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Increased expression levels of monocyte CCR2 and monocyte chemoattractant protein-1 in patients with diabetes mellitus

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Abstract

Increased monocyte recruitment into subendothelial space in atherosclerotic lesions is one of the hallmarks of diabetic angiopathy. The aim of this study was to determine the state of peripheral blood monocytes in diabetes associated with atherosclerosis. Diabetic patients treated with/without an oral hypoglycemic agent and/or insulin for at least 1 year were recruited ($n = 106$). We also included 24 non-diabetic control subjects. We measured serum levels of monocyte chemoattractant protein (MCP)-1, fasting plasma glucose (FPG), HbA1c, total cholesterol, triglyceride, body mass index (BMI), high sensitivity CRP (hs-CRP) and evaluated CCR2, CD36, CD68 expression on the surface of monocytes. Serum MCP-1 levels were significantly ($p < 0.05$) higher in diabetic patients than in normal subjects. In diabetic patients, serum MCP-1 levels correlated significantly with FPG, HbA1c, triglyceride, BMI, and hs-CRP. The expression levels of CCR2, CD36, and CD68 on monocytes were significantly increased in diabetic patients and were more upregulated by MCP-1 stimulation. Our data suggest that elevated serum MCP-1 levels and increased monocyte CCR2, CD36, CD68 expression correlate with poor blood glucose control and potentially contribute to increased recruitment of monocytes to the vessel wall in diabetes mellitus.

Keywords: Diabetes mellitus; MCP-1; CCR2; Angiopathy; Diabetes control

The transmigration of circulating monocytes through the blood vessel wall is one of the earliest events in the development of atherosclerotic lesions, where monocytes are transformed into lipid-laden foam cells, which is followed by atherosclerotic plaque formation [1]. Such transmigration is mediated by the interaction of specific adhesion molecules on vascular endothelial cells with their integrin counter receptors on monocytes. Studies have demonstrated increased interaction of monocytes with vascular cells is linked to the development and progression of atherosclerosis in patients with diabetes mellitus [2,3]. Hyperglycemia is considered to be a major factor and certain key pathways, factors, and mechanisms have been implicated, including oxidant stress [4], advanced glycation end products (AGE) [5], aldose reductase [6], reductive stress [7], carbonyl stress [8], and protein kinase C activity [9]. However, the specific cellular and molecular mechanisms involved in the development of vascular disturbances associated with diabetes mellitus are not fully clear.

One of the key proinflammatory mediators whose expression is stimulated by AGE and/or high glucose signaling pathway is monocyte chemoattractant protein-1 (MCP-1) [10]. MCP-1 is a chemokine responsible for the recruitment of monocytes to sites of inflammation. In animal models, the expression levels of MCP-1 and its monocyte receptor (CCR2) are directly related to the extent of atherosclerosis and macrophage infiltration into the atherosclerotic lesion. Several investigators have reported that

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plasma levels of MCP-1 are associated with cardiovascular risk factors and may be an important biomarker of the preclinical phase of atherosclerosis [1,11]. However, no prospective data are available that evaluated the relation between plasma MCP-1 levels and outcomes in patients with diabetes mellitus. The present study was designed to evaluate the plasma levels of MCP-1 and the expression of CCR2 and scavenger receptors, using samples from diabetic patients. The results suggested that poor glycemic control could induce high expression levels of MCP-1, CCR2, and scavenger receptor and augment oxidized LDL (ox-LDL) uptake in monocytes, which in turn enhances foam cell transformation.

Materials and methods

Study participants. We studied patients who visited our hospital between April 2002 and July 2002. The inclusion criteria were the presence of type 2 diabetes mellitus, treated with/without an oral hypoglycemic agent and/or insulin for at least one year. We excluded patients with the above criteria but had a history of alcohol abuse, evidence of liver disease, or severe cardiac problems. We also included a control group consisting of 24 individuals referred to our department for admission who were free of subjective symptoms and showed no pathologic signs. In the present study, evaluation was based on the criteria for diagnosis of diabetes complications, i.e., retinopathy represented more than stage 1a of Scott classification, regardless of the presence or absence of photocoagulation; neuropathy represented a decrease of the Achilles tendon reflex or CV_{R-R} = less than 2%; nephropathy represented the presence of microalbuminuria with albumin excretion of 30–300 mg/day [12], creatinine level <2 mg/ml; and patients on dialysis were excluded.

The study was performed in accordance with the Declaration of Helsinki. The study protocol was approved by the Human Ethics Review Committee of the Nakama Municipal Hospital, Japan, and an informed consent was obtained from each subject.

Sample analysis. HbA1c, fasting plasma glucose (FPG), high sensitivity c-reactive protein (hs-CRP), hematology, biochemistry, and lipid (triglyceride, total cholesterol) levels were determined using standard laboratory tests (SRL, Tokyo, Japan). In this study, subjects with high hs-CRP value suspicious of inflammation (>2 mg/ml) were excluded from examination. Glycated albumin (GA) was measured by an enzymatic method [13] (SRL). Serum MCP-1 levels were determined by enzyme-linked immunosorbent assay (ELISA) (R&D Systems Inc., Minneapolis, MN).

Preparation of monocytes. Peripheral blood samples were withdrawn from consented healthy volunteers and collected in heparinized polypropylene tubes. Mononuclear cells were isolated by Lymphocyte Separation Medium 50494 (Pharmacia Biotech, Uppsala, Sweden) as described previously [14], further separated into subpopulations by counterflow centrifugal elutriation [15] (Hitachi, Tokyo). The monocyte-enriched fraction was suspended in RPMI-1640 (Nissui, Tokyo) containing 10% fetal bovine serum (FBS) (GIBCO, Grand Island, NY), 100 U/ml penicillin, and 100 μg/ml streptomycin. Flow cytometric analysis indicated that the majority of these cells (>85%) were CD14-positive and representative of highly purified resting monocytes.

Reagents and monoclonal antibodies. The following monoclonal antibodies (mAbs) were purchased from the indicated sources: anti-CD36 mAb CB38, control anti-mouse IgG (PharMingen, San Diego, CA), anti-CD68 mAb KP-1, and anti-CCR2 mAb (Dako Japan, Kyoto, Japan). Ox-LDL was purchased from Biomedical Technologies Inc. (Stoughton, MD).

Immunofluorescence analysis. Staining and flow cytometric analyses of freshly isolated monocytes were carried out using standard procedures, as described previously, using a FACScan (Becton–Dickinson, Mountain View, CA) [16,17]. Cells (2×10^5) were incubated with the corresponding mAbs at saturating concentrations in the presence of goat anti-mouse irrelevant Ab in FACS media consisting of HBSS (Nissui, Tokyo), 0.5% human serum albumin (Green-cross, Osaka, Japan), and 0.2% NaN_3 (Sigma–Aldrich, Tokyo) for 30 min at 4 °C. After washing the cells with FACS media three times, cell samples were immediately analyzed with a FACScan. Quantification of cell surface antigens on single cells was performed using standard beads, QIFIKIT (Dako Japan), as described previously [18,19]. Set-up beads and calibration beads provided by the QIFKIT were treated parallel to the cell samples. Data were used for construction of the calibration curve [mean fluorescence intensity (MFI)] against antibody-binding capacity (ABC). When the green fluorescence laser detector was set at 450 nm level in the used FACScan, $ABC = 414.45 \times \mathrm{Exp}(0.0092 \times MFI)$ ($R2 = 0.9999$). Subsequently, specific antibody-binding capacity (SABC) was obtained after corrections for background, apparent ABC of the negative control mAb. SABC corresponds to the mean number of accessible antigenic sites per cell, referred to as antigen density and expressed in sites/cell.

Stimulation of MCP-1 on monocytes. Resting monocytes isolated by counter flow centrifugal elutriation were suspended in RPMI-1640 medium containing 1% FBS in 30 mm cell culture dishes and were incubated with or without MCP-1 for 12 h at 37 °C in 5% CO_2. The medium was removed and cells were washed by PBS three times for experiments.

Statistical analysis. Data were expressed as means ± SD. Differences between groups were tested for statistical significance using the Student's *t* test. A p value < 0.05 denoted the presence of a statistically significant difference.

Table 1
Patients profiles

	Normal ($n = 24$)	Hyperlipidemia ($n = 7$)	Diabetes ($n = 106$)
Age (years)	58.8 ± 11	62.6 ± 12	65.2 ± 9.6*
Sex (M/F)	8/16	2/5	55/51
FPG (mg/dl)	107 ± 16	112 ± 19	176 ± 76*
HbAlc (%)	5.1 ± 0.4	5.3 ± 0.3	6.9 ± 1.5*
BMI (kg/m^2)	23.5 ± 3.0	24.4 ± 2.4	23.7 ± 3.4
hs-CRP (mg/dl)	0.5 ± 0.5	0.5 ± 0.3	0.7 ± 0.5
TC (mg/dl)	181 ± 33	237 ± 36*	211 ± 50*
TG (mg/dl)	102 ± 61	151 ± 57*	150 ± 79

Mean ± SD, *$p < 0.05$ compared with control.

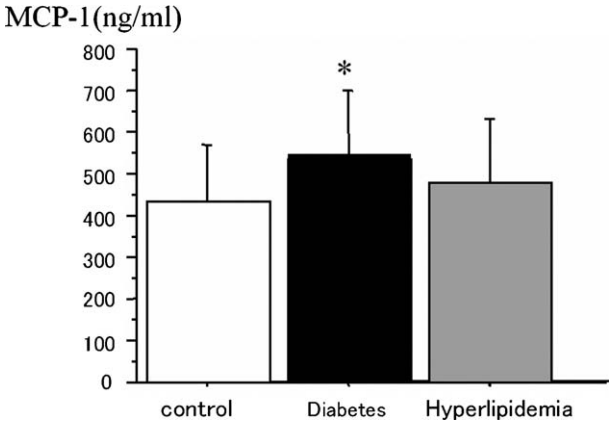

Fig. 1. Correlations between serum concentrations of MCP-1 and diabetic complications. Serum concentrations of MCP-1 were significantly higher in patients with diabetes than in normal control. Serum MCP-1 levels did not correlate significantly with diabetic complications. Data are means ± SD. *$p < 0.05$, compared with the control.