Granulocyte Macrophage–Colony Stimulating Factor Increases the Expression of Histamine and Histamine Receptors in Monocytes/Macrophages in Relation to Arteriosclerosis

Yoshitaka Murata, Akihide Tanimoto, Ke-Yong Wang, Masato Tsutsui, Yasuyuki Sasaguri, Filip De Corte, Hiroshi Matsushita

Objective—To study the effect of granulocyte macrophage–colony-stimulating factor (GM-CSF) on histamine metabolism in arteriosclerosis, the expression of histidine decarboxylase (HDC; histamine-producing enzyme), histamine receptors 1 and 2 (HH1R and HH2R), and GM-CSF was investigated in human and mouse arteriosclerotic carotid arteries. Furthermore, the molecular mechanisms of GM-CSF–induced HDC and HH1R expression in monocyctic U937 cells were investigated.

Methods and Results—Immunohistochemistry showed that atherosclerotic human coronary and mouse ligated carotid arteries contained HDC-expressing macrophages. Gene expression of HDC, HH1R, HH2R, and GM-CSF was also detected in the lesions. In U937 cells, GM-CSF enhanced histamine secretion and gene expression of HDC and HH1R. A promoter assay showed that GM-CSF enhanced gene transcription of HDC and HH1R but not HH2R.

Conclusion—The present results indicate that HDC and HHR are expressed in arteriosclerotic lesion, and that GM-CSF induces HDC and HH1R expression in monocytes. Locally produced histamine might participate in atherogenesis by affecting the expression of atherosclerosis-related genes in monocytes and smooth muscle cells. (Arterioscler Thromb Vasc Biol. 2005;25:430-435.)

Key Words: monocyte/macrophage ■ histamine ■ histidine decarboxylase ■ histamine receptor ■ atherosclerosis

Histidine decarboxylase (HDC) is a rate-limiting enzyme for production of histamine from L-histidine. Expression of HDC is observed in many types of cells including mast cells, T-lymphocytes, monocytes/macrophages, enterochromaffin-like cells, and neuroendocrine cells,1–3 thereby playing an important role in allergy, inflammation, neurotransmission, and gastrointestinal functions via specific histamine receptors (HHRs) that are classified into H1, H2, H3, and H4 types.4,5

We demonstrated previously that HDC is expressed in the macrophages of human atherosclerotic lesions.1 In U937 monocytes, expression of HDC and histamine H1 and H2 receptors (HH1R, HH2R) is regulated during macrophage differentiation.6 In endothelial cells (ECs), histamine induces expression of P-selectin and endothelial NO synthase via HH1R,7,8 which is expressed in ECs and smooth muscle cells (SMCs) of human atherosclerotic lesion.9 Histamine also stimulates SMCs to proliferate and to express matrix metalloproteinase-1 (MMP-1).10 These findings suggest that histamine plays important roles in the functions of monocytes, ECs, and SMCs. However, up to now, no physiological factor(s) regulating the expression of histamine and its receptors in monocytes/macrophages has been demonstrated.

In atheromatous plaques, macrophages are the most abundant cells expressing granulocyte macrophage–colony-stimulating factor (GM-CSF),11 which is a macrophage differentiation and proliferation factor,12 and therefore an atherogenic cytokine.13 We already demonstrated1 that HDC shows a distribution similar to that of GM-CSF in macrophages. However, no study has evaluated the association between histamine and GM-CSF. Here we demonstrated that HDC and HH1R expression are upregulated by GM-CSF at the transcriptional level partly through c-Fos/c-Jun–related mechanisms, and that human advanced atherosclerotic lesion and mouse arteriosclerotic lesion express GM-CSF, HDC, HH1R, and HH2R genes.

Materials and Methods

Immunolocalization of HDC in Human Carotid Artery

Tissues obtained by carotid intimectomy were used for immunohistochemistry2 after fixation in 10% formalin and embedding in paraffin. Sections were then cut and immunostained using rabbit polyclonal anti-HDC antibody (dilution 1:500; PROGEN Biotech-
nik), mouse monoclonal anti-CD68 antibody (dilution 1:100; DAKO), and anti-α-smooth muscle actin (α-SMA; dilution 1:1; DAKO).

Mouse Carotid Ligation

Experiments were performed in 8-week-old male C57BL/6J mice (20 to 25 g). Mice were anesthetized with pentobarbital (50 mg/kg IP), and the left common carotid artery was ligated with a 6-0 silk suture at the site just proximal to the carotid bifurcation. Mice were euthanized by an overdose of pentobarbital (intraperitoneally) at 3 weeks after carotid ligation.14 The aorta was perfused with 10% formalin, and the ligated carotid arteries were resected. Paraffin sections (5-μm thick) were then cut and stained with hematoxylin and eosin and elastica van Giesson stains. For immunostains, sections were incubated with rat monoclonal anti-mouse Mac-3 antibody (dilution 1:25; BD Biosciences), mouse monoclonal anti-α-SMA, and rabbit polyclonal anti-HDC antibody.

Reverse Transcriptase–Polymerase Chain Reaction

Total RNAs extracted from mouse ligated carotid artery with Trizol reagent (GIBCO/BRL) were subjected to RT-PCR for the GM-CSF (448 bp), HDC (310 bp), HH1R (498 bp), and HH2R (601 bp) genes. Primers used were as follows: GM-CSF (GenBank No. X03019), 5'-GGTCTCGAGGAGGATGGG-3'/5'-TGGGCTCTCCATCATTT-TGG-3'; HDC (GenBank No. X75437), 5'-GATCAAGATTTCTACTTGTTGG-3'/5'-GTGTACCATCATCCACTCGTG-3'; HH1R (GenBank No. D50095), 5'-TGCTTCACGCTACCATCCCTGG-3'/5'-GTCTGATGACGTCAAGACG-3'; and HH2R (GenBank No. D50096), 5'-AAATACCACTACGTTGATGGATCAGATTTCTACATCCACTTGG-3'. For HDC gene expression, quantitative real-time PCR was performed by the TaqMan fluorogenic probe method (Applied Biosystems).

Cell Culture

Human monocytic U937 cells (American Type Culture Collection) were maintained in RPMI medium 1640 (GIBCO/BRL) containing 5% FCS (ICN).

Histamine Secretion From U937 Cells

U937 cells (5×10⁶ per well) were stimulated with 10 or 20 ng/mL of GM-CSF (PeproTech) for 72 hours. The conditioned medium was then subjected to ELISA for histamine purchased from SPI Bio according to manufacturer instructions.

Western Blotting for HDC Expressed in U937 Cells

U937 cells were treated with 10, 20, or 50 ng/mL GM-CSF or 20 ng/mL phorbol 12-myristate 13-acetate (PMA; Sigma) for 24 hours. Cells were lysed in a buffer (50 mmol/L Tris-HCl, pH 7.6, 120 mmol/L NaCl, 100 mmol/L NaF, 0.5% NP-40, and 10 μg/mL phenylmethylsulfonyl fluoride), and the soluble fraction after centrifugation (×15 000g) was subjected to SDS-PAGE and Western blotting. As a positive control, a lysate from rat mast cell line RBL-2H3 was used.

Northern Blotting

U937 cells were stimulated with GM-CSF (10 or 20 ng/mL) for 24 hours. Total RNAs were extracted with Trizol reagent and electrophoresed on 1% agarose gel containing 6% formaldehyde. After RNAs were transferred onto a nylon membrane, expression of HDC, HH1R, and HH2R were detected by hybridization with 32P-labeled probes.15 Equal loading of RNAs for each sample was confirmed by methylene blue (0.02% in 0.5 mol/L sodium acetate, pH 5.2) staining of the membrane to visualize 28S ribosomal RNAs.

Luciferase Reporter Assay

Reporter plasmids (pGL3-basic; Stratagene) containing the promoter region of HDC,1 HH1R,16 and HH2R17 were used for the assay. U937 cells (1.4×10⁵ per cuvette) were transfected with 20 μg of reporter constructs along with 0.5 μg of β-galactosidase (β-gal) expression vector by electroporation (950 μf; 300 V Gene-Pulser II, Bio-Rad). Cells were plated into 6 wells of a 24-well plate and incubated with appropriate reagents for the desired periods. Cells were lysed, and the supernatants were mixed with luciferase substrate (Toyo Ink). For cotransfection studies, pSG6–c-Jun and pSG6–c-Fox18 were used (1 μg per cuvette). The dominant-negative c-Jun (dKR)19 expressing vector was generated using a site-directed mutagenesis kit (Stratagene).

Statistical Analysis

Student t test was applied, and differences at P<0.05 were considered significant.

Results

Localization of HDC in Advanced Atherosclerotic Lesion of Human Carotid Artery

We investigated carotid arteries showing advanced atherosclerosis, including accumulation of extracellular lipid and complicated by thrombus and hematoma (type IV, V, and VI lesions), in which macrophages were concentrated at the lateral margins of the lipid core (Figure 1A). The macrophages facing the lipid core or the shoulder region were positive for HDC (Figure 1B). In the complicating lesions (type VI), macrophages located in the thrombus or hematoma also expressed HDC (data not shown). The HDC-positive macrophages were reactive with anti-CD68 antibody but not with anti–α-SMA (Figure 1C and 1D). Together with the findings of the previous study showing the presence of HDC-expressing macrophages in the fatty streaks (type II and
III lesions), these results indicate that histamine-producing macrophages are present at all stages of atherosclerosis.

GM-CSF, HDC, and HHR Expression in Mouse Ligated Artery

The ligated carotid arteries exhibited intimal thickening where mainly SMCs were present. Macrophages were scattered in the intima but predominantly located on the endothelial surface and subendothelial space. These cells were positive for the specific markers $\alpha$-SMA and Mac-3, respectively. The distribution of Mac-3–positive macrophages corresponded to that of the HDC-expressing cells (Figure IA, available online at http://atvb.ahajournals.org).

RT-PCR of material from the ligated arteries showed enhanced expression of the GM-CSF, HH1R, and HH2R, and HDC genes (Figure IB), indicating that the increased gene expression is derived from the thickened intima. Because intimal SMCs in culture express HH1R but not HH2R, the expression of HH1R gene probably originated from SMCs, ECs, and monocytes/macrophages. GM-CSF expression has been demonstrated in SMCs, ECs, and monocytes/macrophages. Thus, among these cells, only monocytes/macrophages are suggested to express the HDC and HHR genes.

GM-CSF–Induced Histamine Production From U937 Cells

GM-CSF increased histamine content in the medium (Figure 2A). Because the difference in cell numbers was <10% in each treatment, the increased histamine was suggested to result from increased histamine secretion. Unlike mast cells, monocytes do not have secretory granules, and their histamine secretion is mediated by transmembrane diffusion. Therefore, histamine secretion from monocytes is mostly dependent on histamine production, which is regulated by the rate-limiting enzyme HDC. This was supported by the finding that U937 cells stimulated with GM-CSF showed increased expression of HDC protein in a dose-dependent manner (Figure 2B). The dose-dependent expression of HDC mRNA was confirmed by real-time quantitative RT-PCR (Figure II, available online at http://atvb.ahajournals.org) and Northern blotting (Figure 2C).

GM-CSF Transactivates HDC Gene Expression in U937 Cells

The human HDC promoter region, spanning the region from $-240$ to $+120$ of transcription initiation site, includes gastrin response element (GAS-RE) motif (+1 to +27), a minimal enhancer element(s) responsive to gastrin and PMA, which contains the proximal GAS-RE1 and the distal GAS-RE2. GM-CSF increased HDC promoter activity in U937 cells (Figure 3A), and the activity was reduced by introduction of a mutation in the GAS-RE motif as well as reduction of the
basal activity (Figure III, available online at http://atvb.aha-journals.org). When U937 cells were transfected with M1 (GAS-RE1 mutation) and M2 (GAS-RE2 mutation), the basal and GM-CSF–induced activity was significantly decreased, indicating that the GAS-RE motif is essential for basal activity and the response to GM-CSF.

Effects of Fos and Jun on HDC Gene Expression

Expression of the HDC gene is regulated through GAS-RE and indirectly via activation of the activator protein-1 (AP-1) pathway in gastric cancer cells.21 In monocytic U937 cells, GM-CSF is known to activate the c-Fos/c-Jun pathway and to increase the expression of c-Jun during macrophage differentiation.22 To study the involvement of Fos/Jun in HDC gene transcription, coexpression of c-Jun, c-Fos, and dKR was introduced. dKR lacks the DNA-binding domain and can dimerize with c-Jun and c-Fos but cannot transactivate.19

dKR showed no effect on the basal promoter activity (Figure 3B, lanes 1 and 2) but exhibited a slight but statistically significant (P<0.01) inhibitory effect on the GM-CSF–induced activity (Figure 3B, lanes 3 and 4). When c-Fos and c-Jun were coexpressed, basal activity was increased, and this upregulation was blocked by the combination of dKR and c-Fos (Figure 3C, lanes 1 and 2). With stimulation by GM-CSF, the coexpression of c-Fos and dKR showed reduced activity than that of c-Fos and c-Jun (Figure 3C, lanes 3 and 4). However, the response to GM-CSF was still preserved even with the expression of dKR (Figure 3C, compare lanes 2 and 4).

Effects of GM-CSF and Fos/Jun on HH1R Expression in U937 Cells

Northern blotting analysis revealed that GM-CSF induced expression of HH1R mRNA in a dose-dependent manner. HH2R mRNA was constitutively expressed and not induced by GM-CSF (Figure 4A). When U937 cells were treated with GM-CSF, HH1R promoter activity was enhanced dose dependently. The basal promoter activity of HH2R gene was higher than that of the HH1R gene but did not respond to GM-CSF (Figure 4B). These results indicate that HH1R gene expression is inducible by GM-CSF, but the HH2R gene is expressed constitutively and is not responsive to GM-CSF.

When U937 cells were transfected with dKR alone, the basal promoter was not reduced (Figure 5A, lanes 1 and 2), but the GM-CSF–induced HH1R promoter activity was downregulated (Figure 5A, lanes 3 and 4). Basal activity was markedly upregulated by coexpression of c-Fos and c-Jun (Figure 5B, lane 1), and GM-CSF increased the activity further (Figure 5B, lane 3). When c-Jun was substituted by dKR, basal activity was decreased (Figure 5B, lane 2), and the GM-CSF induction was abolished (Figure 5B, lane 4). These data indicate that the AP-1 pathway would be directly involved in HH1R gene regulation.

Discussion

In addition to the promotion of macrophage differentiation, GM-CSF regulates MMP expression,23 extracellular matrix production,24 cell adhesion,25 and proliferation.12,26 Because these events are related to the pathogenesis of inflammation, GM-CSF, a proinflammatory cytokine, is thought to be a key molecule involved atherogenesis.13,27 In human atherosclerotic lesions, GM-CSF is expressed in SMCs, ECs, and monocytes/macrophages.11,13,28 In this study, we demonstrated that HDC was expressed in the macrophages (CD68 or Mac-3–positive cells) located in human and mouse arteriosclerotic lesions. Furthermore, the GM-CSF gene was coexpressed with the HDC and HHR genes in mouse ligated carotid arteries. These genes were also detected in human atherosclerotic lesions (data not shown). In monocytic U937 cells, the coordinated expression of HDC and HH1R is enhanced by GM-CSF. Although we demonstrated no direct causal link between atherogenesis and GM-CSF–induced upregulation of HDC and HH1R, these results suggest that GM-CSF not only promotes macrophage differentiation but also regulates histamine metabolism in the lesion. HDC-expressing macrophages might be a potential source of histamine in atherosclerosis, especially contributing to chronic effects of histamine.

Transcriptional regulation of the human HDC and HH1R genes is not yet fully understood. In the present study, we
HH1R mRNA in intimal SMCs. However, little is known about the molecular mechanism(s) of gene regulation by IL-4. 

Aside from the present study, only a few reports have described cytokine regulation of HH1R gene expression. Interleukin-4 (IL-4) is able to upregulate HH1R mRNA in synovial fibroblasts and ECs, whereas platelet-derived growth factor (PDGF)-BB can enhance the expression of HH1R mRNA in intimal SMCs. However, little is known about the molecular mechanism(s) of gene regulation by IL-4 and PDGF-BB, or in monocytes. Although the signaling pathway of GM-CSF in U937 cells is still controversial, it may play an important role in the coordinated expression of HDC and HH1R in monocytes during macrophage differentiation.

The functions of histamine in atherosclerosis still are not clarified. We reported previously that HH1R expression is enhanced in SMCs in human atherosclerotic arteries. In the present and previous studies, we showed that HDC-expressing macrophages (CD68 or Mac-3–positive cells) were located in the atheromatous plaque and neointima of human and mice arteries, respectively. Because histamine is as potent as PDGF-BB in stimulating intimal SMCs to proliferate, and also enhance MMP-1 expression, the localization of HDC-expressing cells in the neointima in arteriosclerotic lesions seems reasonable for explaining the proliferation and migration of medial SMCs on the basis of the “response to injury” hypothesis. This is consistent with a report that an HH1R blocker reduced the intimal thickening by inhibiting intimal cell proliferation in mice with photochemical-induced arteriosclerosis. However, recent studies indicated that the neointimal SMCs are derived from circulating progenitor cells provided by the bone marrow. Thus, the origin of neointimal SMCs is still debatable and remains to be clarified.

In the case of monocytes, we found that HHR switching from HH2R to HH1R during macrophage differentiation is closely related to histamine-mediated gene expression. Histamine downregulates lipopolysaccharide-induced expression of tumor necrosis factor-α via HH2R in monocytes, whereas it upregulates that via HH1R in macrophages. In contrast, monocytic expression of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) and monocyte chemotactant protein-1 (MCP-1) is increased by HH2R activation. After macrophage differentiation, in which HH1R is dominantly expressed, histamine cannot induce LOX-1 and MCP-1. The effects of histamine on monocytes/macrophages would be dependent on the receptor profile. Here we demonstrated that GM-CSF can upregulate HH1R expression. The coordinated expression of HDC and HH1R induced by GM-CSF might contribute to histamine metabolism and the expression of atherosclerosis-related genes in monocytes/macrophages in an autocrine and paracrine manner.

In summary, our studies demonstrated that HDC and HHR are expressed in arteriosclerotic lesion, and that GM-CSF upregulates HDC and HH1R expression in monocytes. Together with our previous studies, these data indicate that locally produced histamine in the neointima might participate in the regulation of atherosclerosis-related genes in monocytes and SMCs and in SMC proliferation.

Acknowledgments
This work was supported in part by a grant from the Smoking Research Foundation, Tokyo, Japan (to A.T.).

References


Granulocyte Macrophage–Colony Stimulating Factor Increases the Expression of Histamine and Histamine Receptors in Monocytes/Macrophages in Relation to Arteriosclerosis
Yoshitaka Murata, Akihide Tanimoto, Ke-Yong Wang, Masato Tsutsui, Yasuyuki Sasaguri, Filip De Corte and Hiroshi Matsushita

Arterioscler Thromb Vasc Biol. 2005;25:430-435; originally published online October 28, 2004;
doi: 10.1161/01.ATV.0000148705.13411.65
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/25/2/430

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2005/01/27/01.ATV.0000148705.13411.65.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/