Molecular Response of Human Monocytes Following Interaction with Colon Cancer Cells by Pre-treatment with Low-dose Lipopolysaccharide

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Abstract. *Background/Aim: The increased mRNA expression of chemotaxis- and angiogenesis-related factors in human monocytes following interaction with colon cancer cells has been shown to be suppressed by pre-treatment with low-dose lipopolysaccharide (LPS) (100 pg/ml). It has been demonstrated that low-dose LPS reduced the expression of RelB, a member of the nuclear factor (NF)-κB transcription factor family, in mouse macrophages and the NF-κB signaling pathway was important for tumor initiation and growth in tumor-associated macrophages. In addition, the signal transducer and activator of transcription 3 (STAT3) regulated innate immunity via Toll-like receptor (TLR)4 signaling. In the present study, the mRNA expression of signaling pathway- and suppression-related genes in human monocytes following a low-dose LPS treatment and subsequent interaction with colon cancer cells was investigated, in order to assess the molecular response. 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. The mRNA expression of various genes was then analyzed using quantitative real-time polymerase chain reaction (PCR). Results: The mRNA expression of RelB, STAT3, interleukin (IL)-10 and transforming growth factor (TGF)-β in THP-1 cells following interaction with DLD-1 cells was suppressed by pre-treatment with low-dose LPS (100 pg/ml). Conclusion: Treating human monocytes with low-dose LPS may be useful for suppressing tumor progression and may be valuable for maintaining homeostasis.*

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Macrophages are found in all tissues and it is thought that monocytes/macrophages are educated by the tissue microenvironment. Thus, macrophages have tissue-specific functions (1-3). They play important roles in development, maintenance of homeostasis, host defense mechanisms, such as phagocytosis of foreign substances, and tissue remodeling (1-3). Tumor-associated macrophages accumulate in tumor tissues and promote tumor progression and metastasis (3-11). Previously, it was demonstrated that the mRNA expression of chemotaxis- and angiogenesis-related factors in human monocytes increased after interaction with colon cancer cells (12-13). It is possible that the inhibitor of mRNA expression of chemotaxis- and angiogenesis-related factors in human monocytes following interaction with colon cancer cells may suppress tumor progression. Therefore, tumor-associated macrophages may be a useful target for colon cancer therapy.

Lipopolysaccharide (LPS) is a component of the outer membrane of Gram-negative bacteria. It is found that circulating high-dose LPS (10-300 ng/ml) can induce systemic pro-inflammatory cytokines and can cause acute shock (14-16). However, our previous report revealed that the increased mRNA expression of chemotaxis- and angiogenesis-related factors in human monocytes following interaction with colon cancer cells was suppressed by pre-treatment with low-dose LPS (100 pg/ml) (17). It is known that macrophages respond to LPS signaling *via* nuclear factor (NF)-κB (3). Recent reports demonstrated that low-dose LPS reduced the expression of *RelB*, a member of the NF-κB transcription factor family, in mouse macrophages and failed to activate the classical NF-κB pathway (16, 18). Prior exposure to LPS can lead to de-sensitization of immune cells to subsequent challenge with LPS; a phenomenon referred to as LPS tolerance. It was revealed that down-regulation of NF-κB signaling pathway may impact LPS tolerance (19). Thus, it is considered that RelB functions as a dual transcriptional regulator during LPS tolerance and severe systemic inflammation by both activating and repressing innate immunity genes (20). Furthermore, low-dose LPS induced priming in mouse macrophages (21-22).

However, the molecular mechanisms for which low-dose LPS affects human monocytes/macrophages are poorly defined. One study reported that unphosphorylated signal transducers and activator of transcription 3 (STAT3) activated transcription by binding to NF-κB (23). In addition, activated STAT3 contributed to the maintenance of NF-κB activity in cancer cells (24) and, also, STAT3 played an essential role in the interleukin (IL)-10 signaling pathway (25-26). In the current study, we investigated the mRNA expression of signaling pathway- and suppression-related genes in human monocytes following a low-dose LPS treatment and subsequent interaction with colon cancer cells to assess molecular response.

Materials and Methods

Cells. DLD-1 cells obtained from the Japan Health Sciences Foundation (Tokyo, Japan) and THP-1 cells obtained from DS Pharma Biomedical (Osaka, Japan) 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 re-suspended 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 2×10^5 cells/ml, whereas THP-1 cells were cultured in the lower chamber at 2×10^5 cells/ml. THP-1 cells were collected at day 5 after the start of co-culture.

RNA extraction. Total RNA from THP-1 cells was extracted 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 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 (13, 27). 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 target expression to the housekeeping gene β-actin (*ACTB*). Data were expressed as change (n-fold) in mRNA expression compared with THP-1 cells incubated without LPS before co-culture.

Results

The mRNA expression of RelB in human monocytes treated with LPS. It was reported that the regulation of RelB expression in mouse monocytes differed in response to high-dose and low-dose LPS treatment (21). We first measured the

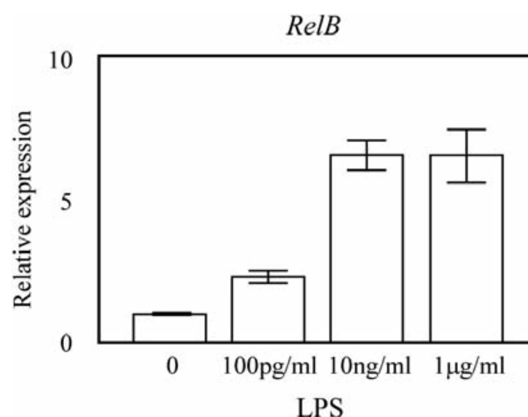

Figure 1. *RelB mRNA expression in THP-1 cells treated with LPS. The RelB mRNA expression in LPS-treated THP-1 cells was analyzed with quantitative real-time PCR. Relative quantification was performed by normalizing RelB expression to the housekeeping gene β-actin (ACTB). Data are expressed as change (n-fold) in mRNA expression compared to that of THP-1 cells incubated without LPS.*

RelB mRNA expression in THP-1 cells 3 h after treatment with high-dose or low-dose LPS. The *RelB* mRNA expression in THP-1 cells increased by 2.3-fold, 6.6-fold and 6.6-fold after treatment with 100 pg/ml, 10 ng/ml and 1 μg/ml LPS, respectively, compared to untreated THP-1 cells. Thus, the *RelB* mRNA expression in THP-1 cells increased to a lower extent at low-dose LPS (100 pg/ml) than at high-dose LPS (10 ng/ml and 1 μg/ml) (Figure 1). Therefore, these results suggest that the *RelB* mRNA expression in human monocytes is regulated by LPS treatment in a dose-dependent manner, similar to mouse monocytes.

The mRNA expression of signaling pathway-related genes in human monocytes treated with LPS. Previous reports demonstrated that the mRNA expression of the chemotaxis-related factor monocyte chemotactic protein-1 (*MCP-1*) and angiogenesis-related factors vascular endothelial growth factor-A (*VEGF-A*), tumor necrosis factor-α (*TNF-α*), *IL-1β* and *IL-8* was increased in THP-1 cells following co-culture with DLD-1 cells (12-13). Furthermore, the mRNA expression of the same genes was decreased in THP-1 cells treated with low-dose LPS on day 5 after co-culture with DLD-1 cells compared to that in THP-1 cells incubated without LPS before co-culture (17). Thus, we investigated the mRNA expression of the signaling pathway-related genes *RelB* and *STAT3* in low-dose LPS-treated THP-1 cells on day 5 after co-culture with DLD-1 cells. The mRNA expression of *RelB* in THP-1 cells increased 1.8-fold without LPS treatment, 1.6-fold with 100 pg/ml LPS, 1.8-fold with 10 ng/ml LPS and 2.1-fold with 1 μg/ml LPS on day 5 after co-culture with DLD-1 cells compared to that in THP-1 cells incubated without LPS before

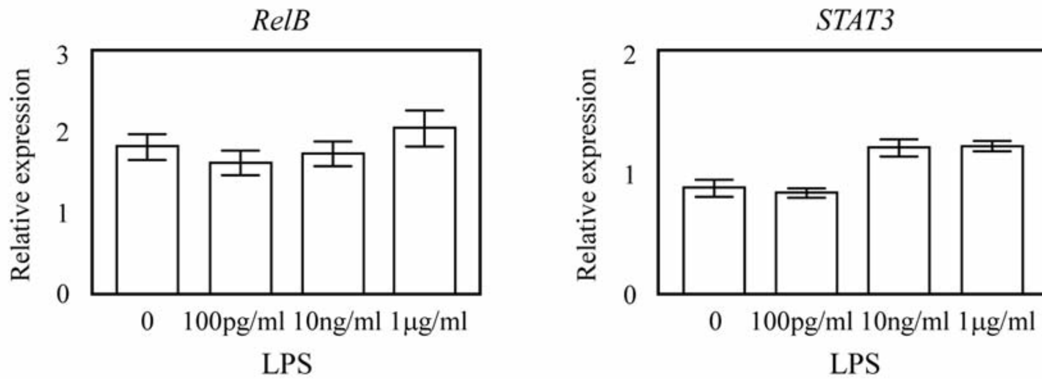

Figure 2. *mRNA expression of signaling pathway-related genes in THP-1 cells treated with LPS. The mRNA expression of target genes in THP-1 cells was analyzed using quantitative real-time PCR after 5 days of co-culture with DLD-1 cells. Relative quantification was performed by normalizing target expression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

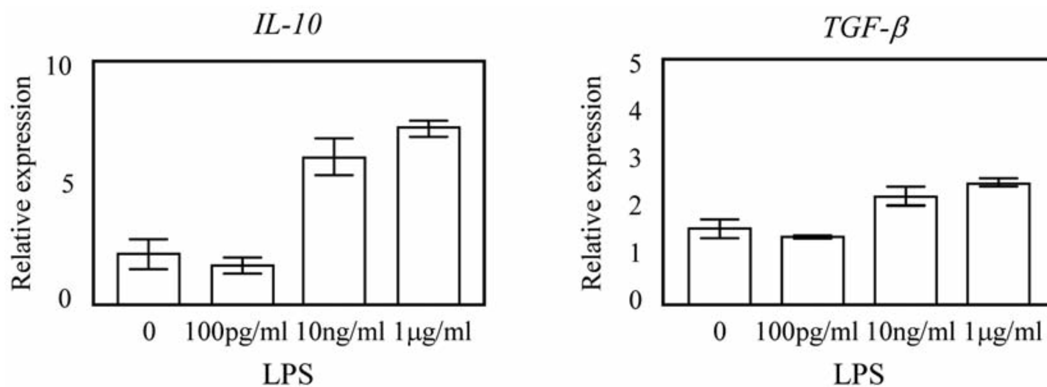

Figure 3. *mRNA expression of suppression-related genes in THP-1 cells treated with LPS. The mRNA expression of target genes in THP-1 cells was analyzed using quantitative real-time PCR after 5 days of co-culture with DLD-1 cells. Relative quantification was performed by normalizing target expression to the housekeeping gene β-actin (ACTB). Data are expressed as change (n-fold) in mRNA expression compared with THP-1 cells incubated without LPS before co-culture.*

co-culture. The mRNA expression of *STAT3* in THP-1 cells increased 0.9-fold without LPS, 0.9-fold with 100 pg/ml LPS, 1.2-fold with 10 ng/ml LPS and 1.2-fold with 1 μg/ml LPS on day 5 after co-culture with DLD-1 cells compared to THP-1 cells incubated without LPS before co-culture (Figure 2). Therefore, these results suggest that the mRNA expression of signaling pathway-related genes in human monocytes following interaction with colon cancer cells is suppressed by pretreatment with low-dose LPS.

The mRNA expression of suppression-related genes in human monocytes treated with LPS. The above data suggest that the mRNA expression of the signaling pathway-related gene *STAT3* in human monocytes following co-culture with colon cancer cells is suppressed by pretreatment with low-dose LPS. In addition, STAT3 plays an essential role in the *IL-10* signaling pathway (25, 26). Thus, we investigated the mRNA expression of the suppression-related genes *IL-10* and transforming growth factor-β (*TGF-β*) in low-dose LPS-treated THP-1 cells on day 5 after co-culture with DLD-1 cells. The mRNA expression of *IL-10* in THP-1 cells increased by 2.1-fold without LPS, 1.6-fold with 100 pg/ml LPS, 5.9-fold with 10 ng/ml LPS and 7.0-fold with 1 μg/ml LPS on day 5 after co-culture compared with that in THP-1 cells incubated without LPS before co-culture. The *TGF-β* mRNA expression in THP-1 cells increased 1.6-fold without LPS, 1.4-fold with 100 pg/ml LPS, 2.2-fold with 10 ng/ml LPS and 2.5-fold with 1 μg/ml LPS on day 5 after co-culture with DLD-1 cells compared to THP-1 cells incubated without LPS before co-culture. The mRNA expression of *IL-10* and *TGF-β* in THP-1 cells increased in an LPS dose-dependent manner on day 5 after co-culture with DLD-1 cells (Figure 3). In addition, the mRNA expression of *IL-10* and *TGF-β* in THP-1 cells treated with low-dose LPS (100

pg/ml) was decreased by 20.5% and 9.7%, respectively, compared to untreated THP-1 cells on day 5 after co-culture with DLD-1 cells. Therefore, these results suggest that the mRNA expression of suppression-related genes in human monocytes following interaction with colon cancer cells is suppressed by pre-treatment with low-dose LPS.

Discussion

It has been previously reported that the mRNA expression of chemotaxis- and angiogenesis-related factors in human monocytes was increased following interaction with colon cancer cells (12, 13). Moreover, it was demonstrated that increased mRNA expression of chemotaxis- and angiogenesis-related genes in human monocytes, following interaction with colon cancer cells, was suppressed by pretreatment with low-dose LPS (17). Recent studies demonstrated that low-dose LPS reduced the levels of RelB in mouse macrophages (16, 18). In addition, it is thought that RelB functions as a dual transcriptional regulator during LPS tolerance and severe systemic inflammation by both activating and repressing innate immunity genes (20). Therefore, it is suggested that low-dose LPS could regulate the fate of monocytes in tumor tissue. This mechanism is unknown. Thus, we investigated the change of *RelB* mRNA expression in low-dose LPS-treated human monocytes. The results demonstrated that the *RelB* mRNA expression in human monocytes was regulated by LPS treatment in a dose-dependent manner (Figure 1). In addition, the *RelB* mRNA expression in human monocytes, following interaction with colon cancer cells, was suppressed by pre-treatment with low-dose LPS (Figure 2). A previous report demonstrated that the NF-κB signaling pathway was important for tumor initiation and growth in tumor-associated macrophages (28-30). These results suggest that treating human tumor-associated macrophages with low-dose LPS is useful to suppress tumor progression.

The current study demonstrated that the *STAT3* mRNA expression in human monocytes, following interaction with colon cancer cells, was suppressed by pre-treatment with low-dose LPS. It is known that LPS signaling is transferred to Toll-like receptor (TLR)4 and that STAT3 regulates innate immunity *via* TLR4 signaling (31). Therefore, treatment with low-dose LPS may affect STAT3 activity in tumor-associated macrophages *via* TLR4 signaling. Herein, it was demonstrated that the *IL-10* mRNA expression in human monocytes, following interaction with colon cancer cells, was suppressed by pre-treatment with low-dose LPS (Figure 3). STAT3 plays an essential role in the IL-10 signaling pathway (25-26). Also, it is possible that decreased *IL-10* expression was caused by decreased *STAT3* expression. Therefore, treating human monocytes with low-dose LPS may be valuable for maintaining homeostasis.

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