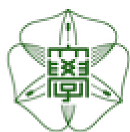
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Letter to the Editor

Ciprofloxacin inhibits lipopolysaccharide-induced toll-like receptor-4 and 8 expression on human monocytes derived from adult and cord blood

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Dear Sir,

Lipopolysaccharide (LPS) interacts with immune cells by binding to CD14 molecules and then interacts with toll-like receptor (TLR)-4 on monocytes and dendritic cells [1-3]. On the other hand, TLR8 recognizes viral single-stranded RNA (ssRNA) [4]. Therefore, TLR4 is involved in immunological events during bacterial infection and TLR8 is involved in immunological events during viral infection. Ciprofloxacin (CIP), a fluorinated 4-quinolone, is one of quinolone antibiotics. CIP reduces the population of intestinal bacteria and influences the development of acute graft-versus-host disease (GVHD) after allogeneic bone marrow transplantation for hematological malignancies [5]. Also, CIP has modulatory actions on immune and inflammatory responses, such as inhibition of the production of TNF-α in LPS-stimulated peripheral blood mononuclear cells (PBMC) [6]. The mechanisms underlying the effects of quinolones on immune modulation might depend on the regulation of intracellular cAMP and NF-kB. CIP induces the production of prostaglandin E2 (PGE2) and increases intracellular cAMP levels. A hyperresponse to LPS by monocytes induces symptoms of septic shock and CIP might therefore contribute to the attenuation of these symptoms after severe infection [7]. Cord blood contains more naïve T cells and Treg precursor cells than dose adult blood [8]. Also, it has been reported that there is a profound defect in IL-12 (p70) synthesis and an increased release of IL-10 in cord blood exposed to LPS compared tothose in adult blood [9]. These facts might

be relevant to the increased vulnerability of human newborns to intracellular pathogens and the low incidence of GVHD after cord blood transplantation (CBT). In this study, we analyzed TLR4, TRL8, ICAM-1 and LFA-1 expression on the surface of CD14-positive monocytes and TLR4, TLR8 and TNF-α mRNA expression after LPS stimulation with CIP using adult peripheral blood mononuclear cells (PBMC) and cord blood mononuclear cells (CB).

Normal adult PBMC and cord blood units were obtained from healthy donors with informed consent from Hokkaido Red Cross Blood Center Sapporo. Cells (1×10^6/ml) were cultured for 3 days with 10 μg/ml LPS (*E.coli* 055:B5, EMD Bioscience, La Jolla, CA, USA) in RPMI-1640 with 10% fetal calf serum (FCS) in the presence or absence of 50 μg/ml ciprofloxacin (CIP, kindly provided by Bayer Yakuhin, Osaka, Japan). After 3 days of culture, cells were harvested and stained with FITC-conjugated anti-TLR4 (HTA125) (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-LFA-1 (HI111) (Becton Dickinson, San Diego, CA, USA) and anti-ICAM-1 (84H10) (Immunotech, Marseille, France) monoclonal antibody (mAb) and with FITC-conjugated goat anti mouse Ig G1 (STAR81F) (Serotec, Oxford, UK) as a secondary antibody for anti-TLR8 (44C143) (IMGENEX, San Diego, CA, USA) mAb and PE-conjugated anti-CD14 (MoP9) (Becton Dickinson, San Diego, CA, USA) mAb. Mean fluorescence intensity (MFI) for TRL4, TRL8, ICAM-1 and LFA-1 on CD14-positive monocytes was analyzed using a FACS Calibur (BD). Statistical analysis was performed using Student's *t*-test.

Total RNA was prepared using a QIAamp RNA Blood Mini Kit (QIAGEN), and cDNA was synthesized from 1.2 μg total RNA using a TaKaRa RNA PCR Kit(AMV) Ver.3.0 following the instructions of the manufacturer (TAKARA, Shiga, Japan). Assays-on-Demand™ Gene Expression products for TLR4, TLR8 and TNF-α were obtained from Applied Biosystems. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an endogenous control. Relative quantification assays for gene expression were performed using an Applied Biosystems 7300 Real-time PCR system (Applied Biosystems).

LPS induced significantly higher expression levels of TLR4, TRL8, ICAM-1 and LFA-1 on monocytes in both PBMC and CB (Table 1). Addition of CIP suppressed the induction of these TRLs and adhesion molecules by LPS. Real-time PCR analysis revealed that LPS induced maximum TLR4 and TNF-α mRNA expression after 6 hours and maximum TRL8 expression after 24 hours in both total mononuclear cells from PBMC and CB. Therefore, LPS-induced signaling may upregulate TLR8 mRNA expression perhaps indirect mechanism. We focused on the expression of TLR4 and TLR8 in this study, however, it would be interesting to evaluate the expression of other TLRs such as TLR2 and TLR9 after LPS stimulation with or without CIP. The magnitude of enhancement of the mRNA expression of these molecules in CB was less than that in PBMC. However, CIP can suppress LPS-induced these mRNA expression in both PBMC and CB. Therefore, CIP has a suppressive

effect on the induction of expression of TRLs, adhesion molecules and TNF-α by LPS.

Also, we estimated TNF-α concentrations using ELISA (Invitrogen-Biosource, CA, USA). We could not detect TNF-α in culture medium before LPS stimulation (<14 pg/mL). TNF-α concentrations 24 hours after LPS stimulation were 2,257±498 pg/mL but 943±279 with CIP in PBMC culture medium (means±SDs, n=4) and also were 3,847±1,248 pg/mL but 1,442±324 with CIP in CB culture medium (n=4). Significant differences were found in the values after addition of CIP compared with the values after stimulation by LPS in both PBMC and CB culture medium ($P<0.01$). There were some discrepancies between the results obtained in real time PCR analysis and the results obtained in FACS analysis. However, ELISA results support some data from real time PCR.

It has been reported that CIP has an immunosuppressive effect on mixed lymphocyte reaction by suppression of IFN-γ and IL-12 production [10]. In this study, CIP reserved the LPS-induced expression of TLR4, TLR8, ICAM-1 and LFA-1 as well as TNF-α production in both PBMC and CB. CIP might exert immune modulatory effects by suppressing the inflammatory responses of both adult and cord blood. The maximal serum concentration of CIP is 5 mg/L in a clinical setting. Therefore, if CIP can be used at a higher concentration, CIP might be able to suppress immune

responses such as GVHD and host-versus-graft disease (HVG) after allogeneic stem cell transplantation including CBT.

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Table 1. Expression of TLRs, adhesion molecules and TNF-α in PBMC and CB after stimulation by LPS with or without CIP.

FACS[a]

		TLR4	TLR8	ICAM-1	LFA-1
	Pre	8.0±1.2	64.0±19.9	11.9±1.2	55.0±6.8
PB	LPS	38.1±4.1	189.9±44.6	166.4±8.8	169.1±6.9
	+CIP	26.3±3.0	94.3±35.7	121.2±9.0	104.0±6.5
	Pre	10.2±1.2	44.2±15.2	20.1±3.5	77.9±11.4
CB	LPS	37.9±3.9	142.6±40.5	163.0±8.7	143.3±12.5
	+CIP	24.4±1.7	58.3±18.8	121.1±5.5	70.4±5.8

Real time PCR[b]

		TLR4	TLR8	TNF-α
	Pre	1.0	1.0	1.0
	6hr LPS	26.3	3.0	15.9
PB	+ CIP	7.3	2.3	5.0
	24hr LPS	6.4	10.6	0.5
	+ CIP	5.3	5.7	1.1
	Pre	1.0	1.0	1.0
	6hr LPS	4.5	0.8	8.9
CB	+ CIP	1.7	0.8	5.1
	24hr LPS	1.1	4.8	0.1
	+CIP	1.3	2.4	0.4

[a]Values indicate mean fluorescence intensity (MFI, means ± SDs, n=6) on CD14-positive cells. Significant differences were found in the values after stimulation by LPS compared with the values before stimulation and in the values after addition of CIP compared with the values after stimulation by LPS ($P<0.05$).
[b] Values indicate fold increase of mRNA compared with that before stimulation. Representative results from several experiments are shown.