Stimulatory Effects of Cardiotrophin 1 on Atherosclerosis

Hanae Konii,* Kengo Sato,* Sayaka Kikuchi, Hazuki Okiyama, Rena Watanabe, Akinori Hasegawa, Keigo Yamamoto, Fumiko Itoh, Tsutomu Hirano, Takuya Watanabe

Abstract—Cardiotrophin 1 (CT-1), an interleukin-6 family cytokine, was recently shown to be expressed in the intima of early atherosclerotic lesions in the human carotid artery. CT-1 stimulates proatherogenic molecule expression in human vascular endothelial cells and monocyte migration. However, it has not been reported whether CT-1 accelerates atherosclerosis. This study was performed to examine the stimulatory effects of CT-1 on human macrophage foam cell formation and vascular smooth muscle cell migration and proliferation in vitro, and on the development of atherosclerotic lesions in apolipoprotein E–deficient (ApoE−/−) mice in vivo. CT-1 was expressed at high levels in endothelial cells and macrophages in both humans and ApoE−/− mice. CT-1 significantly enhanced oxidized low-density lipoprotein–induced foam cell formation associated with increased levels of CD36 and acyl-CoA:cholesterol acyltransferase-1 expression in human monocyte–derived macrophages. CT-1 significantly stimulated the migration, proliferation, and collagen-1 expression in human aortic vascular smooth muscle cells. Four-week infusion of CT-1 into ApoE−/− mice significantly accelerated the development of aortic atherosclerotic lesions with increased monocyte/macrophage infiltration, vascular smooth muscle cell proliferation, and collagen-1 content in the aortic wall. Activation of inflammasome, such as apoptosis-associated speck-like protein containing a caspase recruitment domain, nuclear factor κB, and cyclooxygenase-2, was observed in exudate peritoneal macrophages from ApoE−/− mice infused with CT-1. Infusion of anti–CT-1–neutralizing antibody alone into ApoE−/− mice significantly suppressed monocyte/macrophage infiltration in atherosclerotic lesions. These results indicate that CT-1 accelerates the development of atherosclerotic lesions by stimulating the inflammasome, foam cell formation associated with CD36 and acyl-CoA:cholesterol acyltransferase-1 upregulation in macrophages, and migration, proliferation, and collagen-1 production in vascular smooth muscle cells. (Hypertension. 2013;62:942-950.)

Key Words: atherosclerosis ■ cardiotrophin 1 ■ hypertension ■ macrophages ■ smooth muscle

Atherosclerosis is a pathological injury-to-response process that is initiated by early inflammatory responses of vascular endothelial cells (ECs).1 Endothelial inflammation is characterized by increased production of proatherogenic molecules and inflammatory cytokines in ECs, and monocyte adhesion and infiltration into the neointima lesion, followed by oxidized low-density lipoprotein–induced transformation of macrophages into foam cells.2 Accumulation of cholesterol ester in macrophages is a hallmark of foam cell formation.2 This accumulation depends on the balance between the uptake of oxidized low-density lipoprotein via CD36 and the efflux of free cholesterol controlled by ATP-binding cassette transporter A1 (ABCA1), ATP-binding cassette transporter G1, or scavenger receptor class B type I (SR-BI).3 To protect the cells from the toxicity that would result from excessive free cholesterol accumulation, the free cholesterol is esterified to cholesterol ester by acyl-CoA:cholesterol acyltransferase-1 (ACAT1).3 Cholesterol ester stored in lipid droplets can be removed from cells only after hydrolysis to free cholesterol by neutral cholesterol ester hydrolase.4 Apart from accumulation of macrophage-derived foam cells, the migration and proliferation of vascular smooth muscle cells (VSMCs) followed by extracellular matrix (ECM) production play crucial roles in the development of atherosclerotic lesions.1

Cardiotrophin 1 (CT-1), a 201-aa member of the interleukin-6 cytokine family, was originally cloned from embryoid bodies as a 21.5-kDa protein capable of inducing hypertrophy in neonatal cardiomyocytes.5 Human and mouse CT-1 share 80% amino acid sequence identity and exhibit cross-species activity.6 Subsequent studies confirmed that plasma concentrations of CT-1 are elevated in various cardiorenal diseases, such as hypertension, ischemic heart disease, heart failure, and chronic renal disease.7–10 CT-1 exerts cardiovascular remodeling induced by hypertensive and ischemic heart diseases and congestive heart failure, through its receptor complex glycoprotein 130 (gp130) and leukemia inhibitory factor receptor (LIFR).11–14 Recently, CT-1 has been shown to be expressed in the intima in the early stages of atherosclerotic lesions in human carotid artery.15 CT-1 stimulates the synthesis of inflammatory cytokines and proatherogenic molecules, such as interleukin-6,
monocyte chemoattractant protein-1, and intercellular adhesion molecule-1, in human ECs.\textsuperscript{15} CT-1 also stimulates the synthesis of interleukin-6, interleukin-1β, and tumor necrosis factor-α in human monocytes\textsuperscript{7,16} and induces human monocyte adhesion and migration.\textsuperscript{17} CT-1 increases the proliferation, hypertrophy, and ECM production in rat VSMCs.\textsuperscript{11} However, it has not yet been reported whether CT-1 accelerates the development of atherosclerotic lesions in vivo.

In the present study, we assessed the stimulatory effects of CT-1 on human macrophage foam cell formation, EC proliferation, and VSMC migration, proliferation, and ECM production in vitro, and the development of atherosclerotic lesions in apolipoprotein E-deficient (ApoE\textsuperscript{−/−}) mice, an animal model of atherosclerosis, in vivo.

Methods

Human Cell Culture

This investigation was approved by the Ethics Committee of Tokyo University of Pharmacy and Life Sciences. Informed consent was obtained from all subjects. Human peripheral mononuclear cells were isolated from the blood of 35 healthy volunteers. Monocytes purified using anti-CD14 antibody–conjugated magnetic microbeads (Miltenyi Biotec, Auburn, CA) were seeded onto 3.5-cm dishes (1×10\textsuperscript{5} cells/mL per dish) for immunoblotting analysis and cholesterol esterification assay.\textsuperscript{5,9} Cells were incubated at 37°C in 5% CO\textsubscript{2} for 7 days in RPMI 1640 medium supplemented with 10% human serum, 0.1 mg/mL streptomycin, 100 U/mL penicillin, and the indicated concentrations of recombinant human CT-1 (ITSI Biosciences, Johnstown, PA). The medium in each dish was replaced with fresh medium containing CT-1 every 3 days.

Cholesterol Esterification Assay

Human macrophages differentiated by 7-day culture with the indicated concentrations of recombinant human CT-1 were incubated for 19 hours with 50 μg/mL human oxidized low-density lipoprotein in the presence of 0.1 mmol/L [\textsuperscript{3}H]oleate (PerkinElmer, Yokohama, Japan) conjugated with BSA (Sigma, St Louis, MO).\textsuperscript{11,20} Cellular lipids were extracted and the radioactivity of cholesterol [\textsuperscript{3}H]oleate was determined by thin-layer chromatography.

Migration Assay

Human aortic smooth muscle cells (HASMCs; Lonza, Walkersville, MD) at passage 7 were seeded onto 3.5-cm dishes (5×10\textsuperscript{5} cells/mL per dish). Cells were incubated at 37°C in 5% CO\textsubscript{2} for 7 hours in smooth muscle cell basal medium (Lonza) supplemented with 0.5 ng/mL human epidermal growth factor, 5 μg/mL insulin, 2 ng/mL human fibroblast growth factor, 50 μg/mL gentamicin, 50 ng/mL amphotericin B, and 5% fetal bovine serum (FBS). Then, while HASMCs were incubated in serum-free smooth muscle cell basal medium with or without 0.5 ng/mL of recombinant human CT-1 for 5 hours, photographs of cells were taken at 10-minute intervals. The average migration distance of 10 cells randomly selected in each dish was measured using a BIOREVO BZ-9000 microscope (Keyence, Osaka, Japan).

Proliferation Assay

HASMCs or human EA.hy926 ECs at passage 5–10 were seeded onto 96-well plates (1×10\textsuperscript{4} cells/100 μL per well) and incubated for 24 hours in the same smooth muscle cell basal medium or Dulbecco modified Eagle medium supplemented with 10% FBS, 4.5 mg/mL d-glucose, 0.584 mg/mL L-glutamine, 0.05 mg/mL streptomycin, and 50 U/mL penicillin. HASMCs or EA.hy926 ECs were incubated for 48 hours with the indicated concentrations of recombinant human CT-1 with renewal of each medium. Then, 10 μL of WST-8 solution (Cell Count Reagent SF; Nacalai Tesque, Kyoto, Japan) was added to each well. After 1 hour of incubation, the amount of formazan product was determined by measuring the absorbance at 450 nm using a Sunrise Remote R-micro plate reader (Tecan, Kawasaki, Japan).\textsuperscript{21}

Immunoblotting Analysis

Cells were extracted with 10% SDS containing a protease inhibitor cocktail (Sigma).\textsuperscript{3,19} Aliquots of 20 μg of cell protein were separated by 10% SDS-PAGE and subjected to immunoblotting with the following antibodies: antihuman CT-1 (Bioworld Technology, Louis Park, MN), gp130 (Cell Signaling Technology, Danvers, MA), LIFR, neutral cholesterol ester hydrolase (Abcam, Tokyo, Japan), CD68, ACAT1 (Santa Cruz Biotechnology, Santa Cruz, CA), CD36, scavenger receptor class A (R&D Systems, Minneapolis, MN), ABCA1, ATP-binding cassette transporter G1, SR-BI, collagen-I (Novus Biologicals, Littleton, CO), matrix metalloproteinase-2, fibronectin (GeneTex, Irvine, CA), elastin (Bios, Woburn, MA), apotosis-associated speck-like protein containing a caspase recruitment domain (Millipore, Billerica, MA), nuclear factor κB (NF-κB; Aviva Systems Biology, San Diego, CA), cyclooxygenase-2 (Cayman Chemical, Ann Arbor, MI), glyceraldehyde-3-phosphate dehydrogenase, α-tubulin (GeneTex), or β-actin (Sigma).

Signal Transduction Assay

Signal transduction pathways concerning macrophage foam cell formation and VSMC proliferation were investigated in vitro. Antibodies against human CT-1 (2 μg/mL), gp130 (1 μg/mL), or LIFR (1 μg/mL), otherwise selective inhibitors for MAPK/ERK kinase 1/2 (SL327, 1 μmol/L; Sigma), Janus kinase/signal transducer and activation of transcription-3 (cucurbitacin-I, 10 nmol/L), NF-κB (parthenolide, 1 μmol/L), phosphotyrosinolositol-3 kinase (LY-294002, 100 nmol/L), or Akt1/2 kinase (100 nmol/L; WAKO, Tokyo), were added into CT-1 cells. These suppressive effects could reflect the signal transduction pathways concerned.

Animal Experiments

Animal experiments were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Tokyo University of Pharmacy and Life Sciences. A total of 30 male spontaneously hyperlipidemic ApoE\textsuperscript{−/−} mice were infused with recombinant human CT-1 (100 μg/kg per day) in ApoE\textsuperscript{−/−} mice (C57BL/6. KOR/SlimSc-ApoE\textsuperscript{−/−} mice) at the age of 9 weeks were purchased from Japan SLC Inc (Hamamatsu, Japan) and kept on a normal diet until the age of 13 weeks. Then, a high-cholesterol diet containing 7.5% cocoa butter, 1.25% cholesterol, and 0.5% cholate (Oriental Yeast, Tokyo) was started. Three groups of 17-week-old ApoE\textsuperscript{−/−} mice were infused with recombinant human CT-1 (100 μg/kg per day; BioVendor, Modrice, Czech Republic), antismouse CT-1–neutralizing antibody (10 μg/kg per day; R&D Systems), or saline (vehicle) for 4 weeks by osmotic mini-pumps (Alzet Model 1002; Durect, Cupertino, CA).\textsuperscript{3,22–25} In our preliminary examination, 4-week infusion of recombinant human CT-1 at different doses brought a result that its plasma concentration increased by 7-fold (0.96±0.77 to 6.87±1.71 ng/mL) at 100 μg/kg per day in ApoE\textsuperscript{−/−} mice.

Animal Measurements

After 4 weeks of infusion into ApoE\textsuperscript{−/−} mice, systolic and diastolic blood pressures were measured using the indirect tail-cuff method (Kent Scientific, Torrington, CT). Blood samples were collected after a 4-hour fast. Plasma concentrations of glucose and total cholesterol were measured by enzymatic methods using an autoanalyzer (Hitachi, Tokyo).\textsuperscript{22,25} Plasma CT-1 concentration was measured by ELISA (ELISA kit for human CT-1; Antigenex America, Huntington Station, NY).

Atherosclerotic Lesion Assessment

After 4 weeks of infusion, the ApoE\textsuperscript{−/−} mice were anesthetized with diethylether. The whole aorta was washed by perfusion with PBS and fixed with 4% formaldehyde.\textsuperscript{12,23,25} The aorta was excised from the aortic sinus to the abdominal area and the connective and adipose
tissues were carefully removed. The entire aorta and cross-sections of the aortic sinus were stained with oil red O for assessment of atherosclerotic lesions.\(^{2,3,25}\) Monocytes/macrophages, ECs, VSMCs, collagen-1, and CT-1 expression in the aortic atherosclerotic lesions were visualized by staining with anti–MOMA-2 antibody (Millipore), antipodocalyxin antibody (Life Technologies, Carlsbad, CA), anti-α-smooth muscle actin antibody (Sigma), anticultin-1 antibody (Novus Biologicals), and anti–CT-1 antibody (Biorad), respectively.\(^{23,25}\) The areas of the aortas with atherosclerotic lesions were traced by an investigator blind to the treatment and measured with an image analyzer (Adobe Photoshop, San Jose, CA, and NIH ImageJ, Bethesda, MD).

Mouse Exudate Peritoneal Macrophage Assessment

Exudate peritoneal cells were obtained from ApoE\(^{-/}\) mice as described previously.\(^{2,25}\) Cells were suspended in RPMI 1640 medium supplemented with 10% FBS, 0.1 mg/mL streptomycin, and 100 U/mL penicillin G, and seeded onto 6-cm dishes (4×10\(^6\) cells/2 mL per dish). After a 1-hour incubation at 37°C in 5% CO\(_2\) to allow adhesion, the medium was discarded to remove nonadherent cells. Immediately, adherent macrophages were harvested after washing the dishes with PBS twice for immunoblotting analysis.

Statistical Analysis

All values are expressed as mean±SEM. The data were compared by 2-tailed unpaired Student \(t\) test between 2 groups and 1-way ANOVA followed by Bonferroni post hoc test among \(≥\)3 groups using Statview-J 5.0 (SAS Institute, Cary, NC). \(P\) values <0.05 were considered significant.

## Results

### Expression of CT-1 in Human Vascular Cells and Mouse Atherosclerotic Lesions

Immunoblotting analyses indicated the expression of CT-1 and its 2 receptor subunits, gp130 and LIFR, in a variety of human vascular cells. As shown in Figure 1A, CT-1 was expressed at high levels in human EA.hy926 ECs, human umbilical vein endothelial cells (Kurabo, Osaka), and human monocyte–derived macrophages, but at relatively lower levels in human umbilical artery smooth muscle cells (Lonza) and human monocytes. Both gp130 and LIFR were expressed at high levels in EA.hy926 ECs, human umbilical vein endothelial cells, human umbilical artery smooth muscle cells, monocytes, and macrophages.

As shown in Figure 1B, CT-1 was expressed at high levels in the areas compatible with ECs and monocyte-derived macrophage foam cells within atherosclerotic plaques in the aortic sinus from 21-week-old ApoE\(^{-/}\) mice.

### Effects of CT-1 on Human EC Proliferation

As shown in Figure 2A, CT-1 over the wide range of concentrations tested did not significantly affect the proliferation of human EA.hy926 ECs.

### Effects of CT-1 on Human VSMC Migration and Proliferation

As shown in Figure 2B, CT-1 at 0.5 ng/mL significantly increased the migration of HASMCs by 1.4-fold (\(P<0.0005\)), which was compatible with the 1.7-fold stimulatory effect of 0.5 \(\mu\)mol/L angiotensin II (Sigma; \(P=0.0001\); data not shown).

As shown in Figure 2C, CT-1 resulted in significant increases in the proliferation of HASMCs at the limited concentrations ranging 0.5 to 10 ng/mL (1.35-fold; at least \(P<0.05\)). The effect was comparable with that of 1 \(\mu\)mol/L angiotensin II reported previously (1.7-fold; \(P=0.0001\)).\(^{28}\) The CT-1–induced HASMC proliferation was significantly abolished by anti–CT-1 antibody, anti–LIFR antibody, anti-gp130+anti-LIFR antibodies, SL327, curcubitacin-1, and parthenolide, and partially inhibited by anti-gp130 antibody, LY294002, and Akt1/2 kinase inhibitor (Figure 2D).

### Effects of CT-1 on Human Macrophage Foam Cell Formation

As shown in Figure 2E, CT-1 significantly enhanced oxidized low-density lipoprotein–induced cholesterol ester accumulation in human monocyte–derived macrophages in a concentration-dependent manner. The maximal effect of CT-1 was observed at 50 ng/mL (1.4-fold; \(P<0.05\)), which was compatible with the 1.5-fold stimulatory effect of 1 \(\mu\)mol/L angiotensin II reported previously (\(P<0.005\)).\(^{29}\)

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**Figure 1.** Expression of cardiogrophin 1 (CT-1) and its receptor in human vascular cells and mouse atherosclerotic lesions. A, Aliquots of 50 \(\mu\)g of cellular protein from human monocytes, macrophages, EA.hy926 ECs, human umbilical vein endothelial cells (HUVECs), and human umbilical arterial smooth muscle cells (HUASMCs) were separated by 10% SDS-PAGE and subjected to immunoblotting with antibodies against human CT-1, glycoprotein 130 (gp130), or leukemia inhibitory factor receptor (LIFR). Glyceraldehyde-3-phosphate dehydrogenase (GADPH) served as a loading control. B, Atheromatous plaques of the aortic sinus from 21-week-old ApoE\(^{-/}\) mice were stained with oil red O (a-1, b-1), the endothelial cell marker podocalyxin (a-2), and the monocyte/macrophage marker MOMA-2 (b-2), or antimouse CT-1 antibody (a-3, b-3). Hematoxylin was used for nuclear staining.
Effects of CT-1 on CD36 and ACAT1 Expression in Human Monocyte-Derived Macrophages

As shown in Figure 3, no significant effects of CT-1 on neutral cholesterol ester hydrolase (NCEH), ACAT1, ATP-binding cassette transporter A1 (ABCA1), and scavenger receptor class B type (SR-BI) protein expression were observed at any concentration tested in human monocyte–derived macrophages. However, CT-1 increased CD36 and ACAT1 protein expression in a concentration-dependent manner, with maximal effects observed at 25 ng/mL (1.5-fold and 1.8-fold, respectively; both \( P < 0.05 \)). The CT-1--induced upregulation...
of CD36 and ACAT1 was declined by anti–CT-1 antibody, anti-gp130+anti-LIFR antibodies, LY294002, Akt1/2 kinase inhibitor, and SL327 (Figure 4A). In addition, the stimulatory effects of CT-1 at 25 ng/mL on CD36 and ACAT1 protein expression were enhanced during differentiation from monocytes into macrophages (Figure 4B).

**Effects of CT-1 on ECM Expression in Human VSMCs**

As shown in Figure 4C, CT-1 significantly increased the protein expression of collagen-1 at a concentration-dependent manner, with a significant effect at 5 ng/mL (1.5-fold; P<0.05), but not matrix metalloproteinase-2, fibronectin, and elastin in HASMCs. These findings suggest that CT-1–induced collagen-1 production may also contribute to the development of atherosclerotic plaques.

**Effects of CT-1 on Atherosclerotic Lesion Development in ApoE−/− Mice**

Plasma CT-1 concentration was significantly increased by 3.5- to 5-fold in ApoE−/− mice infused with CT-1 than in others (P<0.05; Table). However, there were no significant differences in body weight, systolic and diastolic blood pressures, or plasma concentrations of glucose and total cholesterol among the 3 groups (Table). Atherosclerotic lesions were markedly observed in the en face aorta and aortic sinus from 21-week-old ApoE−/− mice, and the degree differed among the 3 groups (Figure 5A–5F). Chronic CT-1 infusion significantly enhanced the surface areas of the atherosclerotic lesions by ≈2-fold with significant increases in the atheromatous plaque size, monocyte/macrophage infiltration, VSMC proliferation, and collagen-1 content in the aortic sinus, as compared with saline-infused counterparts (Figure 5P–5T). Neutralization of endogenous CT-1 by chronic infusion of anti–CT-1 antibody significantly reduced only the monocyte/macrophage infiltration within atherosclerotic lesions (Figure 5R).

**Effects of CT-1 on Atherogenic Molecule Expression in Exudate Peritoneal Macrophages From ApoE−/− Mice**

As listed in the Table, the number of exudate peritoneal macrophages from ApoE−/− mice infused with CT-1 was slightly greater than in the others, although the differences were not significant. We assessed the effects of CT-1 on protein expression of foam cell formation–related molecules, such as scavenger receptor class A, CD36, ACAT1, and ABCA1, and inflammasome adaptor molecules, such as apoptosis-associated speck-like protein containing a caspase recruitment domain,30 NF-kB, and cyclooxygenase-2, in exudate peritoneal macrophages from ApoE−/− mice infused with saline, CT-1, or anti–CT-1–neutralizing antibody. As shown in Figure 6, scavenger...
receptor class A, CD36, ACAT1, apoptosis-associated speck-like protein containing a caspase recruitment domain, NF-κB, and cyclooxygenase-2 expression were obviously stimulated by CT-1 but suppressed by anti–CT-1 antibody. The expression of ABCA1 was not markedly changed by CT-1 or anti–CT-1 antibody.

Discussion

This is the first demonstration that CT-1 stimulates human macrophage foam cell formation, human VSMC migration and proliferation, and collagen-1 expression in VSMCs in vitro, and accelerates the development of atherosclerotic lesions with increased monocyte/macrophage infiltration, VSMC proliferation, and collagen-1 content in ApoE−/− mice in vivo. In addition, CT-1 induces inflammatory responses in exudate macrophages in vivo. Endogenous CT-1 expression was observed in ECs and macrophage foam cells within atherosclerotic plaques from ApoE−/− mice. Neutralization of endogenous CT-1 reduces the infiltration of monocytes/macrophages within atherosclerotic lesions in ApoE−/− mice. This finding may be attributable to the anti–CT-1 antibody–induced blockade of stimulatory effects of CT-1 on monocyte–endothelial adhesion and monocyte migration into the vascular wall.15 Our comprehensive research bridges the observation of abundant CT-1 levels in the circulating blood and atherosclerotic lesions in humans and in vitro data for atherogenesis in the vascular cells.8,15–17

CT-1 is expressed in the cardiovascular system as well as the thymus, kidney, liver, muscle, and adipose tissue.31–33 Expression of CT-1 is upregulated by mechanical stretching and atherogenic substances, such as angiotensin II, aldosterone, norepinephrine, fibroblast growth factor-2, heparin-binding epidermal growth factor–like growth factor, glucose, and reactive oxygen species.7,33,34 Vascular media thickness and ECM contents, such as collagen, fibronectin, elastin, and matrix metalloproteinase, in VSMCs were increased by CT-1 treatment in rats.11,12 Media thickness, wall fibrosis, and inflammation were lower in CT-1–deficient mice than in wild-type controls under a normal diet.35 These findings indicated that CT-1 plays an important role in arteriosclerosis that consists of vascular remodeling, inflammation, and calcification with aging. However, the present study is the first to demonstrate that CT-1 also contributes to the development of atherosclerosis, that is, atheromatous plaques, including lipid core. In contrast, a previous study showed that CT-1–deficient mice developed obesity, insulin resistance, and

Table. Characteristics and Laboratory Data

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<td>1.27±0.14</td>
<td>1.03±0.15</td>
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CT-1 indicates Cardiotrophin 1.

*P<0.05 vs others.

Figure 5. Effects of cardiotrophin 1 (CT-1) on the development of atherosclerotic lesions in ApoE−/− mice. The 17-week-old ApoE−/− mice were infused with saline (control) or CT-1 (100 μg/kg per day), antimouse CT-1–neutralizing antibody (10 μg/kg per day) by osmotic mini-pumps for 4 weeks. The excised aorta was opened longitudinally, followed by oil red O staining (A–C). Atherosclerotic lesions in cross-sections of the aortic sinus were stained with oil red O (D–F), antibodies for the monocyte/macrophage marker MOMA-2 (G–I), the vascular smooth muscle cell marker α-smooth muscle actin (J–L), or anticollagen-1 antibody (M–O). Hematoxylin was used for nuclear staining. Data are expressed as means±SEM. *P<0.005; †P<0.05; ‡P<0.0001 vs control.
hypercholesterolemia on a high-cholesterol diet. Further studies are needed to confirm whether CT-1 knockout could counteract the development of atherosclerotic lesions in ApoE−/− mice fed high-cholesterol diet.

Several studies confirmed the trophic properties of CT-1 in other cell types, such as adult cardiomyocytes, cardiac fibroblasts, hepatocytes, and airway smooth muscle cells. CT-1 induces myocardial hypertrophy and confers protection from ischemia/reperfusion injury through its receptor, gp130/LIFR complex, followed by activation of the ERK-1/2, Janus kinase/signal transducer and activation of transcription-3, and phosphatidylinositol-3 kinase/Akt pathways. CT-1 is also known to activate NF-κB and cyclooxygenase-2. Our results and those of other studies showed that CT-1 receptors are present in cardiac fibroblasts, VSMCs, ECs, monocytes, and macrophages. Several lines of evidence have shown that foam cell formation and CD36 and ACAT1 upregulation in macrophages and the migration and proliferation of VSMCs are mediated via ERK-1/2. The results from the present study suggest that CT-1 induces VSMC proliferation via the CT-1 receptor, ERK-1/2, Janus kinase/signal transducer and activation of transcription-3, and NF-κB pathways, and upregulates CD36 and ACAT1 expression via the CT-1 receptor, phosphatidylinositol-3 kinase, Akt1/2, and ERK-1/2 pathways in macrophages.

Plasma CT-1 concentration is ≈1 ng/mL in healthy volunteers but somewhat varies with CT-1 measurement kits. In our study, plasma CT-1 concentration was 2.54±1.04 ng/mL in 5 healthy volunteers. The heart is the main source of circulating CT-1 and further secretes CT-1 after ischemic injury in humans. Plasma CT-1 concentrations are elevated in patients with angina pectoris and unstable angina. Furthermore, plasma CT-1 concentrations are increased in accordance with complications of left ventricular hypertrophy or dysfunction induced by ischemic heart disease, hypertensive heart disease, and heart failure. Plasma CT-1 concentrations are also elevated in patients with metabolic syndrome. Infusion of human CT-1 into ApoE−/− mice reached 6.87 ng/mL in plasma, which was equivalent to the mean to the maximal plasma CT-1 concentrations (3.1–11.5 ng/mL) in patients with unstable angina. Nevertheless, the concentrations of CT-1 required for enhancement of ACAT1 and CD36 expression (25 ng/mL) and foam cell formation (50 ng/mL) in human macrophages were relatively high (2.5–5-fold) compared with the maximal plasma CT-1 concentration in patients with unstable angina. In atherosclerotic plagues, it is mainly ECs and macrophages that generate large amounts of CT-1 in an autocrine/paracrine manner. Therefore, it is not so surprising that local levels of CT-1 were increased by this degree like other vasoactive agents, such as serotonin, angiotensin II, and urotensin II. Cell proliferation and collagen-1 production in VSMCs occurred within the pathophysiological range of 0.5 to 10 ng/mL.

**Perspectives**

The present results indicate that CT-1 accelerates the development of atherosclerotic lesions by stimulating inflammatory responses and foam cell formation associated with CD36 and ACAT1 upregulation in macrophages and inducing the migration and proliferation of VSMCs and collagen-1 production. Thus, developing blockade targets for CT-1 and its receptors may be a novel strategy in the treatment of atherosclerosis and its related diseases. Further studies are needed to identify the roles of CT-1 in other inflammatory vascular diseases, such as ischemia/reperfusion injury, aortitis, and diabetic angiopathy.

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**Disclosures**

None.

**References**

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Novelty and Significance

What Is New?
- Cardiotrophin 1 (CT-1), a cytokine belonging to the interleukin-6 family, has been recently focused in the field of hypertension. This study is the first to demonstrate that CT-1 accelerates the development of atherosclerotic plaques.

What Is Relevant?
- CT-1 is raised in the circulating blood in patients with essential hypertension and plays an important role in hypertension-induced left ventricular hypertrophy and vascular remodeling.

Summary
CT-1 accelerates atherosclerosis by stimulating the inflammatory response, oxidized low-density lipoprotein–induced foam cell formation in macrophages, and the migration, proliferation, and collagen-1 production in vascular smooth muscle cells. Blockade of CT-1 is expected to emerge as a new line of therapy against atherosclerosis in hypertension.
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