Cathepsin G deficiency decreases complexity of atherosclerotic lesions in apolipoprotein E-deficient mice

Naimeh Rafatian,1,2,5 Denuja Karunakaran,3 Katey J. Rayner,3,6 Frans H. H. Leenen,1,5 Ross W. Milne,4,6,7 and Stewart C. Whitman2,5,6†

1Hypertension Unit, University of Ottawa Heart Institute, Ottawa, Ontario, Canada; 2Vascular Biology Unit, University of Ottawa Heart Institute, Ottawa, Ontario, Canada; 3Cardiometabolic microRNA and Epigenetics Laboratory, University of Ottawa Heart Institute, Ottawa, Ontario, Canada; 4Diabetes and Atherosclerosis Laboratory, University of Ottawa Heart Institute, Ottawa, Ontario, Canada; 5Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Ontario, Canada; 6Department of Biochemistry, Microbiology, and Immunology, University of Ottawa, Ottawa, Ontario, Canada; and 7Department of Pathology and Laboratory Medicine, University of Ottawa, Ottawa, Ontario, Canada

Submitted 16 August 2012; accepted in final form 4 August 2013

N. Rafatian et al.

Am J Physiol Heart Circ Physiol 305: H1141–H1148, 2013. First published August 9, 2013; doi:10.1152/ajpheart.00618.2012.—Cathepsin G is a serine protease with a broad range of catalytic activities, including production of angiotensin II, degradation of extracellular matrix and cell-cell junctions, modulation of chemotactic responses, and induction of apoptosis. Cathepsin G mRNA expression is increased in human coronary atheroma vs. the normal vessel. To assess whether cathepsin G modulates atherosclerosis, cathepsin G knockout (Ctsg−/−) mice were bred with apolipoprotein E knockout (Apoe−/−) mice to obtain Ctsg+/−Apoe−/− and Ctsg−/−Apoe−/− mice. Heterozygous cathepsin G deficiency led to a 70% decrease in cathepsin G activity in bone marrow cells, but this reduced activity did not impair generation of angiotensin II in bone marrow-derived macrophages (BMDM). Atherosclerotic lesions were compared in male Ctsg−/−Apoe−/− and Ctsg+/−Apoe−/− mice after 8 wk on a high-fat diet. Plasma cholesterol levels and cholesterol distribution within serum lipoprotein fractions did not differ between genotypes nor did the atherosclerotic lesion areas in either the aortic root or aortic arch. Ctsg−/−Apoe−/− mice, however, showed a lower percentage of complex lesions within the aortic root and a smaller number of apoptotic cells compared with Ctsg+/−Apoe−/− littersmates. Furthermore, apoptotic Ctsg−/− BMDM were more efficiently engulfed by phagocytic BMDM than were apoptotic Ctsg+/+ BMDM. Thus cathepsin G activity may impair efferocytosis, which could lead to an accumulation of lesion-associated apoptotic cells and the accelerated progression of early atherosclerotic lesions to more complex lesions in Apoe−/− mice.

Atherosclerosis; cathepsin G; Apo E; lesion progression

CATHESINS G IS FOUND IN AZUROPHILIC granules of monocytes and neutrophils. It is a neutral serine protease expressed at the promyelocytic and promonocytic stage of hematopoiesis (13, 16), and its expression on the neutrophil cell surface is induced by chemoattractants (23). It is present in arteries (17), and its mRNA is more abundant in human atheromata relative to the normal vessel wall (19, 30). Cathepsin G has a broad range of functions, many of which could potentially enhance or retard development and progression of atherosclerotic lesions. An-

Agiotensin II is a key modulator of atherosclerosis by increasing inflammation and migration of immune cells and causing endothelial dysfunction and smooth muscle cell proliferation (20). Cathepsin G may participate in several steps in the renin angiotensin system, including the activation of prorenin (12) and the conversion of both angiotensinogen and angiotensin I to angiotensin II (23). Cathepsin G can regulate chemotaxis through cleavage of NH2-terminal residues of some chemokines like CCL15 to generate more potent chemotactic factors for monocytes and neutrophils (25). On the other hand, cathepsin G inhibits proatherogenic cytokines TNF-α and IL-8 (1, 5, 21). Cathepsin G can degrade extracellular matrix and basement membrane proteins either directly (7, 8) or through its ability to activate matrix metalloproteinases (26). Through cleavage of extracellular matrix as well as cell surface proteins, cathepsin G can disrupt cell-matrix and cell-cell adhesion, leading to cell detachment and anoikis (4, 24). Finally, cathepsin G has been shown to impair efferocytosis of apoptotic cells by cleavage of a cell surface “eat me” signal (15). Efficient efferocytosis is essential to maintain vascular health and to prevent the progression of early atherosclerosis to an advanced, vulnerable plaque.

Although the presence of cathepsin G has been shown in human atherosclerotic lesions and cathepsin G has a variety of functions that could potentially affect atherosclerosis development, the role of cathepsin G has never been studied in an animal model of human atherosclerosis. In the present study, we compared development of atherosclerotic lesions in male heterozygous cathepsin G knockout mice on an apolipoprotein (apo) E-deficient background (Ctsg+/−Apoe−/− mice) with lesion development in apo E-deficient littersmates that have two functional Ctsg alleles (Ctsg+/+Apoe−/− mice).

MATERIALS AND METHODS

Animals

Ctsg−/− mice were purchased from Medical Research Council Harwell (stock number: FESA: 001531). Apoe−/− mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and maintained in the Animal Care Facility at the University of Ottawa Heart Institute. Both strains of mice have been back-crossed to the C57BL/6 background. Ctsg−/− and Apoe−/− mice were bred, and F1 heterozygotes were mated with Apoe−/− mice to have an F2 generation of breeding mice that were Ctsg+/−Apoe−/−. The Ctsg−/−Apoe−/− mice were embryonically lethal. Therefore, the study was designed for both cathepsin G heterozygous knockout mice and cathepsin G wild-type (WT) mice.
on an Apoe–/– background. This study was approved by the University of Ottawa Animal Care Committee and conforms to the Guide for the Care and Use of Laboratory Animals of the Canadian Council on Animal Care.

For atherosclerosis studies, Ctsg+/+Apoe–/– or Ctsg+/+Apoe–/– male mice at 8 wk of age were fed a standard laboratory mouse diet supplemented with 21% (wt/wt) butterfat, 0.15% (wt/wt) cholesterol, and 19.5% (wt/wt) casein, but without sodium cholate (Harlan Teklad; Madison, WI), for 8 wk. Mice were then anesthetized by intraperitoneal injection of somnitol, and blood was collected by puncture from the left ventricle. Mice were perfused with PBS via a cannula placed in the left ventricle, with the perfusate exiting from the right atrium. Hearts were separated from the aorta at the base, embedded in optimum cutting temperature medium (OCT, Fisher Scientific, Ottawa, Ontario, Canada), and snap-frozen on a precooled metal plate immersed in liquid nitrogen. The imbedded hearts were then kept at −80°C.

Cathepsin G assay. Bone marrow cells were harvested in, and washed with, PBS. Protein was extracted by sonication following resuspension of the cells in assay buffer (1.5 M NaCl, 0.1 M Tris-HCl, pH 7.4) plus 1% Triton X-100 (Sigma, St. Louis, MO). The reaction was in a total volume of 90 μl: 20 μl of sample plus 60 μl of buffer (160 mM Tris-HCl and 1.6 M NaCl, pH 7.4) and 10 μl of the substrate, N-succinyl-Ala-Ala-Pro-Phe-βNA (20 mM in DMSO) (Sigma) at 25°C. The increase in absorbance at 410 nm due to the release of paranitroanilide per unit time was used as a measure of cathepsin G activity (3).

Isolation and Culture of Bone Marrow-Derived Macrophages

Cells were extracted by aspiration of bone marrow cells from mouse femurs and tibias with DMEM containing 10% fetal bovine serum, 15% L929 conditioned media, and 1% penicillin and streptomycin (Invitrogen, Burlington, Ontario, Canada). Cells were counted and plated at a concentration of 2 × 10^6 cells/ml. Cells were differentiated to macrophages after 7 days of incubation at 37°C.

Angiotensin I and II Assays

Bone marrow-derived macrophages (BMDM) were washed, and medium was replaced by serum-free RPMI 1640 for 2 days, either without acetylated low-density lipoprotein (ac-LDL) or with ac-LDL (Biomedical Technologies, Stoughton, MA) at a cholesterol concentration of 50 μg/ml (22). Cell media were collected in 15-ml tubes containing 10% of total volume EDTA-Na2 and 1,10-phenanthroline (inhibitor cocktail) (Sigma). After centrifugation, the media were immediately extracted on Sep-Pak C18 cartridges (Waters, Milford, MA). Cells were collected, washed with PBS, and then lysed by sonication in PBS containing the same inhibitor cocktail. Cell lysates were centrifuged and extracted on Sep-Pak 18 cartridges. Angiotensin peptides were assessed by radioimmunoassay after separation by high-pressure liquid chromatography (18).

Quantification of Atherosclerotic Lesions

Aortic root. The method for quantification of atherosclerotic lesions in tissue sections of the aortic root has been described in detail (10). Briefly, lesion size in the ascending aorta was determined from four, 10-μm Sudan IV-stained serial sections taken at 100-μm intervals with the first section (level 0) being defined as that including the ostia for the coronary arteries. Lesion area was defined as intimal tissue within the internal elastic lamina and was quantified using Image-Pro Plus software (version 6.2, Media Cybernetics, Silver Spring, MD) on images that were created using a digital CoolSNAP cf camera (Roper Scientific, Duluth, GA).

Aortic arch. Advential tissue was removed from formaldehyde-fixed aortas. Aortas were cut open longitudinally through the inner curvature of the aortic arch that extends down the whole length of the aortic tree. The tissue was laid out on a black background, and an image was taken. The extent of grossly discernible lesions vs. total aorta was quantified (10).

Complexity Analysis

Lesions with different layers of macrophages were considered simple lesions, whereas lesions with layers of macrophages together with smooth muscle cells, collagen within the intima, and necrotic cores were counted as complex lesions. Macrophages were identified by immunohistochemistry based on CD68 (ABD Serotec, Raleigh, NC) immunoreactivity. Area covered by smooth muscle cells was determined by immunohistochemistry based on α-smooth muscle actin (Abcam, Toronto, Ontario, Canada). Collagen was stained with Gomori Trichrome. Necrotic core area was analyzed with hematoxylin and eosin staining.

Lipids and Lipoproteins

Plasma (50 μl) was subjected to sepharose 6 fast protein liquid chromatography (FPLC). Cholesterol was measured in total plasma and in 0.5-ml FPLC fractions using commercial enzyme-based kits (Genzyme and Wako Bioproducts, respectively) (31).

Apoptosis

Aortic root cryosections were fixed and postfixed in 1% paraformaldehyde in PBS and acetic acid-ethanol mixture. Slides were stained according to the protocol for cryosections provided with the Apop Tag Red in situ apoptosis detection kit (Millipore, Billerica, MA).

Cleaved caspase 3 (Cell Signaling Technologies, Danvers, MA) was detected in acetic-fixed aortic root cryosections by immunohistochemistry.

Effrcytosis of Apoptotic Macrophages

BMDM were isolated from WT and Ctsg–/– mice (a generous gift from Drs. Rodger P. McEver and Bojing Shao, Oklahoma Medical Research Foundation) as described above and cultured for 7 days. Effrcytosis experiments were performed in a similar manner as previously described (32). Briefly, BMDM from Ctsg–/– and Ctsg+/+ mice were labeled with 5 μM Vybrant CFDA SE Cell Tracer Kit (Life Technologies) per the manufacturer’s instructions. Apoptosis was induced by treating the BMDM with 5 μg/ml staurosporine for 24 h, and >80% cell death was confirmed by trypan blue exclusion (cell viability: 16.8 ± 4.8%) and did not differ between Ctsg–/– and Ctsg+/+ BMDM. The CFDA SE-labeled apoptotic cells (9 × 10^6 cells/well) were added to the phagocytes or Ctsg–/– or Ctsg+/+ BMDM (5 × 10^6 cells/well) for 2 h before the phagocytes were washed vigorously four times with cold PBS before lysis in 0.1% Triton X-100. The fluorescence intensity of the lysates was determined by BioTek Synergy plate reader and corresponds to the degree of engulfment of CFDA SE-labeled apoptotic macrophages. Appropriate controls were also included in the experiment to ensure the CFDA SE-labeled apoptotic macrophages did not adhere to the plate during phagocytosis and autofluorescence was insignificant relative to CFDA SE labeled cells.

Statistical Analysis

Values are reported as means ± SE. One-way repeated-measures ANOVA followed by Newman-Keuls test was used. The level of significance was set at a P value of 0.05.

RESULTS

Characterization of Cathepsin G-Deficient Mice

Cathepsin G deficiency in mice on a WT background did not influence either fetal or postnatal development. However, cathepsin G homozygote knockout mice on an Apoe–/– back-
Ctsg+/−/Apoe−/− mice were born at the expected frequency and developed normally. Bone marrow leukocytes were isolated from Ctsg+/−/Apoe−/− and Ctsg+/+/Apoe−/− littermates, and cathepsin G activity was measured in cell lysates. Cathepsin G activity in bone marrow of Ctsg+/+/Apoe−/− was decreased by almost 70% relative to bone marrow from Ctsg+/−/Apoe−/− littermates (Fig. 1).

Circulating Lipoprotein and Cytokines

After 8 wk on the high-fat diet, plasma cholesterol concentrations were similar in Ctsg+/−/Apoe−/− and Ctsg+/+/Apoe−/− mice (1.348 ± 69 mg/dl) and Ctsg+/−/Apoe−/− (1.281 ± 69 mg/dl) mice. The FPLC serum lipoprotein profiles of the two strains also did not differ (not shown) with the cholesterol being found primarily in fractions containing very low-density, intermediate, and low-density lipoproteins.

Quantification and Characterization of Atherosclerotic Lesions

No differences were observed between Ctsg+/−/Apoe−/− and Ctsg+/+/Apoe−/− mice in either the number of atherosclerotic lesions (not shown) or the area occupied by the lesions within the aortic arch (Fig. 2A). En face analysis of atherosclerosis in the aortic arch also revealed no differences between the two groups (Fig. 2B). Partial cathepsin G deficiency did, however, result in a decreased percentage of lesions that were classified as complex at all levels of the aortic root (Fig. 3A). The advanced lesions in Ctsg+/−/Apoe−/− mice had less collagen (Fig. 3, B–D), fewer smooth muscle cells (Fig. 3, K–M), a higher number of macrophages (Fig. 3, E–G), and a smaller volume of necrotic core (Figs. 3, H–J) compared with Ctsg+/−/Apoe−/− mice. It is notable, however, that when only early lesions were compared, Ctsg+/−/Apoe−/− and Ctsg+/+/Apoe−/− mice did not differ in their respective numbers of CD68-positive macrophages per unit area (181 ± 4 vs. 182 ± 6). Although few lesions in the Ctsg+/−/Apoe−/− mice showed a complex morphology with a fibrous cap and smooth muscle cells, complex lesions for both mouse lines are shown in Fig. 3 to illustrate that, even when complex lesions of similar size are compared, those of Ctsg+/−/Apoe−/− mice appear to have smaller necrotic cores, less collagen, and fewer smooth muscle cells. Consistent with the greater numbers of CD68-positive macrophages in lesions of Ctsg+/+/Apoe−/− mice and the smaller necrotic cores, there were also fewer TUNEL-positive nuclei compared with lesions of Ctsg+/−/Apoe−/− mice (Fig. 4, A–C). The result of cleaved caspase 3 immunohistochemistry confirm the lower incidence of apoptosis in Ctsg+/−/Apoe−/− mice (Fig. 4D).

Angiotensin II Levels in Media and Cell

Angiotensin II levels were quantified in media and cell lysates from Ctsg+/−/Apoe−/− and Ctsg+/+/Apoe−/− BMDM, which had, or had not, been exposed to ac-LDL. No differences in the levels of angiotensin II were detected between Ctsg+/−/Apoe−/− and Ctsg+/+/Apoe−/− independently of whether the cells were, or were not, treated with ac-LDL (not shown). Likewise, lesion-associated immunoreactive angiotensin II appeared to be similar in aortic sections of Ctsg+/−/Apoe−/− and Ctsg+/+/Apoe−/− mice (not shown).

Efferocytosis

Since we observed reduced numbers of apoptotic cells in lesions of the Ctsg+/−/Apoe−/− mice, we determined whether cathepsin G could affect efferocytosis. BMDM isolated from Ctsg+/− and Ctsg+/+ mice were labeled with a fluorescent dye, subjected to apoptosis, and fed to nontreated Ctsg+/− or Ctsg+/+ macrophages, as described in MATERIALS AND METHODS. As shown in Fig. 5B, the degree of efferocytosis of apoptotic Ctsg+/− BMDM by both Ctsg+/+ and Ctsg+/− BMDM was markedly increased (240% and 180%, respectively) compared with that of apoptotic WT BMDM. In contrast, Ctsg+/−/
CATHEPSIN G AND Atherosclerotic Plaque Complexity

**A**

- **Graph**: Percent of complex lesions vs. distance from aortic root (µM).
- **Data**: Ctsg+/+Apoe−/−, Ctg+/−Apoe−/−.

**B**

- Images: Sections of aorta showing different levels of plaque complexity.

**C**

- Images: Sections showing collagen distribution.

**D**

- **Bar Graph**: Percent collagen.

**E**

- Images: Sections showing macrophage distribution.

**F**

- Images: Sections showing necrotic core.

**G**

- **Bar Graph**: Percent macrophage.

**H**

- Images: Sections showing smooth muscle cell (SMC) distribution.

**I**

- Images: Sections showing smooth muscle cell (SMC) distribution.

**J**

- **Bar Graph**: Percent necrotic core.

**K**

- Images: Sections showing collagen distribution.

**L**

- Images: Sections showing collagen distribution.

**M**

- **Bar Graph**: Percent SMC.
and Fig. 3. Analysis of the complexity and composition of atherosclerotic lesions in aortic roots of DISCUSSION

BMDM were not more efficient than Ctsg\(^{+/+}\) BMDM in engulfing either apoptotic Ctsg\(^{-/-}\) or apoptotic Ctsg\(^{+/+}\) BMDM.

DISCUSSION

The present study shows that a 70% reduction in cathepsin G activity does not affect the size of atherosclerotic lesions in the aorta of Apoe\(^{-/-}\) mice fed a high-fat diet for 8 wk, but it does slow the progression of early atherosclerotic lesions to more advanced complex lesions. Compared with lesions in the aortic root of Ctsg\(^{+-/}\) Apoe\(^{-/-}\) littermates, those of Ctsg\(^{-/-}\) Apoe\(^{-/-}\) mice are characterized by a paucity of fibrotic caps, less collagen and smooth muscles cells, more CD68-positive

Fig. 4. Apoptosis in atherosclerotic lesions of Ctsg\(^{-/-}\) Apoe\(^{-/-}\) and Ctsg\(^{+/+}\) Apoe\(^{-/-}\) mice. A: the number of TUNEL-positive nuclei per 1,000 nuclei. Values are means ± SE; n = 8 per group. B and C: TUNEL-positive and total nuclei (Hoechst-staining) in aortic root lesions of Ctsg\(^{-/-}\) Apoe\(^{-/-}\) (B) and Ctsg\(^{+/+}\) Apoe\(^{-/-}\) (C) mice (×100 magnification). D: percent area covered by cleaved caspase 3. Values are means ± SE; n = 6 per group. *Significant difference between Ctsg\(^{-/-}\) Apoe\(^{-/-}\) and Ctsg\(^{+/+}\) Apoe\(^{-/-}\) mice (P < 0.05).
macrophages, fewer numbers of apoptotic cells, and reduced necrotic cores. The difference between Ctg\(^{+/+}\)/Apo\(^{-/-}\) and Ctg\(^{+/+}\)/Apo\(^{-/-}\) mice occurs in the absence of differences in plasma cholesterol concentrations or differences in cholesterol distribution among lipoprotein subfractions. Although cathepsin G has been shown to activate and inactivate cytokines that could potentially contribute to the progression of atherosclerotic lesions, we saw no differences between Ctg\(^{+/+}\)/Apo\(^{-/-}\) and Ctg\(^{+/+}\)/Apo\(^{-/-}\) mice in the serum levels of relevant cytokines (results not shown). We had initially postulated that cathepsin G could potentially promote atherogenesis through its ability to activate the renin angiotensin system. However, BMDM from Ctg\(^{+/+}\)/Apo\(^{-/-}\) and Ctg\(^{+/+}\)/Apo\(^{-/-}\) mice did not differ in their respective production and secretion of angiotensin II, and no differences were observed between Ctg\(^{+/+}\)/Apo\(^{-/-}\) and Ctg\(^{+/+}\)/Apo\(^{-/-}\) mice in lesion-associated immunoreactive angiotensin II. It is, therefore, unlikely that the slower progression of atherosclerosis in Ctg\(^{+/+}\)/Apo\(^{-/-}\) mice can be attributed to impaired cathepsin G-mediated generation of angiotensin II.

By limiting the cellularity of the lesion during the early stages of atherosclerosis, apoptosis is considered beneficial, whereas, in the later stages of atherosclerosis, apoptosis can lead to inflammation, instability, and greater vulnerability to rupture (9, 27). The reduced number of TUNEL-positive nuclei and cleaved caspase 3-stained area that was observed within lesions of Ctg\(^{+/+}\)/Apo\(^{-/-}\)/ mice could reflect a decrease in apoptosis and/or more efficient clearance of apoptotic bodies. When total lesions (early and complex) were analyzed, Ctg\(^{+/+}\)/Apo\(^{-/-}\) mice had increased numbers of lesion-associated CD68-positive macrophages compared with Ctg\(^{+/+}\)/Apo\(^{-/-}\) mice, but this difference was not apparent when early lesions were analyzed in isolation. Apoptosis of macrophages may, therefore, occur coincident with, and possibly contribute to, the transformation of simple lesions into complex lesions. Cathepsin G activity has been shown to promote apoptosis by several mechanisms. Due to its proteolytic activity, cathepsin G can cause the detachment of adhesion-dependent cells from the extracellular matrix and from surrounding cells resulting in anoikis, an anchorage-dependent programmed cell death (4, 6). In the case of cardiomyocytes, activation of protein phosphatase SHP2 by cathepsin G leads to dephosphorylation of focal adhesion kinase (FAK) and impaired activation of the downstream protein kinase B (AKT)-dependent survival pathways (24).

Efferocytosis, the process of engulfment of apoptotic bodies, becomes impaired in the later stages of atherosclerosis. With deficient efferocytosis, apoptotic bodies undergo secondary necrosis, and more apoptotic nuclei are detected in atherosclerotic lesions (28, 29). Cathepsin G has been shown to destroy an “eat me” signal on apoptotic neutrophils and thus prevents their engulfment by macrophages (15). Here, we have demonstrated that apoptotic macrophages from Ctg\(^{-/-}\) mice are efferocytosed more efficiently than phagocytes than are apoptotic Ctg\(^{+/+}\) BMDM (Fig. 5, A and B). In contrast, phagocytes deficient in cathepsin G did not differ in their ability to engulf apoptotic cells (Fig. 5B). This would suggest that apoptotic cells are the primary source of active cathepsin G and/or that cathepsin G may cleave an eat me signal on apoptotic macrophages, as has been reported for neutrophils (15).
proved efferocytosis of apoptotic cells due to the relative deficiency in cathepsin G activity may contribute to the reduced numbers of lesion-associated apoptotic bodies and, ultimately, to the decreased complexity of atherosclerotic plaques that are observed in the Ctsg<sup>−/−</sup>-Apoε<sup>−/−</sup> mice (Fig. 3).

The lesions of Ctsg<sup>−/−</sup>-Apoε<sup>−/−</sup> mice show a decreased content of collagen and decreased numbers of smooth muscle cells, and this appeared to be the case even when complex lesions of a similar size were compared. Decreased cathepsin G-mediated degradation of intracellular matrix or decreased cathepsin-G-mediated activation of other proteases capable of degrading extracellular matrix in lesions of Ctsg<sup>−/−</sup>-Apoε<sup>−/−</sup> mice may impede migration of smooth muscle cells into the intima. Since synthetic smooth muscle cells are major producers of collagen in atherosclerotic lesions (2), the decreased collagen may reflect the decreased numbers of lesion-associated smooth muscle cells. Although collagen is a substrate for cathepsin G, the reduced cathepsin G activity in Ctsg<sup>−/−</sup>-Apoε<sup>−/−</sup> mice did not lead to collagen accumulation in lesions.

The role of several broad range cysteine protease members of the cathepsin family has been investigated in mouse models of human atherosclerosis. Cathepsin S deficiency in bone marrow-derived cells of LDL receptor-deficient (CatS<sup>−/−</sup> × Ldlr<sup>−/−</sup>) mice fed a high-fat diet for 12 wk resulted in atherosclerotic lesions of the aortic root that did not differ in size from those in CatS<sup>−/−</sup> × Ldlr<sup>−/−</sup> mice but showed increased numbers of macrophages, decreased TUNEL-positive cells, decreased collagen, and a reduction in the necrotic core (11). The absence of an effect of leukocyte-specific cathepsin S deficiency on lesion size and changes in plaque morphology shows a remarkable similarity to what we observed with partial cathepsin G deficiency in Apoε<sup>−/−</sup> mice. It is possible that the two proteases participate sequentially in a pathway that promotes the evolution of the atherosclerotic lesion. Likewise, cathepsin K deficiency in bone marrow-derived cells also altered the morphology of aortic root lesions in Ldlr<sup>−/−</sup> mice fed a high-fat diet for 12 wk without changing lesion size (14). Again, lesions were characterized by an increased number of macrophages and decreased collagen but differed in having an increase in both the necrotic core and the TUNEL-positive nuclei. Thus these three members of the protease family appear to influence the progression of the atherosclerotic plaque but not the development of the initial fatty streak and may accomplish this through both mutual and distinct mechanisms. It is also possible that partial cathepsin G deficiency may modulate the expression or activation of other members of the cathepsin family, and this could further contribute to the observed phenotype.

The intrauterine death of the Ctsg<sup>−/−</sup>-Apoε<sup>−/−</sup> fetuses was unanticipated given the viability of Ctsg<sup>−/−</sup> mice when on a WT background. This could suggest a functional cathepsin G-apolipoprotein E interaction during fetal development. Alternatively, cathepsin G deficiency in the context of hyperlipidemia may be incompatible with normal growth of the fetus. Consistent with the latter, we have been unsuccessful to date in obtaining Ctsg<sup>−/−</sup> pups on an LDL receptor-deficient background. Experiments are underway to identify the mechanisms that are involved.

Taken together, our findings indicate that cathepsin G activity may not have a major role in the development of early atherosclerotic lesions but can promote the progression of these early lesions to more complex lesions, possibly by impeding the efferocytosis of apoptotic cells. Over longer periods of time, the larger necrotic zones associated with cathepsin G activity could favor the development of unstable plaques, whereas the increased numbers of lesion-associated smooth muscle cells and increased collagen content could provide a stabilizing influence. Additional experiments are required to elucidate the biological mechanisms and pathways that are involved, as well as the potential clinical consequences.

ACKNOWLEDGMENTS

The authors thank Mirela Hasu and Mohamed Thabet for help in maintaining the mouse colony and mouse dissection, and Dr. Rodger P. McEver at Oklahoma Medical Research Foundation for the generous gift of Ctsg<sup>−/−</sup> mice femurs.

At the request of the authors, readers are herein alerted to the fact that additional materials related to this manuscript (cytokine and angiogenesis II assays, additional photomicrographs) may be found at the institutional website of one of the authors, which at the time of publication is: http://www.ottawaheart.ca/content_documents/Rafatian_et_al_supplementary_figures_27-07-2013.pdf. These materials are not a part of this manuscript and have not undergone peer review by the American Physiological Society (APS). APS takes no responsibility for these materials, for the website address, or for any links to or from it.

GRANTS

Dr. Frans H. H. Leenen holds the Pfizer Chair in Hypertension Research, an endowed chair supported by Pfizer Canada, University of Ottawa Heart Institute Foundation, and Canadian Institutes of Health Research. This work was supported by grant NRA2580062 from Pfizer Investigator Initiated Research to Stewart C. Whitman.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES

H1148

CATHEPSIN G AND ATHEROSCLEROTIC PLAQUE COMPLEXITY


