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Mast Cells Promote Atherosclerosis by Inducing Both an Atherogenic Lipid Profile and Vascular Inflammation

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ABSTRACT

Accumulating in vitro and in vivo studies have proposed a role for mast cells in the pathogenesis of atherosclerosis. Here, we studied the role of mast cells in lipoprotein metabolism, a key element in the atherosclerotic disease. Male mice deficient in low-density lipoprotein receptors and mast cells on a Western diet for 26 weeks had significantly less atherosclerotic changes both in aortic sinus (55%, $P = 0.0009$) and in aorta (31%, $P = 0.049$), as compared to mast cell-competent littermates. Mast cell-deficient female mice had significantly less atherosclerotic changes in aortic sinus (43%, $P = 0.011$). Furthermore, we found a significant positive correlation between the extent of atherosclerosis and the number of adventitial/perivascular mast cells in aortic sinus of mast cell-competent mice ($r = 0.615$, $P = 0.015$). Serum cholesterol and triglyceride levels were significantly lower in both male (63%, $P = 0.0005$ and 57%, $P = 0.004$) and female (73%, $P = 0.00009$ and 54%, $P = 0.007$) mast cell-deficient mice, with a concomitant decrease in atherogenic apoB-containing particles and serum preβ-high-density lipoprotein and phospholipid transfer protein activity in both male (69% and 24%) and female (74% and 54%) mast cell-deficient mice. Serum soluble intercellular adhesion molecule was decreased in both male (32%, $P = 0.004$) and female (28%, $P = 0.003$) mast cell-deficient mice, whereas serum amyloid A was similar between mast cell-deficient and competent mice. In conclusion, mast cells participate in the pathogenesis of atherosclerosis in $ldlr^{-/-}$ mice by inducing both an atherogenic lipid profile and vascular inflammation. J. Cell. Biochem. 109: 615–623, 2010. © 2009 Wiley-Liss, Inc.

KEY WORDS: MAST CELL; ATHEROSCLEROSIS; LIPIDS; MOUSE MODEL

Mast cells are multipotent tissue-dwelling cells that commit to the mast cell lineage already in the bone marrow by expressing CD34 and c-kit [Kirshenbaum et al., 1991, 1999; Rottem et al., 1994]. The mast cell-committed progenitors leave the bone marrow, circulate in the blood as hematopoietic precursor cells [Kitamura and Ito, 2005], and, in the presence of specific chemotactic signals, notably stem cell factor (SCF) and eotaxin [Haley et al., 2000], they adhere to activated endothelial cells via $\alpha_4\beta_1$ integrins, vascular cell adhesion molecule (VCAM-1), and E-selectin [Boyce et al., 2002]. Upon activation of chemokine receptors, notably CXCR2, CCR3, CXCR4, and CCR5 [Ochi et al., 1999], the precursor cells migrate into tissues and differentiate into mature mast cells in the presence of appropriate growth factors, including SCF, interleukin-3 (IL-3), IL-4, IL-6, IL-9, and nerve growth factor (NGF) [Saito et al., 1996; Kinoshita et al., 1999; Kanbe et al., 2000; Matsuzawa et al., 2003; Lappalainen et al., 2007]. The crucial requirement of a functional SCF and c-kit signaling system for mast cell growth and development is emphasized by the

Abbreviations used: ALAT, alanine aminotransferase; apoB, apolipoprotein B; CAD, coronary artery disease; CETP, cholesterol ester transfer protein; CHD, coronary heart disease; FPLC, fast-performance liquid chromatography; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; IFN, interferon; IL, interleukin; LCAT, lecithin–cholesterol acyltransferase; LDL, low-density lipoprotein; NGF, nerve growth factor; PLTP, phospholipid transfer protein; SAA, serum amyloid A; SCF, stem cell factor; sICAM, soluble intercellular adhesion molecule; TNF, tumor necrosis factor; VCAM, vascular cell adhesion molecule; VLA, very late antigen; VLDL, very low density lipoprotein.

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fact that lack of c-kit signaling in mice results in mast cell deficiency [Kitamura et al., 1978; Chabot et al., 1988; Copeland et al., 1990], whereas an elevated expression of c-kit in humans induces mastocytosis [Castells et al., 1996]. Similarly, injection of SCF into the skin of humans results in local accumulation of mast cells [Costa et al., 1996]. In mice, most of the mutations in the c-kit gene, which block the c-kit tyrosine kinase-dependent signaling, cause not only mast cell deficiency but also several other phenotypic abnormalities, such as macrocytic anemia, sterility, and impaired melanogenesis [Nakano et al., 1989; Tsai et al., 2002]. However, in the W-sash (W^{sh}) mice, an inversion mutation in the transcriptional regulatory elements upstream of the c-kit transcription start site seems to affect merely pigmentation and mast cell development [Tono et al., 1992; Nagle et al., 1995], making this particular model more suitable for investigation of mast cell biology in vivo [Grimbaldeston et al., 2005].

Abundant in vitro data have suggested a role for mast cells both in the early and late pathogenesis of atherosclerosis [Lindstedt et al., 2007]. In the early steps of atherogenesis, activated mast cells may increase the amount of low-density lipoprotein (LDL) cholesterol in the vascular intima, by increasing endothelial permeability [Kubes and Granger, 1996], by increasing the intra- and extra-cellular accumulation and retention of LDL [Kovanen, 1993], by decreasing the ability of high-density lipoprotein (HDL) to remove cholesterol from intimal foam cells [Lee et al., 1992, 2003a], and by inactivating phospholipid transfer protein (PLTP) [Lee et al., 2003b] and cholesterol ester transfer protein (CETP) [Lee-Rueckert et al., 2008]. In the late steps of atherogenesis, mast cells may secrete proinflammatory cytokines and proteolytic enzymes that predispose atherosclerotic plaques to erosion and rupture, and thereby contribute to acute coronary syndromes, including myocardial infarction [Lindstedt et al., 2007]. The mechanisms by which mast cells may induce atherothrombotic events include adverse remodeling of the atherosclerotic plaque [Lindstedt and Kovanen, 2004], induction of smooth muscle cell death [Leskinen et al., 2001, 2003], induction of death [Lätti et al., 2003], or detachment [Mäyränpää et al., 2006] of vulnerable endothelial cells, and triggering coronary artery spasm [Kalsner and Richards, 1984; Forman et al., 1985; Laine et al., 1999].

Recent in vivo data using $apoE^{-/-}$ and $ldlr^{-/-}/Kit^{W\text{-}sh/W\text{-}sh}$ mice support the in vitro concept that mast cells participate in the pathogenesis of atherosclerosis by inducing vascular leakage, lipid accumulation, CXCR2/VLA-4-mediated recruitment of leukocytes, intraplaque hemorrhage, macrophage apoptosis, and by releasing proinflammatory cytokines, such as IL-6 and interferon-γ (IFN-γ) [Bot et al., 2007; Lindstedt et al., 2007; Sun et al., 2007]. However, despite increased mast cell-mediated deposition of lipids in the aortic arch and the thoracic-abdominal aortas in mast cell-competent $ldlr^{-/-}$ mice, no significant differences have been observed in the levels of plasma lipids and lipoproteins [Sun et al., 2007]. We demonstrate here that mast cells in both male and female mice on an $ldlr^{-/-}$ background challenged with a Western-type diet, participate in the pathogenesis of atherosclerosis by inducing an atherogenic lipid and lipoprotein profile as well as by triggering vascular inflammation.

MATERIALS AND METHODS

ANIMALS

Animal protocols and characterization of atherosclerotic lesions. $Ldlr^{-/-}$ mice (Jackson Laboratories) were crossbred with $Kit^{W\text{-}sh/W\text{-}sh}$ mice (Jackson Laboratories) to generate mast cell-deficient LDL receptor knockout ($ldlr^{-/-}/Kit^{W\text{-}sh/W\text{-}sh}$) mice and mast cell-competent LDL receptor knockout ($ldlr^{-/-}$) mice susceptible to Western diet-induced atherosclerosis. All mice used in this study were syngeneic in the C57BL/6 background. The experiments were conducted in conformity with the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals, incorporated in the Institute for Laboratory Animal Research (ILAR) Guide for Care and Use of Laboratory Animals, and the protocols were approved by The National Animal Care and Use Committee of Finland. To induce atherosclerosis, 12-week-old male and female mice from both groups were fed a Western diet (21% fat, 0.15% cholesterol; Harlan-Teklad 88137, Madison, WI) for 26 weeks after which the mice were analyzed for the development of atherosclerotic lesions. The vasculature was perfused transcardially with sterile PBS (Cambrex, USA). The heart and the whole aorta were dissected out, and the aorta was fixed in PBS with 4% PFA (Sigma, Germany) overnight. After removal of the adventitia under a preparation microscope, the aorta was pinned to wax plates and stained en face with Sudan IV (Fluka, Germany). The heart including the aortic root was frozen in OCT embedding medium (Sakura Tissue Tek, USA). Ten 10-μm cryosections were collected at 100 μm intervals starting at the level of the aortic cusps. Sections were stained with oil-red-O (Sigma-Aldrich, Inc., St. Louis, MO) and counterstained with hematoxylin, or with toluidine blue (Fluka). Tissue sections were viewed with a Nikon Eclipse E600 microscope (Nikon Co., Tokyo, Japan) and photographed with a digital camera (Spot RT color operated with Spot advanced software, version 4.1; Diagnostic Instruments, Sterling Heights, MI). The total area with positive color was quantified from the images in a blinded fashion using computer-assisted image quantification (Image-Pro Plus software).

Serum and liver analysis. Blood was drawn after an overnight fasting period and serum was obtained by a standard low-speed centrifugation and stored at −80°C. Total cholesterol (CHOD-PAP 1489232 kit; Roche Diagnostics GmbH, Mannheim, Germany), choline-containing phospholipids (990-54009; Wako Chemicals GmbH, Neuss, Germany or Diagnostic Systems, Holzheim, Germany) and triglycerides (GPO-PAP 1488872 kit; Roche Diagnostics GmbH) were measured using enzymatic methods. Soluble intercellular adhesion molecule (sICAM-1; CD-54) was determined by an ELISA kit from Pierce (Thermo Scientific, Rockford, IL) and serum amyloid A (SAA) by an ELISA kit (R&D Systems, Inc., Minneapolis). Alanine aminotransferase (ALAT) activity was determined from serum (15 μl) in a 96-well plate using a Perkin Elmer HTS7000Plus plate reader according to the manufacturer's protocol (ref 981769, Thermo Scientific). Liver samples were snap-frozen and kept at −80°C until analysis. Lipids were extracted using the Folch method [Folch et al., 1957]. Liver extracts were analyzed for total cholesterol, triglycerides, and choline-containing phospholipids as described above.

Size-exclusion chromatography of serum lipoproteins. Serum lipoproteins were fractionated by fast-performance liquid chromatography (FPLC; Merck-HPLC System) using Superose 6 HR 10/30 size-exclusion chromatography column (GE Healthcare, Buckinghamshire, UK). The column was equilibrated with 10 mM Na-phosphate buffer, pH 7.4 containing 140 mM NaCl, and then 110 μl of serum was applied to the column with a flow rate of 0.5 ml/min, fractions of 0.5 ml were collected, and analyzed for cholesterol, triglycerides, phospholipids (as described above), and PLTP activity.

Assay of serum PLTP activity. For the radiometric PLTP activity assay, phosphatidylcholine (PC) liposomes were prepared essentially as described by Damen et al. [1982] and the activity assay was carried out as described by Jauhiainen and Ehnholm [2005]. Prior to analysis, the serum samples were diluted 1:10 with assay buffer and 4 μl of the dilution was used for phospholipid transfer assay.

Quantification of preβ-HDL by crossed immunoelectrophoresis. Freshly isolated serum samples from mice were incubated at +37°C for 3 h in the presence of iodoacetate to measure the ability of serum to generate preβ-HDL in vitro. Iodoacetate (1 mmol/L) was added to prevent maturation of the formed preβ-HDL into α-HDL by serum LCAT activity. The crossed immunoelectrophoresis consisted of agarose electrophoresis of 2 μl serum per well in the first dimension for separation of lipoproteins with preβ- and α-mobility. Electrophoresis in the second dimension (i.e., antigen migration from the first gel into a rabbit anti-mouse apoA-I-antiserum-containing gel) was used to quantitatively precipitate apoA-I. Areas under the preβ-HDL and α-HDL peaks were calculated by multiplication of peak height and width at half peak height. The preβ-HDL area is expressed as a percentage of the sum of α-HDL and preβ-HDL areas. When indicated, preβ-HDL concentrations are also given in absolute amounts (micrograms of apoA-I present in preβ-HDL per milliliter plasma). These values were calculated from the percentage of apoA-I present in preβ-HDL and the total plasma apoA-I concentrations. All other details have been described previously [van Haperen et al., 2000].

STATISTICS

Data are expressed as mean ± SD and a two-tailed Student's *t*-test was used to compare two groups. A level of $P < 0.05$ was considered statistically significant.

RESULTS

Male mice deficient in LDL receptors and mast cells ($ldlr^{-/-}/Kit^{W\text{-}sh/W\text{-}sh}$), which were fed a Western diet consisting of 21% (wt/wt) total fat and 0.15% (wt/wt) cholesterol for 26 weeks, displayed significantly less atherosclerosis (31%, $P = 0.049$) when measured as the percentage of Sudan IV stained lipid in en face prepared aortas, as compared to mast cell-competent ($ldlr^{-/-}Kit^{+/+}$) littermates (Fig. 1A,B). However, no significant differences were observed in the aortic lesion size between mast cell-deficient and mast cell-competent female mice (Fig. 1B). Further analysis of oil-red O-stained atherosclerotic lesion areas in the aortic sinus (Fig. 2A) demonstrated that both male (55%, $P = 0.0009$) and female (43%, $P = 0.011$) mast cell-deficient mice had significantly smaller lesions, as compared to the mast cell-competent mice (Fig. 2B). Furthermore, we found a significant positive correlation between the extent of atherosclerosis and the number of adventitial/perivascular mast cells in aortic sinus of mast cell-competent mice ($r = 0.615$, $P = 0.015$, Fig. 2A,C).

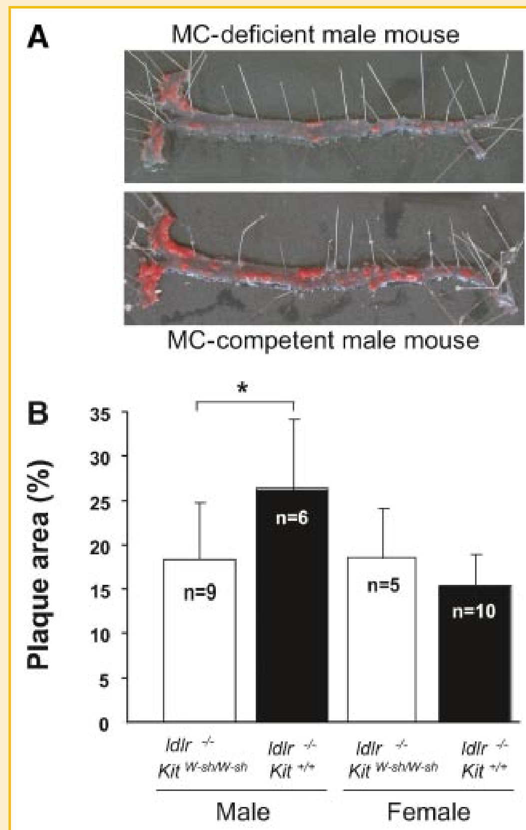

Fig. 1. Analysis of atherosclerosis in the aorta of mast cell-deficient and -competent $ldlr^{-/-}$ mice on a Western diet. Mast cell-deficient and -competent $ldlr^{-/-}$ mice were fed a Western diet for 26 weeks after which the percentage of Sudan IV stained lipid in en face prepared aortas was determined. A: Photographs of en face prepared aortas from mast cell-deficient and -competent $ldlr^{-/-}$ male mice. B: Quantification of the plaque area in aortas from mast cell-deficient and -competent $ldlr^{-/-}$ mice on a Western diet. $^{*}P < 0.05$, n = number of animals in the group. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

To investigate the mechanism of increased accumulation of neutral lipids in the atherosclerotic lesions in the mast cell-competent mice, we first measured the levels of serum lipids. Serum total cholesterol was significantly lower in both male (63%, $P = 0.0005$) and female (73%, $P = 0.00009$) mast cell-deficient mice (Fig. 3A). Similarly, serum triglyceride levels were significantly lower in both male (57%, $P = 0.004$) and female (54%, $P = 0.007$) mast cell-deficient mice, as compared to mast cell-competent animals (Fig. 3B). In addition, the concentration of serum phospholipids was found to be significantly reduced in both male (55%,

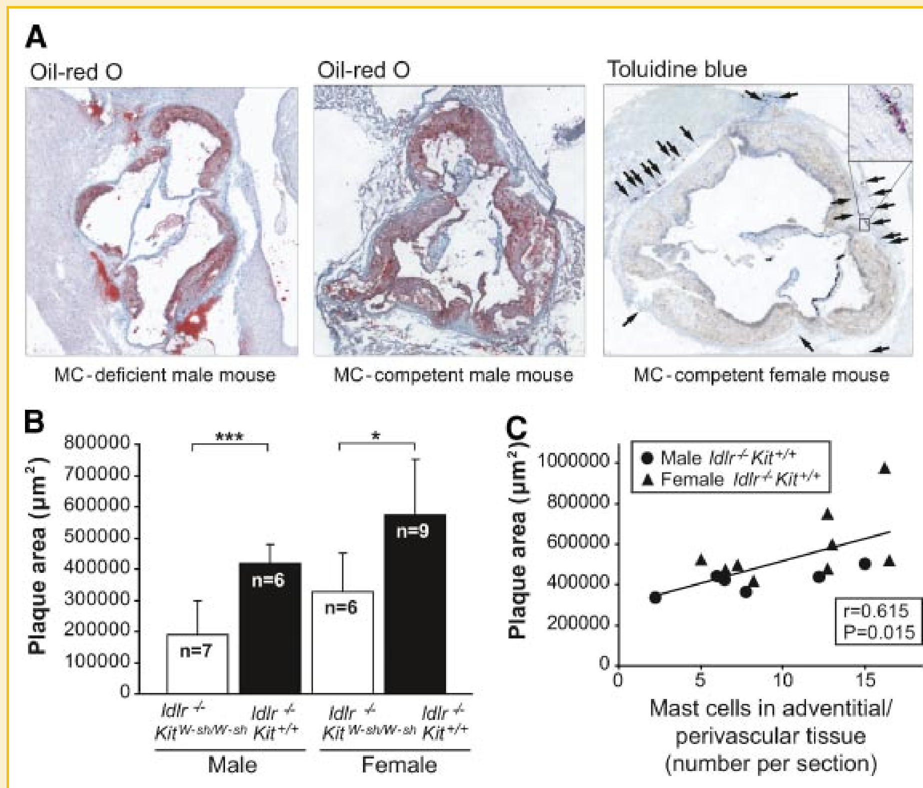

Fig. 2. Analysis of atherosclerosis in the aortic sinus of mast cell-deficient and -competent *Idlr*$^{-/-}$ mice on a Western diet. Mast cell-deficient and -competent *Idlr*$^{-/-}$ mice were fed a Western diet for 26 weeks after which the percentage of oil-red-O stained lipid and mast cells in aortic sinus were determined. A: Light microscopy pictures of oil-red-O stained aortic sinus from mast cell-deficient and -competent *Idlr*$^{-/-}$ male mice, and of toluidine blue stained aortic sinus from mast cell-competent *Idlr*$^{-/-}$ female mouse. Arrows indicate adventitial/perivascular mast cells. B: Quantification of the plaque area in aortic sinus of mast cell-deficient and -competent *Idlr*$^{-/-}$ mice on a Western diet. C: Quantification of mast cells in adventitial/perivascular tissue of aortic sinus in mast cell-competent *Idlr*$^{-/-}$ mice on a Western diet. Values in (B) and (C) are mean of observations in four sections. $^{*}P<0.05$, $^{***}P<0.001$, n = number of animals in the group. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

$P=0.0104$) and female (70%, $P=0.001$) mast cell-deficient mice, as compared to mast cell-competent animals (Fig. 3C). As shown in Figure 4A, liver cholesterol was slightly, but significantly decreased in both male (14%, $P=0.0053$) and female (14%, $P=0.013$) mast cell-deficient animals. Similarly, liver triglycerides were reduced (35%, $P=0.042$) in the female mast cell-deficient mice as compared to control animals, but not in the male animals (Fig. 4B). There were no significant differences in the levels of liver phospholipids between the various groups of mice (Fig. 4C). The serum activity of ALAT, which is often used as a marker of liver steatosis, was clearly reduced both in male (66%, $P=0.006$) and female (73%, $P=0.0005$) mast cell-deficient mice (Fig. 5).

To analyze the distribution of lipids among serum lipoproteins of the mast cell-deficient and competent mice challenged with the Western diet in more detail, we subjected pooled mice sera to Sepharose 6 HR FPLC to fractionate the lipoproteins. As shown in Figure 6, the lipoprotein profile showed increased levels of apoB-containing particles, that is, very low density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), and LDL, which is typical for *ldlr*$^{-/-}$ mice. Interestingly, decreased levels of total serum cholesterol (Fig. 6A), triglycerides (Fig. 6B), and phospholipids (Fig. 6C) in the mast cell-deficient mice were specifically found in these apoB-containing lipoproteins. However, under the feeding conditions, the levels of cholesterol (Fig. 6A) and phospholipids (Fig. 6C) in the HDL fraction were slightly increased in the mast cell-deficient mice.

As the preβ-HDL subfraction of HDL is functional in the reverse cholesterol transport process, we analyzed the levels of serum preβ-HDL in more detail. There was a substantial reduction in the levels of serum preβ-HDL measured at +37°C in male (69%) and female (74%) mast cell-deficient mice (Fig. 7). Moreover, activity of serum PLTP, an important regulator of HDL subclass distribution, was found to be decreased both in male (24%) and female (54%) mice deficient in mast cells (Fig. 7B).

Since mast cells are proinflammatory cells and an increased PLTP activity could be a marker of increased inflammation [Barlage et al., 2001], we analyzed the serum levels of SAA and sICAM, two commonly used markers of systemic and vascular inflammation, respectively. As shown in Figure 8A, there were no significant differences in serum SAA levels between the animal groups. In contrast, serum sICAM levels were markedly decreased in both male (32%, $P=0.004$) and female (28%, $P=0.003$) mast cell-deficient mice (Fig. 8B), reflecting decreased vascular inflammation.

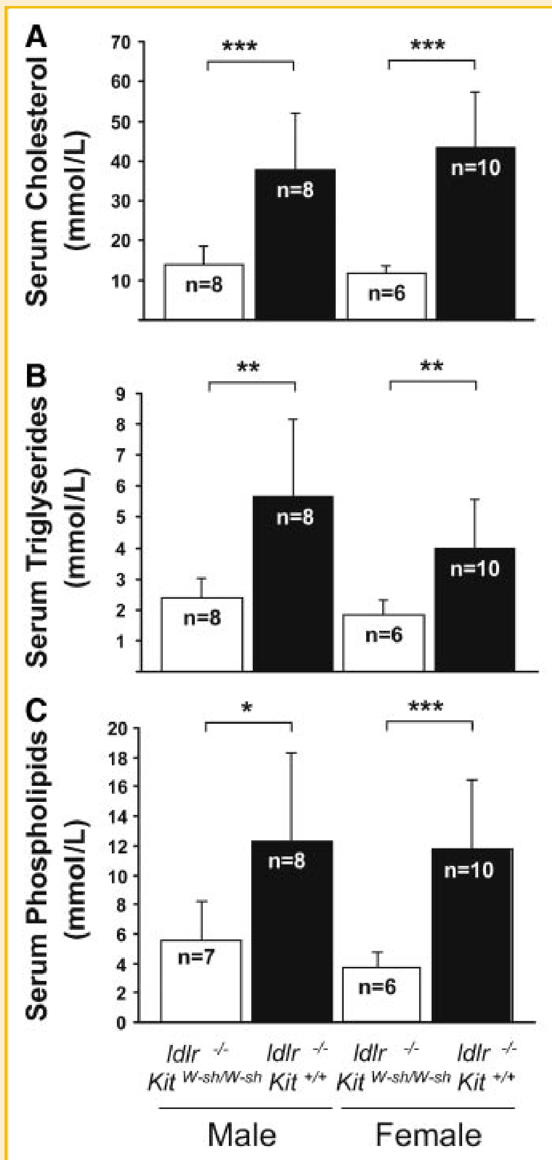

Fig. 3. Determination of serum cholesterol, triglycerides, and phospholipids in mast cell-deficient and -competent $Idlr^{-/-}$ mice on a Western diet. Mast cell-deficient and -competent $Idlr^{-/-}$ mice were fed a Western diet for 26 weeks after which the amount of serum cholesterol (A), triglycerides (B), and phospholipids (C) was determined. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, n = number of animals in the group.

DISCUSSION

In this study, we present further evidence for the role of mast cells in atherogenesis by showing that mast cell-deficient $ldlr^{-/-}$ mice fed a Western diet had less atherosclerosis as compared to mast cell-competent $ldlr^{-/-}$ mice. By inducing an atherogenic serum lipoprotein profile, mast cells were found to trigger vascular inflammation and increase the degree of atherosclerosis in both the aortas and the aortic sinus in male mice, while in the female mice only aortic sinus showed increased atherosclerosis. We also found a significant positive correlation between the extent of atherosclerosis

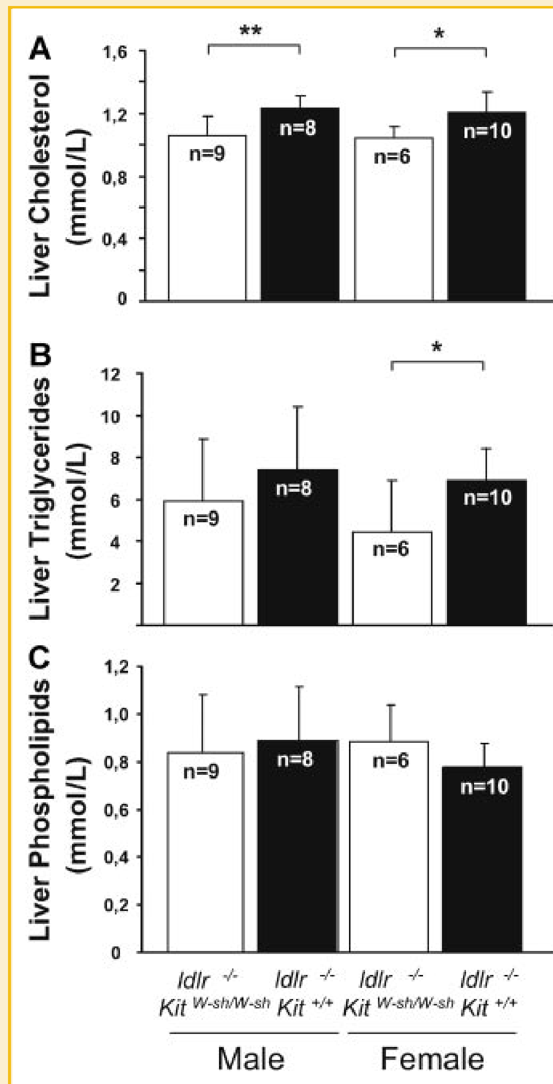

Fig. 4. Determination of liver cholesterol, triglycerides, and phospholipids in mast cell-deficient and -competent $Idlr^{-/-}$ mice on a Western diet. Mast cell-deficient and -competent $Idlr^{-/-}$ mice were fed a Western diet for 26 weeks after which the amount of liver cholesterol (A), triglycerides (B), and phospholipids (C) was determined. $^{*}P<0.05$, $^{**}P<0.01$, n = number of animals in the group.

and the number of adventitial/perivascular mast cells in aortic sinus of mast cell-competent mice. The mast cell-induced atherogenic lipoprotein profile was characterized by an increased concentration of serum cholesterol, triglycerides, and phospholipids, which were found to be present in the pro-atherogenic apoB-containing lipoprotein fractions (VLDL, IDL, LDL). In addition, the level of serum preβ-HDL and the activity of serum PLTP were found to be increased.

MAST CELLS IN MOUSE ARTERIAL WALL

In our previous and present studies, we have failed to observe mast cells in the aortic intima of healthy, normolipidemic mice, an observation that clearly contrasts findings in the normolipidemic human aortic intima, in which mast cells are found abundantly

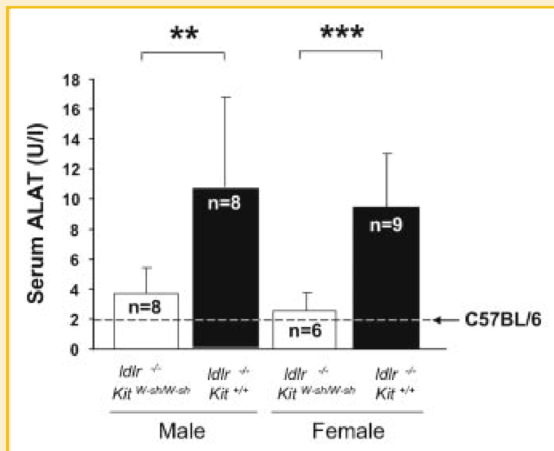

Fig. 5. Determination of serum ALAT activity in mast cell-deficient and -competent $Idlr^{-/-}$ mice on a Western diet. Mast cell-deficient and -competent $Idlr^{-/-}$ mice were fed a Western diet for 26 weeks after which the amount of serum ALAT activity was determined. The basal level of ALAT in C57BL/6 mouse without Western diet is shown with dash line. $^{**}P < 0.01$, $^{***}P < 0.001$, n = number of animals in the group.

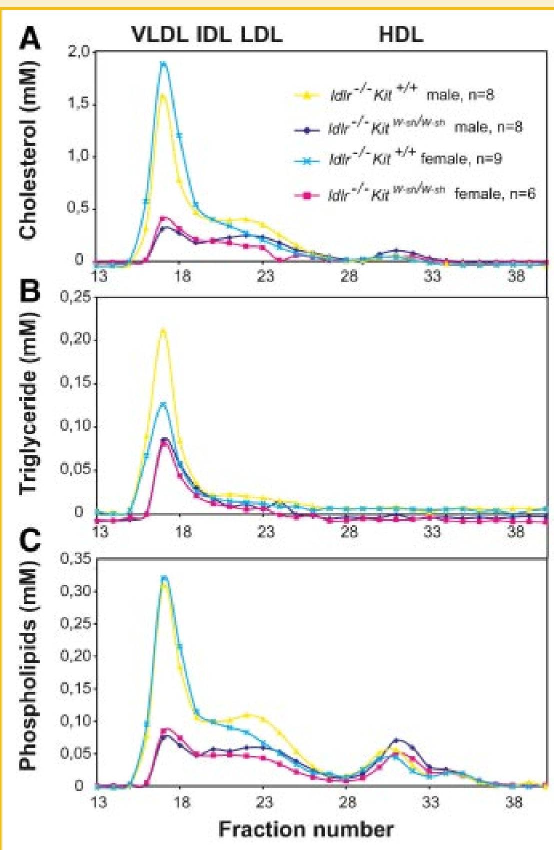

Fig. 6. Analysis of the distribution of lipids among serum lipoproteins of the mast cell-deficient and -competent $Idlr^{-/-}$ mice on a Western diet. Mast cell-deficient and -competent $Idlr^{-/-}$ mice were fed a Western diet for 26 weeks after which the distribution of cholesterol (A), triglyceride (B), and phospholipids (C) among the serum lipoproteins was determined from serum pools of each group of mice. n = number of animals in the group. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

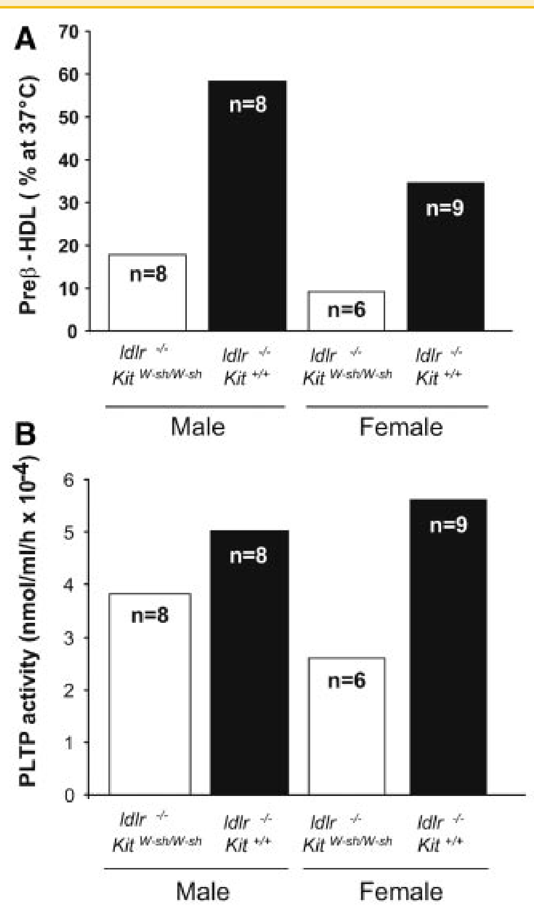

Fig. 7. Analysis of the levels of serum preβ-HDL and activity of PLTP in mast cell-deficient and-competent $Idlr^{-/-}$ mice on a Western diet. Mast cell-deficient and-competent $Idlr^{-/-}$ mice were fed a Western diet for 26 weeks after which the levels of serum preβ-HDL (A) and the activity of PLTP (B) were determined from serum pools of each group of mice. n = number of animals in the group.

[Kaartinen et al., 1994]. The lack of mast cells in normal mouse intima may depend on the fact that it is a very thin layer of extracellular matrix that rarely contains any cells. Moreover, according to our present observations, mast cells were not found in mouse atherosclerotic lesions, suggesting that even diet-induced formation of atherosclerotic lesions in the mouse intima does not induce infiltration of mast cells into the lesions, a finding contrasting a recent study in which surprisingly abundant amounts of mast cells in mouse atherosclerotic lesions were observed [Sun et al., 2007]. In contrast, we show that the adventitial layer of normal mouse aorta contains significant amounts of mast cells, and previous work in $apoE^{-/-}$ mice has clearly revealed a significant role for perivascular mast cells both in the formation of atherosclerotic plaques and in plaque instability [Bot et al., 2007]. Thus, in the hyperlipidemic mouse models currently used for studies on atherogenesis, some of the atherosclerotic processes occurring in the intimal layer may be regulated by the adventitial mast cells via active cross-talk between these two layers, rather than by mast cells in the lesions themselves. Indeed, previous results in humans,

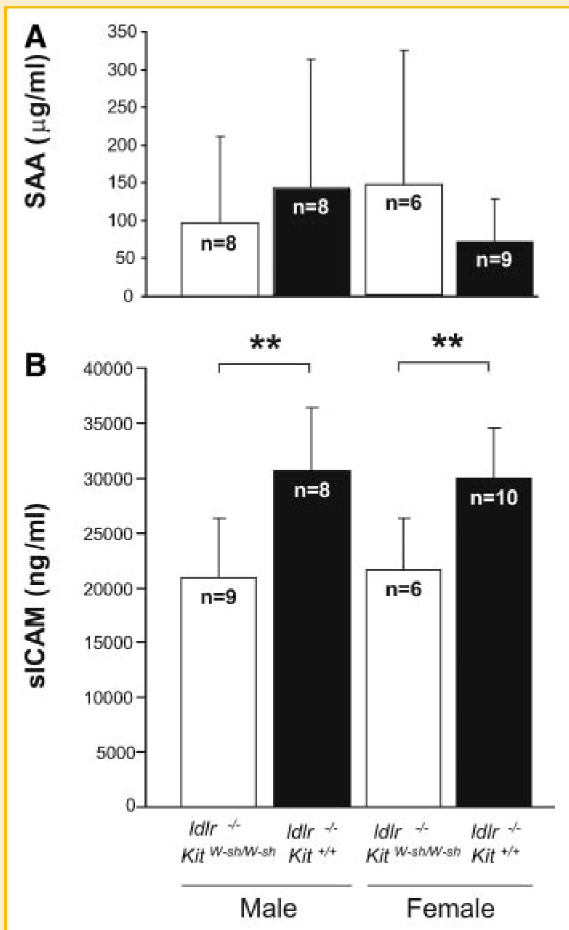

Fig. 8. Analysis of the levels of serum amyloid A (SAA) and soluble intercellular adhesion molecule (sICAM) in mast cell-deficient and -competent *Idlr*$^{-/-}$ mice on a Western diet. Mast cell-deficient and -competent *Idlr*$^{-/-}$ mice were fed a Western diet for 26 weeks after which the total levels of serum amyloid A (SAA) (A) and soluble intercellular adhesion molecule (sICAM) (B) were determined. $^{**}P < 0.01$, n = number of animals in the group.

showing an association between myocardial infarction and mast cells in the adventitia of infarct-related human coronary arteries [Laine et al., 1999], support a role for adventitial mast cells in the progression of atherogenesis.

MAST CELLS IN ATHEROSCLEROSIS

In the present study, we chose to crossbreed *ldlr*-deficient mice with mast cell-deficient mice ($Kit^{W\text{-}sh/W\text{-}sh}$) to obtain a model that would be suitable for studying the role of mast cells in atherosclerosis in vivo. Indeed, parallel studies by Sun et al. [2007] have shown that this type of double knockout animals develop less atherosclerosis due to a reduced production and release of pro-inflammatory cytokines, notably IL-6 and IFN-γ. Our present study showing decreased levels of serum sICAM in the double knockouts confirms the involvement of mast cells in vascular inflammation in this particular mouse model. However, in contrast to previous results [Sun et al., 2007], our present results demonstrate significant changes in serum lipid and lipoprotein profiles. The present data show that mast cell-deficient ($Kit^{W\text{-}sh}/Kit^{W\text{-}sh}$) mice on an *ldlr*$^{-/-}$ background have lower levels of serum total cholesterol, triglycerides, and phospholipids, thus displaying a less atherogenic lipoprotein profile. The observed reduction in triglyceride and phospholipid levels in the mast cell-deficient mice may suggest higher levels of lipase activities and call for future studies in this important field of lipoprotein metabolism. Interestingly, it was recently shown that T-cell-derived lymphotoxin and LIGHT, two proinflammatory members of the TNF-alpha cytokine family, are critical regulators of key enzymes that control lipid metabolism [Lo et al., 2007]. LIGHT overexpression in LDLr$^{-/-}$ mice caused significant elevation of serum cholesterol and triglyceride levels with decreased expression and activity of hepatic lipase that could explain the hyperlipidemia observed in LIGHT transgenic mice. Since mast cell-deficient mice also show reduced serum levels of cholesterol and triglycerides, it is possible that mast cell deficiency could attenuate T-cell LIGHT expression, and thereby lead to increased activity of hepatic lipase. However, this was not the case since the levels of hepatic lipase did not differ between the control and mast cell knockout mice (data not shown).

In addition, low levels of apoB-containing particles could be due to changes in the production/secretion of VLDL–LDL particles from the liver or reduction in hepatic triglyceride synthesis that in turn would affect VLDL assembly. Reduction of their levels in circulation will ultimately cause attenuation of atherosclerosis. This was recently demonstrated in LDLr$^{-/-}$ ApoB$^{100/100}$ Mttp$^{flox/flox}$ Mx1-Cre mouse model via a genetic switch to lower plasma lipoproteins [Skogsberg et al., 2008]. However, transcriptional changes in genes involved in hepatic lipid or apoB biosynthesis or those involved in β-oxidation were not studied here. Moreover, since mast cells are abundant in the intestine, they may affect the intestinal absorption of cholesterol. Thus, the observed hyperlipidemia and increased hepatic fat in the mast cell competent mice could conceivably be caused by increased mast cell-mediated cholesterol absorption.

The role of mast cells in atherogenesis in vivo has also been studied in Steel-Dickie (Sl/Sl^{d}) mice that are mast cell-deficient due to a mutation causing a deletion of the transmembrane and cytoplasmic domains of SCF [Dileepan et al., 2004]. In this particular study, the mice that were fed a high-fat diet containing 22.5% cocoa butter, 3.75% cholesterol, 1.5% sodium cholate, and 0.375% cholic acid (so called Paigen diet) for 17 weeks had an increased intima-media thickness and an elevated granulopoiesis and erythropoiesis, no differences in total plasma cholesterol levels, but reduced concentrations of plasma triglycerides [Dileepan et al., 2004]. However, in our present study using a Western diet with 0.15% cholesterol and no cholate, we observed a reduction in all measured serum lipids in the mast cell-deficient mice, but did not observe any significant differences in the leukocyte count (data not shown). Moreover, it is difficult to carry out direct comparisons between these two studies, since the atherosclerosis-inducing diets differed substantially and included potentially hepatotoxic substances, such a cholic acid.

MAST CELL EFFECTS ON HDL METABOLISM IN VIVO

By affecting the levels of preβ-HDL and the activities of PLTP mast cells may influence the reverse cholesterol transport homeostasis.

Surprisingly, in contrast to previous in vitro studies [Lee et al., 2003b], mouse mast cells seem to increase the activity of serum PLTP and the serum levels of preβ-HDL. Indeed, the increased amount of preβ-HDL may be explained by the observed increased activities of PLTP in these mice. In terms of mast cell-induced induction of preβ-HDL, this effect would be considered anti-atherosclerotic, since preβ-HDL is one of the major acceptors of cholesterol in peripheral tissue, and thus, a regulator of reverse cholesterol transport in the body. However, the fact that these mice still have increased levels of atherosclerosis, suggests that the protective role of the formed preβ-HDL is insufficient. Interestingly, also human studies have documented an increased plasma level of small preβ-HDL particles in men with CHD as compared to healthy men [Asztalos et al., 2004]. Mast cell-deficient mice have decreased levels of PLTP activity and less inflammation. High levels of plasma PLTP activity correlates with increased coronary artery disease (CAD) in humans [Schlitt et al., 2003]. The PLTP activity, however, may also have proatherogenic effects [Schlitt et al., 2005], and increased PLTP levels have been shown to correlate with increased inflammation, which supports our present results of vascular inflammation. However, unlike systemic PLTP which has been associated with an increase in atherosclerotic lesion areas, PLTP might be antiatherogenic when its expression is restricted to macrophages [Valenta et al., 2006]. However, recently Vikstedt et al. [2007] reported opposite findings demonstrating that macrophage-derived PLTP is a significant contributor to plasma PLTP activity and deficiency of PLTP in macrophages leads to lowered atherosclerotic lesion development in LDLr-deficient mice on Western-type diet. Therefore, at the time being, the role of PLTP in atherosclerosis is not resolved.

In conclusion, deficiency of mast cells in mice with the $ldlr^{-/-}$ background on a Western diet attenuates the pathogenesis of atherosclerosis by inducing a less atherogenic lipid profile as well as by reducing vascular inflammation.

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