Increased Expression of Adhesion Molecules in Uremic Atherosclerosis in Apolipoprotein-E–Deficient Mice

SUSANNE BRO,*‡ FLEMMING MOELLER,‡ CLAUS B. ANDERSEN,§ KLAUS OLGAARD,* and LARS B. NIELSEN‡

Abstract. Chronic renal failure markedly accelerates atherosclerosis in apolipoprotein-E–deficient mice, but the mechanism is unknown. The recruitment of inflammatory cells in the arterial wall by vascular adhesion molecules plays a key role in the formation of classical atherosclerosis. This study examines whether the expression of vascular adhesion molecules is increased in uremic atherosclerosis. Uremia was induced by 5/6 nephrectomy; control mice were sham-operated. After 2 wk of uremia, no lesion formation could be demonstrated in uremic or control mice. After 12 wk, aortas from uremic mice had a 9.8-fold increase of the aortic plaque area fraction compared with control mice (P < 0.0001). The aortic expression of intercellular adhesion molecule-1 (ICAM-1) mRNA in uremic mice was 215 ± 31% (P < 0.05) and 243 ± 55% (P < 0.05) of that in controls after 2 and 12 wk, respectively (n = 9 × 4). In contrast, aortic expression of vascular cell adhesion molecule-1 (VCAM-1) mRNA in uremic mice was unchanged after 2 wk but increased to 237 ± 40% (P < 0.01) of that in control mice after 12 wk. On immunohistochemistry of aortas from uremic mice, ICAM-1 was predominantly present in endothelial cells both in nonlesioned and lesioned aortas, whereas VCAM-1 was predominantly present in the medial smooth muscle cell layer in the lesions. The plasma concentration of soluble ICAM-1 (sICAM-1) (but not of sVCAM-1) was slightly elevated after 2 wk of uremia. In contrast, both sICAM-1 and sVCAM-1 plasma concentrations were markedly higher in uremic than control mice after 12 wk. These results suggest that uremic atherosclerosis is preceded by an upregulation of ICAM-1 expression in arterial endothelium and that formation of early uremic lesions is accompanied by upregulation of VCAM-1 expression in the medial smooth muscle cell layer.

The prevalence of arterial disease is extremely high in chronic renal failure (CRF) (1–3). Uremic patients have impaired endothelium-dependent vasodilation (4) and increased arterial stiffness (5). Histologic examinations of arterial biopsies from uremic patients have shown atherosclerosis-like lesions in the intima as well as medial calcifications (6,7). Recently, independent results from three groups (including ours) have established that nephrectomy-induced uremia confers markedly accelerated formation of intimal atherosclerosis-like lesions in mice with genetical hyperlipidemia (i.e., apolipoprotein-E–deficient [apo-E–/–] mice) (8–10). This new mouse model represents a platform for investigating the pathogenesis of uremic atherosclerosis. The uremic lesions are characterized by marked accumulation of macrophages, nitrotyrosine (a marker of reactive oxygen species–protein interaction) (8,9), and high-level expression of receptors for advanced glycation end products (RAGE) (9). Remarkably, the accelerated lesion formation in the uremic mice cannot be fully explained by risk factors that are normally believed to cause vascular disease in uremic patients (e.g., changes in plasma lipoproteins, BP, and plasma homocysteine) (8). This finding is in line with a recent clinical study that showed a disappointing small effect on cardiovascular disease in uremic patients by treatment with a cholesterol-lowering statin (11). Thus, uremia appears to have direct adverse effects on the arterial wall. The identification of the molecular responses of the arterial wall to uremia may help to identify new and specific approaches for preventing cardiovascular disease in uremic patients.

It is well established that inhibition of the expression of adhesion molecules retards development of classical atherosclerosis in animal models, probably by slowing down the recruitment of inflammatory cells from the blood (12–14). Patients with CRF display increased plasma concentrations of soluble portions of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (15). Moreover, uremic medium causes increased expression of mRNA for ICAM-1, VCAM-1, and E-selectin and enhanced shedding of the soluble parts of these adhesion molecules when added to cultures of vascular endothelial cells (16). Thus it is tempting to speculate that the propensity to accelerated lesion formation in uremia may involve increased expression of adhesion molecules. It is unknown, however, whether uremia affects the expression of adhesion molecules in the arterial wall at the sites of lesion formation.
To improve the understanding of how uremia affects the arterial wall and how uremia may accelerate atherosclerosis, we have examined aortic expression of three adhesion molecules involved in classical atherosclerosis (ICAM-1, VCAM-1, and E-selectin) in uremic and control apo-E−/− mice before and upon the formation of arterial lesions.

Materials and Methods

Animals

Male apo-E−/− mice (C57BL/6J B6apoE<sup>−/−</sup>), backcrossed >10 generations onto the C57BL/6 background; Taconic M&B Laboratory Animals and Services for Research, Ry, Denmark) were kept (five mice per cage) on a 12-h light/dark cycle in a temperature-controlled room at 21°C to 23°C with free access to water and a standard mouse diet (Altromin 1314; Altromin, Lage, Germany). The experiments were performed according to the principles stated in the Danish law on animal experiments and were approved by the Animal Experiments Inspectorate, Ministry of Justice, Denmark.

Surgical Procedures

Renal failure was induced by a two-step surgical procedure. At age 8 wk, the upper and lower poles of the right kidney were resected leaving an intact kidney segment. At age 10 wk, the left kidney was removed after ligation of the renal blood vessels and the ureter. Control mice underwent sham-operations both at 8 wk of age (exposure of right kidney) and at 10 wk of age (exposure of left kidney). The kidneys were approached through a dorsal midline incision of the abdominal wall and how uremia may accelerate atherosclerosis.

Analysis of Aortic Atherosclerosis

After the surgical procedures, the kidneys were approached through a dorsal midline incision of the abdominal wall. The kidneys were removed after ligation of the renal blood vessels and the ureter. The remaining part of the right kidney was used for studies of arterial mRNA and protein expression, and the left kidney was used for studies of arterial lesion development. At age 10 wk, the left kidney was resected leaving an intact kidney segment. After kidney manipulation or exposure, the muscles and fascia were sutured, and the skin incision was closed with metal clips.

Anesthesia was achieved with a mixture of fentanyl 0.079 mg/ml, fluanisone 2.5 mg/ml, and midazolam 1.25 mg/ml (Hypnorm/Dormicum) at a dose of 0.08 to 0.10 ml/10 g body wt, subcutaneously. Analgesia (buprenorphine 0.001 mg/10 g body wt, subcutaneously) at a dose of 0.08 to 0.10 ml/10 g body wt, subcutaneously twice daily for 3 d) was used after the surgical procedures.

At the end of the study, each mouse was anesthetized, and the thorax was quickly opened. A small incision was made in the right cardiac auricle, and a cannula was inserted into the left ventricle. Through the left ventricle, the circulation was perfused with 0.9% NaCl (0.9 mol/L) until the eluate became clear. The heart and aorta were dissected free to the iliac arteries and removed en bloc. For immuno-histochemistry, heart with the aortic root was separated from more distal aorta; the rest of the aorta was snap-frozen in liquid N<sub>2</sub> and stored at −80°C until RNA isolation or quantification of atherosclerotic lesions.

Experimental Protocol

For studies of arterial mRNA and protein expression, 36 mice were randomly allocated to 5/6 nephrectomy or sham-operated. Half of the uremic mice (n = 9) and half of the sham-operated mice (n = 9) were killed 2 wk after the second operation. The other half of the mice (n = 9 × 2) were killed 12 wk after the second operation. Arterial lesion formation was studied in additional 17 mice that were either 5/6 nephrectomized or sham-operated and killed 12 wk after the second operation. Fasting blood samples from the retro-orbital venous plexus were collected from uremic and control mice after 2 and 12 wk.

Analysis of Aortic Atherosclerosis

After thawing, the aortas were carefully freed of connective and adipose tissue under a dissection microscope, opened longitudinally, and placed between a microscope slide and a cover slip. The intimal surface was scanned with an Agfa Snapscan e50 flatbed scanner (Agfa-Gevaert, Glostrup, Denmark). Aortic total area and lesion area were determined by digital image analysis with the Multi-Analyzer/PC version 1.1 software from Bio-Rad Laboratories (Hercules, CA).

Aortic lipids were quantified with thin layer chromatography (TLC) as described previously (17,18). All samples were analyzed in triplicate on separate TLC plates. The precision and accuracy of this assay have been reported elsewhere (19,20).

Plasma Biochemistry

Blood was collected in heparinized microtubes (Capiject; Terumo Medical, Elkton, MD) and subjected to centrifugation at 2000 × g for 10 min at 4°C. Plasma was stored at −20°C. Plasma urea, creatinine, total calcium, and phosphate concentrations were measured by a Hitachi Automatic analyzer 917 using reagents from Roche A/S (Hvidovre, Denmark). Plasma urea is a more sensitive marker of uremia than plasma creatinine in mice, as the picric acid analysis (Jaffe’s method) tends to overestimate creatinine levels due to the presence of interfering substances (21). The detection limit of the creatinine analysis was 0.009 mmol/L. Plasma concentrations of soluble ICAM-1 (sICAM-1) and soluble VCAM-1 (sVCAM-1) were measured with monoclonal antibody-based sandwich ELISA kits (catalog nos. MVC00 and MIC100; R&D Systems Europe, Abingdon, Oxon, UK). The intra-assay coefficients of variation were <5% for both ELISA assays.

RNA and cDNA Preparation

Total RNA was isolated from each individual mouse aorta with the TRIzol reagent (Invitrogen, Life Technologies, Taastrup, Denmark) after homogenization with a Polytron PT1200CL (Buch & Holm, Herlev, Denmark). RNA purity and concentration were determined by absorbance measurements at 260 nm and 280 nm. The yield was 17.5 ± 1.3 µg RNA per aorta (n = 36). RNA integrity was ensured by analysis on an Agilent 2100 Bioanalyzer (Agilent Technologies Denmark A/S, Naerum, Denmark) using an RNA 6000 Nano Assay Kit (Agilent Technologies). cDNA was made from 0.5 µg RNA with 20 U M-MuLV reverse transcriptase (Roche) and random hexamers (Roche) in a 10-µl reaction at 37°C for 60 min.

Real-Time PCR

Real-time PCR in a LightCycler (Roche) was used to determine the mRNA expression of ICAM-1, VCAM-1, E-selectin, and β-actin in mouse aortas. The primers for ICAM-1, VCAM-1, and β-actin have been described previously (22). The forward and reverse primer sequences for E-selectin were: 5'-ATCTGGTGGCGATTCAGAAC-3', 5'-TGTTTGTGTGGTTCGACTCGA-3', and the size of the PCR product was 177 bp. Each reaction mixture (20 µl) contained cDNA synthesized from 10 ng of total RNA, 0.5 µmol/L of each primer, 2 to 4 mmol/L MgCl<sub>2</sub>, and 2 µl of LightCycler Faststart DNA Master SYBR Green I mix (Roche).

The real-time PCR protocol consisted of an initial step at 95°C for 10 min followed by 40 cycles: 95°C for 15 sec, annealing at 60 to 61°C for 5 sec, and elongation at 72°C for 7 to 8 sec. The fluorescence reading temperatures were 82 to 84°C. The temperature ramp was 20°C/s. Standard curves were made by serial dilution of a pool of wild-type mouse heart cDNA. cDNA was run in duplicates or triplicates. To account for differences in cDNA preparation and cDNA amplification efficiency, the mRNA expression of each of the adhesion molecules was normalized by β-actin.
Immunohistochemistry

The heart, including the proximal ascending aorta, was embedded in OCT compound (Tissue-Tek; Sakura Finetec Inc., Vaerloese, Denmark), frozen on dry ice, and stored at −80°C until sectioning. Four-micrometer-thick cryossections were cut from the cardiac end of the aortic root and transferred to slides (SuperFrost Plus; Menzel-Glaser, Germany), air-dried, and fixed in 10% acetone for 10 min at room temperature. The sections were rinsed in TRIS-buffered saline, pH 7.6 (Bie & Berntsen, Roedovre, DK) and preincubated in peroxidase-blocking solution (DakoCytomation, Glostrup, DK) for 8 min before incubation for 60 min at room temperature with biotinylated monoclonal antibodies (ICAM-1: CD54, catalog no. 553250, dilution 1:100; VCAM-1: CD106, catalog no. 553331, 1:100; CD31, catalog no. 553371, 1:100 from PharMingen, San Diego, CA; and α-smooth muscle actin, catalog no. MS-113-B, 1:25, NeoMarkers, LabVision Corporation, Fremont, CA) that had been diluted with background-reducing components from DakoCytomation. After rinsing with TRIS-buffer, sections were treated with peroxidase-labeled streptavidin (DakoCytomation) for 30 min followed by 2% 3,3'-diaminobenzidine (DakoCytomation) in substrate buffer (DakoCytomation). Mayer’s hematoxylin was used as counterstain. Sections were dehydrated through a series of alcohol dilutions and cover-slipped in Permtex mounting media (Histolab Products AB, Gothenburg, Sweden). Mouse thymus, spleen, and skin tumor were used as positive controls. Negative controls included omission of the primary antibody.

Statistical Analyses

Results were analyzed by unpaired t tests. Data are means ± SEM, with n indicating the number of mice studied. P < 0.05 was considered significant.

Results

Induction of Uremia by Subtotal Nephrectomy

Subtotal 5/6 nephrectomy of apo-E−/− mice conferred uremia with 103 to 127% and 49 to 57% increases of plasma urea (P < 0.001) and creatinine concentrations (P < 0.001), respectively (Table 1). The plasma calcium concentration also increased in uremic mice after 2 and 12 wk (P < 0.001), whereas the plasma phosphate concentration was only increased in the uremic mice after 12 wk (P < 0.05) (Table 1).

Accelerated Lesion Formation in Uremic Mice

No lesion formation was observed neither macroscopically nor by sectioning the aortic root from uremic mice after 2 wk. After 12 wk of uremia, however, the total aortic plaque area fraction was markedly larger in uremic compared with control mice (0.039 ± 0.005 (n = 11) versus 0.004 ± 0.001 (n = 6) (P < 0.0001) (Figure 1A). Examination of the aortic root cross-sections from 12 wk uremic mice (n = 5) by light microscopy revealed lesions resembling early atherosclerotic plaques with accumulation of lipid-filled macrophages in the intima (Figure 2). No plaques were observed in aortic roots from 12 wk control mice (n = 5). The lipid composition of the aortas was examined by a TLC-based method. After 12 wk, aortas from uremic mice (n = 10) contained significantly more free cholesterol (6.75 ± 0.58 versus 2.79 ± 0.18 nmol/mg wet weight, P < 0.001) and cholesterol esters (7.16 ± 1.06 versus 2.56 ± 0.09 nmol/mg wet weight, P < 0.01) than aortas from control mice (n = 6) (Figure 1B). Aortic phosphatidylcholine and sphingomyelin concentrations were significantly elevated in uremic compared with control mice (P < 0.05 and P < 0.01, respectively), whereas aortic phosphatidylcholine and triglyceride concentrations did not differ between the two groups (Table 2).

Soluble Adhesion Molecules in Plasma

The plasma concentration of sICAM-1 was slightly but significantly higher in uremic (n = 9) compared with control mice (n = 9) after 2 wk of uremia (P < 0.05). The plasma sVCAM-1 concentration was not affected after 2 wk of uremia (Figure 3). After 12 wk, the plasma concentrations of both sICAM-1 and sVCAM-1 displayed a pronounced elevation in uremic (n = 9) compared with control mice (n = 9) (P < 0.01 and P < 0.0001, respectively) (Figure 3).

Changes in Vascular Expression of Adhesion Molecules during Development of Uremic Atherosclerosis

On real-time PCR, the aortic expression of ICAM-1 mRNA in uremic mice was 215 ± 31% (P < 0.05) and 243 ± 55% (P < 0.05) of that in control mice after 2 and 12 wk, respectively (n = 9 in each group) (Figure 4A). The aortic expression of VCAM-1 mRNA in uremic mice was unchanged after 2 wk, but was 237 ± 40% (P < 0.01) of that in controls after 12 wk (Figure 4B). E-selectin mRNA expression did not differ between aortas from uremic and control mice at 2 or 12 wk (Figure 4C).

Table 1. Effect of renal failure on plasma indices of uremia

<table>
<thead>
<tr>
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<th>2 wk</th>
<th>Controls</th>
<th>12 wk</th>
<th>Controls</th>
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<tbody>
<tr>
<td>Urea</td>
<td>26.6 ± 2.0d</td>
<td>11.7 ± 0.6</td>
<td>23.7 ± 0.6d</td>
<td>11.7 ± 0.3</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.049 ± 0.003d</td>
<td>0.033 ± 0.001</td>
<td>0.047 ± 0.001d</td>
<td>0.030 ± 0.001</td>
</tr>
<tr>
<td>Calcium</td>
<td>2.70 ± 0.05d</td>
<td>2.30 ± 0.03</td>
<td>2.66 ± 0.06d</td>
<td>2.33 ± 0.02</td>
</tr>
<tr>
<td>Phosphate</td>
<td>2.64 ± 0.12</td>
<td>2.30 ± 0.15</td>
<td>2.77 ± 0.17b</td>
<td>2.30 ± 0.08</td>
</tr>
<tr>
<td>Ca × P</td>
<td>7.12 ± 0.39c</td>
<td>5.31 ± 0.37</td>
<td>7.38 ± 0.58c</td>
<td>5.35 ± 0.18</td>
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Values are mean ± SEM. b P < 0.05; c P < 0.01; d P < 0.001 compared with controls.
Immunohistochemistry was used to examine the localization of ICAM-1 and VCAM-1 in the arterial wall of uremic mice. After 2 wk, staining of ICAM-1 was seen on the luminal surface of the nonlesioned arteries that co-localized with the staining of an endothelial cell marker (CD31) (Figure 5, A and C). After 12 wk, expression of ICAM-1 was most abundant on the CD31-positive endothelial cells covering lesions, but there was also a staining in α-actin-positive cells in the basal layer of the plaques (Figure 5, B and E). VCAM-1 was not detectable in nonlesioned aortas from uremic mice after 2 wk. After 12 wk, however, there was a strong staining of α-actin-positive cells in the smooth muscle cell rich medial layer beneath the intimal lesions (Figure 5, D and E). A weaker staining of VCAM-1 associated with CD31-positive endothelial cells at the luminal surface of plaques after 12 wk was also found.

**Discussion**

The expression of inducible adhesion molecules in the arterial wall represents a key event in the development of classical atherosclerosis. Adhesion molecules on endothelial cells, such as E-selectin, ICAM-1, and VCAM-1 contribute to lesion initiation by mediating the recruitment of mononuclear white blood cells to the intima (23,24). The adhesion process starts with leukocyte rolling along the endothelial surface. This is mainly due to selectin ligation, whereas the subsequent firm adhesion depends on interactions between Ig-like molecules (ICAM-1, VCAM-1) on the endothelium and integrins on the leukocyte surface. Knockout mice that lack E-selectin, ICAM-1, or VCAM-1 and mice treated with antibodies against adhesion molecules display reduced atherosclerosis (12–14,25–27).

The uremic mice had a striking 9.8-fold increase of the mean total aortic plaque area fraction (0.039) compared with control mice (0.004) after 12 wk. Histologic examinations revealed lipid-filled macrophages in intimal lesions resembling the early-stage lesions of classical atherosclerosis. There was an accumulation of free and esterified cholesterol in uremic aortas, which is pathognomonic of classical atherosclerosis (28). The uremic aortas also displayed some accumulation of phospholipids and unchanged triglyceride content, as described in human atherosclerotic lesions (29). Thus both the morphologic and biochemical analyses in this study suggest that accelerated initiation and expansion of lesions rather than specific lesion composition is the key characteristic of early uremic atherosclerosis in apo-E−/− mice. This notion is in accordance with our former study, where we studied mice that had been uremic for 22 wk and had extensive aortic atherosclerosis with a mean total plaque area of 0.27 (8). In that study, we observed aortic lesions with extracellular lipids, lipid-filled macrophages, lymphocytes, and collagen-rich connective tissue, i.e. characteristics of more advanced classical atherosclerosis. Remarkably, aortic calcifications are virtually absent in uremic apo-E−/− mice (8,9), despite significantly elevated plasma concentrations of calcium and phosphate (8). As previously discussed (8), the explanation for the elevated plasma calcium concentration in uremic mice and dogs (30) remains enigmatic.

A primary aim of the present study was to assess whether uremia increases the expression of ICAM-1, VCAM-1, and/or E-selectin in lesion prone arteries. It is well established that classical atherosclerotic lesions have increased expression of ICAM-1 and VCAM-1 compared with nonlesioned arterial wall (22,31,32); we therefore chose to examine aortic gene expression 2 wk after surgery. At this time point, we sought to avoid the biases from differences in lesion extent and at the same time minimized a potential effect of surgical stress on the pattern of gene expression. Interestingly, the aortic expression of ICAM-1 mRNA was significantly higher in uremic compared with sham-operated control mice, despite the absence of aortic lesions. Immunohistochemistry showed distinct ICAM-1 staining of the endothelial surface. The results suggest that uremia-related factors increase the expression of ICAM-1 gene...
Figure 2. Representative micrographs showing cross-sections of the aortic root from an apo-E−/− mouse 12 wk after induction of uremia (A) and an apo-E−/− mouse 12 weeks after sham-operation (B). (A) Hematoxylin-stained cross-section of the aortic root from an apo-E−/− mouse with 12 weeks of uremia and early plaque formation. The plaque consists of lipid-filled macrophages. (B) Hematoxylin-stained cross section of the aortic root from an apo-E−/− mouse 12 weeks after sham-operation. No plaque formation could be demonstrated. Lu, aortic lumen. Magnification, ×250.

Table 2. Lipid composition (µg/mg wet weight) of mouse aortas after 12 wk of uremia

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Uremic</th>
<th>Controls</th>
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<tbody>
<tr>
<td>Phosphatidylcholine</td>
<td>1.89 ± 0.13b</td>
<td>1.42 ± 0.0904</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>9.67 ± 1.64</td>
<td>12.49 ± 3.06</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>1.95 ± 0.10</td>
<td>1.77 ± 0.09</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>0.88 ± 0.07c</td>
<td>0.53 ± 0.04</td>
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* Aortic lipids in apo-E−/− mice 12 wk after induction of uremia (n = 10) or sham-operation (controls, n = 6). Values are mean ± SEM. b P < 0.05; c P < 0.01 compared with controls.

The expression of ICAM-1 is regulated transcriptionally in a nuclear factor-κB (NF-κB)–dependent fashion (33). NF-κB is a key transcription factor in vascular inflammation and is activated by proinflammatory cytokines, angiotensin II, atherogenic lipoproteins, advanced glycation end products, and reactive oxygen intermediates (34). We suspect that NF-κB activation is involved in mediating the increase of ICAM-1 expression in the nonlesioned intima because the above-mentioned stimuli are all characteristically in excess in uremia. Uremic patients often have elevated plasma or tissue concentrations of proinflammatory cytokines (IL-6, TNFα, and IL-1β), advanced glycation end products, and reactive oxygen intermediates (34). We suspect that NF-κB activation is involved in mediating the increase of ICAM-1 expression in the nonlesioned intima because the above-mentioned stimuli are all characteristically in excess in uremia. Uremic patients often have elevated plasma or tissue concentrations of proinflammatory cytokines (IL-6, TNFα, and IL-1β) (35,36), advanced glycation end products (37), and of angiotensin II (38), and they frequently display evidence of increased oxidative stress (39,40). NF-κB also controls VCAM-1 expression (41). Nevertheless, we did not see any effect of uremia on the aortic VCAM-1 mRNA expression after 2 wk. Hence the data imply that additional mechanisms that specifically regulate ICAM-1 expression are activated in uremia.

Hypertension is yet another prevalent cardiovascular risk factor in humans with uremia and is associated with increased sICAM-1 and sVCAM-1 (42,43). In contrast to humans, uremic apo-E−/− mice do not develop hypertension (8,9); therefore, elevated BP cannot explain the elevated CAM expression in the present study.

ICAM-1 is expressed in a variety of nonvascular cell types, e.g., lymphocytes, tissue macrophages, epithelial cells, keratinocytes, fibroblasts, smooth muscle cells, hepatocytes, and chondrocytes (31,44,45), providing multiple sources for soluble ICAM-1–derived molecules. Interestingly, the increase of aortic ICAM-1 mRNA expression was much more pronounced than that of plasma sICAM-1 after 2 wk. Thus the data may suggest that uremia has a specific and rapid impact on the arterial ICAM-1 gene expression. It is tempting to speculate that the increased endothelial ICAM-1 expression may help to explain the initiation of lesion formation in uremic aortas. The macrophages in early atherosclerotic lesions are derived from blood monocytes (23) and ICAM-1 plays an essential role in their recruitment into classical atherosclerotic lesions (24,27). The sustained increase of ICAM-1 expression mainly in the endothelium (after 12 wk) suggest that increased monocyte/macrophage recruitment might also have accelerated the continuous growth of the early uremic lesion above what is seen in classical atherosclerosis.

In contrast to ICAM-1, the VCAM-1 mRNA expression was unaffected in uremic mice after 2 wk. However, after 12 wk, aortas from uremic mice had a 2.4-fold increased expression of VCAM-1 mRNA. The increased expression of VCAM-1 may to some extent reflect the pronounced difference in lesion formation between uremic and control mice. Thus, the aortic mRNA expression of VCAM-1 is increased in lesioned aortas of hypercholesterolemic rabbits, LDL-receptor–deficient mice and apo-E−/− mice compared with control animals (32). This finding may to some extent reflect the modulatory effect of monocytes/macrophages and lymphocytes within the developing lesions (46).

It is however likely that uremia per se also has a general effect on VCAM-1 expression in the vasculature, because the plasma sVCAM-1 concentration increased in parallel with the aortic VCAM-1 mRNA content after 12 wk. In uremic mice, the aortic VCAM-1 protein was predominantly localized in the medial smooth muscle cell layer beneath the foam cell lesions with a concomitant but less pronounced increase of VCAM-1.
expression on the endothelium covering foam cell lesions. This VCAM-1 expression pattern in early uremic lesions is similar to that in classical atherosclerotic lesions of apo-E−/− mice (Moeller and Nielsen, unpublished observation). Thus, increased VCAM-1 expression may participate in accelerating the growth of the early lesions in uremic mice in a manner that is similar to the proposed role of VCAM-1 in the expansion of classical lesions.
Figure 5. Representative micrographs showing the expression of ICAM-1 and VCAM-1 proteins in aortic root cross-sections from apo-E−/− mice after induction of uremia. (A) Weak expression of ICAM-1 on the intact endothelium after 2 weeks of uremia. (B) ICAM-1 expression in endothelial cells covering the lesion and in smooth muscle cells in the basal layer of the plaque after 12 weeks of uremia. (C) CD31-positive endothelial cells covering lesions after 12 wk of uremia. (D) VCAM-1 expression in medial smooth muscle cells beneath the intimal lesion, as well as in α-actin-negative smooth muscle cells migrating into the lesion and in endothelial cells covering the plaque after 12 wk of uremia. (E) α-actin-positive medial smooth muscle cells beneath the intimal lesion after 12 wk of uremia. (F) Aortic lesion after 12 wk of uremia. Negative control (no primary antibody). Magnification, ×250.
In summary, the present investigation has shown that accelerated atherosclerosis in uremic apo-E−/− mice is preceded by an upregulation of ICAM-1 expression, and accompanied by an upregulation of both ICAM-1 and VCAM-1 expression. The augmented inflammatory response in the arterial wall may be an important impetus for accelerated atherogenesis in uremia.

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