Published in final edited form as:

Atherosclerosis. 2010 May ; 210(1): 57–62. doi:10.1016/j.atherosclerosis.2009.10.029.

Moderate Kidney Disease Inhibits Atherosclerosis Regression

Manish P. Ponda^{1,2}, Irina Barash², Jonathan E. Feig³, Edward A. Fisher^{3,*}, and Edward Y. Skolnik^{2,*}

¹ Laboratory of Biochemical Genetics and Metabolism, Rockefeller University, New York, NY

² Division of Nephrology, Department of Medicine, New York University School of Medicine, New York, NY

³ Marc and Ruti Bell Vascular Biology Program, Leon H. Charney Division of Cardiology, Department of Medicine, New York University School of Medicine, New York, NY

Abstract

Chronic kidney disease (CKD) accelerates cardiovascular disease. The mechanisms that explain this independent, excess risk associated with CKD have not been fully elucidated.

Objectives—We propose that impaired regression of atherosclerosis in renal disease represents a novel risk factor for the heightened morbidity and mortality and resistance to treatment observed in patients with CKD.

Methods and Results—Using a transplant model to study atherosclerosis regression, we transplanted atheromatous aortic segments generated in Apolipoprotein E knock-out (ApoE^{−/−}) mice, into either control or moderately uremic, normolipidemic, wild-type mice. In non-uremic mice, lesions regressed 55%, whereas lesions in uremic mice increased in size by 17% ($P < 0.01$ for control vs. uremic). The lesions in uremic mice were also characterized by a greater presence of macrophages (36,300 μm^2 vs. 12,600 μm^2 , $P < 0.01$). This finding was despite upregulation of chemokine receptor 7 (CCR7), normally a migration factor, in uremic lesion macrophages. Gene expression analysis of lesion macrophages showed relative down-regulation of serum response factor (SRF) target genes in the uremic group, consistent with impaired CCR7 signaling.

Conclusion—Moderate kidney disease inhibits regression of atherosclerosis in a mouse transplant model. This inhibition may be a result of impaired CCR7 signaling.

Keywords

kidney disease; atherosclerosis; atherosclerosis regression; chemokine receptor; serum response factor

Correspondence to MPP: The Rockefeller University, 1230 York Avenue, Box 179, New York, NY 10065, mponda@rockefeller.edu.

*Co-corresponding authors

Statement of Originality

We certify that this manuscript represents entirely original work that has not been presented or published before except in abstract form.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

CKD afflicts millions of people, with a high proportion of individuals with moderate to advanced CKD (stages 3 to 5)[1]. The majority of CKD patients die of accelerated cardiovascular diseases and not renal failure *per se*. While this population suffers from co-morbid conditions, such as diabetes and hypertension, their dramatically increased cardiovascular mortality cannot be fully explained by these and other “traditional” risk factors, such as age, gender, smoking, cholesterol levels, obesity and physical inactivity[2].

Atherosclerosis is a major contributor to cardiovascular disease and is strongly associated with CKD in humans[3,4]. Animal models corroborate the clinical data – when renal disease is superimposed upon mouse models of atherosclerosis, the phenotype is accelerated[5,6]. While potential pathomechanisms for the progression of atherosclerosis in clinical and experimental renal disease have been identified, options for therapy and secondary prevention are limited. Current therapy is based on regimens used in the general population, without specifically targeting kidney-disease specific mechanisms^[7–9]. For example, in hemodialysis patients, statins are able to impressively lower LDL cholesterol, but unlike other at-risk populations, these patients do not experience improved survival.

One promising area of investigation is the regression of atherosclerosis[10]. Regression can occur experimentally and clinically when plasma lipids are substantially lowered[11]. Because atherosclerotic plaques are dynamic, impaired plaque regression may be an additional cardiovascular risk factor in patients with renal disease and an explanation for the typical resistance to LDL-lowering. To gain better insight into whether kidney disease impacts the regression of atherosclerosis, we studied the phenomenon in our well-validated transplant model of atherosclerosis regression[12–14].

Methods

Animals and Surgical Procedures

To generate uremic mice, renal disease was induced in male C57BL/6 wild-type mice (Jackson Labs) through surgical reduction of approximately 2/3 renal mass (n = 10). Control animals underwent a sham operation where both renal hila were exposed (n = 10). One month after induction of renal disease or sham operation, these mice served as “recipients” of an atherosclerotic aortic segment. The “donors” of this segment were male ApoE^{-/-} mice on a C57BL/6 background (Jackson Labs). To generate the atherosclerotic segment, at 4 weeks of age, these donor mice (n=30) were weaned on to a “Modified Western Diet”, consisting of: 21% fat, 19.5% casein and 0.3% cholesterol. The donor mice were maintained on this diet for 16 weeks. At this point, 10 animals were sacrificed and their aortic arches were used as baseline controls. The atheromatous aortic arches of remaining donors were surgically transplanted into the infra-renal aortae of age-matched “recipients” as previously described[12]. The recipients had been maintained on a standard chow diet. After receiving the atherosclerotic segment, the recipients continued to receive a standard chow diet for 5 days. At this point, the animals were sacrificed. All procedures were approved by the Institutional Animal Use and Care Committee.

Baseline Characteristics

Blood pressure was measured using the CODA non-invasive blood pressure system (Kent Scientific), using volume pressure recording software. Blood was obtained from the retro-orbital venous plexus prior to sacrifice. Samples were centrifuged and serum isolated and frozen at -80°C until analyzed. Urea nitrogen, calcium and phosphorus were measured using commercially available assays (Quantichrom Urea Assay Kit, Quantichrom Calcium Assay Kit; Quantichrom Phosphate Assay Kit; BioAssay Systems). The HDL component of

serum cholesterol was isolated through ultracentrifugation. Total and HDL cholesterol were measured using the Enzychrom Cholesterol Assay Kit (BioAssay Systems).

Frozen Sections

Prior to removing the transplanted segment for analysis, the animals were perfused with an RNase inhibitor solution. After removal, the transplanted segments were bisected, immediately embedded in Optimal Cutting Temperature (OCT) medium and frozen at -80°C . Frozen sections (6 μm thick) were cut from each segment and mounted on to Superfrost Plus Slides (Fisher) using a cryostat.

Lesion Analysis

At least 4 slides, representing distinct areas of the transplanted segment, were used per animal for lesion morphometry and macrophage content analysis. Remaining slides were kept at -80°C for further analyses. Slides were stained by immunohistochemistry (IHC). After blocking in 4% normal rabbit serum, slides were exposed to rat anti-mouse CD68 antibody (Serotec). Biotinylated rabbit anti-rat secondary antibodies (Vector Labs) were used in conjunction with the Vector ABC- Alkaline Phosphatase kit and Vector Red substrate (Vector Labs). Slides were counterstained with hematoxylin and dehydrated. Coverslips were mounted with Permount (Fisher). Using a Leica photomicroscope, cross-sections were digitally imaged and analyzed using ImagePro Plus software. An average of 20 aortic cross-sections were analyzed per animal. The plaque area and CD68 positive area were averaged for each animal and for the two groups. Intra-plaque CCR7, CCL19 and CCL21 expression was determined through IHC staining using either rabbit anti-mouse CCR7 (AbCam) or goat anti-mouse CCL19 or CCL21 antibodies (R&D Systems) and the VectorABC-AP kit and Vector Red substrate (Vector Labs) as above. Apoptosis was detected using anti-cleaved caspase 3 antibodies (Cell Signaling).

RNA Isolation

To obtain mRNA from lesion macrophages, we performed laser capture micro-dissection (LCM) utilizing a portion of the saved frozen sections for each animal using our previously published protocol[15]. To preserve RNA integrity, slides were rapidly stained with hemotoxylin and eosin and then dehydrated. Using a PixCell II LCM Instrument, lesion macrophages were laser captured onto Macro LCM Caps (Molecular Devices). We used slides that had already been stained for CD68 as guides to identify the macrophage-rich areas of the plaques. RNA was extracted from tissue captured onto the LCM Caps using the PicoPure RNA Isolation Kit (Molecular Devices). RNA quality was checked using an Agilent 2100 Bioanalyzer. 3–5ng of RNA was linearly amplified to cDNA using the WT-Ovation RNA Amplification System (NuGen).

Microarray Analysis

5mg of amplified cDNA was hybridized to a GeneChip Mouse Genome 430 2.0 Array (Affymetrix) and processed using an Affymetrix GeneChip System. Data were analyzed using GeneSpring (Agilent Technologies) and Gene Set Enrichment Analysis (GSEA; Broad Institute) software. GSEA analysis was conducted using the transcription factor target gene set database ($n = 583$ gene sets), gene set permutation (6000 permutations). Nominal p-values for the gene sets shown were reported by GSEA as 0.000, which reflects $< 1/\#$ of permutations, and thus are more accurately shown as < 0.0002 . The Molecular Signatures Database can be found at: <http://www.broad.mit.edu/gsea/msigdb/>

To validate microarray results, 100ng of cDNA was used as template for quantitative PCR using a TaqMan-based assay and ABI Prism 7700 Sequence Detection System (Applied

Biosystems). Data were normalized to 28S rRNA expression and then averaged within groups (data not shown).

Statistical Analysis

Data were analyzed by unpaired, two-tailed Student's t test with statistical significance attributed to $P < 0.05$.

Results

Metabolic Parameters of Uremic and Control Mice

Through surgical reduction of nephron mass (approximately 2/3 nephrectomy), we generated uremic mice to approximate moderate kidney disease. Using blood urea nitrogen (BUN) as a marker of renal function, we were able to achieve significant impairment of kidney function in the uremic mice as compared to controls (50 ± 3 mg/dl vs. 28 ± 2 mg/dl; $P < 0.001$; Table 1). In pilot experiments, we found this level of renal function to remain stable for > 3 months. We also measured serum total cholesterol, high-density lipoprotein (HDL) cholesterol, calcium and phosphorus in addition to blood pressure in uremic and control mice, but found no differences in these parameters.

Moderate Kidney Disease Inhibits Regression of Atherosclerotic Lesions

To determine how kidney disease affected atherosclerotic plaque size, we measured cross-sectional area of intraluminal plaques in frozen sections of aortic arches. There was marked intra-luminal plaque in the aortic arches of ApoE^{-/-} mice fed a Modified Western Diet for 16 weeks, typical of this classic mouse model of hypercholesterolemia and atherosclerosis (baseline group, Figure 1). Average cross-sectional lesion size in this group was $115,000 \mu\text{m}^2$. Consistent with prior reports, the average plaque sizes were significantly smaller 5 days after the aortic segments were transplanted into normal wild-type mice ($52,300 \mu\text{m}^2$, $P < .01$ vs. baseline). However, in aortic segments transplanted into uremic wild-type animals, there was no change in average plaque size ($135,000 \mu\text{m}^2$, $P < 0.01$ vs. non-uremic, $P = \text{NS}$ vs. baseline).

Atherosclerotic Lesion Macrophage Content

Lesion macrophage emigration is an early and necessary event in atherosclerosis regression[13,15]. Therefore, we sought to determine if the failure of plaque regression in uremic animals was associated with increased lesion macrophage content (Figure 2). There was a significantly greater degree of macrophage-specific CD68⁺ staining in lesions of uremic animals compared to non-uremic animals ($36,300 \mu\text{m}^2$ vs. $12,600 \mu\text{m}^2$, $P < 0.01$). Even accounting for total lesion area, there was a greater proportion of macrophage staining in uremic animals as compared to non-uremic controls (28% vs. 18%, $P = 0.02$). There was no difference in the presence of apoptotic cells ($<5\%$ for both groups).

CCL19, CCL21 and their receptor CCR7

Lesion macrophages require intact chemokine receptor 7 (CCR7) signaling, via its ligands CCL19 and CCL21, in order to emigrate from atherosclerotic plaques under regressive conditions[15]. Therefore, to determine if differences in lesion macrophage content between groups was a result of CCR7 ligand availability, we measured intra-plaque levels of CCL19 and CCL21. There was no difference between uremic and non-uremic groups for the levels of expression or their pattern of distribution. The ligands co-localized to CD68⁺ areas of the plaque (Figure 3A). Because decreased CCR7 expression could also account for the accumulation of lesion macrophages in the uremic group, we compared differences in

receptor expression. Contrary to our expectation, there was a marked increase in CCR7 expression in the uremic group compared to the non-uremic group (Figure 3B).

Serum Response Factor Gene Targets are Downregulated in Uremic Lesions

The abrupt change from the hyperlipidemic environment of ApoE^{-/-} “donors” into a normolipidemic environment of non-uremic recipients changes lesion macrophage gene expression[15]. These changes are consistent with lesion regression and offer mechanistic insight. For example, genes involved in cholesterol efflux, such as LXR α and ABCA1 are upregulated. In contrast, pro-inflammatory genes such as MCP-1 and VCAM-1 are downregulated.

To determine if gene regulation differs between uremic and non-uremic lesion macrophages, we characterized their gene expression profile using microarray analysis. No individual transcript was found to be significantly up- or down-regulated after correction for multiple testing comparisons. Therefore, we proceeded to analyze the microarray results using GSEA, to find potential patterns of genes involved in common signaling pathways. Gene cluster analysis demonstrated relative down-regulation of serum response factor (SRF) target genes in the uremic group (Table 2).

Discussion

We studied the effect of moderate kidney disease on the regression of atherosclerosis in a mouse model. We generated a phenotype of moderate kidney disease with approximately 40–50% of normal kidney function. Surgical reduction of nephron mass in a mouse does not perfectly mimic human CKD, where hypertension, diabetes and proteinuria are often co-existent. C57BL/6 mice are notoriously resistant to induction of progressive renal disease and often require multiple insults to achieve hypertension and glomerular sclerosis[16,17]. However, our model and human CKD both exhibit solute retention. This is relevant when considering that glomerular filtration rate is inversely related to risk of cardiovascular disease, independent of co-morbid conditions[18].

While we wanted to generate a phenotype that was severe enough to influence our endpoints, we purposefully avoided a more aggressive reduction in kidney function. This enabled us to achieve solute retention without other, potentially confounding, metabolic derangements attendant with more severe renal disease, such as dyslipidemia and hyperphosphatemia. This also enabled us to avoid other confounders of vascular biology such as angiotension infusion, which is used to generate hypertensive renal injury in concert with mineralocorticoid administration[17]. Perhaps most importantly, it shows that our findings may be applicable to a wider spectrum of renal disease and not necessarily restricted to progressive or advanced CKD. With respect to progression of atherosclerosis, even uninephrectomy has shown a deleterious effect in mouse models[5,19].

We report the novel finding that uremia inhibits atherosclerosis regression. Further, we demonstrated that this impairment is associated with macrophage accumulation in the atherosclerotic lesions of uremic animals. The accumulation of macrophages may represent increased influx, decreased efflux, or a combination of the two. Impaired emigration is consistent with prior work showing that macrophage efflux is an early and necessary event in atherosclerosis regression[13].

CCR7 signaling plays a critical role in macrophage emigration and plaque regression under non-uremic conditions. Thus, it was surprising to find CCR7 markedly upregulated in uremic lesion macrophages. This finding suggests that uremia may impair regression of atherosclerosis by interfering with CCR7 signal transduction. The increase in CCR7

expression observed in the uremic animals may reflect a feedback mechanism in an attempt to overcome this inhibition. Given that the CCR7 ligands, CCL19/21, are present within the plaques, it is unlikely that ligand availability is responsible for impaired CCR7 signaling. Based on these findings, we hypothesized that the uremic environment impaired signaling downstream of CCR7. In a nonbiased screen to assess whether transcriptional profiles are different in lesion macrophages from control and uremic animals, we found a relative downregulation of SRF target genes in the uremic animals.

SRF is a highly conserved transcription factor, whose specific DNA binding activity is modulated through interactions with cofactors[20]. These cofactors are broadly categorized as Rho-dependent or Ras-dependent. The Ras-dependent cofactors include Elk-1 and SAP, which are activated by ERK. Several previous studies have shown that ERK activation is critical for CCR7-stimulated macrophages migration *in vitro*[21], raising the possibility that impaired SRF and ERK activation may explain the defect in emigration of lesion macrophages in uremic animals. Therefore, we propose that a retained solute in uremia interferes with CCR7 activation of ERK/SRF, thereby preventing lesion macrophages from coordinating a structural response to CCR7 signaling and the subsequent macrophage emigration out of atherosclerotic plaques.

We acknowledge that mechanisms independent of CCR7 signaling may also contribute to the observed phenotype. Macrophage apoptosis may be accelerated in uremic lesions; an effect observed *in vitro* [22]. We did not detect increased apoptosis in uremic lesions. However, this may be a relatively early event and not detectable after 5 days in a uremic milieu as in our model. Further, reverse cholesterol transport may be impaired in uremic macrophages. Indeed, renal disease can decrease expression of ATP-binding cassette transporter A1 (ABCA1)[23]. Carbamylation of HDL has also been implicated in the pathogenesis of atherosclerosis in patients with renal disease[24]. Indeed, low HDL levels or dysfunctional HDL and impaired reverse cholesterol transport may be of greater importance than lowering LDL in dialysis patients, where conventional therapy has failed to show any survival benefit [8,9,25].

Conclusion

We describe a novel pathomechanism for accelerated atherosclerosis in renal disease. Because atherosclerotic plaques are dynamic – the net result of progressive and regressive forces – even a subtle decline in the rate of regression could lead to significant increases in plaque size over time. Impaired regression in uremia may involve a defect in CCR7 signaling.

Acknowledgments

We thank Dr. Jan L. Breslow for helpful discussions and review of the manuscript, and Dr. Jianhua Liu and Mr. Victor Joaquin for technical assistance.

These studies were supported by funds from Rockefeller University (MPP) and NIH grant HL84312 (EAF).

References

1. Coresh J, Selvin E, Stevens LA, Manzi J, Kusek JW, Eggers P, Van Lente F, Levey AS. Prevalence of chronic kidney disease in the United States. *Jama*. 2007; 298:2038–2047. [PubMed: 17986697]
2. Beddhu S, Cheung AK, Larive B, Greene T, Kaysen GA, Levey AS, Rocco M, Sarnak M, Toto R, Eknoyan G. Inflammation and inverse associations of body mass index and serum creatinine with mortality in hemodialysis patients. *J Ren Nutr*. 2007; 17:372–380. [PubMed: 17971309]

3. Reis SE, Olson MB, Fried L, Reeser V, Mankad S, Pepine CJ, Kerensky R, Merz CN, Sharaf BL, Sopko G, Rogers WJ, Holubkov R. Mild renal insufficiency is associated with angiographic coronary artery disease in women. *Circulation*. 2002; 105:2826–2829. [PubMed: 12070108]
4. Chen R, Kumar S, Timmis A, Feder G, Yaqoob MM, Hemingway H. Comparison of the relation between renal impairment, angiographic coronary artery disease, and long-term mortality in women versus men. *Am J Cardiol*. 2006; 97:630–632. [PubMed: 16490426]
5. Bro S, Bentzon JF, Falk E, Andersen CB, Olgaard K, Nielsen LB. Chronic renal failure accelerates atherogenesis in apolipoprotein E-deficient mice. *J Am Soc Nephrol*. 2003; 14:2466–2474. [PubMed: 14514724]
6. Massy ZA, Ivanovski O, Nguyen-Khoa T, Angulo J, Szumilak D, Mothu N, Phan O, Daudon M, Lacour B, Druke TB, Muntzel MS. Uremia accelerates both atherosclerosis and arterial calcification in apolipoprotein E knockout mice. *J Am Soc Nephrol*. 2005; 16:109–116. [PubMed: 15563564]
7. Shepherd J, Kastelein JJ, Bittner V, Deedwania P, Breazna A, Dobson S, Wilson DJ, Zuckerman A, Wenger NK. Intensive lipid lowering with atorvastatin in patients with coronary heart disease and chronic kidney disease: the TNT (Treating to New Targets) study. *J Am Coll Cardiol*. 2008; 51:1448–1454. [PubMed: 18402899]
8. Wanner C, Krane V, Marz W, Olschewski M, Mann JF, Ruf G, Ritz E. Atorvastatin in patients with type 2 diabetes mellitus undergoing hemodialysis. *N Engl J Med*. 2005; 353:238–248. [PubMed: 16034009]
9. Fellstrom BC, Jardine AG, Schmieder RE, Holdaas H, Bannister K, Beutler J, Chae DW, Chevaile A, Cobbe SM, Gronhagen-Riska C, De Lima JJ, Lins R, Mayer G, McMahon AW, Parving HH, Remuzzi G, Samuelsson O, Sonkodi S, Sci D, Suleymanlar G, Tsakiris D, Tesar V, Todorov V, Wiecek A, Wuthrich RP, Gottlow M, Johnsson E, Zannad F. Rosuvastatin and cardiovascular events in patients undergoing hemodialysis. *N Engl J Med*. 2009; 360:1395–1407. [PubMed: 19332456]
10. Williams KJ, Feig JE, Fisher EA. Cellular and molecular mechanisms for rapid regression of atherosclerosis: from bench top to potentially achievable clinical goal. *Curr Opin Lipidol*. 2007; 18:443–450. [PubMed: 17620862]
11. Williams KJ, Feig JE, Fisher EA. Rapid regression of atherosclerosis: insights from the clinical and experimental literature. *Nat Clin Pract Cardiovasc Med*. 2008; 5:91–102. [PubMed: 18223541]
12. Cheresnev I, Trogan E, Omerhodzic S, Itskovich V, Aguinaldo JG, Fayad ZA, Fisher EA, Reis ED. Mouse model of heterotopic aortic arch transplantation. *J Surg Res*. 2003; 111:171–176. [PubMed: 12850459]
13. Llodra J, Angeli V, Liu J, Trogan E, Fisher EA, Randolph GJ. Emigration of monocyte-derived cells from atherosclerotic lesions characterizes regressive, but not progressive, plaques. *Proc Natl Acad Sci U S A*. 2004; 101:11779–11784. [PubMed: 15280540]
14. Trogan E, Fayad ZA, Itskovich VV, Aguinaldo JG, Mani V, Fallon JT, Cheresnev I, Fisher EA. Serial studies of mouse atherosclerosis by in vivo magnetic resonance imaging detect lesion regression after correction of dyslipidemia. *Arterioscler Thromb Vasc Biol*. 2004; 24:1714–1719. [PubMed: 15256400]
15. Trogan E, Feig JE, Dogan S, Rothblat GH, Angeli V, Tacke F, Randolph GJ, Fisher EA. Gene expression changes in foam cells and the role of chemokine receptor CCR7 during atherosclerosis regression in ApoE-deficient mice. *Proc Natl Acad Sci U S A*. 2006; 103:3781–3786. [PubMed: 16537455]
16. Susztak K, Bitzer M, Meyer TW, Hostetter TH. Animal models of renal disease. *Kidney Int*. 2008; 73:526–528. [PubMed: 18274542]
17. Kirchhoff F, Krebs C, Abdulhag UN, Meyer-Schwesinger C, Maas R, Helmchen U, Hilgers KF, Wolf G, Stahl RA, Wenzel U. Rapid development of severe end-organ damage in C57BL/6 mice by combining DOCA salt and angiotensin II. *Kidney Int*. 2008; 73:643–650. [PubMed: 18033241]
18. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med*. 2004; 351:1296–1305. [PubMed: 15385656]

19. Buzello M, Tornig J, Faulhaber J, Ehmke H, Ritz E, Amann K. The apolipoprotein e knockout mouse: a model documenting accelerated atherogenesis in uremia. *J Am Soc Nephrol.* 2003; 14:311–316. [PubMed: 12538731]
20. Posern G, Treisman R. Actin' together: serum response factor, its cofactors and the link to signal transduction. *Trends Cell Biol.* 2006; 16:588–596. [PubMed: 17035020]
21. Riol-Blanco L, Sanchez-Sanchez N, Torres A, Tejedor A, Narumiya S, Corbi AL, Sanchez-Mateos P, Rodriguez-Fernandez JL. The chemokine receptor CCR7 activates in dendritic cells two signaling modules that independently regulate chemotaxis and migratory speed. *J Immunol.* 2005; 174:4070–4080. [PubMed: 15778365]
22. D'Intini V, Bordon V, Bolgan I, Bonello M, Brendolan A, Crepaldi C, Gastaldon F, Levin NW, Bellomo R, Ronco C. Monocyte apoptosis in uremia is normalized with continuous blood purification modalities. *Blood Purif.* 2004; 22:9–12. [PubMed: 14732806]
23. Zuo Y, Yancey P, Castro I, Khan W, Motojima M, Ichikawa I, Fogo AB, Linton MF, Fazio S, Kon V. Renal dysfunction potentiates foam cell formation by repressing ABCA1. *Arterioscler Thromb Vasc Biol.* 2009; 29:1277–1282. [PubMed: 19667109]
24. Wang Z, Nicholls SJ, Rodriguez ER, Kumm O, Horkko S, Barnard J, Reynolds WF, Topol EJ, DiDonato JA, Hazen SL. Protein carbamylation links inflammation, smoking, uremia and atherogenesis. *Nat Med.* 2007; 13:1176–1184. [PubMed: 17828273]
25. Ponda MP, Barash I. Lipid metabolism in dialysis patients-the story gets more complicated. *Semin Dial.* 2008; 21:390–394. [PubMed: 18764791]

Figure 1.

(A) Average atherosclerosis lesion area from baseline ApoE^{-/-} controls (left), wildtype control recipients (center) and wildtype uremic recipients (right). As expected, non-uremic mice showed significant regression of atherosclerotic lesions after 5 days. Uremic mice showed no regression in the same time frame. (B) Representative cross-sections stained with anti-CD68. Bar = 200µm.

Figure 2. Macrophage Content as determined by average CD68 positivity of aortic-cross sections from baseline ApoE^{-/-} controls (left), wildtype control recipients (center) and wildtype uremic recipients (right). At baseline, atherosclerotic plaques were highly enriched with CD68⁺ macrophages. After 5 days in a non-uremic wildtype environment, there was significant depletion of these cells. A trend toward fewer lesion macrophages was seen in the uremic group, though these mice still had significantly more macrophages after 5 days than in the control group.

Figure 3.

(A) Aortic cross-sections of intra-plaque staining of CCL19 (top row) or CCL21 (bottom row) from aortic-cross sections of baseline controls (left), normal control recipients (center) and uremic recipients (right). Both ligands showed equivalent staining in all 3 groups; (B) serial sections stained for CD68 (top row) and CCR7 (bottom row) under baseline (*left*), normal control (*center*) and uremic (*right*) conditions. CCR7 was much more highly expressed in uremic lesions than in controls or at baseline. CCR7 staining co-localized with CD68⁺ areas of the plaques.

Table 1

Baseline characteristics. Uremic mice had significantly higher serum creatinine and BUN levels, though other metabolic parameters known to influence atherosclerosis, including blood pressure, serum total and HDL cholesterol, calcium and phosphorus were similar to control mice. Values are mg/dl unless otherwise indicated.

	Non-uremic control Mean±SEM (n=9)	Uremic Mean±SEM (n=10)	P value
BUN	28±2	50±3	< .001
Blood Pressure (mmHg)	97/74 ±2/1	98/76 ±2/1	NS
Total Cholesterol	106±8	105±8	NS
HDL	56±4	60±3	NS
Calcium	10.0±0.7	10.6±0.5	NS
Phosphorus	3.3±0.2	3.5±0.2	NS

GSEA Results showing differential regulation of transcription factor target gene sets as defined by the Molecular Signatures Database. SRF target genes are significantly down-regulated in the uremic group compared to controls. “Size” represents the number of genes analyzed for each gene set. Ranks determined by normalized enrichment score (NES) with negative values indicating down regulation in uremic vs. non-uremic animals. Also shown are nominal p-values (NOM p-val) and false discovery rate (FDR) q-value statistics. Only gene sets with FDR <1% are shown.

Table 2

	GENE SET NAME	SIZE	ES	NES	NOM p-val	FDR q-val
1	V\$SRF_Q5_Q1	156	-0.46	-2.02	<0.0002	0.004
2	V\$SRF_Q6	180	-0.45	-1.99	<0.0002	0.003
3	V\$SRF_C	153	-0.44	-1.92	<0.0002	0.006
4	V\$SRF_Q4	156	-0.43	-1.89	<0.0002	0.007
5	CCAWWNAAGG_V\$SRF_Q4	63	-0.49	-1.85	<0.0002	0.008
6	WWTAAGGC_UNKNOWN	103	-0.44	-1.83	<0.0002	0.010
7	V\$FOX_Q2	130	-0.43	-1.82	<0.0002	0.009