Homocysteine Mediated Expression and Secretion of Monocyte Chemoattractant Protein-1 and Interleukin-8 in Human Monocytes

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Abstract—Homocysteine (Hcy) is an independent risk factor for cardiovascular disease. Monocyte chemoattractant protein-1 (MCP-1) and interleukin-8 (IL-8) are major chemokines for leukocyte trafficking and have been identified in atheromatous plaques. MCP-1 and IL-8 have been found to express mainly by macrophages in human lesion. We undertook this study to determine whether Hcy could induce the secretion of chemokines from human monocytes and, if so, to explore the mediating mechanism. We found that clinically relevant levels of Hcy (10 to 1000 μmol/L) increased the protein secretion and mRNA expression as well as activity of MCP-1 and IL-8 in cultured primary human monocytes. These effects of Hcy were primarily mediated by reactive oxygen species (ROS) through NAD(P)H oxidase, because Hcy could upregulate the production of ROS and the inhibitors of protein kinase C, calmodulin, free radical scavengers, or NAD(P)H oxidase abolished Hcy-induced ROS production and MCP-1 and IL-8 secretion in these cells. Furthermore, the inhibitors of mitogen-activated protein kinase (p38 and extracellular signal-regulated kinase 1/2) and nuclear factor-κB or the activator of peroxisome proliferator-activated receptor γ (PPARγ) significantly decreased Hcy-induced MCP-1 and IL-8 secretion in these cells. These data indicate that pathophysiological levels of Hcy can alter human monocyte function by upregulating MCP-1 and IL-8 expression and secretion via enhanced formation of intracellular ROS originated from NAD(P)H oxidase source via calmodulin or protein kinase C signaling pathways and that Hcy-induced ROS subsequently activates mitogen-activated protein kinase (p38 and ERK1/2) and nuclear factor-κB in a PPARγ activator–sensitive manner. Thus, activation of PPARγ may become a therapeutic target for preventing Hcy-induced proatherogenic effects. (Circ Res. 2003;93:311-320.)

Key Words: homocysteine ■ monocytes ■ atherosclerosis ■ monocyte chemoattractant protein-1 ■ interleukin-8

Hcy is a sulfur-containing amino acid formed during the metabolism of methionine. Hyperhomocysteinemia is found in 30% of patients with premature atherosclerosis of carotid and peripheral arteries. Elevated plasma Hcy levels have been implicated as an independent risk factor for coronary heart disease. Therefore, intensive studies have been focused on whether hyperhomocysteinemia is the cause or merely a marker for cardiovascular disease.

Chemokines belong to a superfamily of structurally related small chemotactic cytokines involved in leukocyte trafficking. They play a key role in atherogenesis. Monocyte chemoattractant protein-1 (MCP-1), a prototype of CC chemokines, and interleukin (IL)-8, a prototype of CXC chemokines, were found to be highly expressed in human atherosclerotic lesions and postulated to be crucial in leukocyte recruitment into the arterial wall and developing lesions. More importantly, both IL-8 and MCP-1 convert monocyte rolling to firm adhesion on endothelial monolayers. This strongly implies a role of MCP-1 and IL-8 in atherosclerotic pathogenesis.

Previous studies have suggested that Hcy induces expression and secretion of MCP-1 and IL-8 in human aortic endothelial cells and MCP-1 in human smooth muscle cell line. Because MCP-1 and IL-8 have been shown to be expressed mainly by macrophages in human lesions, activated macrophages in plaque in response to pathologic agents, such as Hcy, may play an important role in the production of MCP-1 and IL-8. In monocyctic cell lines, it remains controversial as to whether Hcy could induce the secretion of MCP-1 or IL-8 from monocytes. We undertook the present study to determine whether Hcy induces MCP-1 and IL-8 secretion in human monocytes and, if so, to explore the mediating mechanisms. Our results indicate that Hcy at clinically relevant concentrations not only promotes MCP-1 and IL-8 mRNA expressions but also induces secretion of MCP-1 and IL-8 in human monocytes. Therefore,
hyperhomocysteinemia might promote atherogenesis by inducing chemokine production from monocytes and macrophages, thereby leading to infiltration of leukocytes to site of vascular injury and resulting in vascular inflammation.

Materials and Methods

Cell Culture
Human blood from a healthy donor was drawn into heparinized syringes. The whole blood was separated into peripheral blood mononuclear cells and neutrophils by using the density gradient from Nycoprep 1.077 (Life Technologies), and then monocytes were isolated from peripheral blood mononuclear cells by adherence to serum-coated culture flask for 2 hours. Adherent cells were then detached and resuspended in RPMI-1640 medium containing 5% autologous plasma. Isolated cells were plated in flat-bottom 24-well culture plates at a density of 5×10⁵ (monocytes). Cellular viability was determined with trypan blue exclusion. Only cell preparations with a 95% viability or greater were used.

Measurement of MCP-1 and IL-8

Protein Secretion
Cultured human monocytes were treated for certain times with L-Hcy or with other pharmacological reagents. The supernatant of cultured monocytes was harvested and transferred to other polypropylene tubes and stored at −30°C for not more than 1 week before measurement of chemokines and cytokines. Tumor necrosis factor (TNF)-α, IL-β, MCP-1, and IL-8 protein concentrations in cultured monocyte supernatants were determined by ELISA (R&D Systems).

MCP-1 Chemotaxis Assays
Chemotaxis in response to endogenous MCP-1 was studied in human peripheral blood monocytes. Human monocytes were treated with 100 μM/L Hcy for 4 days. Conditioned medium was collected from monocyte cultures treated with 100 μM/L Hcy for 4 days. Data are expressed as mean±SEM. *P<0.05 compared with corresponding untreated groups. The data were representative of at least 4 independent experiments.

Measurement of MPO Release
Conditioned medium was collected from monocyte cultures treated with 100 μM/L Hcy for 4 days. Cultured neutrophils were incubated with conditioned medium for 24 hours. MPO release from cultured neutrophils was determined by MPO assay kits (Nanjing Jiancheng Chemical Co). Anti–hIL-8 monoclonal antibody 4 μg/mL (R&D Systems) was added to culture medium for 30 minutes to neutralize secreted IL-8 and to confirm MCP-1–specific effects. Normal rabbit IgG was used as the negative control. Medium from untreated monocytes was used to determine basal migration.

RNase Protection Assays
Total RNA was isolated from cultured monocytes with TriZOL Reagent (Life Technologies). Assays were performed with a nuclease protection assay kit (Riboquant, Pharmingen). In brief, the isolated RNA (2 μg) was hybridized with 32P end-labeled MCP-1 or IL-8 oligonucleotide probes overnight at 30°C followed by nuclease digestion. A GAPDH rRNA oligonucleotide probe was used as an internal control. After digestion, the protected fragments were resolved on a denaturing 12% polyacrylamide gel containing 8 mol/L urea followed by transferring to a filter paper, which was later exposed to an x-ray film. The bands corresponding to MCP-1, IL-8, or GAPDH mRNA were analyzed using a gel documentation system (Cold-Spring Eletro Doc and Analyst Edas 290). Values were expressed as relative expression of MCP-1 or IL-8 mRNA normalized to GAPDH rRNA levels.

Measurement of Intracellular ROS Generation
Hcy was added to the cells together with a ROS probe dye 2′, 7′-dichlorofluorescin diacetate (DCFH-DA, 5 μmol/L). Determination of intracellular oxidant production in monocytes was based on the oxidation of DCFH-DA by intracellular ROS, resulting in the formation of the fluorescent compound 2′, 7′-dichlorofluorescin.
14 DCF fluorescence was monitored with a confocal laser-scanning microscope (Leica).

**Measurement of LDH Release**

Cultured human monocytes were treated with Hcy (10 to 1000 μmol/L) for 32 hours or 4 days. Cell suspensions were collected for determination of LDH. LDH activity was measured with the spectrophotometric enzyme activity method and expressed as units per liter of medium.

**Chemicals**

L-Hcy, L-cysteine, L-methionine, catalase (CAT), superoxide dismutase (SOD), SOD/CAT-polyethylene glycol (PEG-SOD/PEG-CAT), genistein, tyrphostin, pyrrolidine dithiocarbamate (PDTC), rotenone, antimycin A, oxypurinol, allopurinol, and indomethacin were purchased from Sigma. Calphostin C, RO-31-8220, W7, diphenyleneiodonium (DPI), phenylarsine (PAO), MK-886, SB 203580, PD 98059, curcumin, ciglitazone, and troglitazone were purchased from Calbiochem Co. 2’7’-DCFH-DA was obtained from Molecular Probes. Other chemicals were purchased from the Chinese Chemical Co.

**Statistical Method**

Results are expressed as mean±SEM. The number of experimental samples used in each group is presented in the figure legends. The data were analyzed using one-way ANOVA and additionally analyzed using the Student Newman-Keuls test for multiple comparisons within treatment groups or t test for between two groups. P<0.05 was considered significant.

**Results**

**Effect of Hcy on MCP-1 and IL-8 Secretion From Cultured Human Monocytes**

To determine whether Hcy is able to induce the secretion of MCP-1 and IL-8 in human monocytes, cells were isolated, cultured, and treated with Hcy. The amounts of MCP-1 and IL-8 secreted from cultured monocytes were determined by ELISA. Treatment of cells with 100 μmol/L Hcy induced a time-dependent accumulation of MCP-1 and IL-8 secretion, plateauing within 4 days in cultured monocytes (Figures 1A and 1B). As shown in Figures 1C and 1D, the secretions of MCP-1 and IL-8 in the monocytes treated with various concentrations of Hcy (10 to 1000 μmol/L) for 4 days were significantly elevated compared with the control. The maximum level of MCP-1 or IL-8 secretion was observed with 100 μmol/L Hcy. These observations indicate that relatively low concentration of Hcy, within the pathophysiological range, can directly trigger the secretion of chemokines, MCP-1, and IL-8 in cultured human monocytes.

**Effect of Hcy on MCP-1 and IL-8 mRNA Expressions**

To determine whether Hcy modulates the expression of MCP-1 and IL-8 mRNA, total RNA was isolated from
cultured monocytes treated with Hcy at various times and at different concentrations. After normalization against GAPDH mRNA, the RNase protection assays revealed that the expressions of MCP-1 and IL-8 mRNA were significantly enhanced after Hcy treatment. The increase in the levels of MCP-1 and IL-8 mRNA after incubation with 100 μmol/L Hcy reached the maximum at 8 hours of incubation (Figures 2A through 2C). As shown in Figures 2D through 2F, when cultured monocytes were incubated with Hcy (10 to 1000 μmol/L) for 8 hours, the expressions of MCP-1 and IL-8 were significantly increased compared with untreated cells. Hcy, at a concentration as low as 10 μmol/L, significantly enhanced the expressions of MCP-1 and IL-8 mRNA. The present results demonstrate that Hcy not only increases the production of MCP-1 and IL-8 proteins but also augments their mRNA levels in cultured human monocytes.

Biological Activities of Secreted MCP-1 and IL-8 From Cultured Monocytes

Monocyte migration toward conditioned medium collected from cultured human monocytes treated with 100 μmol/L Hcy for 4 days was increased by 2.3-fold relative to untreated cells (Figure 3A). Migration was inhibited by preincubation with anti-rhMCP-1 antibodies but not by rabbit IgG. This suggests that MCP-1 induced by Hcy treatment acts as a chemotactic factor of monocytes. In addition, when human neutrophils were stimulated by conditioned medium, MPO release was increased compared with that of untreated cells (Figure 3B). MPO release was inhibited by preincubation with anti-rhIL-8 antibodies but not with rabbit IgG. Hcy could not directly stimulate migration of monocytes or production of MPO by neutrophils. These data indicate that IL-8 is a factor triggering MPO release from human neutrophils. Thus, MCP-1 and IL-8 proteins secreted by Hcy-treated monocytes exhibit biological activities.

Effect of Hcy on Intracellular ROS Levels

To examine whether L-Hcy, L-cysteine, or L-methionine induces ROS production in cultured human monocytes, we measured intracellular ROS level using the redox-sensitive fluorescent dye DCFH-DA. The fluorescence intensity was significantly increased at 27 minutes and continually increased up to 50 minutes after adding 100 μmol/L Hcy (Figure 4A). When monocytes were cultured with Hcy (10 to 1000 μmol/L) for 50 minutes, Hcy promoted the formation of ROS (2- to 4-fold) in a concentration-dependent manner (Figure 4B). Other compounds with thiol (-SH), such as L-cysteine, and without thiol (-SH), such as L-methionine, did not affect the formation of ROS (Figure 4C). L-Cysteine slightly increased IL-8 secretion without activating MCP-1 secretion (data not shown). These results suggest that Hcy can induce the formation of ROS in human monocytes.

Mechanisms Underlying Hcy-Induced ROS Formation

Previously studies have suggested that the activation of protein kinase C (PKC) and calmodulin (CaM) is involved in ROS production in monocytes. To understand the mechanism of Hcy-induced ROS formation, cells were pretreated with PKC inhibitors (calphostin C, 50 to 500 nmol/L; RO-31-8220, 10 to 100 nmol/L), CaM inhibitor (W7, 28 to 280 μmol/L), or mitogen-activated protein kinase (MAPK) inhibitors (SB203580, 0.6 to 6 μmol/L; PD98059, 2 to 20 μmol/L; curcumin, 2 to 20 μmol/L) for 60 minutes before the cells were stimulated by Hcy (100 μmol/L) for 50 minutes. CaM and PKC inhibitors could partially inhibit Hcy-induced ROS formation in monocytes (Figure 4D). In contrast, MAPK inhibitors had no such effect (data not shown).

To explore the enzymatic sources of ROS in response to Hcy, the monocytes were pretreated with the inhibitors of NAD(P)H oxidase (DPI or PAO, 1 to 10 μmol/L), xanthine oxidase (oxypurinol or allopurinol, 10 to 100 μmol/L),
cyclooxygenase (indomethacin, 1 to 10 μmol/L), 5-lipoxygenase (MK-886, 1 to 10 μmol/L), or complex I or III of the mitochondrial respiratory chain (rotenone, 10 to 100 μmol/L; antimycin A, 1 to 10 μmol/L) for 60 minutes, respectively, before 100 μmol/L Hcy stimulation for 50 minutes. NAD(P)H oxidase inhibitors DPI and PAO completely blocked Hcy-induced ROS formation (Figure 4E) and MCP-1 and IL-8 secretions (Figures 5A and 5B). However, other inhibitors had no significant effects (data not shown). These data suggest that the major enzyme responsible for Hcy-induced chemokine secretion is NAD(P)H oxidase in human monocytes.

Role of ROS in Hcy-Induced Secretion of MCP-1 and IL-8

To test additionally the role of ROS in Hcy-induced chemokine secretion, human monocytes were pretreated for 60 minutes with antioxidant reagent DMSO at 1/100 vol/vol (a scavenger of OH·), CAT and PEG-CAT at 250 U/mL for both (scavenger of H2O2), SOD and PEG-SOD at 500 U/mL for both (scavenger of O2·-), or a combination of both PEG-CAT and PEG-SOD. The cultured monocytes were then stimulated with Hcy at 100 μmol/L for 50 minutes. As shown in Figures 5C and 5D, DMSO, PEG-CAT, PEG-SOD, and the combination of both PEG-CAT and PEG-SOD not only completely inhibited Hcy-induced ROS formation (data not shown) but also significantly inhibited Hcy-induced secretion of MCP-1 and IL-8. DMSO gave a larger inhibition than other antioxidant reagents. However, the combination of both PEG-CAT and PEG-SOD gave a larger inhibition than other antioxidant reagents. However, the combination of both PEG-CAT and PEG-SOD gave a larger inhibition than other antioxidant reagents. However, the combination of both PEG-CAT and PEG-SOD gave a larger inhibition than other antioxidant reagents. However, the combination of both PEG-CAT and PEG-SOD gave a larger inhibition than other antioxidant reagents. However, the combination of both PEG-CAT and PEG-SOD gave a larger inhibition than other antioxidant reagents. However, the combination of both PEG-CAT and PEG-SOD gave a larger inhibition than other antioxidant reagents.
Signaling Mechanisms Involved in Hcy-Induced Secretion of MCP-1 and IL-8

A substantial body of evidence indicated that activation of MAPK, nuclear factor (NF)-κB, and gene expression of chemokine might be regulated by ROS. Recently, it has been shown that a nuclear factor peroxisome proliferator-activated receptor (PPAR) can reduce inflammation by inducing NF-κB binding to NF-κB. Therefore, we hypothesized that PKC- and CaM-mediated ROS formation may activate MAPK and NF-κB nuclear transcription factors, contributing to Hcy-induced chemokine expression and secretion in human monocytes. The monocytes were pretreated with the inhibitors of CaM (W7, 28 to 280 μmol/L), PKC (calphostin C, 50 to 500 nmol/L; RO-31-8220, 10 to 100 nmol/L), protein tyrosine kinase inhibitors (genistein, 2.6 to 26 μmol/L; tyrphostin, 0.5 to 5 μmol/L), extracellular signal-regulated kinase (ERK) 1/2 MAPK (PD98059, 2 to 20 μmol/L), p38 MAPK (SB203580, 0.6 to 6 μmol/L), c-Jun NH2-terminal kinase MAPK (curcumin, 2 to 20 μmol/L), NF-κB (PDTC, 1 to 10 μmol/L), or the activator of PPARγ (ciglitazone, 30 μmol/L; troglitazone, 10 μmol/L) for 60 minutes and then stimulated by Hcy 100 μmol/L for 4 days, respectively. As shown in Figures 6A through 6D, CaM, PKC, and MAPK (p38 and ERK1/2) inhibitors significantly inhibited Hcy-induced MCP-1 and IL-8 productions in human monocytes. In addition, NF-κB inhibitor and PPARγ activator also prevented Hcy-induced MCP-1 and IL-8 productions in these cells (Figures 7A through 7D). However, the inhibitors of protein tyrosine kinase and c-Jun NH2-terminal kinase MAPK had no direct effect on chemokine production in the cells (data not shown). In addition, inhibition of NF-κB by PDTC or activation of PPARγ by ciglitazone or troglitazone inhibited Hcy-induced ROS formation significantly (data not shown). These data provide evidence that Hcy-induced secretion of MCP-1 and IL-8 is mediated by a signaling pathway sequentially involving PKC/CaM–NAD(P)H oxidase–ROS–MAPK(p38 and ERK1/2)–NF-κB in a PPARγ activator–sensitive manner (Figure 8). In the range of concentration used, Hcy did not significantly increase LDH release compared with the control (data not shown), indicating that Hcy has no obvious toxic effect on human monocytes.

Discussion

The results of the present study demonstrate for the first time that Hcy not only promotes secretion of MCP-1 and IL-8 but also induces their gene expressions in cultured human monocytes. The intracellular production of NAD(P)H oxidase–dependent ROS such as O2•−, OH•, and H2O2 activates a signaling pathway involving PKC/CaM–NAD(P)H oxidase–ROS–MAPK(p38 and ERK1/2)–NF-κB, mediating Hcy-induced chemokine MCP-1 and IL-8 secretions from human monocytes. Furthermore, activation of PPARγ can inhibit Hcy-induced effects in these cells.

Whereas previous studies have provided controversial reports on the effect of Hcy-induced chemokine formation in
monocytic cell lines, we have shown that Hcy (10 to 1000 μmol/L) can markedly increase MCP-1 and IL-8 secretions in primary cultured human monocytes. This finding has important clinical implications. This conclusion is supported by the following evidence. First, plasma concentration of Hcy in patients suffering from homozygous homocystinuria is as high as 500 μmol/L. Patients with plasma Hcy level >10 μmol/L are at increased risk for vascular disorders. This is at the range used in the present study. Second, in the present study, we have also produced mechanistic insights regarding signaling pathways responsible for Hcy-induced chemokine secretion in human monocytes.

First, we have tested whether ROS acts as a mediator in Hcy-induced expression of chemokines and found that Hcy (100 μmol/L) elevates MCP-1 and IL-8 secretions and increases intracellular ROS. Although previous studies have reported that Hcy induces ROS formation in the endothelium, the significance of those studies has been significantly compromised in most of the patients with vascular disorders. This is at the range used in the present study. Second, in the present study, we have also produced mechanistic insights regarding signaling pathways responsible for Hcy-induced chemokine secretion in human monocytes.

Second, we have distinguished the potential enzymatic sources of ROS, including the NADH/NAD(P)H oxidases, xanthine oxidase, the mitochondrial respiration, arachidonic acid pathway enzymes lipoxygenase, and cyclooxygenase. The present study has provided evidence that the major enzyme responsible for Hcy-induced chemokine secretion is NAD(P)H oxidase but not others in human monocytes. Thus, the predominant superoxide-producing enzyme in response to Hcy is an NADH/NAD(P)H oxidase in human monocytes.

Furthermore, our results indicate that protein expressions of MCP-1 and IL-8 induced by Hcy in human monocytes can be inhibited by antioxidants DMSO, PEG-SOD, and PEG-CAT. This finding argues against previous report that the ROS was not involved in Hcy-induced secretion of chemokines from endothelial cells. Because membrane-impermeable SOD and CAT cannot inhibit the secretion of MCP-1 or IL-8 but membrane-permeable PEG-conjugated SOD and CAT are able to inhibit the secretion of MCP-1 and IL-8 induced by Hcy, Hcy-triggered ROS formation is originated from intracellular human monocytes. To additionally discriminate the specific ROS induced by Hcy, we compared effects of DMSO, a scavenger of OH radicals, with effects of PEG-SOD or PEG-CAT, scavengers of O₂⁻ or H₂O₂, and found that DMSO fully blocks Hcy-induced MCP-1 and IL-8 secretions but PEG-SOD and PEG-CAT only exhibit partial inhibitions. These data suggest that although 3 types of ROS, O₂⁻, OH⁻, and H₂O₂, are involved.
OH’ radicals plays an essential role in Hcy-induced chemokine production in human monocytes.

Some evidence indicates that PPARγ activation reduces the inflammation and chemokine secretion via antagonizing NF-κB activity\(^{29}\) and may modulate ROS generation.\(^{30}\) Therefore, we have also examined the effects of NF-κB inhibition and PPARγ activation on Hcy-induced response. Indeed, we have shown that NF-κB inhibition and PPARγ activation obviously inhibit Hcy-induced ROS formation and chemokine production. Recently, our initial data showed that Hcy downregulated PPARγ mRNA levels after Hcy (100 μmol/L) treatment for 4 to 16 hours in cultured human monocytes (unpublished data). These results suggest that Hcy-induced chemokine production can be regulated by nuclear transcription factors of NF-κB by competing with peroxisome proliferators.\(^{31}\) Thus, the activation of PPARγ may have a beneficial effect in patients with atherosclerotic disorders.

It has been previously demonstrated that MCP-1 is highly expressed in human atherosclerotic lesions and involved in monocyte recruitment into the arterial wall and the developing lesion.\(^{5}\) Although it is still controversial whether IL-8 induces monocyte chemotaxis,\(^ {6,32}\) IL-8 is considered a chemoattractant for T lymphocytes\(^ {33}\) and acts as an angiogenic factor to induce vascular smooth muscle cell proliferation and migration.\(^ {34,35}\) In this study, we have provided direct evidence that Hcy-induced MCP-1 and IL-8 increase human monocyte function. In addition, MPO release induced by IL-8 might oxidize LDL in the human artery wall.\(^ {36}\) Furthermore, IL-8 and MCP-1 can convert monocytes rolling to firm adhesion to endothelial monolayers and expressing E-selectin under normal flow conditions.\(^ {6}\) Therefore, enhanced production of MCP-1 and IL-8 in response to Hcy in monocytes might contribute to the development of vascular inflammation and atherosclerotic lesion.

Although the molecular mechanism underlying Hcy-induced atherosclerosis has been the subject of intensive investigation, most previous studies have focused on the influence of Hcy on endothelial cells and vascular smooth

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**Figure 7.** Effects of NF-κB blocker or PPARγ agonists on Hcy-induced secretion of MCP-1 and IL-8 in cultured human monocytes. Cells were pretreated with NF-κB inhibitor PDTC (2 to 20 μmol/L) (A and B) or PPARγ agonists ciglitazone (30 μmol/L) and troglitazone (10 μmol/L) (C and D) for 60 minutes and then stimulated by Hcy 100 μmol/L for 4 days, respectively. Data are expressed as mean±SEM. *P<0.05 compared with Hcy-treated alone. Data are representative of at least 4 independent experiments.

**Figure 8.** Schematic diagram showing signaling pathways involved in Hcy-induced MCP-1 and IL-8 production in human monocytes.
muscle cells.\textsuperscript{7,8} Our recent studies have shown that Hcy increases cultured primary T and B lymphocyte proliferation\textsuperscript{10,38} and increases MCP-1 and IL-8 secretion in monocytes. Our unpublished data suggest that the MCP-1 level is apparently elevated both in the plasma and the monocyte in response to low-dose endotoxin in the patients with angiographically confirmed coronary artery disease with mild hyperhomocysteinemia (unpublished data). This suggests that Hcy may also act directly on the immune cells to initiate and promote the progression of atherosclerosis. When we were preparing this manuscript, Holven et al\textsuperscript{39} reported that folic acid treatment reduced MCP-1 and IL-8 release from peripheral blood mononuclear cells in hyperhomocysteinemic patients. They suggested that Hcy might exert its atherogenic effect by enhancing inflammatory response. Thus, our findings indicate that Hcy might play an important role in the progression of vascular inflammation and atherogenesis. Similar to the situation in the hypercholesterolemia,\textsuperscript{10,11} Hcy might work through a mechanism involving upregulation of MCP-1 and IL-8 expressions by monocytes and macrophages in human vascular lesion, in which an influx of monocyte and T-cells into the plaque was present. Elucidation of the role of Hcy in the expression of MCP-1 and IL-8 in vivo, especially in the development of atherosclerotic plaque in patients with vascular disorder, should provide new insight to our understanding of hyperhomocysteine-induced atherosclerosis.

Previous reports have suggested that cytokines, such as IL-1\textbeta and TNF-\alpha, induce long-lasting IL-8 and MCP-1 productions.\textsuperscript{40} However, our preliminary study revealed that Hcy did not enhance the secretion of TNF-\alpha or IL-1\textbeta in cultured human monocytes (data not shown). This indicates that IL-1\textbeta and TNF-\alpha are not essential for Hcy-induced expression of MCP-1 or IL-8 in cultured human monocytes. In conclusion, this study demonstrates that Hcy significantly enhances MCP-1 and IL-8 in healthy human monocytes that can increase leukocyte chemotaxis. The intracellular oxidative products and subsequent activation of multiple signaling mediators, including MAPKs and NF-\kappaB, are involved in the effects of Hcy in human monocytes. These findings reveal a novel role of Hcy in the pathogenesis of human vascular disease.

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