Histamine H1 Receptor Promotes Atherosclerotic Lesion Formation by Increasing Vascular Permeability for Low-Density Lipoproteins

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Objective—Enhanced endothelial permeability leading to intimal accumulation of low-density lipoproteins (LDL) stimulates the formation of atherosclerotic lesions. Histamine is known to increase vascular permeability. Whether this affects the formation of atherosclerotic lesions, however, remains elusive.

Methods and Results—Apolipoprotein E–null (ApoE−/−) mice treated with a histamine H1 receptor but not an H2 receptor antagonist developed 40% fewer atherosclerotic lesions in the aorta than placebo-treated controls. Similarly, genetic deletion of the H1 but not the H2 receptor resulted in a 60% reduction of lesions compared with ApoE−/− controls. The H1 receptor enhanced LDL permeability and lipid accumulation in the aorta, whereas plasma lipoprotein levels remained unaltered. In contrast, the H1 receptor did not affect proliferation and migration of vascular smooth muscle cells. Bone marrow transplantation confirmed that the formation of atherosclerotic lesions depended on the H1 receptor in vascular cells, whereas its presence in bone marrow-derived cells was irrelevant for plaque development. Mice expressing the H1 receptor exhibited higher levels of the chemokine (C-C motif) ligand 5 and higher numbers of macrophages and T-helper lymphocytes in plaques, higher numbers of circulating lymphocytes, and larger spleens.

Conclusion—These data indicate that H1 but not H2 receptor activation drives the formation of atherosclerotic lesions through an increased vascular permeability for LDL, which is associated with an enhanced secondary aortic and systemic inflammation. These data open novel perspectives for the prevention and treatment of atherosclerotic vascular disease. (Arterioscler Thromb Vasc Biol. 2010;30:923-930.)

Key Words: histamine • H1 receptor • atherosclerosis • vascular permeability

Histamine (2-[4-imidazole]-ethylamine) is a biogenic amine involved in inflammatory responses, and it plays a dominant role in acute allergic reactions.1,2 In this context, it increases vascular permeability, resulting in the formation of erythema and edema. In the arterial system, histamine has been shown to participate in the response to acute injury by promoting arterial remodeling and enhancing neointima formation. Indeed, mice lacking L-histidine decarboxylase, the rate-limiting enzyme for histamine synthesis, exhibit reduced neointimal thickening after balloon injury.3 There is increasing evidence that histamine may also modulate the response to chronic arterial injury, such as atherosclerotic lesion formation.3–5 An enhanced vascular permeability is indeed an early alteration of arterial function during the formation of such lesions and represents an important prerequisite for the accumulation of low-density lipoproteins (LDL) in the developing plaque.6–8 Once LDL are present in the vessel wall, they become oxidized and thereby exhibit chemotactic and proinflammatory properties, leading to monocyte accumulation, foam cell formation, and progression of lesion formation.9 However, whether histamine enhances vascular permeability in the setting of chronic arterial injury and whether such an effect would promote the formation of atherosclerotic lesions has not yet been examined.

Histamine exhibits its actions through 4 G-protein-coupled membrane receptors, of which the H1 and H2 receptor are expressed in the vessel wall.4 Most of the deleterious vascular effects of histamine, such as vasospasm, tissue factor induction, and intimal thickening after acute arterial injury, are mediated by the H1 receptor;10–11 similarly, the increase in vascular permeability induced by histamine is mediated via this receptor.12,13 These observations indicate an important
role of the H1 receptor in the vascular response to acute injury. The present study was therefore designed to investigate, using a combined pharmacological and genetic approach, (1) whether histamine promotes atherosclerotic lesion formation, (2) whether an increased vascular permeability is involved, and (3) whether these effects are mediated by the H1 receptor.

Materials and Methods

Animal Experiments
Animals were kept in individually ventilated cages with free access to food and water and maintained at 24°C on a 12-hour light/dark cycle. All animal experiments were approved by the Cantonal Veterinary Inspection Office (Zurich, Switzerland).

Pharmacological Approach
Six-week-old apolipoprotein E–null (Apoe−/−) littermate male mice were treated for 12 weeks with a high-cholesterol diet (Clinton-Cybulski diet, 1.25% cholesterol, Research Diets, No. D12108; Supplemental Table I, available online at http://atvb.ahajournals.org). In parallel, the histamine H1 receptor (H1R) blocker mepyramine (10 mg/kg per day, Sigma) or the H2 receptor (H2R) blocker ranitidine (100 mg/kg per day, Sigma) was administered in drinking water.

Genetic Approach
H1R and H2R knockout mice were kindly provided by Prof. T. Watanabe (Graduate School of Medicine, Kyoto University, Yoshida-Konoe machi, Sakyo-ku, Kyoto, Japan). These animals were crossed with ApoE−/− mice (purchased from Jackson Laboratory, Bar Harbor, Maine). An ApoE−/−H1R−/− histamine H2R−/− breeding pair was used to generate ApoE−/−H1R−/−H2R−/−, ApoE−/−H1R−/−H2R+/−, and ApoE−/−H1R+/−H2R−/− breeding pairs, so that all experimental animals originated from the same founder ApoE−/−H1R−/−H2R−/− breeding pair. All the animals were on C57BL/6 background, and only homozygous knockout mice were used for experiments. Six-week-old double-knockout male mice and ApoE−/− controls were treated with a high-cholesterol diet for 12 weeks.

Plasma Cholesterol, Triglycerides, and Histamine
Plasma cholesterol was determined using Infinity Cholesterol (Thermo Electron Corp, No. 52621), plasma triglycerides were determined using Infinity Triglycerides (Thermo Electron Corp, No. TR22421) and MC Cal (Abbott, No. 1E65-02), and plasma histamine was measured using an inhibition ELISA from LDN GmbH (Nordhorn, Germany).

Quantification of Atherosclerosis
Animals were euthanized by cervical dislocation. Blood was collected via a right ventricular puncture into a syringe flushed with heparin (Braun, No. 46613). Aortas were harvested, fixed with 4% paraformaldehyde (Sigma), and stained with Oil Red O (ORO; Sigma, No. O9755). AnalysisFIVE was used for quantification of ORO-positive area. Values are presented as percentage of plaque area (ie, ORO-positive area divided by total aortic surface area).

Immunohistochemistry
Specimens were frozen at −80°C in O.C.T. compound (Tissue-Tek, No. 62550-01), cut into 8-μm sections, and fixed with 4% paraformaldehyde. Frozen sections were stained with anti-mouse CD3 (Serotec), CD8 (Santa Cruz), CD68 (BD Biosciences), histidine decarboxylase, or CD117 (both from Antibodies-Online, Aachen, Germany) antibodies followed by incubation with alkaline phosphatase–conjugated secondary antibody (Jackson Immunoresearch or DAKO). Percentages of stained area were quantified with the AnalysisFIVE program.

RNA Expression
Total RNA was isolated using RNeasy Mini Kit (Qiagen, No. 74105). cDNA was generated using Ready-To-Go You-Prime First-Strand Beads (Amersham Bioscience, No. 27-9264-01) and first-strand cDNA primer pd(N), Quantitative real-time polymerase chain reaction was performed using SybrGreen Jump start kit (Sigma, No. S-4438). Primers were designed to detect mouse chemokine (C-C motif) ligand 5 (CCCL5), H1R, H2R, and histidine decarboxylase. Data were normalized for murine ribosomal S12 and analyzed by the comparative ΔCt method. Great care was taken to remove all adventitial tissue before processing for RNA analysis.

Antibody Array
Mouse Atherosclerosis Antibody Array (Tebu-Bio, No. 126AAM-ATH-1L) was used according to the manufacturer’s instructions.

Permeability Assay
An in vivo permeability assay was performed as described.16 Animals were anesthetized with 80 mg/kg intraperitoneal pentobarbital. To analyze aortic permeability, 100 μL of buffer containing 5×107 cpm 125I-LDL or 1% Evans blue dye was injected retroorally 30 minutes before the aortas were harvested. Aortas were washed with ice-cold PBS, and the radioactive signal was normalized to blood radioactivity and aorta weight. All measurements were done in triplicate.

Fluorescence-Activated Cell Sorting
Splenocytes were isolated using a 70-μm nylon mesh and stained with fluorescently labeled anti-mouse CD3 (clone 17A2), CD4 (clone RM4–5), CD8 (clone H35–17.2), CD19 (clone 1D3), and CD44 (clone IM7) antibodies (all from Pharmingen) for 30 minutes at 4°C. Data were collected using DiVAIL (BD Biosciences), and fluorescence-activated cell sorting analysis was performed using FlowJo software.

5′-Bromo-2′-Deoxyuridine Incorporation
Vascular smooth muscle cell (VSMC) proliferation in vivo was determined by 5′-bromo-2′-deoxyuridine (BrdUrd) incorporation. Osmotic pumps (Alzet, No. 1007D) containing BrdUrd (Serva; infusion rate, 13 mg/kg per day) were implanted under anesthesia between the shoulder blades. Incorporation of BrdUrd was monitored for 7 days after pump implantation in the region of the aortic root (BD Pharmingen, No. 550803). Slides were costained using anti–smooth muscle actin-α antibody conjugated with alkaline phosphatase (Sigma, No. A5691).

Bone Marrow Transplantation
Bone marrow cells were isolated by flushing femur and tibia bones with ice-cold PBS, ApoE−/− and ApoE−/−H1R−/− recipient mice were lethally irradiated with 1’100 rads (split dose, within 1 hour) and underwent intravenous rescue injection with 2×106 BM cells. After 3 weeks of reconstitution time, mice were put on a high-cholesterol diet for 12 weeks.

Statistical Analysis
Results are expressed as mean±SEM. The unpaired Student t test was applied for comparison of 2 groups, ANOVA for 3 or more groups. For each experiment, P<0.05 was considered statistically significant.

Results
H1R Promotes the Formation of Atherosclerotic Lesions
To assess the role of histamine receptor activation in the formation of atherosclerotic lesions, 6-week-old mice were...
fed a high-cholesterol diet for 12 weeks and treated with the H1 receptor antagonist mepyramine or the H2 receptor antagonist ranitidine throughout this time period. Animals treated with mepyramine but not ranitidine showed a reduction of nearly 40% in atherosclerotic lesions (mepyramine: \( P<0.05 \) versus control; ranitidine: \( P=\text{not significant (NS)} \) versus control; Figure 1A and 1B). This observation was confirmed by a genetic approach involving ApoE/H1 receptor double-knockout (ApoE\(^{-/-}\)/H1R\(^{-/-}\)) and ApoE/H2 receptor double-knockout (ApoE\(^{-/-}\)/H2R\(^{-/-}\)) animals. ApoE\(^{-/-}\)/H1R\(^{-/-}\) mice exhibited a 60% decrease in atherosclerotic lesions compared with ApoE\(^{-/-}\) controls (\( P<0.01 \); Figure 1C and 1D). In contrast, plaque formation in ApoE\(^{-/-}\)/H2R\(^{-/-}\) mice did not differ from ApoE\(^{-/-}\) controls (\( P=\text{NS} \); Figure 1C and 1D). Plasma levels of cholesterol and triglycerides, as well as the respective subfractions, did not differ between ApoE\(^{-/-}\)/H1R\(^{-/-}\) mice and ApoE\(^{-/-}\) controls (Table). Moreover, plasma levels of aspartate aminotransferase and alanine aminotransferase were within the normal range (data not shown), and histological analysis did not reveal any sign of structural abnormality indicative of liver destruction after the high-cholesterol diet (Supplemental Figure I). Deletion of the H1 receptor did not affect blood pressure regulation over the entire feeding period (telemetric analysis; data not shown).

The expression of H1 receptor, H2 receptor, and histidine decarboxylase in mouse aorta was assessed using quantitative real-time polymerase chain reaction. Development of atherosclerotic lesions did not affect the aortic expression of H1 and H2 receptor (data not shown). In contrast, histidine decarboxylase expression was 2.5-fold higher in aorta of atherosclerotic ApoE\(^{-/-}\) animals compared with healthy age-matched wild-type controls (n=6 to 8; \( P<0.05 \); data not shown). Histamine plasma levels increased after 12 weeks of a high-cholesterol diet; however, no significant difference was observed between ApoE\(^{-/-}\) compared with ApoE\(^{-/-}\)/H1R\(^{-/-}\) mice (Supplemental Figure II). Expression of the mast cell marker CD117 did not differ significantly in apoE\(^{-/-}\) and ApoE\(^{-/-}\)/H1R\(^{-/-}\) mice after 12 weeks of a high-cholesterol diet (Supplemental Figure III).

| Table. Plasma Triglyceride and Plasma Cholesterol Levels, Daily Food Intake, and Weight of ApoE\(^{-/-}\) and ApoE\(^{-/-}\)/H1R\(^{-/-}\) Mice After 12 Weeks of a High-Cholesterol Diet |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                | Triglyceride, mmol/L | Cholesterol, mmol/L | Daily Food Intake, g | Weight, g       |
| ApoE\(^{-/-}\)                  | 0.61±0.14          | 17.23±7.21       | 2.75±0.32         | 28.38±2.38      |
| ApoE\(^{-/-}\)/H1R\(^{-/-}\)    | 0.84±0.31          | 16.24±4.42       | 2.73±0.40         | 31.22±3.71*     |

\( n=12 \) (ApoE\(^{-/-}\)) and \( n=13 \) (ApoE\(^{-/-}\)/H1R\(^{-/-}\)).

\( *P=0.0342 \).
H1R Increases Vascular Permeability to LDL

LDL is an important trigger of atherosclerotic lesion formation. 125I-LDL levels were more than 2-fold higher in the aortic wall of 18-week-old ApoE−/− animals fed a high-cholesterol diet compared with age- and diet-matched ApoE−/− H1R−/− mice (P<0.05; Figure 2A). Similar to mice on a high-cholesterol diet compared with age- and diet-matched ApoE−/− H1R−/− mice under these conditions (P<0.07; data not shown). There was a strong tendency toward higher levels of Evans blue dye in the aortas of ApoE−/− mice compared with the aortas of ApoE−/−H1R−/− mice under these conditions (P=0.07; data not shown). These mice exhibited atherosclerotic changes in their descending aortas, as assessed by ORO en face staining (Figure 2A, right). The ORO-positive area in aortic cross sections at the level of the aortic valve was 24.9±3.2% and 16.5±1.7% of plaque area in ApoE−/− and ApoE−/−H1R−/− mice, respectively (P<0.05; Figure 2B). Similar to mice on a high-cholesterol diet, 18-week-old ApoE−/− animals fed a normal diet showed more than 1.5-fold higher 125I-LDL levels in the aortic wall compared with ApoE−/−/H1R−/− mice (P<0.05; Figure 2C, left), whereas simultaneously measured 125I-LDL plasma levels did not differ (P=NS; data not shown). These mice did not exhibit any atherosclerotic changes in their descending aortas as assessed by ORO en face staining (Figure 2C, right).

H1R Does Not Affect VSMC Proliferation

VSMC proliferation contributes to the progression of atherosclerotic lesions. BrdUrd incorporation over a time period of 7 days was applied to monitor VSMC proliferation during atherosclerosis progression in mice fed a high-cholesterol diet for 8 weeks. BrdUrd-positive cells occupied 1.4±0.2% of plaque area in ApoE−/− and 1.6±0.2% in ApoE−/−H1R−/− mice (P=NS; data not shown). Aortic tissue sections were double-stained with anti-smooth muscle α-actin to specifically investigate the proliferation of VSMC. The number of double-positive cells normalized to plaque area was 6.5×10−5±1.1×10−5 cells/μm² in ApoE−/− and 5.0×10−5±0.7×10−5 cells/μm² in ApoE−/−H1R−/− mice (P=NS; Figure 3A). In line with this, the total area occupied by smooth muscle α-actin-positive cells did not differ in the 2 groups of animals (P=NS; Figure 3B and 3C).

H1R on Vascular but Not on Bone Marrow-Derived Cells Promotes the Formation of Atherosclerotic Lesions

Vascular inflammation is an important event in the formation of atherosclerotic plaques. To determine whether the effect of the H1 receptor on lesion formation is mediated by vascular cells or by cells of the hematopoietic system, bone marrow transplantation experiments were performed. No difference in plaque formation was observed when reconstitution of...
ApoE<sup>−/−</sup> recipient mice with ApoE<sup>−/−</sup> bone marrow (8.0±1.0% aortic plaque area) was compared with that of ApoE<sup>−/−</sup> recipients with ApoE<sup>−/−</sup>H1R<sup>−/−</sup> bone marrow (6.4±0.6%; P=NS; Figure 4). Similarly, plaque formation did not differ when ApoE<sup>−/−</sup>H1R<sup>−/−</sup> recipient mice were reconstituted with ApoE<sup>−/−</sup> bone marrow (3.3±1.1% aortic plaque area) or with ApoE<sup>−/−</sup>H1R<sup>−/−</sup> bone marrow (2.6±0.3%; P=NS; Figure 4). In contrast, ApoE<sup>−/−</sup> recipients exhibited significantly more atherosclerosis than ApoE<sup>−/−</sup>H1R<sup>−/−</sup> recipients irrespective of whether these mice were reconstituted with ApoE<sup>−/−</sup> or ApoE<sup>−/−</sup>H1R<sup>−/−</sup> bone marrow (P<0.01 for ApoE<sup>−/−</sup> recipients versus ApoE<sup>−/−</sup>H1R<sup>−/−</sup> recipients with either type of bone marrow for reconstitution; Figure 4).

**H1R Is Associated With Increased Aortic and Systemic Inflammation**

In ApoE<sup>−/−</sup> mice fed a high-cholesterol diet for 12 weeks, immunohistochemistry revealed more CD68<sup>+</sup> cells in plaques compared with ApoE<sup>−/−</sup>H1R<sup>−/−</sup> mice, indicating that lesions contained a higher number of macrophages in the presence of the H1 receptor (P<0.01; Figure 5A). A similar pattern was observed with CD3<sup>+</sup> T lymphocytes (P<0.01; Figure 5A), as well as with CD4<sup>+</sup> helper T cells (P<0.05; Figure 5A), whereas the number of CD8<sup>+</sup> cytotoxic T cells did not differ (P=NS; Figure 5A). This increase in the content of macrophages and lymphocytes resulted in an enhanced cell density at the expense of extracellular matrix.

Expression of the chemokine CCL5 (RANTES) was higher in plaques of ApoE<sup>−/−</sup> mice compared with ApoE<sup>−/−</sup>H1R<sup>−/−</sup> mice at both the mRNA level and the protein level (P<0.01; Figure 5B and Supplemental Table II). In contrast, CCL2 and CCL3 RNA levels were similar between the 2 groups (n=6 to 8, P=0.21, data not shown).

Similar to the higher number of lymphocytes in plaques of ApoE<sup>−/−</sup> as opposed to ApoE<sup>−/−</sup>H1R<sup>−/−</sup> mice, the blood lymphocyte count was 2.86×10^9±0.27×10^9 cells/µL in ApoE<sup>−/−</sup> mice and 1.71×10^9±0.28×10^9 cells/µL in ApoE<sup>−/−</sup>H1R<sup>−/−</sup> animals (P<0.01; Figure 5C). In contrast, no difference in the number of blood monocytes, neutrophils, basophils, or eosinophils was observed (P=NS; Figure 5C). The plasma level of tumor necrosis factor α did not differ

Figure 3. H1R does not affect VSMC proliferation. A, No difference in the number of smooth muscle α-actin–BrdUrd double-positive cells was observed in the atherosclerotic plaques of ApoE<sup>−/−</sup>H1R<sup>−/−</sup> mice compared with those of ApoE<sup>−/−</sup> controls after 8 weeks of a high-cholesterol diet (n=7; P=NS). B, No difference in the smooth muscle α-actin–positive area was observed in the atherosclerotic plaques of ApoE<sup>−/−</sup>H1R<sup>−/−</sup> mice compared with those of ApoE<sup>−/−</sup> controls (n=7; P=NS). C, Immunohistochemical staining for smooth muscle α-actin in the atherosclerotic plaques of ApoE<sup>−/−</sup>H1R<sup>−/−</sup> mice and ApoE<sup>−/−</sup> controls. Scale bars=100 µm.

Figure 4. H1R on vascular cells but not bone marrow-derived cells enhances the development of atherosclerosis. Shown are percentage of plaque area (A) and representative en face ORO-stained aortic sections (B) after indicated transplantation protocol. See text for details. **P<0.01, n=4 to 6.
between ApoE−/− mice and ApoE−/−H1R−/− mice (n=4; P=NS; data not shown).

The spleen of ApoE−/− animals was 40% larger than that of ApoE−/−H1R−/− mice (P<0.05; Figure 5D); splenic morphology and histological appearance were similar (Supplemental Figure 1). Size, morphology, and cellularity of inguinal, axillary, and brachial lymph nodes did not differ (P=NS; data not shown).

Discussion

The H1 receptor plays a major role in the vascular response to histamine. Histamine-induced vascular contractions,11,17 as well as expression of tissue factor12,13, a key trigger of the coagulation cascade, are mediated via this receptor. In line with these findings, the present data indicate that activation of the H1 receptor, but not the H2 receptor, promotes the formation of atherosclerotic lesions. Hence, H1 receptor activation is involved in the vascular response to chronic injury. The more pronounced effect of genetic deletion of the H1 receptor on plaque formation compared with pharmacological blockade may be attributable to a higher efficacy of the genetic compared with the pharmacological approach. In addition, off-target effects of mepyramine on plaque formation cannot be ruled out. Such first-generation H1R blockers are indeed known to reduce blood pressure because of their vasodilating properties, and they may exert other off-target effects as well. However, the main action would be expected to occur through H1 receptor inhibition even if such effects were present, because genetic deletion of the receptor resulted in a pronounced attenuation of plaque formation without altering blood pressure.

ApoE−/− mice fed a high-cholesterol diet were used to study atherosclerotic lesion formation. Although very high plasma lipid levels are obtained in these mice, they are a well-recognized model of atherogenesis used by many groups.18,19 Nevertheless, additional studies in other animal models and eventually in humans may be required to confirm the involvement of the H1 receptor in slowly progressing atherogenesis. The analysis of plaque formation focused on the descending aorta, where a moderate degree of lesion formation was observed and differences in lesion size could be determined in a reliable manner. A higher atheroma burden was present in the aortic root, one of the major predilection sites for the development of atherosclerosis. Lesion size did not differ significantly between ApoE−/− and ApoE−/−H1R−/− mice in this part of the aorta (n=10; P=0.18; data not shown), although there was a clear tendency for smaller lesions in the ApoE−/−H1R−/− mice. This

Figure 5. Enhanced inflammation in the presence of the H1 receptor. A, The number of CD68-positive (n=9 to 10; P<0.005), CD3-positive (n=12 to 13; P<0.05), and CD4-positive cells (n=10; P<0.05) was higher in the atherosclerotic plaques of ApoE−/− compared with ApoE−/−H1R−/− mice after 12 weeks of a high-cholesterol diet. The number of CD8-positive cells was similar (n=12; P=NS).

The number of circulating lymphocytes was higher in aorta of ApoE−/− mice compared with ApoE−/−H1R−/− mice after 12 weeks of a high-cholesterol diet (n=10; P<0.05). C, The number of circulating lymphocytes was higher in ApoE−/− mice compared with ApoE−/−H1R−/− mice after 12 weeks of a high-cholesterol diet (left panel; n=8 to 9; P<0.01); in contrast, no difference in the number of monocytes, neutrophils, eosinophils, or basophils was observed (Table). D, Spleen weight was higher in ApoE−/− mice compared with ApoE−/−H1R−/− mice after 12 weeks of a high-cholesterol diet (left panel; n=10; P<0.05).
effect is presumably related to the very advanced degree of plaque formation in the aortic root, which is consistent with the rapid progression of lesion formation in this model. In line with this interpretation, it is reasonable to assume that the pathogenic events during plaque formation in the aortic root differ in a quantitative manner from those in the remaining aorta.

Plasma levels of cholesterol and triglycerides, as well as the respective subfractions, did not differ between ApoE<sup>−/−</sup>/H1R<sup>−/−</sup> mice and ApoE<sup>−/−</sup> controls, indicating that the effect of H1 receptor activation does not depend on plasma lipoproteins. The H1 receptor thus modulates vascular lesion formation independent of changes in lipid metabolism. Although food intake was similar in the different groups of animals, ApoE<sup>−/−</sup>/H1R<sup>−/−</sup> mice were slightly heavier than ApoE<sup>−/−</sup> controls, which may be related to the known reduced physical activity of ApoE<sup>−/−</sup>/H1R<sup>−/−</sup> animals. However, the weight difference between the 2 groups of mice likely did not affect the formation of atherosclerotic lesions, because the heavier group would be expected to exhibit more rather than fewer lesions.

The formation of atherosclerotic lesions did not affect the aortic expression of the H1 and H2 receptors. Thus, the development of atherosclerosis by itself did not interfere with the target of the experimental intervention. In contrast, expression of histidine decarboxylase and histamine plasma levels increased following 12 weeks of a high-cholesterol diet, indicating an upregulation of histamine expression and secretion during atherogenesis. This observation is consistent with recent reports implying an active role of histamine in the immunologic response during atherogenesis. The higher number of macrophages and T lymphocytes in atherosclerotic lesions of mice expressing the H1 receptor indicates that inflammation of plaques is more active in the presence of this receptor. The increased levels of the potent chemokine CCL5 may well account for the higher number of leukocytes in these plaques. These data are in line with the concept that increased vascular permeability for LDL is the driving force for plaque formation through the H1 receptor. Indeed, when LDL accumulate in the vessel wall, they become oxidized and thereby highly chemotactic for leukocytes; in addition, they induce the production of CCL5, which further increases the chemotactic activity in the developing plaques. CCL5 may also be released from platelets under these conditions. Because leukocytes and platelets can release histamine, a positive feedback loop may be induced at this stage, resulting in the further recruitment of inflammatory cells.

VSMC proliferation contributes to progression of atherosclerotic plaques. The H1 receptor does not affect VSMC proliferation during the formation of atherosclerotic lesions. Both VSMC proliferation and VSMC-positive plaque area were similar, suggesting that the H1 receptor does not affect VSMC migration either. Therefore, the H1 receptor does not promote the formation of atherosclerotic lesions via proliferation or migration of VSMC. Previously, histamine was observed to stimulate proliferation of cultured VSMC; however, these experiments were performed in vitro and do not reflect the complex events occurring during plaque formation in vivo. Although VSMC do not contribute to the effect of the H1 receptor on plaque formation via proliferation or migration, they may still be involved in lesion progression via their metabolic functions.

Macrophage-derived, lipid-loaded foam cells are the most abundant cell type present in atherosclerotic plaques. However, the bone marrow transplantation experiments demonstrate that the H1 receptor expressed in vascular cells rather than in bone marrow-derived cells promotes the formation of atherosclerotic lesions. These data are in line with the observation that the H1 receptor enhances vascular permeability for LDL, because the latter is regulated primarily by vascular cells. This notion supports the concept that the increased vascular permeability is the driving force for plaque formation through the H1 receptor. Thus, histamine promotes plaque formation via the H1 receptor on vascular cells only, although bone marrow-derived cells still participate in the formation of atherosclerotic lesions and may even release histamine under these conditions. Indeed, a reduced neointimal thickening after acute vascular injury was demonstrated in mice lacking L-histidine decarboxylase, the rate-limiting enzyme for histamine synthesis, and bone marrow-derived cells expressing the enzyme enhanced neointima formation compared with cells lacking it. These data indicate that bone marrow-derived cells are a source of histamine in vivo, suggesting that they may release histamine during the formation of atherosclerotic lesions as well. In this case, however, the cells would contribute to plaque formation only by releasing histamine, not by responding to it.

Although fewer T lymphocytes compared with macrophages are found in atherosclerotic plaques, T lymphocytes are involved in atherosclerotic lesion formation. CD4<sup>+</sup> cells appear to be particularly important in orchestrating the immunologic response during atherogenesis. The formation of atherosclerotic lesions did not affect the aortic expression of the H1 and H2 receptors. Thus, the development of atherosclerosis by itself did not interfere with the target of the experimental intervention. In contrast, expression of histidine decarboxylase and histamine plasma levels increased following 12 weeks of a high-cholesterol diet, indicating an upregulation of histamine expression and secretion during atherogenesis. This observation is consistent with recent reports implying an active role of histamine in the immunologic response during atherogenesis. The higher number of macrophages and T lymphocytes in atherosclerotic lesions of mice expressing the H1 receptor indicates that inflammation of plaques is more active in the presence of this receptor. The increased levels of the potent chemokine CCL5 may well account for the higher number of leukocytes in these plaques. These data are in line with the concept that increased vascular permeability for LDL is the driving force for plaque formation through the H1 receptor. Indeed, when LDL accumulate in the vessel wall, they become oxidized and thereby highly chemotactic for leukocytes; in addition, they induce the production of CCL5, which further increases the chemotactic activity in the developing plaques. CCL5 may also be released from platelets under these conditions. Because leukocytes and platelets can release histamine, a positive feedback loop may be induced at this stage, resulting in the further recruitment of inflammatory cells.

The higher blood lymphocyte count and spleen weight in ApoE<sup>−/−</sup> compared with ApoE<sup>−/−</sup>/H1R<sup>−/−</sup> mice indicate a higher degree of systemic inflammation in the presence of the H1 receptor, reflecting the difference in plaque burden between these groups of mice. The animals had a similar number of blood lymphocytes and a similar spleen weight before initiation of the high-cholesterol diet (P=NS; data not shown), underscoring that these differences indeed occur secondary to the development of atherosclerosis. Careful examination of spleen, liver, and lymph node histology did
not reveal any difference between ApoE\(^{-/-}\) and ApoE\(^{+/+}\) H1R\(^{-/-}\) mice, supporting this interpretation.

In summary, this study demonstrates by pharmacological and genetic intervention that histamine enhances the development of atherosclerosis via the H1 receptor but not the H2 receptor. The H1 receptor promotes the formation of atherosclerotic lesions when it is expressed in vascular cells, whereas its presence in bone marrow-derived cells is irrelevant for plaque formation. The effect of the H1 receptor occurs through an increased vascular permeability, leading to LDL accumulation and secondary vascular inflammation. These data open interesting perspectives for the prevention and treatment of atherosclerotic vascular disease.

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**Disclosures**

None.

**References**

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Supplemental Table I: Detailed description of the diet

<table>
<thead>
<tr>
<th>%</th>
<th>gm</th>
<th>kcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>22.6</td>
<td>20.0</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>45.2</td>
<td>40.0</td>
</tr>
<tr>
<td>Fat</td>
<td>20.1</td>
<td>39.9</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>kcal/gm</td>
<td>4.52</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>gm</th>
<th>kcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein, Alcohol Extracted</td>
<td>200</td>
<td>800</td>
</tr>
<tr>
<td>L-Cystein</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Corn Starch</td>
<td>212</td>
<td>848</td>
</tr>
<tr>
<td>Maltodextrin 10</td>
<td>71</td>
<td>284</td>
</tr>
<tr>
<td>Sucrose</td>
<td>113</td>
<td>452</td>
</tr>
<tr>
<td>Cellulose, BW200</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Soybean Oil</td>
<td>25</td>
<td>225</td>
</tr>
<tr>
<td>Cocoa Butter</td>
<td>155</td>
<td>1395</td>
</tr>
<tr>
<td>Mineral Mix S10021</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Dicalcium Phosphate</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Calcium Carbonate</td>
<td>5.5</td>
<td>0</td>
</tr>
<tr>
<td>Potassium Citrate</td>
<td>16.5</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin Mix V10001</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>Choline Bitrate</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Cholestrol</td>
<td>11.25</td>
<td>0</td>
</tr>
<tr>
<td>Blue Dye, FD&amp;C #1</td>
<td>0.05</td>
<td>0</td>
</tr>
<tr>
<td>Yellow Dye, FD &amp; C #5</td>
<td>0.05</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>897.35</strong></td>
<td><strong>4056</strong></td>
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</table>
Supplemental Table II: Antibody array demonstrating expression of cytokines and adhesion molecules in aortas of ApoE<sup>-/-</sup> and ApoE<sup>-/-</sup> control mice after 12 weeks of high-cholesterol diet (n=6). * p < 0.05

<table>
<thead>
<tr>
<th>Gene</th>
<th>ApoE&lt;sup&gt;-/-&lt;/sup&gt;</th>
<th>ApoE&lt;sup&gt;-/-&lt;/sup&gt;-H1R&lt;sup&gt;-/-&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>b FGF</td>
<td>7.79 ± 0.61</td>
<td>10.64 ± 2.29</td>
</tr>
<tr>
<td>CD40 *</td>
<td>27.61 ± 3.39</td>
<td>14.85 ± 11.92</td>
</tr>
<tr>
<td>RANTES *</td>
<td>33.05 ± 5.38</td>
<td>18.93 ± 12.11</td>
</tr>
<tr>
<td>Eotaxin</td>
<td>6.41 ± 0.72</td>
<td>6.72 ± 0.65</td>
</tr>
<tr>
<td>GCSF</td>
<td>21.98 ± 5.28</td>
<td>12.96 ± 8.76</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>24.12 ± 6.32</td>
<td>15.47 ± 10.18</td>
</tr>
<tr>
<td>IFN-g</td>
<td>13.90 ± 2.74</td>
<td>10.26 ± 4.51</td>
</tr>
<tr>
<td>IL-1a</td>
<td>20.67 ± 5.73</td>
<td>14.33 ± 7.03</td>
</tr>
<tr>
<td>IL-1b</td>
<td>17.94 ± 5.92</td>
<td>11.97 ± 6.73</td>
</tr>
<tr>
<td>IL-2</td>
<td>16.38 ± 5.14</td>
<td>11.81 ± 6.76</td>
</tr>
<tr>
<td>IL-3</td>
<td>22.92 ± 5.91</td>
<td>14.49 ± 10.78</td>
</tr>
<tr>
<td>IL-4</td>
<td>17.08 ± 5.16</td>
<td>13.73 ± 6.93</td>
</tr>
<tr>
<td>IL-5</td>
<td>18.79 ± 5.91</td>
<td>12.64 ± 9.13</td>
</tr>
<tr>
<td>IL-6</td>
<td>13.78 ± 3.80</td>
<td>10.31 ± 4.69</td>
</tr>
<tr>
<td>IL-13</td>
<td>21.96 ± 6.94</td>
<td>15.91 ± 9.64</td>
</tr>
<tr>
<td>L-Selectin</td>
<td>19.81 ± 6.12</td>
<td>13.51 ± 7.73</td>
</tr>
<tr>
<td>MCP-1</td>
<td>8.425 ± 1.47</td>
<td>10.02 ± 1.17</td>
</tr>
<tr>
<td>M-CSF</td>
<td>21.02 ± 5.85</td>
<td>16.24 ± 7.98</td>
</tr>
<tr>
<td>MIP-3a</td>
<td>22.51 ± 7.59</td>
<td>14.18 ± 10.12</td>
</tr>
<tr>
<td>P-Selectin</td>
<td>14.74 ± 3.97</td>
<td>13.67 ± 3.60</td>
</tr>
<tr>
<td>TNF-a</td>
<td>20.86 ± 4.47</td>
<td>13.77 ± 7.03</td>
</tr>
<tr>
<td>VEGF</td>
<td>6.31 ± 0.90</td>
<td>6.53 ± 0.48</td>
</tr>
</tbody>
</table>
Supplemental Figure I: Liver, lymph nodes, and spleen do not differ histologically between ApoE\(^{-/-}\) and ApoE\(^{-/-}\) H1R\(^{-/-}\) mice.

Representative hematoxylin-eosin sections are shown. Scalebars = 200 µm
Supplemental Figure II: High cholesterol diet increases histamine plasma levels

Plasma histamine was measured before and after high cholesterol diet in both ApoE−/− and ApoE−/− H1R−/− mice. *** p< 0.001
Supplemental Figure III: Expression of neither the mast cell marker CD117 (upper panel) nor of histamine decarboxylase (lower panel) differs between ApoE\(^{-/-}\) and ApoE\(^{-/-}\)H1R\(^{-/-}\) mice after 12 weeks of high cholesterol diet.

Aortas of mice were stained with an antibody for CD117 (upper panel) or histamine decarboxylase (HDC, lower panel). Slides are representative of at least 7 experiments. Scalebars = 1 mm