Materials and Methods:

Human studies

Subjects: Patients (n=37) diagnosed with coronary heart disease in the Department of LuHe hospital (Beijing, China) were included in the study. General information including medical history, medication use, total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, glucose, blood pressure and white blood cell count were obtained. We excluded individuals with white blood cell (WBC) count ≥ 10,000cells/μl or ≥ 75% granulocytes, suggestive of acute inflammation. All patients signed an informed consent, approved by the Ethics Committee in LuHe hospital.

HSPC measurement in patients: Peripheral blood (PB) (2-5 ml) was collected from patients with low HDL (< 1.04 mmol/l) or normal HDL (1.04 ~ 1.53 mmol/l). Mononuclear cells in the PB (PBMNC) were isolated by Ficoll (GE Heslthcare, Belgium) and stained with an anti-human Lineage cocktail APC (BD), anti-human CD38 APC (eBioscience), anti-human CD34PE (BD) and anti-human CD45RA PerCP-Cy5.5 (eBioscience). The frequency of HSPCs (Lin^- $CD34^+$ $CD38^-$ $CD45RA^{-/low}$ cells) was determined on a Gallios apparatus (Beckman Coulter).

SR-BI expression on human HSPC**:** hPBMC were stained with rabbit anti-mouse SR-BI (1 μg/1x10^6 cells, Clone Ab3, Abcam) for 20 min and then goat anti-rabbit Alexa 488 1/400 (Invitrogen), anti-human Lineage APC, anti-human CD38 APC, and anti-human CD34 PE for 20 min. SR-BI expression on HSPC was quantified by FACS.

Murine studies

Mice, diet and treatments: Wild type C57BL/6J (CD45.2) and B.6SJL-PTPRCA (CD45.1) were used at the age of 8-12 weeks. SR-BI knockout ($SR\text{-}BI^{-/-}$) mice and $SR\text{-}BI^{+/+}$ littermates were kindly provided by Prof. Deneys van der Westhuyzen from Kentucky University. Homozygous LDL receptor knockout ($LDLr^{-/-}$) and $apoAI^{tm1/unc}$ mice were purchased from Jackson Laboratory (Bar Harbor, Maine, U.S.A.). Mice homozygous for the $apoAI^{tm1/unc}$ targeted mutation are in C57BL/6 background and have no apoA-I protein in the plasma. $LDLr^{-/-}$ mice were backcrossed with C57BL/6J mice for at least 6 generations to achieve 98.44% C57BL/6J background and then crossed with $apoAI^{tm1/unc}$ mice for at least 9 generations to yield $LDLr^{-/-}$ $apoAI^{-/-}$ double knockout (DKO) mice, as previously described.[1] At the age of 8-12 weeks, $SR\text{-}BI^{-/-}$ mice and control littermates were fed on HFD (34% fat, 1% cholesterol, Catalog no. D12492 mod, BioServices, the Netherlands) or chow diet for 8-10 weeks. For apoA-I studies, $SR\text{-}BI^{-/-}$ and $SR\text{-}BI^{+/+}$ mice, or DKO mice, fed with HFD for 11 and 9 weeks respectively, received during the last 3 weeks subcutaneous saline or lipid-free human apoA-I injection (500 μg per injection, 2 injections per week).[2] For ROS inhibitor experiments, $SR\text{-}BI^{+/+}$ and $SR\text{-}BI^{-/-}$ mice were fed with a HFD and received *i.p*. injection of saline or N-acetylcysteine (NAC, Sigma-Aldrich, 1 mg/kg, daily) for 12 weeks. In total, 287 mice including 243 males and 44 females were used in this study. All the mice used were males except the following experiments: (1) the frequency of LT HSC, LSK and GMP in BM in Figure 1, in which 8 of 10 $SR\text{-}BI^{+/+}$ mice on chow diet were females and 8 of10 $SR\text{-}BI^{-/-}$ mice were females; (2) NAC injection experiments in Figure 5, in which 9 of 13 were $SR\text{-}BI^{+/+}$ females and 10 of 18 $SR\text{-}BI^{-/-}$ were females; and (3) the effect of apoA-I injection on irradiated $LDLr^{-/-}$ mice that were transplanted with $SR\text{-}BI^{+/+}$ BMC or $SR\text{-}BI^{-/-}$ BMC, in which 6 $LDLr^{-/-}$ mice were males and 6 $LDLr^{-/-}$ recipients were females. All mice were maintained in the animal facility of the KU Leuven or Beijing University. All experiments were carried out with approval of the ethics committees of the KU Leuven and of the Beijing University.

Lipoprotein separation by gel filtration: Murine plasma (50 μl) was separated on fast performance liquid chromatography (FPLC) as described before.[3] Cholesterol and cholesterol esters content of plasma and gel filtration fractions were quantified.[3]

Lipoprotein fractionation: Plasma lipoproteins were fractionated and cholesterol content was measured as described before.[4]

White blood cell count: Leukocytes, lymphocytes, monocytes and granulocytes in the PB were enumerated with an Ac-Tdiff hematology analyzer as described before (Beckman Coulter; Brea, CA, U.S.A.).

Lineage negative cell and LSK cell isolation: Lin- cells were isolated from BM cells using the Lineage cell depletion Kit (Miltenyi Biotech, Bergisch Gladbach, Germany). Lin- cells were stained with an anti-Sca-1, anti-Lin and anti-cKit antibodies (Abs)(see table xx) and LSK cells were isolated on a FACS Aria III (Becton Dickinson, NJ, U.S.A.).

ELISA for pAkt: To study the phosphorylation status of Akt in LSK cells, BM LSK cell from SR-BI$^{+/+}$ and SR-BI$^{-/-}$ mice on HFD were isolated by FACS. LSK cells were maintained in SFEM (Stem Cell Technologies, Vancouver, Canada) supplemented with SCF (50 ng/ml, Oxon, UK), TPO (100 ng/ml, R&D Systems) and IL-3 (10 ng/ml, R&D Systems) for 4 days. Cells were collected and enumerated. Equal amount of cells were added to a 96-well plate, fixed and stained with a primary Ab against pAkt and a secondary Ab according to the instruction. pAkt levels were measured by optical density (OD). Data are expressed as optical density (OD)/1x10^6 LSK cells.

ELISA for Serum Amyloid A (SAA): Plasma samples were subjected to SAA ELISA kit (Invitrogen) and SAA was measured according to the manual.

Flow cytometry: Details regarding antibodies used are as in Table 1**.** After red blood cell lysis, WBCs were stained with anti-mouse CD11b PE, anti-mouse Gr-1 APC and anti-mouse F4/80 APC-Cy7 and analysed by FACS. To quantify different HSPC populations in BM, spleen and PB, splenocytes and PB cells were stained with anti-Lineage cocktail, anti-Sca-1, anti-cKit Abs with or without anti-CD150 and anti-CD48 Abs, or anti-FcR Abs as described before.[3] Granulocyte monocyte progenitors (GMP) (CD34$^+$ FcR$^+$ Lin$^-$ Sca-1$^-$ cKit$^+$ cells), HSPC (or LSK cells; Lin$^-$ Sca-1$^+$ cKit$^+$ cells) and LT-HSC (briefly HSCs; CD150$^+$ CD48$^-$ LSK cells) were quantified by FACS.

To study LSK cell proliferation, BrdU was injected *i.p.* in mice 12 hours before sacrifice. SR-BI$^{+/+}$ and SR-BI$^{-/-}$ BMCs were stained with antibodies against BrdU, cKit, Sca-1 and Lineage antigens, and BrdU incorporating LSK cells were quantified by FACS.[3, 5]

To assess Akt and p38 MAPK phosphorylation in LSK cells, BM cells were fixed, permeabilized and then stained with anti-Sca-1, anti-Lin and anti-cKit Abs combined with anti-pAkt or anti-p38MAPK, Abs[3]. The percentage of pAkt+ LSK or p38MAPK+ LSK cells in LSK population was quantified by FACS.

To measure reactive oxygen species (ROS) content in LSK cells, BM cells were first stained with anti-Sca-1, anti-Lin and anti-cKit Abs for 20 min. After washing with PBS, cells were incubated with 2'-7'-dichloroflurescene diacetate (DCF-DA) 10 µM at 37°C for 10 min. ROS^{high} LSK cells were quantified by FACS.

To evaluate chimerism, red blood cells in PB were lysed. White blood cells were stained with combinations of anti-CD45.1, anti-CD45.2, anti-CD4, anti-CD8, anti-B220, anti-CD11b PE, and anti-Gr-1 Abs.

FACS antibodies list is shown in Table 1.

All FACS analyses were performed using appropriate isotype control Abs. To obtain reliable quantifications, at least 100,000 events were acquired for BM cells and 10,000 events were acquired for Lin$^{-/low}$ cells. Data were acquired using a FACSCanto (BD), FACS Aria III (BD) or Gallios (Beckman Coulter) apparatus and analyzed by FlowJo.

Table 1. FACS antibodies list. Fluorescent-conjugated primary antibodies that were used in flow cytometry are summarized below:

antibodies	clones/catalogue number	company
anti-mouse Sca-1 PerCP-Cy5.5, & FITC	D7	eBioscience
anti-mouse Sca-1 PE	D7	BD
anti-mouse cKit PE	2B8	eBioscience
anti-mouse cKit APC-H7	2B8	BD
anti-mouse lineage cocktail APC	M1/70, 145-2C11, RB6-8C5, TER-119, RA3-6B2	BD
anti-mouse CD11b PE	M1/70	BD
anti-mouse CD45.1 FITC	A20	BD
anti-mouse CD45.2 PerCP-Cy5.5	104	BD
anti-mouse CD45R/B220 APC	Ly-5	BD
anti-mouse Gr-1 APC	RB6-8C5	BD
anti-mouse CD4 PE	GK1.5	BD
anti-mouse CD8 APC-Cy7	53-6.7	eBioscience
Anti-mouse F4/80 APC-Cy7	BM8	eBioscience
Phospho-Akt Alexa Fluor 555 conjugated	D13.14.4E	Cell Signaling technology (Bioké, Leiden, the Netherlands)
Phospho-p38 MAPK Alexa Fluor 555 conjugated	3D7	Cell Signaling technology (Bioké, Leiden, the Netherlands)

HSPC cultures:

LSK cells from SR-BI$^{+/+}$ and SR-BI$^{-/-}$ mice on HFD were cultured in SFEM medium supplemented with 50 ng/ml SCF and 100 ng/ml TPO with PBS or 10 µM SB203580 (pP38MAPK inhibitor, Calbiochem) or 10 µM Ly 204950 (pAkt inhibitor, Calbiochem) for 5 days and the cell number numerated. Data are expressed as fold reduction (inhibition vs. no inhibition).

LSK cells were isolated from the BM of SR-BI$^{+/+}$ and SR-BI$^{-/-}$ mice on HFD by FACS and seeded at 1000 LSK cells per well in 96-well plate, and cultured in SFEM containing 50

ng/ml SCF, 100 ng/ml TPO and 10 ng/ml IL-3. Cells were collected and stained with anti-CD11b and anti-F4/80 Abs for FACS analysis.

Colony forming cell (CFC) frequency in BM of SR-BI$^{+/+}$ and SR-BI$^{-/-}$ mice on chow or HFD were enumerated by seeding 1x10^4 BMC in methylcellulose assays and CFCs were scored 10 days after seeding.

qRT-PCR: Total RNA from sorted LSK cells were extracted using RNAeasy microkit (Qiagen, Valencia, CA). mRNA was reverse transcribed to cDNA using Ovation RNA Amplification System V2 (Nugen, The Netherlands). Primers used in this study are as following:

ABCA1: forward 5'-TAGCAGCACCGTGTCTTGTC-3' and

reverse: 5'-TACGGCAGCACATAGGTCAG-3';

β-actin: forward 5'-CGTGGGCCGCCCTAGGCACCA-3' and

reverse:5'-TGGCCTTAGGGTTCAGGGGGG-3'.

Transplantation studies:

To compare the number of HSPC in BM of SR-BI$^{+/+}$ and SR-BI$^{-/-}$ mice on chow diet, SR-BI$^{+/+}$ or SR-BI$^{-/-}$ BM cells (CD45.2+) were mixed in limiting dilution studies with CD45.1 BM cells in the ratios of 1:3, 1:1 or 3:1 and injected into irradiated CD45.1 recipients via tail vein. Four and sixteen weeks after transplantation, blood cells were stained with anti-CD45.2 and anti-CD45.1 to evaluate the chimerism. Data were expressed as the ratio between CD45.2 and CD45.1.

In other studies, SR-BI$^{+/+}$ or SR-BI$^{-/-}$ BM cells mixed with equal amounts of CD45.1 BM cells were injected into irradiated LDLr$^{-/-}$ recipients. Following transplantation, LDLr$^{-/-}$ recipients were first fed on chow diet for 8 weeks and switched to HFD for another 8 weeks, and chimerism, expressed as the ratio of CD45.2 and CD45.1, was assessed at 16 weeks.

LDLr$^{-/-}$ recipients were lethally irradiated and then received 7 x 10^6 SR-BI$^{+/+}$ or SR-BI$^{-/-}$ BMC via tail vein. Five days after transplantation, mice were placed on HFD for 8 weeks. Starting from 5th weeks of HFD, mice were subcutaneously injected with 500 µg purified human apoA-I twice per week for three weeks. After sacrifice, BMC were stained with an Ab cocktail containing anti-mouse Lineage APC, anti-mouse Sca-1 FITC and anti-mouse cKit PE. LSK cells were quantified by FACS.

Histology and immunostaining of hearts and aortas: After perfusion with saline and 4% paraformaldehyde, hearts and aortas were dissected. Cryosections of 7µm thickness were obtained. Morphometric analysis was performed on H&E stained slides using KS300 software (Carl Zeiss, Oberkochen, Germany). Alternatively, to quantify plaque area, cryosections were stained with oil red O (ORO) staining (counterstained with hematoxylin) as described before.[6] Cryosections were incubated with rat anti-mouse CD45 (5 µg/ml, Biolegend, San Diego, U.S.A.) and biotin CD45.2 (5 µg/ml, BD) overnight and then labeled with goat anti-rat Alexa 488 1/500 (Invitrogen) and streptavidin 555 1/500 (Invitrogen). For negative controls, slides were probed with rat anti-mouse IgG (5 µg/ml, DAKO) overnight and then labeled with goat anti-rat Alexa 488 1/500 and streptavidin 555 1/500. CD45.2-derived inflammatory cells in the atherosclerotic plaques were identified and quantified using the Axiovision software (Axioimager Z1 microscope, Carl Zeiss) on Z-Stack images after extended focus computation (Carl Zeiss).

Statistics: Data were expressed as mean ± SD. The mouse data were first tested for normal distribution by D'Agostino-Pearson Omnibus test (when there were no less than 8 values per group) or Shapiro-Wilk test (when there were no less than 7 values per group). For data confirmed normal distribution, in the experiment where there 2

experimental groups, unpaired, 2-tailed Student's t test was used; In the experiments where there were no less than 3 groups, One-way ANOVA with Dunnett was used when comparing treated groups against control and ANOVA with Bonferroni was applied when comparing all groups. The mouse data that did not pass Normality test or less than 7 values per group, nonparametric Mann Whitney analysis was used to compare 2 groups and nonparametric Kruskal-Wallis analysis was used to compare 3 or more than 3 groups. For human study, nonparametric Spearman analysis was performed by SAS. A P value less than 0.05 was considered significant.

References:

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