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The effect of ABCG1 deficiency on atherosclerotic lesion development in LDL receptor knockout mice depends on the stage of atherogenesis

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ABSTRACT

Objective: As ABCG1 plays a role in cholesterol efflux, macrophage ABCG1 expression has been suggested to protect against atherosclerosis. However, we and others observed varying effects of ABCG1 deficiency on atherosclerotic lesion size. The objective of this study was to define the effect of ABCG1 deficiency during atherosclerotic lesion progression in LDL receptor knockout ($LDLr^{-/-}$) mice.

Methods and results: $ABCG1^{-/-}/LDLr^{-/-}$ and $ABCG1^{+/+}/LDLr^{-/-}$ littermates were fed a Western-type diet for 10 and 12 weeks in order to study the effect of ABCG1 deficiency in the exponential phase of atherosclerotic lesion formation. At 10 weeks of diet feeding, a significant 1.5-fold increase in early atherosclerotic lesion size ($130 \pm 12 \times 10^3$ μm^2) was observed in $ABCG1^{-/-}/LDLr^{-/-}$ mice compared to $ABCG1^{+/+}/LDLr^{-/-}$ mice ($88 \pm 11 \times 10^3$ μm^2; $p < 0.05$). Interestingly, in more advanced lesions, induced by 12 weeks of WTD feeding, $ABCG1^{-/-}/LDLr^{-/-}$ mice showed a significant 1.7-fold decrease in atherosclerotic lesion size ($160 \pm 20 \times 10^3$ μm^2 vs $273 \pm 19 \times 10^3$ μm^2 in control mice; $p < 0.01$), indicating that in the $ABCG1^{-/-}/LDLr^{-/-}$ mice progression of lesion formation is retarded as compared to $ABCG1^{+/+}/LDLr^{-/-}$ mice. In addition, correlation analysis performed on 7 independent published studies and the current study confirmed that ABCG1 is atheroprotective in early lesions, while the development of advanced lesions is stimulated.

Conclusions: It appears that the effect of ABCG1 deficiency on lesion development in $LDLr^{-/-}$ mice depends on the stage of atherogenesis, whereby the absence of ABCG1 leads to increased lesions at sizes $< 167 \times 10^3$ μm^2 while in more advanced stages of atherosclerosis enhanced apoptosis and/or compensatory mechanisms lead to retarded lesion progression.

1. Introduction

Reverse cholesterol transport (RCT), defined as the transport of excess cholesterol from peripheral tissues back to the liver for biliary excretion, plays an important protective role in the development of atherosclerosis [1,2]. Previously, the ATP-binding cassette (ABC) transporter A1 has been reported to play an important role in the prevention of atherosclerosis by facilitating cholesterol and phospholipid efflux from macrophages to lipid-poor or lipid-free apolipoprotein AI (apoAI) [3–6]. Similar to ABCA1, ABCG1 has been implicated in macrophage lipid homeostasis by actively effluxing cellular cholesterol to mature HDL [7,8]. As cholesterol efflux from macrophages is an important protective mechanism to prevent excessive cellular lipid accumulation, macrophage ABCG1 expression was expected to protect against atherosclerosis. However, independent groups have shown that ABCG1 might be pro-atherogenic as well as anti-atherogenic. Previously, we reported that both total body and macrophage ABCG1 deficiency led either to a significantly increased susceptibility to atherosclerotic lesion development [9,10] or to no change in lesion size [11,12]. In contrast, the group of Edwards et al. [13,14] and Tall et al. [15] reported decreased atherosclerosis in LDL receptor knockout ($LDLr^{-/-}$) mice transplanted with $ABCG1^{-/-}$ bone marrow cells, which was explained by accelerated apoptosis of $ABCG1^{-/-}$ macrophages or compensatory upregulation of ABCA1 expression and apoE secretion in macrophages lacking ABCG1. Thus, the role of macrophage ABCG1 in the development of atherosclerosis still remains uncertain.

The aim of this study was to assess the effect of ABCG1 deficiency on different stages of atherosclerotic lesion development and especially during the exponential phase of lesion formation in order to unravel the mechanism by which ABCG1 deficiency affects atherogenesis. Upon atherogenic diet feeding, total body

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ABCG1$^{-/-}$ mice develop only modest atherosclerotic lesions, therefore, we generated ABCG1/LDLr double knockout (ABCG1$^{-/-}$/LDLr$^{-/-}$) mice to perform this lesion stage dependent study.

2. Materials and methods

For detailed methodology, please refer to the data supplement.

ABCG1$^{+/-}$ mice, obtained from Deltagen Inc., San Carlos, CA, were cross-bred with single LDLr$^{-/-}$ mice to generate ABCG1$^{-/-}$/LDLr$^{-/-}$ mice on a C57Bl/6 background.

To induce atherosclerosis development, the mice were fed Western-type diet (WTD), containing 15% (w/w) cocoa butter and 0.25% (w/w) cholesterol (Diet W, Ab Diets, Woerden, The Netherlands) for 10 and 12 weeks after which the mice were euthanized and atherosclerotic lesion development was quantified in oil red O-stained cryosections. Furthermore, bone marrow cells were isolated from ABCG1$^{+/+}$/LDLr$^{-/-}$ and ABCG1$^{-/-}$/LDLr$^{-/-}$ and differentiated into bone marrow-derived macrophages to evaluate macrophage cholesterol efflux.

3. Results

3.1. Effect of ABCG1 deficiency on serum lipid levels and lipid homeostasis in tissues

On regular chow diet, containing 4.3% fat and no added cholesterol, no significant difference in total serum cholesterol levels were observed between ABCG1$^{+/+}$/LDLr$^{-/-}$ and ABCG1$^{-/-}$/LDLr$^{-/-}$ mice (Table 1). Fractionation of serum lipoproteins, however, showed a moderate shift of HDL cholesterol to the LDL and VLDL fraction in ABCG1$^{-/-}$/LDLr$^{-/-}$ mice (HDL: 75 ± 4 compared to 90 ± 3 mg/dL for ABCG1$^{+/+}$/LDLr$^{-/-}$ mice, $p < 0.05$; VLDL: 36 ± 3 compared to 19 ± 3 mg/dL, $p < 0.001$; and LDL: 123 ± 5 compared to 108 ± 4 mg/dL for ABCG1$^{+/+}$/LDLr$^{-/-}$ mice, $p < 0.05$) (Table 1 and Fig. 1A). To induce atherosclerotic lesion development, ABCG1$^{-/-}$/LDLr$^{-/-}$ and control mice were fed a WTD for 10 and 12 weeks, which induced approximately a 5-fold increase in serum cholesterol concentrations in both ABCG1$^{+/+}$/LDLr$^{-/-}$ and ABCG1$^{-/-}$/LDLr$^{-/-}$ mice. Both after 10 weeks and 12 weeks on the WTD, total serum cholesterol levels did not differ between the groups. Lipoprotein profiles of mice fed the WTD for 10 and 12 weeks were essentially identical. Therefore, the representative 12 weeks WTD profile of ABCG1$^{-/-}$/LDLr$^{-/-}$ and control mice on WTD is shown in Fig. 1A. A moderate increase in VLDL (~25%) and LDL (~23%) cholesterol levels was observed in ABCG1$^{-/-}$/LDLr$^{-/-}$ mice compared to control animals, which only reached significance for LDL after 12 weeks WTD feeding ($p < 0.01$) (Table 1). This increase in VLDL and LDL was associated with a moderate non-significant decrease in HDL (~16%) levels in ABCG1$^{-/-}$/LDLr$^{-/-}$. Furthermore, during the course of the experiment, the weight gain curve did not show significant differences between ABCG1$^{-/-}$/LDLr$^{-/-}$ and ABCG1$^{+/+}$/LDLr$^{-/-}$ mice both after 10 or 12 weeks on WTD (data not shown).

ABCG1 deficiency has been shown to coincide with increased secretion of apoE by macrophages and elevated serum apoE levels [15]. Immunoblotting for apoE was performed to analyze the association of ABCG1 deficiency with serum apoE levels of the LDLr$^{-/-}$ mice fed WTD for 10 and 12 weeks. No significant effect of ABCG1 deficiency was observed on serum apoE levels between the ABCG1$^{-/-}$/LDLr$^{-/-}$ and ABCG1$^{+/+}$/LDLr$^{-/-}$ mice fed WTD (Fig. 1B). In addition, ABCG1 deficiency in macrophages has been shown to be correlated with an induction of ABCA1 expression [15]. However, immunohistochemical staining of aortic root cryosections showed no apparent increase in ABCA1 expression in macrophages located in atherosclerotic lesions of ABCG1$^{-/-}$/LDLr$^{-/-}$ mice, either after 10 or 12 weeks WTD feeding (data not shown).

Abnormal lung morphology was observed in ABCG1$^{-/-}$/LDLr$^{-/-}$ mice compared with control mice. Both after 10 and 12 weeks of diet feeding, ABCG1 deficiency resulted in accumulation of large amounts of lipids in the subpleural regions of the lungs (Supplemental Fig. IA). In addition, spleens of ABCG1$^{-/-}$/LDLr$^{-/-}$ showed lipid accumulation in the red pulp regions, while no accumulation was observed in spleens of control mice (Supplemental Fig. IB).

3.2. Effect of ABCG1 disruption on atherosclerotic lesion formation

To define the role of ABCG1 in the exponential phase of atherogenesis, atherosclerotic lesion development was analyzed in the aortic root of ABCG1$^{+/+}$/LDLr$^{-/-}$ and ABCG1$^{-/-}$/LDLr$^{-/-}$ mice after 10 and 12 weeks of WTD feeding. Representative photomicrographs of the aortic root of control mice and mice deficient for ABCG1 are shown in Fig. 2A. After 10 weeks WTD feeding, a significant 1.5-fold increase in atherosclerotic lesion size was observed in the aortic root of ABCG1$^{-/-}$/LDLr$^{-/-}$ mice ($130 \pm 12 \times 10^3$ μm^2 [$n = 8$] compared to $88 \pm 11 \times 10^3$ μm^2 [$n = 7$] for ABCG1$^{+/+}$/LDLr$^{-/-}$ mice; $p < 0.05$). *In vitro* studies using bone marrow-derived macrophages of ABCG1$^{+/+}$/LDLr$^{-/-}$ and ABCG1$^{-/-}$/LDLr$^{-/-}$ mice showed that disruption of ABCG1 results in a 15% decrease in cholesterol efflux to HDL ($p < 0.001$), whereas the cholesterol efflux to ApoAI was unaffected (Fig. 2B). Additional 2 weeks of WTD feeding resulted in rapid progression of atherosclerotic lesion development in control mice (3.1-fold) (Fig. 2C). Interestingly, atherogenesis in the ABCG1-deficient mice appeared to be attenuated from 10 till 12 weeks and only a 1.2-fold increase in lesion size is noticed over this period. As a result, ABCG1-deficient mice showed a 1.7-fold smaller atherosclerotic lesions as compared to control mice after 12 weeks WTD feeding ($160 \pm 20 \times 10^3$ μm^2 [$n = 8$] compared to $273 \pm 19 \times 10^3$ μm^2 [$n = 9$]; $p < 0.01$, respectively) (Fig. 2A).

Disruption of ABCG1 in LDLr$^{-/-}$ mice also affected the composition of atherosclerotic lesions. Immunostaining for macrophages showed less staining in atherosclerotic lesions of ABCG1$^{-/-}$/LDLr$^{-/-}$ mice fed WTD for 10 weeks ($65 \pm 2\%$ of atherosclerotic area compared with $81 \pm 4\%$ in control mice; $p < 0.01$) (Fig. 3A). The lower macrophage content, coincided with an almost significantly larger percentual necrotic core area after 10 weeks on WTD ($22 \pm 5\%$ of atherosclerotic area compared with $8 \pm 3\%$ in control mice; $p = 0.06$). Additional 2 weeks of WTD feeding resulted in an increase in absolute macrophage area and necrotic core area (Fig. 3A). However, no significant differences could be observed between lesions of ABCG1$^{+/+}$/LDLr$^{-/-}$ and ABCG1$^{-/-}$/LDLr$^{-/-}$ mice. Furthermore, Masson Trichrome-staining showed no significant differences in collagen accumulation in the atherosclerotic plaques between the ABCG1-deficient mice and control mice after 10 weeks and 12 weeks of WTD feeding (data not shown).

As *in vitro* studies have recently demonstrated that macrophage ABCG1 deficiency is associated with increased susceptibility to apoptosis in response to the altered cellular lipid homeostasis [13], we examined the apoptotic macrophage content of the lesions by TUNEL staining. Lesions of ABCG1-deficient mice fed the WTD for 10 weeks showed no differences in TUNEL-positive macrophages compared with lesions of control mice (Fig. 3B). After 12 weeks of WTD feeding, a 2.5-fold increase in TUNEL-positive macrophages was observed in lesions of ABCG1$^{-/-}$/LDLr$^{-/-}$ mice compared with ABCG1$^{+/+}$/LDLr$^{-/-}$ animals ($p < 0.05$, $n = 7$–8). The decrease in atherosclerotic lesion size observed in ABCG1$^{-/-}$/LDLr$^{-/-}$ mice fed

Table 1
Serum lipid levels in ABCG1$^{-/-}$/LDLr$^{-/-}$ and control mice on chow and WTD.

Mice	Time (weeks)	Diet	Free cholesterol (mg/dL)	Total cholesterol (mg/dL)	VLDL-C (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)
ABCG1$^{+/+}$/LDLr$^{-/-}$	0	Chow	96 ± 4	230 ± 12	19 ± 3	108 ± 4	90 ± 3
	10	WTD	289 ± 32	1030 ± 36	361 ± 82	369 ± 42	58 ± 4
	12	WTD	289 ± 16	1010 ± 82	441 ± 53	374 ± 24	65 ± 6
ABCG1$^{-/-}$/LDLr$^{-/-}$	0	Chow	91 ± 4	233 ± 9	36 ± 3***	123 ± 5*	75 ± 4*
	10	WTD	292 ± 23	1206 ± 107	492 ± 81	451 ± 33	58 ± 6
	12	WTD	332 ± 20	1047 ± 111	536 ± 57	467 ± 19**	54 ± 5

Data represent the means ± SEM of 8 mice.
Abbreviations: WTD, Western-type diet; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; C, cholesterol.
* Statistical significance of $p < 0.05$ compared with ABCG1$^{+/+}$/LDLr$^{-/-}$ mice.
** Statistical significance $p < 0.01$ compared with ABCG1$^{+/+}$/LDLr$^{-/-}$ mice.
*** Statistical significance $p < 0.001$ compared with ABCG1$^{+/+}$/LDLr$^{-/-}$ mice.

WTD for 12 weeks, might therefore be a result of increased susceptibility to apoptosis of ABCG1-deficient macrophages inside the lesions.

Overall, these findings indicate that the effect of ABCG1 deficiency on atherosclerotic lesion development depends on the stage of atherogenesis. ABCG1 expression protects against early atherosclerotic lesion development, by facilitating cholesterol efflux from macrophages to HDL. In the more advanced lesions, however, accumulation of cholesterol due to impaired cholesterol efflux from ABCG1-deficient macrophages, eventually, will lead to increased macrophage apoptosis and a reduced further progression of atherogenesis.

4. Discussion

Several pathways are involved in the efflux of cholesterol from macrophages, including aqueous diffusion, SR-BI mediated cholesterol efflux, efflux dependent on macrophage apoE secretion, and active cholesterol efflux mediated by ABCA1 [16–18]. Also ABCG1 has been implicated in cellular lipid homeostasis by its property to actively efflux cholesterol to mature HDL [8,19]. Studies using genetically engineered mice have established the physiological importance of ABCG1. Targeted disruption of ABCG1 in mice resulted in age-related progressive pulmonary lipidosis when fed a regular chow diet [20–22]. In addition, overexpression of ABCG1 protected against diet-induced lipid deposition within multiple tissues [8]. These findings imply a critical role for ABCG1 in maintaining normal lipid metabolism in the lung, thereby preventing inflammatory responses triggered by massive cholesterol and/or cholesterol metabolite accumulation.

Although the critical role of ABCG1 in lung lipid homeostasis is clearly established, contradictory findings on the role of macrophage ABCG1 in the development of atherosclerosis have been reported by different groups/laboratories [9–15,23–25].

Transgenic mice overexpressing human ABCG1 showed either no effect [24] or increased atherosclerosis [25]. In contrast,

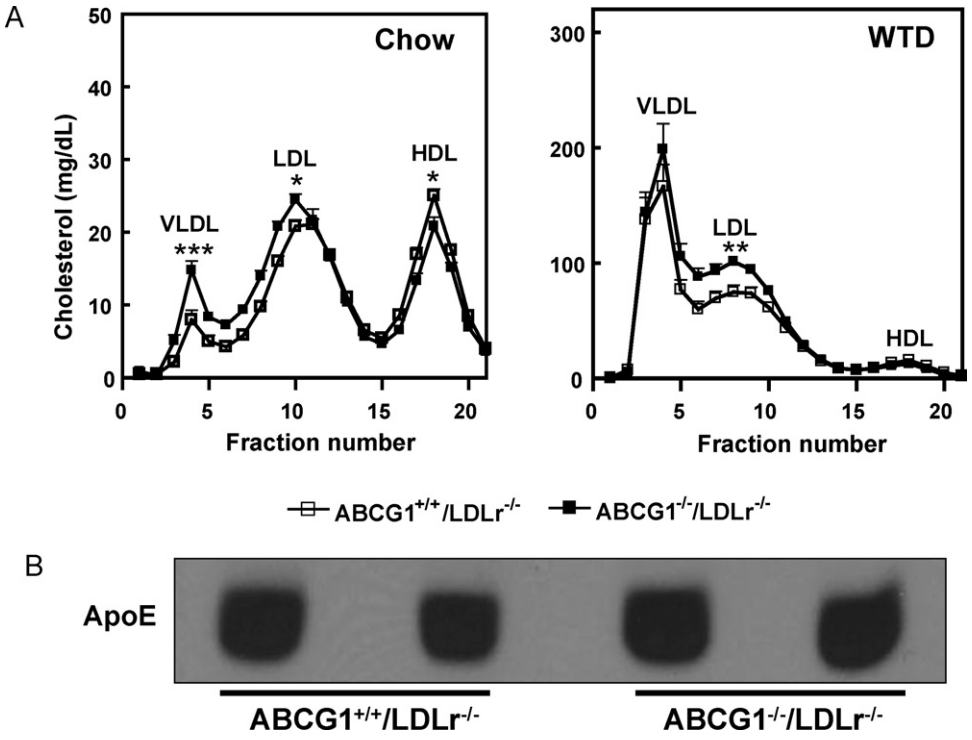

Fig. 1. The effect of ABCG1 deficiency on serum cholesterol distribution and apoE levels in LDLr$^{-/-}$ mice. (A) Blood samples were drawn after an overnight fasting period while feeding a regular chow diet and after 12 weeks on WTD. Sera from individual mice were loaded onto a Superose 6 column, and fractions were collected. Fractions 2–5 represent VLDL, fractions 6–14 represent LDL, and fractions 15–20 represent HDL. The distribution of cholesterol over the different lipoproteins in ABCG1$^{+/+}$/LDLr$^{-/-}$ (□) and ABCG1$^{-/-}$/LDLr$^{-/-}$ (■) mice is shown. Values represent the mean ± SEM of 8 mice per group. (B) A representative immunoblot of apoE in serum of ABCG1$^{+/+}$/LDLr$^{-/-}$ and ABCG1$^{-/-}$/LDLr$^{-/-}$ mice after 12 weeks of WTD feeding.

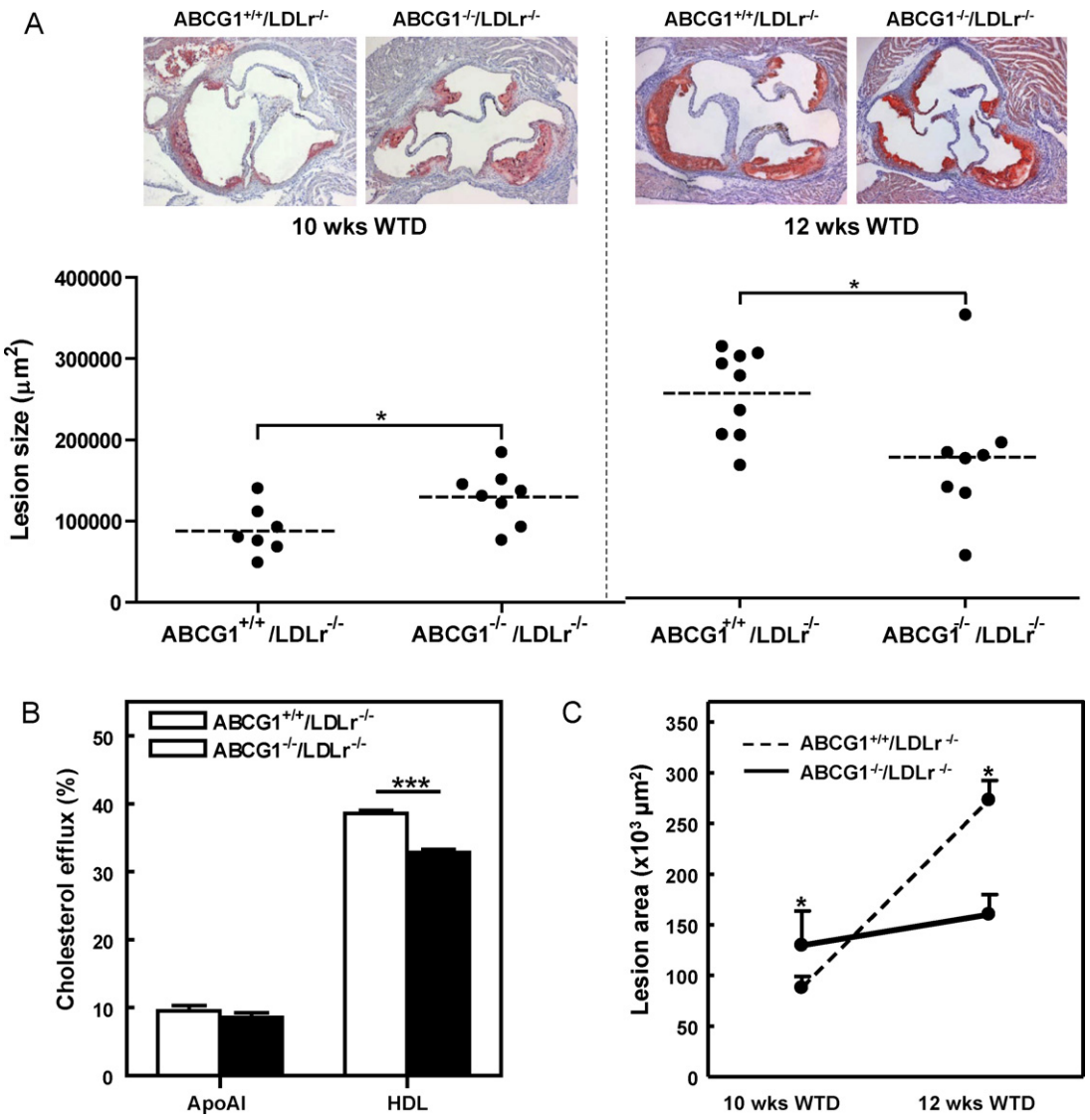

Fig. 2. The effect of ABCG1 deficiency on atherosclerotic lesion formation and cholesterol efflux in LDLr$^{-/-}$ mice. (A) Atherosclerotic lesion formation was determined in the aortic root at the level of the tricuspid valves of ABCG1$^{+/+}$/LDLr$^{-/-}$ and ABCG1$^{-/-}$/LDLr$^{-/-}$ mice fed a WTD for 10 and 12 weeks (separated by the dotted line). Mean lesion area of each individual mouse is shown. The horizontal dotted lines represent the means of each group of 7–9 mice. Representative photomicrographs of oil red O-stained lesions are shown (magnification 50×). (B) Cholesterol efflux to HDL is impaired in ABCG1-deficient macrophages. ApoA-I (10 μg/mL) and HDL (50 μg/mL) induced cellular cholesterol efflux from ^{3}H-cholesterol-labeled bone marrow-derived macrophages of ABCG1$^{+/+}$/LDLr$^{-/-}$ and ABCG1$^{-/-}$/LDLr$^{-/-}$ mice. Basal efflux to BSA (in the absence of added acceptors) has been subtracted from the data shown. Values represent the mean ± SEM of 4 animals. (C) Progression of atherosclerotic lesions of ABCG1$^{+/+}$/LDLr$^{-/-}$ and ABCG1$^{-/-}$/LDLr$^{-/-}$ mice from 10 to 12 weeks WTD feeding is shown. Values represent the mean ± SEM of 7–9 mice. Statistically significant difference $^{*}p < 0.05$ and $^{***}p < 0.001$ as compared to ABCG1$^{+/+}$/LDLr$^{-/-}$ controls.

Westerterp et al. [26] recently reported an atheroprotective role of vascular ABCG1, which is likely related to its role in the preservation of endothelial NO synthase activity. Furthermore, independent studies, using ABCG1-deficient mice or LDLr$^{-/-}$ mice transplanted with bone marrow cells of ABCG1-deficient mice, have shown that macrophage ABCG1 might be proatherogenic [9,10,12] as well as antiatherogenic [13–15]. In the present study, we show that ABCG1 deletion in LDLr$^{-/-}$ mice can both induce and attenuate atherosclerotic lesion development. ABCG1 deficiency led to a significant 48% increase in atherosclerotic lesion size after only 10 weeks Western-type diet feeding, while a significant 32% decrease in lesion size was observed after 12 weeks WTD feeding. These data imply that the effect of ABCG1 deficiency on atherosclerotic lesion development in LDLr$^{-/-}$ mice depends on the stage of atherogenesis.

The reduced atherosclerosis in LDLr$^{-/-}$ mice transplanted with ABCG1$^{-/-}$ bone marrow was suggested to be a result of compensatory induction of apoE secretion and ABCA1 expression in ABCG1-deficient macrophages [15]. In this study, however, both at 10 weeks and 12 weeks of WTD feeding, ABCG1$^{-/-}$/LDLr$^{-/-}$ mice showed no compensatory increase in serum apoE levels, although ABCG1$^{-/-}$/LDLr$^{-/-}$ mice did exhibit a decrease in atherosclerotic lesion development at 12 weeks of WTD feeding. These findings are in agreement with our previous studies showing that apoE mRNA and protein expressions were not affected upon deletion of ABCG1 [10,12]. In addition, no apparent increase in ABCA1 expression was observed in ABCG1-deficient macrophages in atherosclerotic lesions of LDLr$^{-/-}$. Furthermore, accelerated apoptosis was proposed as a mechanism for the reduced atherosclerosis susceptibility of LDLr$^{-/-}$ mice lacking ABCG1 in macrophages [13,14]. In agreement, in the present study, the more advanced atherosclerotic lesions of ABCG1$^{-/-}$/LDLr$^{-/-}$ mice fed WTD for 12 weeks were decreased in size and showed a significant increase in TUNEL-positive macrophages as compared to control mice. Macrophage apoptosis is an important

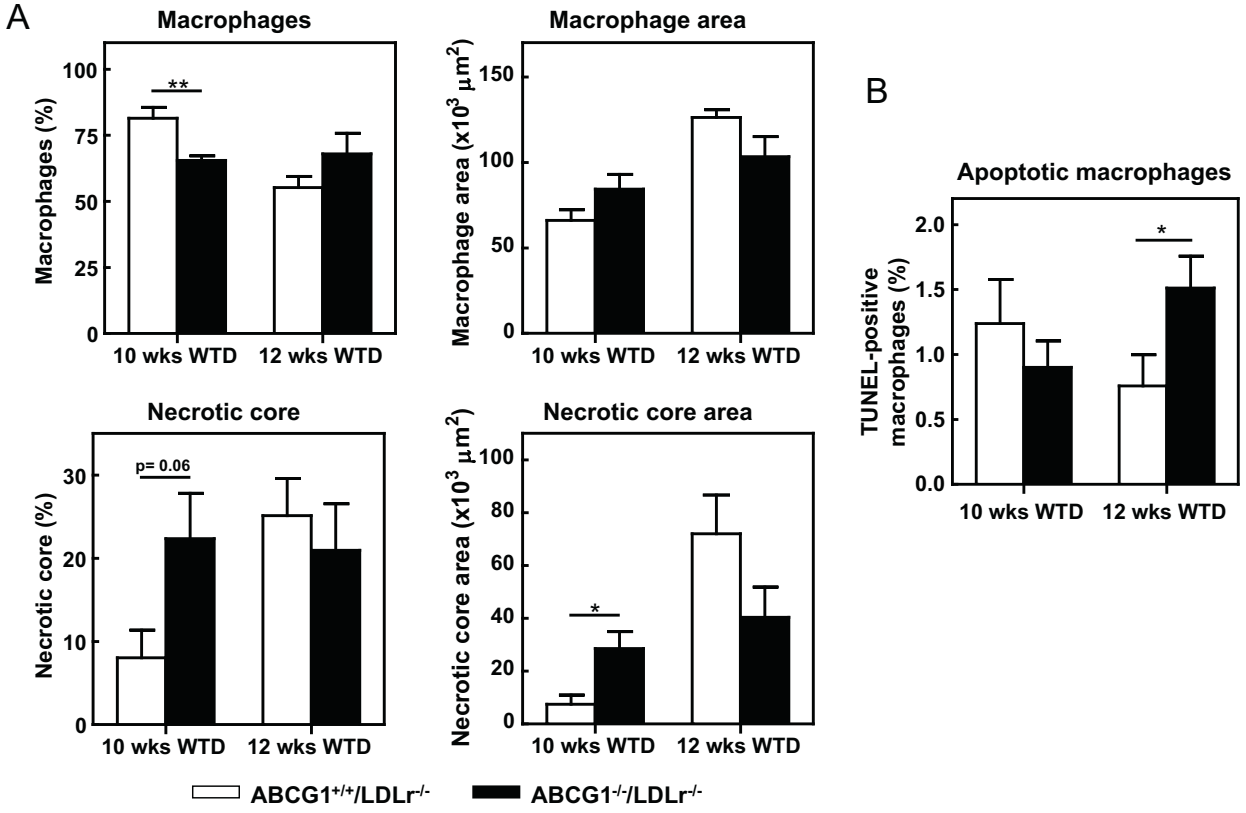

Fig. 3. Disruption of ABCG1 affects the composition of atherosclerotic lesions. (A) Quantification of lesion macrophages and necrotic core in ABCG1$^{+/+}$/LDLr$^{-/-}$ (open bars) and ABCG1$^{-/-}$/LDLr$^{-/-}$ (closed bars) after 10 and 12 weeks of WTD feeding are depicted. The macrophage lesion area and necrotic core area were histochemically quantified and expressed as % area of the lesions that is composed of macrophages or necrotic core (left) and expressed as absolute area (μm^2) (right). (B) Quantification of apoptotic macrophages after 10 and 12 weeks of WTD feeding using the TUNEL staining. Statistically significant difference $^*p < 0.05$ and $^{**}p < 0.01$ as compared to ABCG1$^{+/+}$/LDLr$^{-/-}$ controls.

feature of atherosclerotic plaque development and occurs during all stages of atherosclerosis with increasing frequencies as the plaque develops [27]. Research directed at understanding the functional consequences of macrophage death in atherosclerosis has revealed opposing roles for apoptosis in atherosclerotic plaque progression. Under normal physiologic conditions, apoptotic cells are rapidly cleared by phagocytes, a process called efferocytosis. In early atherosclerotic lesions, macrophage apoptosis, followed by efferocytosis limits lesion cellularity and suppresses plaque progression [28,29]. In advanced lesions, efferocytosis is defective and under these conditions macrophage apoptosis thus promotes the development of the necrotic core [30,31].

We show that lesions of ABCG1$^{-/-}$/LDLr$^{-/-}$ mice fed WTD for 10 weeks exhibited an increase in necrotic core in the absence of an increase in TUNEL-positive macrophages. In addition, in atherosclerotic lesions induced by 12 weeks WTD feeding, ABCG1 deficiency did not affect necrotic core size, despite a significant increase in macrophage apoptosis. These findings suggest that analyses of macrophage apoptosis are a snapshot and that there may be additional determinants of the lesional necrotic core area. Necrosis can be either secondary to apoptosis (secondary necrosis) or a primary process [32]. In particular, necrotic cores are formed by multiple processes, including accumulation of both intracellular and extracellular lipid [33,34]. Several studies have reported that ABCG1$^{-/-}$ macrophages are indeed more susceptible to oxLDL-induced apoptosis as compared to ABCG1-expressing cells [13,14,35]. Efflux of 7-ketocholesterol, the main oxysterol present in oxLDL, is completely dependent on expression of ABCG1 and not on the expression of ABCA1 [35]. Therefore, ABCG1-deficient macrophages show increased accumulation of 7-ketocholesterol upon oxLDL loading, which is cytotoxic to the cell and induces accelerated apoptosis [14,35] indicating that ABCG1 is essential for the prevention of oxLDL-induced apoptosis.

Our findings suggest that in addition to accelerated apoptosis, ABCG1 deficiency also leads to increased necrotic core formation. Under normal physiological conditions, necrotic core formation becomes more prominent in advanced lesions. In absence of ABCG1, however, necrotic core formation is already evident in early atherosclerotic lesions.

The current study indicates that the effect of ABCG1 deficiency on atherosclerotic lesion size depends on the stage of lesion development. To investigate the differential effects found on atherosclerosis susceptibility upon disruption of ABCG1, correlation analysis was performed on published studies of the independent groups, the current study, and one other unpublished study of our group. Experimental details of these studies are represented in Table 2. Although different diets and protocols were used in the individual studies, a high correlation ($R = 0.92$) can be found when the fold increase/decrease in atherosclerotic lesion size of ABCG1$^{-/-}$ mice compared to ABCG1$^{+/+}$ mice is plotted against the atherosclerotic lesion size of ABCG1$^{+/+}$ mice (Fig. 4). Based on this clear correlation, we propose that the effect of ABCG1 deficiency on lesion development depends on the stage of atherogenesis. In early atherosclerotic lesions (lesions $< 167 \times 10^3\ \mu m^2$), ABCG1 deficiency causes an increase in atherosclerotic lesion development (ratio > 1.0), most likely as a direct result of the impaired cholesterol efflux to HDL in ABCG1-deficient macrophages. This indicates that ABCG1 expression is protective in early atherosclerotic lesion development. Interestingly, at atherosclerotic lesion sizes above $167 \times 10^3\ \mu m^2$, the role of ABCG1 in

Table 2
Overview of experimental details of the different studies on the role of ABCG1 in atherosclerosis.

Author	Baldan 'et al. [13]	Lammers et al. (unpublished)	Tarling et al. [14]	Meurs et al. (present study)	Ranalletta et al. [15]	Tarling et al. [14]	Out et al. [11]	Ranalletta et al. [15]	Out et al. [9]	Out et al. [9]	Lammers et al. [12]	Meurs et al. (present study)	Yvan-Charvet et al. [23]	Out et al. [10]
Total body (TB) or macrophage deficiency (Mφ)	Mφ deficiency	Mφ deficiency	TB deficiency	TB deficiency	Mφ deficiency	Mφ deficiency	Mφ deficiency	Mφ deficiency	Mφ deficiency	Mφ deficiency	Mφ deficiency	TB deficiency	Mφ deficiency	TB deficiency
Diet	21% fat 1.25% chol	21% fat 1.25% chol	21% fat 0.2% chol	15% fat 0.25% chol	21.2% fat 0.2% chol	21% fat 0.2% chol	15% fat 0.25% chol	21.2% fat 0.2% chol	15% fat 0.25% chol	15% fat 0.25% chol	21% fat 1.25% chol	15% fat 0.25% chol	1.25% chol 7.5% fat 0.5% cholic acid	15% fat 1% chol 0.5% cholate
Time diet (weeks)	16	12	16	12	11	12	8	7	6	12	6	10	12	12
ABCG1$^{+/+}$ lesion area (μm^2)	500,000	674,600	700,000	272,600	238,100	550,000	113,000	77,900	4,9000	123,000	96,000	87,800	9100	24,000
ABCG1$^{-/-}$ Lesion Area (μm^2)	300,000	459,800	475,000	187,000	180,000	480,000	118,000	84,000	65,000	167,000	137,000	129,600	15,400	46,000
ABCG1$^{+/+}$ TC (mg/dL)	1083	1326	1065	1011	1182	1105	760	911	676	632	1018	1030	241	210
ABCG1$^{-/-}$ TC (mg/dL)	1043	1176	935	1047	1172	1132	800	829	511	670	1031	1206	215	220
Lesion Area relative to ABCG1$^{+/+}$	0.60	0.68	0.68	0.69	0.76	0.87	1.04	1.08	1.33	1.36	1.43	1.48	1.70	1.92

Abbreviations: Mφ, macrophage; TB, total body; chol, cholesterol; TC, total cholesterol.

Fig. 4. Relative increase/decrease in atherosclerotic lesion size of ABCG1$^{-/-}$/LDLr$^{-/-}$ mice compared to ABCG1$^{+/+}$/LDLr$^{-/-}$ mice plotted to atherosclerotic lesion size of ABCG1$^{+/+}$/LDLr$^{-/-}$ mice. Data from bone marrow transplantation studies in LDLr$^{-/-}$ mice and total body studies by different groups, the present study, and unpublished studies are included (experimental details represented in Table 2). In early atherosclerotic lesions (lesions < 167 × 10^3 μm^2), ABCG1 deficiency causes an increase in atherosclerotic lesion development (ratio > 1.0), while at atherosclerotic lesion sizes above 167 × 10^3 μm^2, enhanced apoptosis and/or compensatory mechanisms lead to retarded lesion progression. The numbers given in the graph represent the reference numbers of the different studies.

atherogenesis switches from anti-atherosclerotic to pro-atherosclerotic. In more advanced lesions, the persistent impaired cholesterol efflux from ABCG1-deficient macrophages is likely to induce accumulation of (oxy)sterols, which leads to enhanced apoptosis/compensatory mechanisms and, subsequently, decreased atherosclerotic lesion size (ratio < 1.0). Previously, we [10] have also reported a highly significant correlation when the fold increase/decrease in atherosclerotic lesion size of ABCG1$^{-/-}$ as compared with ABCG1$^{+/+}$ is plotted against total serum cholesterol whereby at about 900 mg/dL serum cholesterol a switch from ABCG1's protective function to lesion formation was noticed. When recent published studies of the independent groups, the current study, and two other unpublished studies of our group are included, again a high correlation between the fold increase/decrease in atherosclerotic lesion size and total serum cholesterol is observed with a switch at 1000 mg/dL serum cholesterol from an atheroprotective function of ABCG1 to a proatherogenic function ($p = 0.0075$; $R = 0.73$). Therefore, under normal physiological levels of cholesterol, the role of ABCG1 in atherogenesis is likely to be protective. Furthermore, since higher serum cholesterol levels are associated with a more rapid development of atherosclerotic lesions, this correlation is probably also a direct effect of the stage of atherosclerotic lesion development.

In conclusion, our results indicate that the effect of ABCG1 on lesion development depends on the stage atherogenesis, whereby the absence of ABCG1 leads to increased lesions at sizes < 167 × 10^3 μm^2 while in more advanced stages of atherosclerosis enhanced apoptosis and/or compensatory mechanisms lead to retarded lesion progression.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2011.11.024.

References

[1] Glomset JA. The plasma lecithins: cholesterol acyltransferase reaction. J Lipid Res 1968;9:155–67.
[2] von Eckardstein A, Nofer JR, Assmann G. High density lipoproteins and arteriosclerosis. Role of cholesterol efflux and reverse cholesterol transport. Arterioscler Thromb Vasc Biol 2001;21:13–27.
[3] Higgins CF. ABC transporters: from microorganisms to man. Annu Rev Cell Biol 1992;8:67–113.
[4] van Eck M, Bos IS, Kaminski WE, et al. Leukocyte ABCA1 controls susceptibility to atherosclerosis and macrophage recruitment into tissues. Proc Natl Acad Sci USA 2002;99:6298–303.
[5] Aiello RJ, Brees D, Bourassa PA, et al. Increased atherosclerosis in hyperlipidemic mice with inactivation of ABCA1 in macrophages. Arterioscler Thromb Vasc Biol 2002;22:630–7.
[6] Oram JF, Lawn RM, Garvin MR, Wade DP. ABCA1 is the cAMP-inducible apolipoprotein receptor that mediates cholesterol secretion from macrophages. J Biol Chem 2000;275:34508–11.
[7] Wang N, Lan D, Chen W, Matsuura F, Tall AR. ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins. Proc Natl Acad Sci USA 2004;101:9774–9.
[8] Kennedy MA, Barrera GC, Nakamura K, et al. ABCG1 has a critical role in mediating cholesterol efflux to HDL and preventing cellular lipid accumulation. Cell Metab 2005;1:121–31.
[9] Out R, Hoekstra M, Hildebrand RB, et al. Macrophage ABCG1 deletion disrupts lipid homeostasis in alveolar macrophages and moderately influences atherosclerotic lesion development in LDL receptor-deficient mice. Arterioscler Thromb Vasc Biol 2006;26:2295–300.
[10] Out R, Hoekstra M, Meurs I, et al. Total body ABCG1 expression protects against early atherosclerotic lesion development in mice. Arterioscler Thromb Vasc Biol 2007;27:594–9.
[11] Out R, Hoekstra M, Habets K, et al. Combined deletion of macrophage ABCA1 and ABCG1 leads to massive lipid accumulation in tissue macrophages and distinct atherosclerosis at relatively low plasma cholesterol levels. Arterioscler Thromb Vasc Biol 2008;28:258–64.
[12] Lammers B, Out R, Hildebrand RB, et al. Independent protective roles for macrophage Abcg1 and ApoE in the atherosclerotic lesion development. Atherosclerosis 2009.
[13] Baldan A, Pei L, Lee R, et al. Impaired development of atherosclerosis in hyperlipidemic Ldlr$^{-/-}$ and ApoE$^{-/-}$ mice transplanted with Abcg1$^{-/-}$ bone marrow. Arterioscler Thromb Vasc Biol 2006;26:2301–7.
[14] Tarling EJ, Bojanic DD, Tangirala RK, et al. Impaired development of atherosclerosis in Abcg1$^{-/-}$ ApoE$^{-/-}$ mice: identification of specific oxysterols that both accumulate in Abcg1$^{-/-}$ ApoE$^{-/-}$ tissues and induce apoptosis. Arterioscler Thromb Vasc Biol 2010;30:1174–80.
[15] Ranalletta M, Wang N, Han S, Yvan-Charvet L, Welch C, Tall AR. Decreased atherosclerosis in low-density lipoprotein receptor knockout mice transplanted with Abcg1$^{-/-}$ bone marrow. Arterioscler Thromb Vasc Biol 2006;26:2308–15.
[16] Mendez AJ. Cholesterol efflux mediated by apolipoproteins is an active cellular process distinct from efflux mediated by passive diffusion. J Lipid Res 1997;38:1807–21.
[17] Duong M, Collins HL, Jin W, Zanotti I, Favari E, Rothblat GH. Relative contributions of ABCA1 and SR-BI to cholesterol efflux to serum from fibroblasts and macrophages. Arterioscler Thromb Vasc Biol 2006;26:541–7.
[18] Babiker A, Andersson O, Lund E, et al. Elimination of cholesterol in macrophages and endothelial cells by the sterol 27-hydroxylase mechanism. Comparison with high density lipoprotein-mediated reverse cholesterol transport. J Biol Chem 1997;272:26253–61.
[19] Klucken J, Buchler C, Orso E, et al. ABCG1 (ABC8), the human homolog of the *Drosophila* white gene, is a regulator of macrophage cholesterol and phospholipid transport. Proc Natl Acad Sci USA 2000;97:817–22.
[20] Baldan A, Gomes AV, Ping P, Edwards PA. Loss of ABCG1 results in chronic pulmonary inflammation. J Immunol 2008;180:3560–8.
[21] Baldan A, Tarr P, Vales CS, et al. Deletion of the transmembrane transporter ABCG1 results in progressive pulmonary lipidosis. J Biol Chem 2006;281:29401–10.
[22] Wojcik AJ, Skaflen MD, Srinivasan S, Hedrick CC. A critical role for ABCG1 in macrophage inflammation and lung homeostasis. J Immunol 2008;180:4273–82.
[23] Yvan-Charvet L, Ranalletta M, Wang N, et al. Combined deficiency of ABCA1 and ABCG1 promotes foam cell accumulation and accelerates atherosclerosis in mice. J Clin Invest 2007;117:3900–8.
[24] Burgess B, Naus K, Chan J, et al. Overexpression of human ABCG1 does not affect atherosclerosis in fat-fed ApoE-deficient mice. Arterioscler Thromb Vasc Biol 2008;28:1731–7.
[25] Basso F, Amar MJ, Wagner EM, et al. Enhanced ABCG1 expression increases atherosclerosis in LDLr-KO mice on a western diet. Biochem Biophys Res Commun 2006;351:398–404.
[26] Westerterp M, Koetsveld J, Yu S, et al. Increased atherosclerosis in mice with vascular ATP-binding cassette transporter G1 deficiency—brief report. Arterioscler Thromb Vasc Biol 2010;30:2103–5.
[27] Kockx MM. Apoptosis in the atherosclerotic plaque: quantitative and qualitative aspects. Arterioscler Thromb Vasc Biol 1998;18:1519–22.
[28] Arai S, Shelton JM, Chen M, et al. A role for the apoptosis inhibitory factor AIM/Spalpha/Api6 in atherosclerosis development. Cell Metab 2005;1:201–13.
[29] Liu J, Thewke DP, Su YR, Linton MF, Fazio S, Sinensky MS. Reduced macrophage apoptosis is associated with accelerated atherosclerosis in low-density lipoprotein receptor-null mice. Arterioscler Thromb Vasc Biol 2005;25:174–9.
[30] Schrijvers DM, De Meyer GR, Kockx MM, Herman AG, Martinet W. Phagocytosis of apoptotic cells by macrophages is impaired in atherosclerosis. Arterioscler Thromb Vasc Biol 2005;25:1256–61.
[31] Henson PM, Bratton DL, Fadok VA. Apoptotic cell removal. Curr Biol 2001;11:R795–805.
[32] Majno G, Joris I. Apoptosis, oncosis, and necrosis: an overview of cell death. Am J Pathol 1995;146:3–15.
[33] Tabas I, Marathe S, Keesler GA, Beatini N, Shiratori Y. Evidence that the initial up-regulation of phosphatidylcholine biosynthesis in free cholesterol-loaded macrophages is an adaptive response that prevents cholesterol-induced cellular necrosis. Proposed role of an eventual failure of this response in foam cell necrosis in advanced atherosclerosis. J Biol Chem 1996;271:22773–81.
[34] Warner GJ, Stoudt G, Bamberger M, Johnson WJ, Rothblat GH. Cell toxicity induced by inhibition of acyl coenzyme A: cholesterol acyltransferase and accumulation of unesterified cholesterol. J Biol Chem 1995;270:5772–8.
[35] Terasaka N, Wang N, Yvan-Charvet L, Tall AR. High-density lipoprotein protects macrophages from oxidized low-density lipoprotein-induced apoptosis by promoting efflux of 7-ketocholesterol via ABCG1. Proc Natl Acad Sci USA 2007;104:15093–8.