Abstract—To clarify the role of histamine-producing cells and its origin in atherosclerosis, we investigated histidine decarboxylase (HDC; histamine-producing enzyme) expression in murine arteries with vascular injuries after the animal had received transplanted bone marrow (BM) from green fluorescent protein (GFP)–transgenic mice. The neointima in the ligated carotid arteries contained BM-derived HDC+ cells that expressed macrophage (Mac-3) or smooth muscle cell antigen (α-SMA). In contrast, the HDC+ BM-derived cells, which were positive for Mac-3, were mainly located in the adventitia in the cuff replacement model. In apolipoprotein E-knockout mice on a high cholesterol diet, BM-derived cells expressing Mac-3 in the atheromatous plaques were also positive for HDC. In comparison with wild-type mice, HDC−/− mice showed reduced neointimal thickening and a decreased intima-to-media ratio after ligation and cuff replacement. These results indicate that histamine produced from BM-derived progenitor cells, which could transdifferentiate into SMC- or macrophage-like cells, are important for the formation of neointima and atheromatous plaques. (Circ Res. 2005;96:974-981.)

Key Words: histamine ■ histidine decarboxylase ■ progenitor cells ■ bone marrow ■ vascular injury

Histidine decarboxylase (HDC) is a rate-limiting enzyme for the production of histamine from L-histidine. Histamine plays an important role in allergy, inflammation, neurotransmission, and gastrointestinal functions by acting via specific histamine receptors.1,2 With regards to the atherosclerotic coronary artery, histamine is a vasoconstrictor, and the accumulation of activated mast cells in the adventitia and ruptured plaques in acute coronary syndrome has been reported.3–5 Another histamine-producing cell in the atherosclerotic lesion is the macrophage,6 which is present in all stages of atherosclerosis and is a major cellular constituent of atheromatous plaques. Previously, we demonstrated that HDC is expressed in CD68+ foam cells (macrophages) of human atherosclerotic lesions.6 In monocytic human U937 cells, the expression of HDC and histamine H1 receptor (HH1R) is induced during macrophage differentiation,6,7 and granulocyte macrophage-colony stimulating factor, one of the proinflammatory and macrophage-differentiation factors produced in the atherosclerotic lesions,8 is also able to induce HDC and HH1R.9

As a longer-term effect, histamine stimulates cultured human intimal smooth muscle cells (SMCs) to proliferate and to express matrix metalloproteinase-1 (MMP-1).10 Histamine also upregulates the gene expression of endothelial nitric oxide synthase (eNOS) in vascular endothelial cells (ECs).11 These histamine effects are all mediated via HH1R, which is expressed in the ECs, foam cells, and SMCs of human atherosclerotic lesions.12 In the case of monocytes, we have reported that histamine upregulates lipopolysaccharide-induced expression of tumor necrosis factor-α (TNF-α) during macrophage differentiation and switching of the histamine receptor from histamine H2 receptor (HH2R) to HH1R.7 Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) and monocyte chemoattractant protein-1 (MCP-1) expression are increased by HH2R activation.13,14 Together, these findings indicate that histamine regulates the expression of atherosclerosis-related genes in SMCs, ECs, and monocytes. However, at present, no in vivo study providing direct and convincing evidence that histamine is involved in the pathogenesis of atherosclerosis has been reported. One notable report indicated that photochemical-induced intimal thickening of mouse femoral arteries is inhibited by HH1R antagonist.15

To further evaluate the long-term effect of histamine on arteriosclerosis, we investigated the intimal hyperplasia of arteries induced by ligation- and cuff-induced vascular inj-
ries in HDC knockout (HDC<sup>−/−</sup>) and wild-type (WT) mice. Histamine involvement in hyperlipidemia-induced vascular injuries was also studied in apolipoprotein E (apoE) knockout (apoE<sup>−/−</sup>) mice on a high-cholesterol diet. In this study, we demonstrate that HDC<sup>+</sup> cells were mainly localized in the neointima after carotid ligation and in the aortic intima of apoE<sup>−/−</sup> mice but that HDC<sup>+</sup> cells infiltrated the adventitia in the cuffing model. Furthermore, a transplantation of bone marrow (BM) from green-fluorescent protein (GFP)–transgenic mice<sup>17</sup> revealed that most of the HDC<sup>+</sup> cells were GFP<sup>+</sup> and also expressed the markers for macrophages or SMCs. These results indicate that histamine produced by BM-derived progenitor cells, which could potentially transdifferentiate into vascular cells, is important for neointima and atheroma formation. Finally, we demonstrated that the neointimal formation in the HDC<sup>−/−</sup> mice was much less than that in the WT mice in the ligation- and cuff-induced vascular injury models.

**Materials and Methods**

**Animals**

Experiments (5 to 10 mice were used for each experiment and treatment) were performed on 8-week-old male C57BL/6 WT and HDC<sup>−/−</sup> mice (KBT, Toyama, Japan)<sup>16</sup>, weighing 20 to 25 g. The mice were anesthetized with an intramuscular injection of a mixture of ketamine (50 mg/kg) and medetomidine (1 mg/kg) for the operation described. To investigate ligation-induced vascular injuries, the left common carotid artery was ligated with a 6-0 silk suture at the site just proximal to the carotid bifurcation. For the study of cuff-induced vascular injuries, the right femoral artery was dissected from the animals just proximal to the carotid bifurcation. The animals were euthanized by an overdose of IP injected pentobarbital (Nembutal, 80 mg/kg, Dainippon Sumitomo, Osaka, Japan) at the desired periods of time. All protocols were approved by the Ethics Committee of Animal Care and Experimentation, University of Occupational and Environmental Health, Japan, and were performed according to the Institutional Guidelines for Animal Experiments and the Law (no. 105) and Notification (no. 6) of the Japanese Government.

**Histopathological Examination**

The aorta was perfused with 10% paraformaldehyde, ligated carotid arteries were resected, and paraffin sections (3 μm thick) were made for hematoxylin and eosin (HE) staining. For the histological evaluation, the vascular lesions were classified into early, intermediate, and advanced stages. The early stage was defined as endothelial attachment of small numbers of cells, whereas the intermediate stage was defined as increased intimal thickness [up to intima-to-media (IM) ratio, 0.5]. The advanced stage showed pronounced neointimal formation (IM ratio, more than 0.5) with increased deposition of extracellular matrix and lipid. For immunostaining, the mirror sections were incubated with rat monoclonal anti–mouse Mac-3 antibody (dilution 1:25, BD Biosciences), mouse monoclonal anti–α-SMA antibody (dilution 1:1, Dako), and rabbit polyclonal anti-HDC antibody (dilution 1:500, PROGEN Biotechnik), and then incubated with secondary antibody (Envision system, Dako). Anti–histamine rabbit polyclonal antibody (dilution 1:100, PROGEN Biotechnik) was also used for the detection of histamine in the arterial wall.

**Morphometrical Analysis**

The areas of vascular lumen, intima, and media in the histological sections were measured by using NIH image for quantitative evaluation of neointimal formation.

**Reverse Transcriptase-Polymerase Chain Reaction**

Total RNAs extracted from the mouse arteries with Trizol reagent (Gibco BRL) were subjected to reverse transcriptase-polymerase chain reaction (RT-PCR) for HDC by using the primer pair 5′-GATCAGATTCTACCTGTGG-3′ and 5′-GTGTAACCATCACCAC-TTCG-3′ to amplify 310-bp fragments. The amounts of PCR products were measured by using a densitometer and normalized by those of β-actin.

**BM Transplantation**

BM cells obtained from GFP-transgenic mice (under control of the β-actin promoter<sup>17</sup>) on a C57BL/6 background were transplanted into lethally irradiated WT mice. Four weeks after the transplantation, ligation and cuff replacement procedures or 6-week consumption of the high-cholesterol diet (1%) was undertaken as described earlier. At the desired periods of time, the mice were euthanized, and their arteries were processed as described. The paraffin sections were immunostained to study the colocalization of HDC and GFP by using anti-HDC and monoclonal anti-GFP (dilution 1:2000, Sigma) antibodies. For fluorescence detection of GFP, the tissue samples were embedded in a low-temperature polymerized resin, and sections were cut and observed under a fluorescence microscope.

**Figure 1.** Vascular lesions in ligated carotid arteries of wild-type mice. Three weeks after the ligation of the carotid arteries in WT mice, expression of HDC was evaluated by RT-PCR and immunohistochemical analyses. A, RT-PCR demonstration of increased HDC mRNA expression in the ligated carotid arteries (L1 and L2) in comparison with the expression in nonligated control arteries (C1 and C2). Expression levels of HDC mRNA were normalized by those of β-actin mRNA. B, Hematoxylin and eosin (HE) and immunohistochemical staining of the normal and ligated carotid arteries after 3 weeks. HE section demonstrates normal and thickened intima. Immunohistochemical results show HDC<sup>+</sup>/histamine<sup>+</sup> cells positive for Mac-3 or α-SMA mainly located in the neointima and also in the media.
BM transplantation from wild-type to HDC−/− mice was also performed, and ligation and cuff replacement were introduced to rescue the phenotype of HDC−/− mice.

**Statistical Analysis**

Student t test was applied for the statistics, and a probability value of less than 0.05 was taken as being significant.

**Results**

**Ligation-Induced Vascular Injuries and HDC Expression**

To investigate the relation between histamine expression and arteriosclerosis, we induced vascular injuries in mouse carotid arteries by carotid ligation. Three weeks after the ligation, the arteriosclerotic arteries showed increased expression of HDC mRNA in comparison with nonligated control carotid arteries (Figure 1A). Histological examination revealed that the intima was markedly thickened and contained HDC−/− cells, which were positive for α-SMA (marker for SMCs) or Mac-3 (marker for macrophages) and histamine (Figure 1B). Small numbers of HDC+ and Mac-3+ or α-SMA+ were scattered.

**Cuff-Induced Vascular Injuries and HDC Expression**

Next, we studied another model for mechanically producing vascular injuries, ie, induced by femoral cuff replacement. At 3 weeks after the cuffing, the arteriosclerotic arteries showed increased gene expression of HDC in comparison with control arteries (Figure 2A). The histology showed infiltration of mononuclear cells on the endothelial surface and in the adventitia, in which these cells were HDC+ and positive for Mac-3 and histamine but α-SMA expression was inconspicuous (Figure 2B).

**Hyperlipidemia-Induced Vascular Injuries in ApoE−/− Mice and HDC Expression**

The mechanical vascular injury models do not include foam cells in the arteriosclerotic lesions, whereas apoE−/− mice develop a foam cell-rich atheromatous plaque.18 To further evaluate the involvement of histamine metabolism in atherosclerosis, we investigated the distribution of the HDC+ cells in apoE−/− mice on a high-cholesterol diet. After 6 weeks on this high-cholesterol diet, the animals showed atheromatous plaques with increased expression of HDC mRNA (Figure 3A). The atheromatous plaques included foam cells that had accumulated, and these cells were positive for HDC, histamine, and Mac-3 (Figure 3B).

**Origin of HDC+ Cells in Ligation-Induced Vascular Lesions**

Because the immunohistochemical study demonstrated the presence of HDC+ cells that were positive for either α-SMA or Mac-3 or both, these HDC+ cells were suggested to be heterogeneous in phenotype. To evaluate BM origin of HDC+
cells in the vascular lesions, WT mice were subjected to ligation-induced vascular injury after transplantation with BM from GFP-transgenic mice. Then, histological examination was performed during the lesion development. Neointimal formation and adventitial thickening, which were identical to those observed in the mice with ligation only, were gradually enhanced after the ligation (Figure 4). In the early stage of the lesion development, the cells on the endothelium or in the slightly thickened intima were positive for GFP and HDC. In the intermediate and advanced stage, the markedly thickened neointima consisted of many GFP^+^ cells, which were positive for HDC. Because the amount of extracellular matrix had increased, these GFP^+^ cells seemed to be scattered in the intima, especially at the advanced stage. At 3 weeks after the ligation, more than 80% (81.5±8.9%, n=6) of the intimal cells were GFP^+^ in the media and adventitia, small numbers of GFP^+^ cells were detected. The distribution of GFP^+^ cells corresponded fairly well to that of HDC^+^ cells in the neointima.

**Origin of HDC^+^ Cells in Cuff-Induced Vascular Lesions**

In the cuff-induced injuries with BM transplantation, both intimal hyperplasia and adventitial inflammation were identical to those in mice with cuff replacement only (Figure 5). During the lesion development caused by cuffing injuries, GFP^+^ cells were mainly located in the inflamed adventitia, and their distribution corresponded to that of HDC^+^ cells. In the early to intermediate stages, a few GFP^+^ cells on the endothelial surface and in the media were also positive for HDC. However, in the advanced stage, few GFP^+^ cells were detected in the thickened intima. At 3 weeks after the cuff replacement, nearly 70% (68.3±12.9%, n=6) of the adventitial cells were GFP^+^.

**Distribution of BM-Derived Cells and HDC Expression in ApoE^−/−^ Mice**

The foam cells were detected in the early to advanced stages of the lesion development. In the early stage, small numbers of GFP^+^ cells were scattered in the intima and media in the early to intermediate stage, but the neointima in the advanced stage shows no GFP^+^ cells. HDC indicates immunohistochemical demonstration of HDC expression. Distributions of GFP^+^ and that of HDC^+^ cells correspond fairly well.
of foam cells were located in the subendothelial space. In the intermediate and advanced stages, these cells accumulated in the atheromatous plaques located in the intima and media (Figure 6). These foam cells were positive for both HDC and GFP. More than 80% (82.5 ± 4.5%, n = 6) of the intimal cells at the advanced stage were GFP positive. A few GFP+ cells were observed in the adventitia in the early stage and in the media in intermediate to advanced stage.

Ligation- and Cuff-Induced Vascular Injuries in HDC−/− Mice
To obtain direct evidence that histamine production by the vascular cells is related to arteriosclerosis, we examined HDC−/− histamine-deficient mice, in which ligation- and cuff-induced vascular injuries were produced. The ligated carotid arteries after 3 weeks showed a reduced neointimal formation in the HDC−/− mice in comparison with that in the WT mice. When the HDC−/− mice were transplanted with BM from WT mice, the intima was increased in thickness (Figure 7A). The IM ratio for both WT and HDC−/− mice increased during the lesion progression but that of the HDC−/− mice was a significantly lower value at 3 weeks after the ligation. BM transplantation from WT mice resulted in increased IM ratio (Figure 7B).

The femoral arteries with cuff replacement at 2 to 3 weeks showed decreased intimal thickening in the HDC−/− mice but still-persistent adventitial inflammation. When the HDC−/− mice were transplanted with BM from WT mice, the intima was increased in thickness (Figure 8A). The IM ratio for the HDC−/− mice was significantly lower than that for the WT mice at 2 to 3 weeks. BM transplantation from WT mice resulted in increased IM ratio (Figure 8B).

Discussion
Previous studies in our series of experiments to examine the role of histamine in atherosclerosis have shown that histamine stimulates cultured human intimal SMCs to proliferate via H1R,10 and histological examinations of human atherosclerotic aortas and coronary arteries revealed the expression of H1R and HDC in the intimal SMCs and foam cells (macrophages), respectively.6,9,12 Human monocytic U937 and THP-1 cells are known to express both H1R and H2R.
which expression profile is regulated by their differentiation status. Because these results indicate that histamine metabolism might be involved in the pathogenesis of atherosclerosis, the present study was performed by using the HDC\(^{-/-}\) histamine-deficient mice to investigate the relation between the vascular cell–derived histamine and vascular injuries. The HDC\(^{-/-}\) mice, when compared with the WT mice, showed a 49% and 26% decrease in IM ratio in the ligation- and cuff-induced vascular injury model, respectively. The reduction of IM ratio was inhibited by BM transplantation from WT mice. The HDC\(^{-/-}\)/histamine\(^{+}\) cells, which derived from the BM progenitor cells, were mainly located in the neointima and adventitia. Thus, we clearly demonstrated that histamine is associated with the process of arteriosclerosis during ligation- and cuff-induced vascular injuries.

We used 2 different mouse models of mechanically induced vascular injuries, ie, carotid ligation and femoral cuff replacement. In the ligation model, a reduced shear stress and increased blood flow turbulence are associated with the lesion formation. In contrast, adventitial inflammation, which is accompanied by enhanced expression of inflammatory cytokines, is a major cause of the neointimal formation in the cuffing model. Although a different pathogenesis would be associated with these 2 models, the intimal thickening is suggested to be a common histopathological feature. The significant reduction in neointimal hyperplasia by HDC gene knockout indicated an essential role for histamine in the pathogenesis of neointimal formation induced by the ligation and cuff replacement. Because mast cells were not detected in the vascular lesions (data not shown) and histamine is known to be produced from the BM-derived progenitor cells, the HDCC/histamine\(^{+}\) cells in the neointima and adventitia would be a potentially major source of histamine in the vascular wall.

The “Response to Injury” hypothesis that the intimal SMCs migrate from the medial layer has been widely and long accepted; however, recent studies indicate the possibility that the neointimal cells are derived from circulating progenitor cells provided from BM.23–27 Our previous studies also demonstrated that human intimal SMCs and ECs express hematopoietic lineage markers such as stem cell factor and c-kit.28–29 In agreement with these reports, the present study using the ligation model showed that most of the intimal HDC\(^{-/-}\) cells were positive for GFP, indicating the BM origin of the HDC\(^{+}\) cells. Because histamine is able to stimulate SMCs to proliferate and migrate,10,30 and also monocytes/macrophages to enhance cytokine expression, histamine produced from the HDC\(^{-/-}\) BM-derived cells would stimulate preexisting SMCs or BM-derived macrophage- and SMC-like cells in the intima in both an autocrine and paracrine manner.

On the other hand, in the cuffing model, the HDC\(^{+}\) BM-derived cells were mainly located in the adventitia but rarely detected in the neointima. Another group has reported similar results comparing the distribution of BM-derived cells in the neointima in wire-mediated, ligation-, and cuff-induced vascular injury models. They did not include any description of the presence of the BM-derived cells in the adventitia; however, the neointima in the wire and ligation models contained BM-derived cells but not that in the cuffing model. Adventitial inflammation is a major cause of the neointimal formation in the cuffing model.20,32 Therefore, histamine-induced upregulation of monocyctic or macrophage expression of inflammatory cytokines, including TNF-\(\alpha\), MCP-1, and vascular endothelial growth factor (VEGF),7,14,33 could potentially influence the inflammatory condition in the adventitia. The origin of the neointimal cells in the cuffing model has not been determined (but is suggested to be from preexisting intimal cells or from migrating cells from the media); however, the role of histamine produced in the adventitia might include upregulation of inflammatory reactions, which would result in the neointimal formation. Further study would be necessary to understand the mechanism(s) of neointimal formation in cuff-induced vascular injuries.

The BM-derived HDC\(^{+}\) cells were heterogeneous in phenotype, expressing either the macrophage (Mac-3) or SMC (\(\alpha\)-SMA) marker or both in the present study. Although the exact origin of the SMC-like or macrophage-like cells and other vascular cells is still controversial and the fate of the

Figure 8. Cuff-induced vascular injuries in HDC-KO mice. Cuffed femoral arteries in HDC\(^{-/-}\) mice were histologically examined at 1, 2, and 3 weeks after the cuffing, and the findings were compared with those for control arteries. A, In the HDC\(^{-/-}\) mice, the femoral arteries showed reduced intimal thickening 3 weeks after the cuff replacement. BM transplantation (BMT) from WT mice resulted in increased intimal thickness (HE stain). B, Morphometrical analysis of histological sections of the cuffed- and control arteries. Intima-to-media ratio (IM ratio) of the HDC\(^{-/-}\) mice showed significantly lower values than that for the wild-type (WT) mice at 2 and 3 weeks after the cuffing. BM transplantation (BMT) from WT mice resulted in increased IM ratio (*\(P<0.05, n=6\)).
BM-derived cells in the arteries is unknown, our results suggest that the HDC⁺ cells were derived from the donor BM and that the common progenitor cells could potentially transdifferentiate into macrophage- and SMC-like vascular cells. Similar transdifferentiation of BM-derived progenitor cells into various types of vascular cells, including SMC- and macrophage-like cells, has been reported in animal models of graft vasculopathy, postangioplasty restenosis, hyperlipidemia-induced atherosclerosis, and cuff-induced vascular injury.25–27,34

Because apoE−/− mice contain the entire spectrum of lesions observed during atherogenesis similar to those in humans,18 HDC expression in BM-derived Mac-3⁺ macrophage-like cells but not in α-SMA⁺ SMC-like cells in the plaques of apoE−/− mice suggests a similar expression profile of HDC in the human lesions. In fact, in human atherosclerosis, HDC was only expressed in CD68⁺ foamy macrophages but not in α-SMA⁺ cells in all stage of aortic and carotid atherosclerosis.6,9 Furthermore, expression of HDC mRNA in cultures of human intimal SMCs was not detected by RT-PCR (data not shown).

In conclusion, we have provided evidence that histamine locally produced from BM-derived progenitor cells might contribute to neointimal formation in ligation-, cuff-, and hyperlipidemia-induced vascular injury models. Histamine produced from HDC⁺ progenitor cells in the intima would directly influence the intimal lesion formation. In contrast, those in the adventitia would influence the inflammatory condition of the adventitia and subsequent intimal hyperplasia. Furthermore, our results indicate the possibility that the BM-derived progenitor cells could transdifferentiate into macrophage-like or SMC-like cells in the arterial wall. Investigating the origin, site of homing, and functions of vascular cells will be important for better understanding the pathogenesis of vascular injury.

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References


Role of Histamine Produced by Bone Marrow–Derived Vascular Cells in Pathogenesis of Atherosclerosis

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