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Abca1 deficiency protects the heart against myocardial infarction-induced injury

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ABSTRACT

Background and aims: We explored the role of ATP-binding cassette transporter A1 (Abca1), in post-myocardial infarction (MI) cardiac injury.
Methods: In *Abca1*$^{-/-}$ mice, wild type (WT) mice, and WT mice transplanted with *Abca1*$^{-/-}$ or WT bone marrow, an MI was induced *in vivo*. Furthermore, an *ex vivo* MI was induced in isolated *Abca1*$^{-/-}$ and WT hearts.
Results: Twenty-four hours and two weeks after *in vivo* MI induction, MI size was reduced in *Abca1*$^{-/-}$ (−58%, $p = 0.007$; −59%, $p = 0.03$) compared to WT. *Ex vivo* MI induction showed no effect of *Abca1*$^{-/-}$ on infarct size. Interestingly, two weeks after MI, *Abca1*$^{-/-}$ mice showed higher circulating levels of B-cells (+3.0 fold, $p = 0.02$) and T-cells (+4.2 fold, $p = 0.002$) compared to WT. Bone marrow-specific *Abca1*$^{-/-}$ tended to reduce infarct size (−43%, $p = 0.12$), suggesting a detrimental role for hematopoietic Abca1 after MI.
Conclusions: Although Abca1 has a protective role in atherosclerosis, it exerts detrimental effects on cardiac function after MI.

1. Introduction

ATP-binding cassette transporter A1 (Abca1) utilizes ATP to transport cholesterol across membranes. Abca1 is an important determinant of circulating high-density lipoprotein (HDL) levels, and exerts several cardioprotective and anti-atherogenic functions [1,2]. Hence, up regulation of Abca1 is considered an important therapeutic strategy to prevent atherosclerotic cardiovascular disease. However, the role of Abca1 during myocardial infarction (MI), an acute cardiovascular event often resulting from rupture of advanced atherosclerotic lesions [3], is currently unknown.

In humans, mutations in the *ABCA1* gene are associated with extremely low levels of HDL [4]. Notably, low HDL levels correlate with an increased MI risk in humans [5]. The inflammatory response following MI is a critical factor in the balance between adverse ventricular remodeling on one hand, and cardiac repair on the other hand [6]. Abca1A has important anti-inflammatory properties, due to its key role in modulating the cholesterol content of plasma membranes and intracellular compartments [7,8]. Furthermore, in response to binding of lipid-poor apoA-I, Abca1 acts as an anti-inflammatory mediator by inducing signaling through the Janus kinase 2/signal transducer and activator of transcription 3 (Jak2/Stat3) pathway, an important regulator of cytokine signaling [9]. Abca1 is thus anticipated to be cardioprotective during MI, both directly by its anti-inflammatory effects through the Jak2/Stat3 pathway as well as indirectly by

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generating HDL.

To investigate this hypothesized role of Abca1 in MI recovery, we performed permanent coronary artery ligation experiments in total body and bone marrow-specific *Abca1*$^{-/-}$ mice and the respective wild type (WT) controls and experiments on isolated hearts from *Abca1*$^{-/-}$ and WT mice.

Our results show that Abca1 has unanticipated unfavorable cardiac effects after MI.

2. Materials and methods

An expanded methods section is available in the Supplementary data.

2.1. Animals

Female WT mice (C57BL/6J background) and *Abca1*$^{-/-}$ mice were used. For the 24-h and 2-week experiment, 6 and 8 WT mice and 6 and 4 *Abca1*$^{-/-}$ mice were used, respectively. Animal experiments were approved by the Ethics Committee for Animal Experiments of Leiden University.

2.2. Bone marrow transplantation

To generate bone marrow chimeras, bone marrow from WT mice and *Abca1*$^{-/-}$ mice was transplanted into WT mice as previously described [10]. Twelve WT chimeras and 8 *Abca1*$^{-/-}$ chimeras were used. Briefly, irradiated WT recipients received 5×10^6 bone marrow cells. After 8 weeks myocardial infarctions were induced or mice were subjected to a sham operation.

2.3. Induction of myocardial infarctions

Mice were anesthetized, artificially ventilated and the left anterior descending coronary artery was ligated. All mice that had ischemia, confirmed by bleaching of the left ventricle (LV) and the emergence of arrhythmias, were included in the study.

2.4. Infarct size and immunohistochemistry

Twenty-four hours or two weeks after MI the mice were sacrificed, hearts were removed and subsequently cut into four equal thick slices. The two lower slices were used for infarct quantification either by Evans Blue (5% solution injected i.v. 20 min before sacrifice, 24 h post-MI) or immunohistochemically after staining with Sirius red for collagen (2 weeks post-MI). Total LV wall area and infarct area were measured. Infarct areas were normalized to total LV areas and averaged for individual hearts.

2.5. Flow cytometry

Upon sacrifice blood was collected by retro-orbital venous plexus puncture. Subsequently, 200,000 blood cells were stained with the appropriate antibodies.

2.6. Ex vivo langendorff perfusion

Hearts from 5 WT and 7 *Abca1*$^{-/-}$ mice were removed and placed in ice cold Krebs–Henseleit buffer. Cannulated hearts were perfused with Krebs–Henseleit buffer in a retrograde fashion with a constant pressure. Hearts were exposed to 20 min stabilization, 35 min of no-flow global ischemia and 45 min of reperfusion. Hearts were frozen, cut into 6–7 slices and incubated with triphenyltetrazolium chloride to stain viable myocardium. Total myocardium and infarcted areas were measured.

2.7. Statistical analysis

Statistically significant differences were tested using the unpaired Student's *t*-test. The probability level for statistical significance was set at 0.05. Results are expressed as average ± SEM.

3. Results

To investigate the effects of Abca1 deficiency on MI-induced injury *in vivo*, we subjected *Abca1*$^{-/-}$ and WT mice to acute coronary artery ligation. Surprisingly, despite the anticipated cardioprotective functions of Abca1, *Abca1*$^{-/-}$ mice displayed a substantial 58% reduction in MI size as compared to WT mice as measured by absence of blood circulation in the infarcted area (Evans Blue unstained area, $p = 0.007$; Fig. 1A and B) 24 h after MI induction. The smaller infarct size observed *Abca1*$^{-/-}$ mice after coronary artery ligation is most likely not (primarily) caused by Abca1 deficiency in cardiomyocytes, as supported by an unaltered infarct size after *ex vivo* MI induction using the Langendorff system (Fig. 1C and D).

To examine the effect of Abca1 deficiency on long term remodeling after MI, *Abca1*$^{-/-}$ and WT mice were subjected to 2 week coronary artery ligation. Comparable to the acute effects observed after MI, *Abca1*$^{-/-}$ mice showed a 59% reduction in collagen-rich scar formation at 2 weeks after coronary ligation as compared to WT mice (Sirius red positive area, $p = 0.03$; Fig. 2A and B). To investigate a possible role of circulating leukocytes, FACS analysis was performed. No differences pre-MI were found in T-lymphocyte ($CD3^+$, $CD4^+$, and $CD8^+$), B-lymphocyte ($CD19^+$), dendritic cell ($CD11c^+$) or monocytes/macrophage ($F4/80^+$) numbers between *Abca1*$^{-/-}$ and WT mice (Fig. 2C). Importantly, 2 weeks after MI induction a striking 4.2-fold increase in $CD3^+$ T-lymphocytes ($p = 0.002$; Fig. 2D) in *Abca1*$^{-/-}$ mice was observed. This phenomenon was accompanied with increased $CD4^+$ T-helper lymphocytes (4.6-fold increase; $p = 0.002$) and $CD8^+$ cytotoxic T-lymphocytes (3.6-fold increase; $p = 0.002$). In addition, *Abca1*$^{-/-}$ mice displayed a clear 3.0-fold increase in $CD19^+$ B-lymphocytes after MI ($p = 0.02$). In contrast, monocyte/macrophage ($F4/80^+$) and dendritic cell ($CD11c^+$) numbers did not differ between both genotypes upon MI. Together, these data indicate that the induction of MI in *Abca1*$^{-/-}$ mice primarily increased common lymphoid progenitor-derived cells, such as T- and B-lymphocytes, rather than common myeloid progenitor-derived cells, including monocytes/macrophages and dendritic cells.

To establish the importance of Abca1 deficiency in the hematopoietic lineages for cardioprotection after MI, coronary artery ligation was performed in WT mice, transplanted with bone marrow from *Abca1*$^{-/-}$ vs WT mice. Quantification of infarct size two weeks after MI showed a clear trend towards a reduction in MI size (−43%; $p = 0.12$, Fig. 2E and F) in mice transplanted with *Abca1*$^{-/-}$ bone marrow as compared to WT bone marrow. These data suggest that the detrimental role of Abca1 in post MI cardiac remodeling is, at least in part, mediated by hematopoietic Abca1.

4. Discussion

The current study is the first to show that mice lacking Abca1 are protected against cardiac damage after coronary artery ligation. This finding is particularly important as *Abca1*$^{-/-}$ mice are virtually depleted of HDL, which is commonly accepted to have protective effects after MI [11,12]. *Abca1*$^{-/-}$ mice had a smaller infarct size after coronary artery ligation. This effect was present both 24 h and 2 weeks post-MI induction, suggesting that Abca1 exerts both acute and persistent detrimental effects on cardiac injury. MI induction in isolated hearts showed no effect of Abca1 deficiency on infarct size.

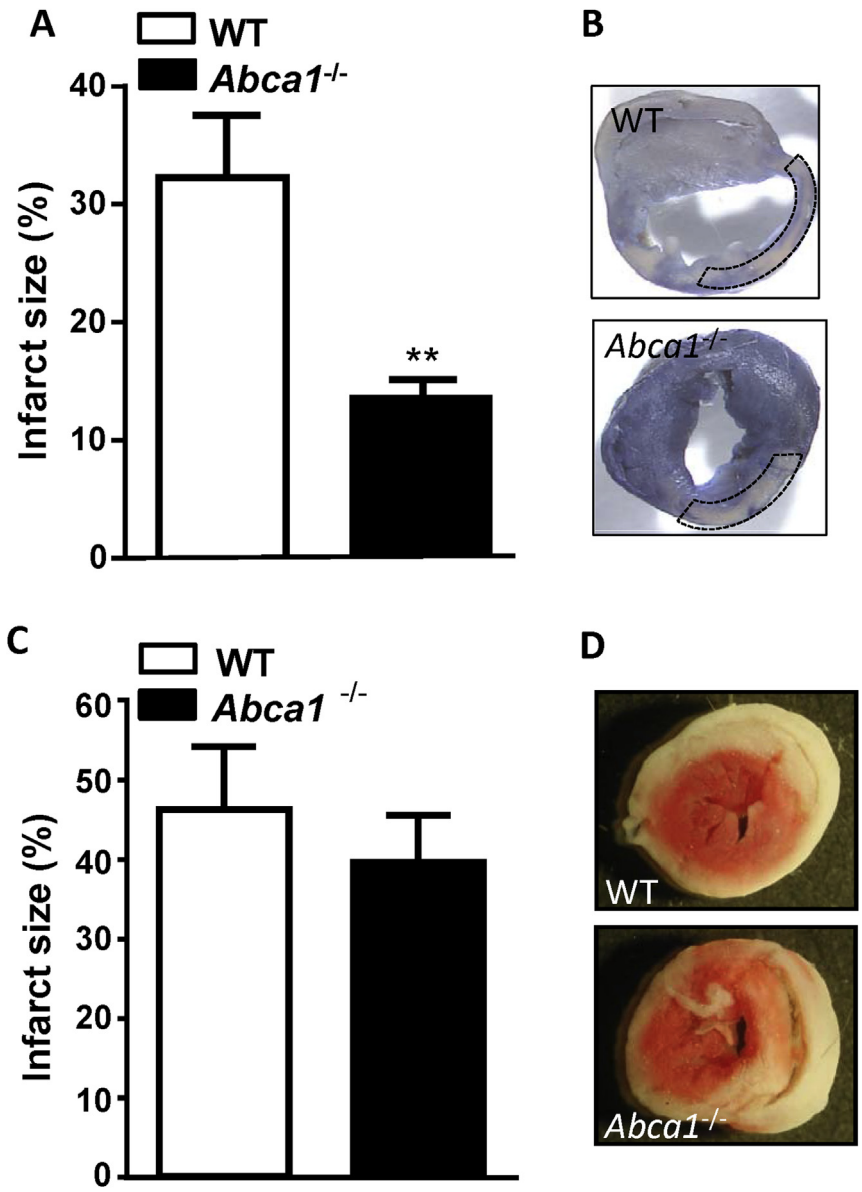

Fig. 1. Acute coronary artery ligation in *Abca1*$^{-/-}$ mice results in reduced MI size *in vivo*, but is unaltered after *ex vivo* ischemia reperfusion. (A) Twenty-four hours after induction of myocardial infarction (MI) infarct size was determined. (B) Representative cross sections are shown, stained with Evans blue to visualize the infarcted area (dotted line, white area). For *ex vivo* MI induction, isolated hearts were stabilized for 20 min in a Langendorff perfusion system, followed by 35 min of no-flow global ischemia, and 45 min of reperfusion. (C) Infarct size was normalized to total left ventricular area and averaged for individual hearts. (D) Representative cross sections, stained with Triphenyltetrazolium chloride to determine viable myocardium (dotted line, red staining). Values are means ± SEM (n ≥ 5 mice per group). $^{**}p < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Furthermore, mice lacking Abca1 in bone marrow-derived cells displayed a similar trend to a reduced infarct size as found in whole body *Abca1*$^{-/-}$ mice, suggesting a detrimental role for hematopoietic but not cardiomyocyte Abca1 after MI.

Induction of MI in *Abca1*$^{-/-}$ mice resulted in a substantial increase in circulating B- and T-lymphocytes after 2 weeks. Hofmann et al. previously showed that T-cell receptor activation by released cardiac autoantigens is a prerequisite for proper wound healing after MI [13]. In line, CD4^{+} T-lymphocytes, are protective mediators of myocardial perfusion injury after MI, likely by modulating monocyte influx [14,15], Moreover, intramyocardial injection of B-lymphocytes into the early post-ischemic myocardium preserves cardiac function [16], underlining the protective roles of B- and T-lymphocytes upon MI.

Cholesterol enrichment of B- and T-lymphocytes is known to initiate cellular activation [17,18]. One could therefore hypothesize that Abca1 deficiency-induced inability to efflux cholesterol enhances the activation status of immune cells towards a more efficient repair of the MI-induced damage. In line, Wilhelm et al. observed increased circulating lymphocyte counts in Western-type diet fed *Ldlr*$^{-/-}$ mice lacking apoA-I, the major apolipoprotein of HDL [17]. Absence of HDL may thus, at least in part, have promoted the observed secondary effects on lymphocyte numbers upon MI in *Abca1*$^{-/-}$ mice. However, further studies are needed to determine exactly how Abca1-expressing leukocytes exert their detrimental effects during cardiac wound healing after MI.

In conclusion, despite its protective role in atherosclerosis, Abca1 has adverse effects on cardiac function after MI, likely due to a direct effect of Abca1 function in bone marrow-derived cells. Importantly, although Abca1 is considered a potential therapeutic

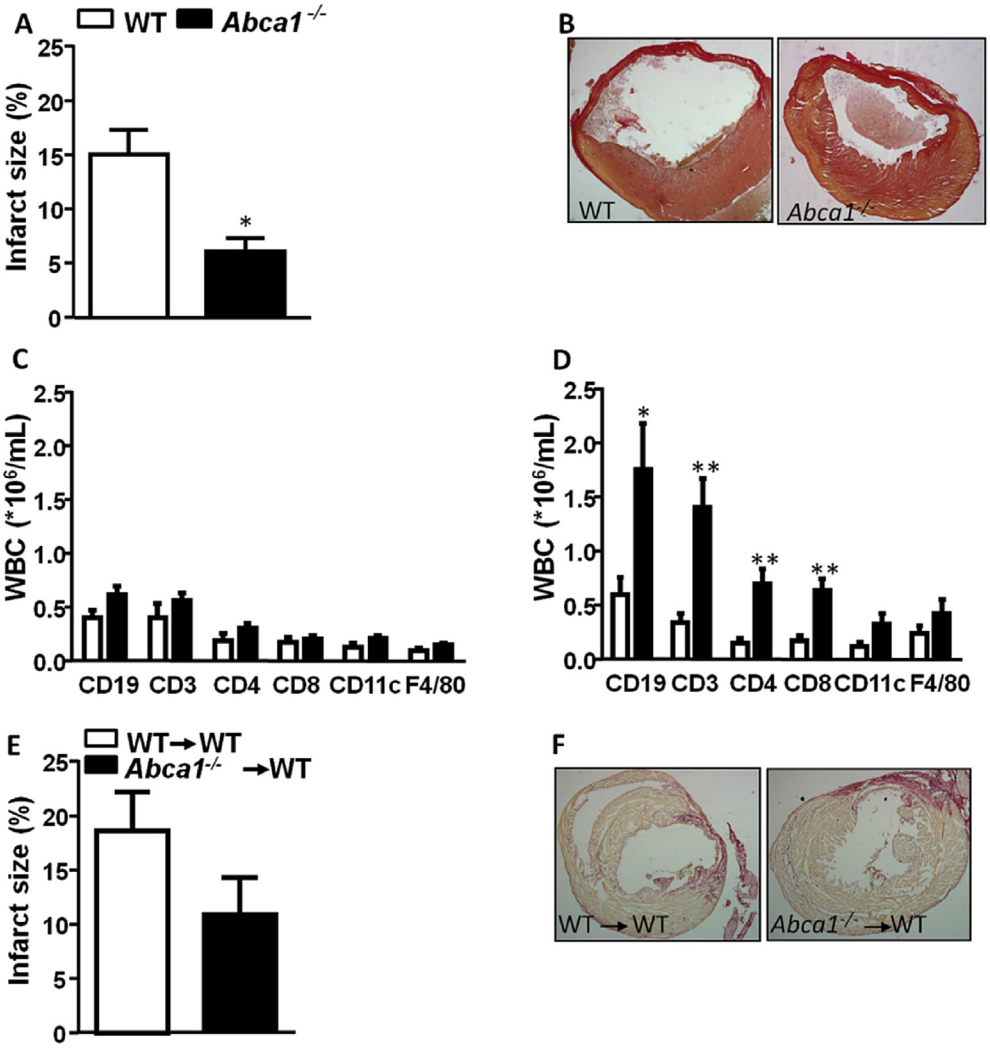

Fig. 2. Myocardial infarction (MI) size reduction two weeks after coronary artery ligation in *Abca1*$^{-/-}$ mice is accompanied by increased circulating T- and B-cells, and is at least in part mediated by hematopoietic Abca1 expression. (A) Two weeks after induction of MI, infarct size was determined. (B) Representative cross sections are shown, stained with Sirius red to visualize the collagen-rich infarcted area. For FACS analysis, isolated white blood cells (WBC) were stained for T-cells (CD3$^+$, CD4$^+$, and CD8$^+$), B-cells (CD19$^+$), monocytes/macrophages (F4/80$^+$) and dendritic cells (CD11c$^+$) and analyzed pre- (C) and post-MI (D). For MI induction in *Abca1* chimeras, WT mice were transplanted with bone marrow from WT or *Abca1*$^{-/-}$ mice. After 8 weeks of recovery, MI was induced by ligation of the coronary artery. (E) Infarct size was determined 2 weeks post-MI. (F) Representative cross sections, stained with Sirius red to visualize the collagen-rich infarcted area (red staining). WT→WT, WT mice transplanted with WT bone marrow, *Abca1*$^{-/-}$ →WT, WT mice transplanted with *Abca1*$^{-/-}$ bone marrow. Values are means ± SEM (n ≥ 4 per group). Values are means ± SEM (n ≥ 4 mice per group). $^*p < 0.05$, $^{**}p < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

target to treat atherosclerosis, strategies aiming at up regulation of Abca1 function should be pursued with care in light of its potential adverse effects on cardiac damage following MI.

Conflict of interest

The authors declared that they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2016.06.023.

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