Renal Dysfunction Potentiates Foam Cell Formation by Repressing ABCA1

Yiqin Zuo, Patricia Yancey, Iris Castro, Wasif Khan, Masaru Motojima, Iekuni Ichikawa, Agnes B. Fogo, MacRae F. Linton, Sergio Fazio, Valentina Kon

Objective—Patients with chronic kidney disease (CKD) have the highest risk for atherosclerotic cardiovascular disease (CVD). Current interventions have been insufficiently effective in lessening excess incidence and mortality from CVD in CKD patients versus other high-risk groups. The mechanisms underlying the heightened risk remain obscure but may relate to differences in CKD-induced atherogenesis, including perturbation of macrophage cholesterol trafficking.

Methods and Results—We examined the impact of renal dysfunction on macrophage cholesterol homeostasis in the apoE−/− mouse model of atherosclerosis. Renal impairment induced by uninephrectomy dramatically increased macrophage cholesterol content, linked to striking impairment of macrophage cholesterol efflux. This blunted efflux was associated with downregulation of the cholesterol transporter ATP-binding cassette transporter A1 (ABCA1) and activation of the nuclear factor-kappa B (NF-κB). Treatment with the angiotensin receptor blocker (ARB) losartan decreased NF-κB and restored cholesterol efflux.

Conclusions—Our findings show that mild renal dysfunction perturbs macrophage lipid homeostasis by inhibiting cholesterol efflux, mediated by decreased ABCA1 transporter and activation of NF-κB, and that ARB can restore cholesterol efflux. (Arterioscler Thromb Vasc Biol. 2009;29:1277-1282.)

Key Words: renal impairment ▪ atherosclerosis ▪ macrophage ▪ ATP-binding cassette transporter A1 ▪ angiotensin

Patients with chronic kidney disease (CKD), including those with mild renal dysfunction, have very high risk for cardiovascular disease (CVD).1–3 Our limited understanding of the underlying mechanisms of CKD-driven vasculopathy constrains development of specific therapeutic approaches. While risk reductions, through current therapeutic interventions, have halved the overall CVD morbidity and mortality in the general population,4 poor CV outcomes continue to escalate in CKD patients.1 Further confounding this issue is variability in the response of CKD patients to therapeutic interventions on lipids. Although lipid-lowering agents prevent CV events in patients with mild to moderate CKD,5–10 their effectiveness appears more equivocal as renal damage progresses to end-stage renal disease.5,11,12 These observations suggest that renal dysfunction may exert a unique influence over the process of atherogenesis, and may influence response to standard lipid therapy.

Although renal damage can cause dyslipidemia, the increased incidence of CVD in CKD cannot be attributed to higher serum cholesterol per se.13 Recent studies indicate that multiple local vascular perturbations influence cholesterol trafficking and foam cell formation, a key process in atherogenesis.14–16 There is currently little information about foam cell formation in the setting of renal dysfunction. The formation of macrophage foam cells reflects a failure of cholesterol export mechanisms to keep pace with internalization of cholesterol from lipoproteins and cellular debris.14 Thus, macrophage cholesterol homeostasis is critically dependent on lipid efflux, which involves mobilization of excess cholesterol from intracellular pools to the plasma membrane and transfer to suitable external cholesterol acceptors.14–16 The key pathway for cholesterol movement out of macrophages involves an energy-dependent efflux linked to the ATP-binding cassette transporter A1 (ABCA1).16,17 However, little is known about the status and regulation of this critical pathway in CKD.

Although we previously showed that renal impairment induced by uninephrectomy (UNx) in apoE−/− mice dramatically ameliorates atherogenesis that was responsive to inhibition of angiotensin II actions, the underlying mechanism remained unclear.18 Notably, unlike other atherosclerosis-inducing conditions such as diabetes,19,20 where specific metabolites (ie, glucose or advanced glycation end products) can influence macrophage lipid homeostasis in vitro, renal impairment does not have such a specific metabolite. We therefore used in vivo as well as ex vivo approaches to study the effects on macrophage lipid homeostasis in this model.11,22 Because angiotensin II (AII) inhibition is a mainstay in...
treatment of atherosclerotic heart disease and CKD is an AII-responsive state, we also evaluated the effects of AII inhibition on macrophage lipid homeostasis and regulation of the efflux pathway in this setting.

Methods
For a detailed description please see the supplemental materials (available online at http://atvb.ahajournals.org).

Animals and Experimental Design
Female apoE−/− or wild-type (WT) mice on C57BL/6 background (Jackson Laboratories, Bar Harbor, Maine) were maintained on normal mouse chow. Animal care and procedures were carried out in accordance with National Institutes of Health and Vanderbilt University animal care facility guidelines. At 9 weeks of age, apoE−/− and WT mice underwent uninephrectomy (UNx) or sham operation performed under isoflurane inhalation anesthesia. After 1 week, UNx and sham mice were begun on HFD or continued on the regular chow diet. ApoE−/− and WT mice on C57BL/6 background were maintained on normal mouse chow. Animal care and procedures were carried out in accordance with National Institutes of Health and Vanderbilt University animal care facility guidelines. At 9 weeks of age, apoE−/− and WT mice underwent uninephrectomy (UNx) or sham operation performed under isoflurane inhalation anesthesia. After 1 week, UNx and sham mice were begun on HFD or continued on the regular chow diet.

Macrophage Cholesterol Content and Cholesterol Efflux
At termination of the study, total cholesterol (TC) and free cholesterol (FC) content was assessed in freshly isolated peritoneal macrophages, harvested by peritoneal lavage 3 days after peritoneal injection of 3% thioglycollate. Cells from each mouse were seeded and, after 4-hour incubation, baseline cholesterol mass determined and peritoneal macrophages harvested as described.21,25

Results
Macrophage Cholesterol Content
Uninephrectomy in high-fat fed apoE−/− mice caused a dramatic expansion in macrophage cholesterol, including a 2.5-fold increase in total cholesterol (TC), a 1.8-fold increase in free cholesterol (FC), and a 3.0-fold increase in cholesterol ester (CE; Table). Treatment with losartan reduced cellular cholesterol by 76%. The cellular cholesterol differences were not directly explained by serum lipid levels (Table). To evaluate whether reduction in renal mass affects cellular cholesterol in the absence of in vivo cholesterol loading, we also assessed the cellular lipid characteristics in macrophages of apoE−/− mice with UNx or sham operation maintained on regular chow diet. ApoE−/− UNx mice on chow also showed a marked increase (3-fold) in macrophage cholesterol, reflecting largely an increase in FC (Table). Again, the cellular cholesterol differences in UNx versus sham on chow were not explained by serum lipid levels (Table). ARB treatment significantly reduced cellular TC, FC, and CE (Table), in the absence of any reduction in serum cholesterol.

Macrophage Cholesterol Efflux
Uninephrectomy resulted in blunted macrophage cholesterol efflux, regardless of diet. In the presence of apolipoprotein AI (apoAI) as an extracellular cholesterol acceptor, cellular cholesterol levels in macrophages from apoE−/− UNx+HFD remained elevated compared to cells harvested from apoE−/− sham+HFD (Figure 1A) attributable to strikingly repressed efflux (5.4±0.9% in UNx+HFD versus 31.6±3.5 in sham+HFD, P<0.05). ARB treatment in these UNx mice partially restored efflux (to 16.1±2.1%, P<0.05 versus UNx+HFD). Similarly, repressed efflux was seen in macrophage of UNx maintained on chow, (UNx, 7.9±2.7% versus sham, 17.7±2.9; P<0.05; Figure 1B). In vivo treatment of apoE−/− UNx mice with losartan also significantly restored efflux, to 12.0±2.1% (P<0.05 versus UNx). Similar trends

| Table. Serum Lipids and Macrophage Cholesterol Content |
|---------------------------------|---------------------------------|---------------------------------|
| **High-Fat Western Diet**       | **Normal Chow Diet**            | **High-Fat Western Diet**       |
| Serum Lipids                    | Cellular Cholesterol            | Serum Lipids                    |
| Cholesterol                     | Triglycerides                   | Cholesterol                     |
| Sham                            | 1123±131                       | 397±31                          |
| UNx                             | 1480±80*                       | 381±55                          |
| UNx+L                           | 824±79†                        | 447±52                          |
| **TC**                          | 272±39                         | 87±8                            |
| **FC**                          | 112.6±25.1                     | 204.2±28.4                      |
| **CE**                          | 45.0±3.9                       | 67.6±22.7                       |
| **TC**                          | 21.0±0.7                       | 21.0±0.7                       |
| **FC**                          | 18.8±0.5                       | 18.8±0.5                       |
| **CE**                          | 2.2±0.3                        | 2.2±0.3                        |

Data are mean±SEM in mg/dl for serum lipid and in μg/mg cell protein for macrophage cholesterol content. TC indicates total cholesterol; FC, free cholesterol; CE, cholesteryl ester; L, losartan.

*P<0.05 vs sham; †P<0.05 vs UNx.
were observed when the macrophages were incubated with HDL as cholesterol acceptor, but the differences did not reach statistical significance (data not shown).

**Macrophage ATP-binding Cassette Transporter (ABC) A1 and ABCG1**

To elucidate mechanisms for the reduced cholesterol efflux in macrophages of UNx mice, we examined the expression of pivotal transporters involved in cholesterol efflux. As noted above, regardless of diet, UNx increased cellular cholesterol level which is expected to increase ABCA1 expression. Notably, however, the increase is stunted. Thus, although the level of cellular cholesterol in UNx on chow is similar to that seen in mice with intact kidneys on HFD, the level of ABCA1 expression is strikingly less in macrophages from UNx mice (Table and Figure 3). Greater cellular cholesterol loading with HFD feeding further emphasizes this point, as comparison of UNx on HFD had dramatically lower level of ABCA1 expression than shams on normal chow. Notably, increased efflux of UNx on HFD had dramatically lower level of ABCA1 mRNA expression as well in UNx than in sham-operated WT (by 38%, *P*<0.05).

**Macrophage NF-κB**

The NF-κB pathway is involved in the downregulation of ABCA1 in cholesterol-loaded cells and can be activated in renal disease. We found significantly increased macrophage nuclear NF-κB activity assessed by electrophoretic mobility-shift assay (EMSA) in apoE−/− UNx versus apoE−/− sham macrophages, with almost 2-fold increase in the protein levels of RelA and p50 DNA binding subunits of NF-κB (*P*<0.05 UNx versus sham; Figure 4A and 4B). Ex vivo exposure of UNx macrophages to an NF-κB antagonist, ammonium pyrrolidinedithiocarbamate (PDTC), significantly increased ABCA1 protein levels (Figure 4C). The upregulation of both p65 and p50 NF-κB subunits (Figure 4B) was inhibited by treatment with ARB. Taken together, these data even in nonhyperlipidemic wild-type mice (WT), ABCA1 mRNA expression was also lower in UNx than in sham-operated WT (by 38%, *P*<0.05).

**Figure 2.** Uninephrectomy depresses macrophage ABCA1 expression which is restored by losartan. A, Western blot of ABCA1/β-actin in macrophages of apoE−/− sham on chow, sham+HFD, UNx+chow, UNx+HFD. B, Semiquantitative data of ABCA1. Sham on chow (n=6), sham+HFD (n=5), UNx on chow (n=9), UNx+HFD (n=6). *P*<0.05 vs sham; †*P*<0.05 vs sham+HFD. C, Change in ABCA1 expression with losartan (L) (UNx on chow+L (n=5) and UNx+HFD+L (n=4) vs no treatment. §*P*<0.05 vs UNx on chow, §§*P*<0.05 vs UNx+HFD.

**Figure 3.** Uninephrectomy stunts macrophage ABCA1 expression. Macrophage cholesterol content vs ABCA1 expression assessed in macrophages from apoE−/− groups of sham on normal chow, sham+HFD, UNx on chow, and UNx+HFD groups.

**Figure 4.** Uninephrectomy increases macrophage NF-κB which can be lessened by losartan, whereas antagonism of NF-κB restores ABCA1 expression. A, Electrophoretic mobility-shift assay (EMSA) of NF-κB (RelA, NF-κB (p65, Rel A), NF-κB (p50) in macrophages of sham (n=5) and UNx (n=6). Faint band is a nonspecific loading control. B, Semiquantitative data of protein expressions for NF-κB (p65, Rel A), NF-κB (p50) in macrophages of sham (n=3), UNx (n=6), and UNx treated with losartan (L) (UNx+L, n=5). *P*<0.05 vs sham, †*P*<0.05 vs UNx. C, Western blot of ABCA1 in macrophages of UNx with ex vivo exposure to the NF-κB antagonist, PDTC (n=5) or without (n=4). *P*<0.05 vs UNx.
suggest that UNx suppresses ABCA1 through activation of NF-κB, and that ARB restores ABCA1 by decreasing activation of NF-κB.

Discussion

The present study makes the novel observation that in vivo, renal impairment induced by reduction in renal mass markedly increases macrophage lipid content. We further show that the mechanisms for this involve reduction in cholesterol efflux from the macrophages. The underlying molecular mechanism involves decreased macrophage ABCA1 transporter and activated NF-κB. Antagonizing activated macrophage NF-κB with ARB or a specific NF-κB inhibitor reversed these events. These data indicate that the dramatic acceleration in atherosclerosis observed in the setting of renal dysfunction may be related to abnormal macrophage cholesterol homeostasis attributable, at least in part, to repression of ABCA1 caused by activated NF-κB, which can be ameliorated by a novel effect of ARB treatment.

Because foam cell formation depends on perturbations in macrophage cholesterol homeostasis, we investigated whether renal dysfunction affects macrophage lipid metabolism in this setting. We show that uninephrectomy in apoE−/− mice on high-fat Western diet causes significant accumulation of macrophage cholesterol, compared with mice with intact kidneys. The cellular lipid expansion was not simply a reflection of the in vivo plasma lipid environment, as the plasma cholesterol in UNx was only 30% higher than sham while the macrophage cholesterol in UNx was increased by 250% (Table). A similar step-up between plasma and cellular cholesterol was seen in UNx versus shams with intact kidneys. The cellular lipid expansion was not simply a parallel plasma lipid levels, particularly in the presence of renal dysfunction.5,33–35

Our studies further show that in vivo treatment with an ARB, losartan, reduced the cholesterol content in macrophages of UNx mice on either diet. Epidemiological studies have documented fewer cardiovascular events and increased survival with ARB in the general population, and in patients with early renal damage.22,36 The benefits of ARB in various studies have occurred in the absence of any plasma lipid-lowering effects and have previously been ascribed to modulation of macrophage infiltration, endothelial cell activation, or vascular oxidant stress.18,37 We now show that ARB directly modulates macrophage cholesterol handling, thereby providing a mechanism for our previous observation.18 In this previous study with the UNx model, we showed that ARB treatment lessened atherosclerosis while decreasing blood pressure, whereas a nonspecific vasodilator (hydralazine) reduced blood pressure without affecting atherosclerosis. This suggests that the vascular effects of an ARB are not dependent on systemic hemodynamics.18 Nonetheless, in the current study we cannot exclude possible hemodynamic effects on macrophage cholesterol accumulation.

Patients with renal damage have increased activity of scavenger receptors, which promote increased cellular uptake of cholesterol; however, once upregulated, macrophages do not downregulate scavenger receptors or inflow of cholesterol by this pathway.38 Cholesterol efflux in this setting is therefore a pivotal step in determining whether intracellular lipid homeostasis is maintained or whether the macrophage will turn into a foam cell.39 Our data show that a modest reduction in renal mass decreases macrophage cholesterol efflux. This effect is linked to repression of the macrophage ABCA1 transporter (Figure 3). UNx also reduced ABCA1 gene expression in normolipemic wild-type mice. This effect is also apparent in humans, in that plasma from CKD patients downregulates ABCA1 in cultured endothelial cells.40 Our study makes the novel observation that repression of the ABCA1 transporter underlies the decreased efflux and expanded cholesterol content in macrophages in renal dysfunction. UNx did not significantly change ABCG1 levels despite its established effects on cholesterol efflux. These findings reiterate the knowledge that changes in ABCA1 versus ABCG1 are not necessarily coordinated in a parallel fashion.19,29,41 Our studies also show that in vivo treatment with ARB restored macrophage ABCA1 expression in renal dysfunction. Although ARB increased macrophage ABCA1 expression, macrophage cholesterol efflux was not completely normalized. These results suggest possible involvement of other mechanisms, including oxidative stress or perturbation in intracellular lipid trafficking. However, in cultured human macrophages exposed to the ARB telmisartan cholesterol efflux was increased via PPARγ-dependent pathway together with upregulation of ABCA1, suggesting a direct ARB effect on macrophages.42 These results complement previous in vivo and in vitro findings that exogenous AII downregulates ABCA1.29,42,43 Taken together, these observations suggest a pivotal importance of the AII-ABCA1 interaction for macrophage cholesterol homeostasis in the setting of renal dysfunction, providing a basis for possible modulation of excess CVD risk in this setting.

We then explored potential mechanisms of interactions between AII and ABCA1. ABCA1 is markedly upregulated by oxysterols via activation of liver X receptor (LXR),44 and is downregulated by inflammatory stimuli, including lipopolysaccharide and interleukin-1β through the NF-κB signaling pathway.30,31,45 The relationship between the NF-κB system, ABCA1, AII, and lipid accumulation in the setting of renal damage remains unclear. We show that UNx macrophages have significantly elevated NF-κB activity (Figure 4) together with a corresponding increase in protein expression of NF-κB DNA binding subunits, p65 (RelA) and p50 (Figure 4A and 4B). We also show that specific antagonism of the NF-κB activation pathway in macrophages lessens the repression of the ABCA1 transporter (Figure 4C). Further, ARB significantly decreased upregulation of RelA and p50, suggesting this as a potential key regulatory step (Figure 4B).

In summary, our study shows for the first time that loss of the renal parenchyma disrupts macrophage cholesterol homeostasis by repressing ABCA1 through activation of the NF-κB pathway. Further, ARB downregulates NF-κB subunits, upregulates ABCA1, and thus lessens macrophage cholesterol burden. These advantageous effects of ARB may decrease atherogenesis and provide further support for the use of ARB to decrease CV risk in CKD patients.
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Disclosures
None.

References


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An erratum has been published regarding this article. Please see the attached page for:
/content/29/12/e133.full.pdf
In the article that appeared on page 1277 of the 29.9 issue, an author was missing a middle initial. The author should be listed as Wasif N. Khan. The error has been noted in the online version of the article, which is available at http://atvb.ahajournals.org/.

Reference:
**Reagents.** High fat western diet (HFD) was purchased from Harlan (#TD 88137). It is an adjusted calories diet and 42% calories comes from fat. Attached is a detailed component of the diet from the manufacturer. ApoAI was from Calbiochem and HDL was from Intracel. NF-κB antagonist (PDTC) was from Sigma. N-Acetyl-Leu-Leu-norleucinal (ALLN) was from Sigma. Antibodies used in this study and sources are as follows: ABCA1 from Novus Biologicals (# NB 400-105); ABCG1 from Gene Tex (# GTX30598); NF-κB (p65, RelA), Calbiochem; NF-κB p50, Santa Cruz Biotechnology; antibody to β-actin, Sigma; horseradish peroxidase-conjugated goat anti-rabbit IgG and anti-mouse IgG, Amersham Biosciences.

**Ex Vivo Studies.** To evaluate the effect of NF-κB on ABCA1, macrophages were incubated with an NF-κB antagonist, (0.5µM PDTC) or control (DMEM) (1). The dose and incubation of PDTC were based on our preliminary cell survival assays. Twenty hours later, macrophages were lysed for ABCA1 protein expression level.

**Western Blotting.** We performed western blotting as described (2,3).

**Real-time PCR Analysis.** We performed Real-time PCR as described (4). The primer sets were synthesized by ABI (ABCA1: Mm00442646_m1, ABCG1:Mm00437390_m1).

**Electrophoretic mobility-shift assay.** For the NF-κB EMSA assays, the mice were on normal chow diet. NF-κB activity was determined by EMSA as previously described by us (1,5,6).

**ABCA1 degradation study.** To study the calpain-mediated proteolysis of ABCA1, experiments were performed according to the method described by Arakawa et al (7). In brief, freshly isolated peritoneal macrophages from UNx and sham-operated mice were
incubated with ALLN (50µM) or (DMEM). One hour later, macrophages were lysed and the ABCA1 protein expression level assessed by western blot as described above.

**Statistical analysis.** Results are expressed as mean±standard error of the mean (SEM) where each mean represents at least 6 experiments performed in triplicate using cells isolated from at least four-six individual mice (not pooled cells) in each group unless otherwise noted in the figure legends. Statistical difference was assessed by single factor variance (ANOVA); Levene’s test was used to test for the homogeneity of variances; post-hoc comparisons were performed with least significant difference (LSD) or Tamhane’s T2 test depending upon the results of homogeneity test. A p value <0.05 was considered to be significant.

References


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