 cells increased, whereas *MCP-1* mRNA expression in DLD-1 cells did not increase after co-culture (13). This confirmed that MCP-1 protein present in the co-culture supernatant is derived from THP-1 cells. This result suggests that the increase in MCP-1 protein expression in human monocytes following interaction with colon cancer cells is suppressed by low-dose LPS.

*mRNA expression of angiogenesis-related factors in human monocytes following LPS treatment*. We have previously reported that mRNA expression of angiogenesis-related factors, such as vascular endothelial growth factor *(VEGF)-A,* tumor necrosis factor *(TNF)-α* interleukin *(IL)-1β*, and *IL-8*, increased in THP-1 cells at day 5 after the start of co-culture with DLD-1 cells (12). Therefore, we examined the effects of low-dose LPS on mRNA expression of angiogenesis-related factors in THP-1 cells after five days of co-culture with DLD-1 cells. In THP-1 cells previously treated with low-dose LPS (100 pg/ml), *VEGF-A* mRNA expression decreased by 12.0%, *TNF-α* mRNA expression decreased by 13.0%, *IL-1β* mRNA expression decreased by 27.0%, and *IL-8* mRNA expression decreased by 39.0% compared with those in untreated THP-1 cells after five days of co-culture with DLD-1 cells (Figure 3). This suggests that the increase in mRNA expression of angiogenesis-related factors in human monocytes following interaction with colon cancer cells is suppressed by low-dose LPS.

## Discussion

We previously demonstrated that *MCP-1* mRNA expression increased in human monocytes but not in colon cancer cells after co-culture. Thus, we hypothesized that human

monocytes may migrate into and accumulate in tumor tissues in response to macrophage-derived MCP-1 (12). In addition, tumor-associated macrophages accumulated in tumor tissues are considered to promote the progression of malignancies (1, 4-11). The findings of this study suggested that the increase in MCP-1 expression in human monocytes following interaction with colon cancer cells is suppressed by low-dose LPS. Thus, it is possible that low-dose LPS may act on human tumor-associated macrophages to suppress the migration and accumulation of human monocytes in tumor tissue, and to suppress the progression of malignancies. Therefore, low-dose LPS treatment of human monocytes may be effective for prevention and therapy of colon cancer.

We have previously reported that mRNA expression of angiogenesis-related factors increased in human monocytes following interaction with colon cancer cells (12). It is believed that angiogenesis-related factors are involved in the progression and metastasis of cancer. Thus, we examined changes in mRNA expression of angiogenesis-related factors in human monocytes, following the interaction with colon cancer cells after exposure to low-dose LPS. The results indicate that the increase in expression of angiogenesis-related factors in human monocytes following interaction with colon cancer cells is suppressed by low-dose LPS. It is possible that the effect of low-dose LPS on human tumor-associated macrophages may suppress tumor progression and metastasis. Therefore, low-dose LPS treatment of human tumor-associated macrophages may be a useful therapy for colon cancer.

It has been previously reported that the NF-κB signaling pathway in tumor-associated macrophages is important for tumor initiation and growth (25-27). Recent studies have demonstrated that low-dose LPS did not induce activation of NF-κB but reduced the levels of RelB, which is another member of the NF-κB family, in mouse macrophages (22-24). In addition, it was reported that RelB/p50 regulated TNF production in LPS-stimulated human macrophages (28). The mechanism underlying the effects of low-dose LPS has not been clarified in human monocytes. Therefore, further investigation to clarify these mechanism is required.

Although a high level of LPS can cause acute septic shock, it has been suggested that LPS aids in the treatment of various diseases, such as cancer and allergies, by controlling innate immunity involving macrophages (16, 19-21). LPS is reported to be an exo-hormone and not only an endotoxin (29). Macrophages are also associated with the correct regulation of immune balance. Moreover, it is reported that oral administration of LPS may be effective for prevention against various diseases (21). Therefore, oral administration of low-dose LPS may be useful not only for colon cancer prevention and therapy but also for the maintenance of homeostasis.

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