Syndecan-1 Displays a Protective Role in Aortic Aneurysm Formation by Modulating T Cell–Mediated Responses

Jiantao Xiao, Julianty Angsana, Jing Wen, Sumona V. Smith, Pyong Woo Park, Mandy L. Ford, Carolyn A. Haller, Elliot L. Chaikof

Objective—Chronic inflammation drives progressive and pathological remodeling inherent to formation of abdominal aortic aneurysm (AAA). Syndecan-1 (Sdc-1) is a cell surface heparan sulfate proteoglycan that displays the capacity to modulate inflammatory processes within the vascular wall. In the current investigation, the role of Sdc-1 in AAA formation was examined using 2 models of experimental aneurysm induction, angiotensin II infusion and elastase perfusion.

Methods and Results—Sdc-1 deficiency exacerbated AAA formation in both experimental models and was associated with increased degradation of elastin, greater protease activity, and enhanced inflammatory cell recruitment into the aortic wall. Bone marrow transplantation studies indicated that deficiency of Sdc-1 in marrow-derived cells significantly contributed to AAA severity. Immunostaining revealed augmented Sdc-1 expression in a subset of AAA localized macrophages. We specifically characterized a higher percentage of CD4⁺ T cells in Sdc-1-deficient AAA, and antibody depletion studies established the active role of T cells in aneurysmal dilatation. Finally, we confirmed the ability of Sdc-1 macrophage to modulate the inflammatory chemokine environment.

Conclusion—These investigations identify cross-talk between Sdc-1-expressing macrophages and AAA-localized CD4⁺ T cells, with Sdc-1 providing an important counterbalance to T-cell–driven inflammation in the vascular wall. (Arterioscler Thromb Vasc Biol. 2012;32:386-396.)

Key Words: aneurysms • aortic diseases • glycosaminoglycan • leukocytes • macrophages

Abdominal aortic aneurysm (AAA) is a common and life-threatening clinical condition. In the United States, with approximately 15 000 deaths each year, AAA ranks as the 13th leading cause of death.¹ Current treatment strategies for AAA are largely confined to endovascular or open surgical intervention or physician surveillance. Despite improvement in minimally invasive surgical techniques, the risk of periprocedural morbidity and mortality and subsequent secondary reintervention remains significant.² Nonsurgical treatment options would be desirable. Indeed, a number of approaches have been proposed to prevent progression of aneurysmal disease during the period of aneurysm surveillance, including hemodynamic control and inhibition of inflammation and protease activity.³,⁴ However, a clinically effective pharmacotherapeutic that limits or reverses aortic dilatation has yet to be identified. Defining endogenous mediators that serve to dampen the inflammatory response within the setting of AAA may identify new avenues for medical therapy.

The syndecans are a family of 4 cell surface proteoglycans (syndecan-1 [Sdc-1], Sdc-2, Sdc-3, and Sdc-4) that display the capacity to modulate proinflammatory and proteolytic processes within the vascular wall. The biological function of syndecans is primarily exerted via pendant glycosaminoglycans, such as heparan sulfate (HS) and chondroitin sulfate, which sequester and regulate the activity of heparin-binding growth factors, proinflammatory chemokines, and proteases. Intact syndecan ectodomains can be released from the cell surface through proteolytic shedding. This process, which is upregulated within the context of inflammation, allows syndecans to exert a biological effect beyond the confines of the plasma membrane.⁵ Detailed reviews of syndecan biology can be found elsewhere.⁶,⁷ Within the syndecan family, Sdc-1 is emerging as an important regulator of inflammation. Several studies have documented the protective role of Sdc-1 in animal models with strong inflammatory components, such as nephritis,⁸ toxic shock,⁹ allergic lung inflammation,¹⁰ and myocardial infarction.¹¹ Within these diverse investigations lies a common thread suggesting that in an environment of exaggerated inflammation, Sdc-1 can promote a dampened response by interfering with the inflammatory signaling cascade.

In adult tissue, Sdc-1 is found predominately expressed on epithelial cells and noncirculating plasma cells. Expression can...
also be induced in additional cell types, such as endothelial cells, smooth muscle cells, fibroblasts, and macrophages. Macrophage specific Sdc-1 expression is of particular interest, as induction is governed through the cAMP/protein kinase A signaling cascade.\textsuperscript{12,13} The recent availability of new cAMP analogs, which explicitly target protein kinase A or guanine nucleotide exchange factor, is generating increased interest in deciphering the specificity of these downstream effectors.\textsuperscript{14} Notably, cAMP/protein kinase A specific activation in macrophages has been reported to be inhibitory toward the production of inflammatory mediators.\textsuperscript{15} Thus, it appears likely that Sdc-1 is induced on macrophages in the context of a broad signaling program designed to dampen an inflammatory response.

We have previously characterized the spatiotemporal expression for Sdc-1, -2, and -4 in a murine model of angiotensin II induced AAA.\textsuperscript{16} We observed that the expression of Sdc-1 is augmented during the course of aneurysm formation. In addition, Sdc-1 expression in the aneurysm wall seems restricted to a subset of macrophages. In this investigation, we sought to define the functional role of Sdc-1 in experimental AAA formation. We report that Sdc-1 has an important protective function in aneurysm formation that appears mediated, at least in part, through an effect on AAA-localized T cells.

### Methods

**Elastase Perfusion Model**

C57BL/6J mice (Jackson Laboratory) and Sdc-1\textsuperscript{1−/−} mice (backcrossed 10 times onto a C57BL/6J background, provided by Dr Pyong Woo Park, Baylor College of Medicine, Houston, TX) were crossed 10 times onto a C57BL/6J background, provided by Dr.\textsuperscript{16} All mice were genotyped by polymerase chain reaction (PCR) followed by alkaline phosphatase streptavidin (Vector Laboratories).

**Generation of ApoE\textsuperscript{-/-}/Sdc-1\textsuperscript{1−/−} Mice and Angiotensin II Infusion Model**

Apolipoprotein E (ApoE)\textsuperscript{-/-}/Sdc-1\textsuperscript{1−/−} (C57BL/6J background) double knockout mice were generated by standard crossbreeding experiments. Sdc-1\textsuperscript{1−/−} breeder males were mated with ApoE\textsuperscript{-/-} females (Jackson Laboratory), and all mice were genotyped by polymerase chain reaction (PCR). Male mice were subsequently maintained on Paigen atherogenic diet (Research Diets) and received a subcutaneous infusion of angiotensin II (0.75 mg/kg per day) over a 2-week period by mini-osmotic pump (Alza Scientific Products). Systolic blood pressure before and after the implantation of miniosmotic pump (Alza Scientific Products). Systolic blood pressure was measured by tail-cuff method (Visitech Systems). The incidence of AAA formation, incidence of fatal aortic rupture, and final aortic diameter at 2 weeks were determined. Total serum cholesterol was measured with Amplex Red (Molecular Probes).

**Light Microscopy and Immunohistochemistry**

Immunohistochemistry was performed as described previously.\textsuperscript{16} The following antibodies were used: Sdc-1 (N-18, Santa Cruz Biotechnology), neutrophil (NIMP-R14, Abcam), macrophage (Mac3, BD Biosciences), CD4 (RM4-5, BD), CD8 (53-6.7, BD Biosciences), Foxp3 (FJK-16s, eBioscience). Sections were incubated with biotinylated secondary antibodies (Vector Laboratories) followed by alkaline phosphatase streptavidin (Vector Laboratories). Negative controls with isotype IgG were prepared for each specimen.

Spleen sections were used as a positive control tissue for identification of Foxp3-positive cells. Foxp3-positive cells were counted in each aortic section by a trained laboratory technician blinded to sample classification. At least 4 sections from each of 3 animals at each time point in both groups were examined. A mean value for positively stained cells was determined for each animal, and a mean for each animal group was then calculated. Acu-Sstain elastic stain kit (Sigma-Aldrich) was used for elastin degradation studies. Double fluorescent immunostaining was performed as described previously.\textsuperscript{16}

**Flow Cytometry**

Aortas (pooled from 3–6 individual mice) were excised from below the infrarenal arteries to just above the bifurcation after the blood content was flushed. Tissue was finely minced and shaken for 60 minutes at 37°C in 1 mL of RPMI 1640 supplemented with 10% fetal calf serum, 62.5 U/mL collagenase VII (Sigma-Aldrich), and 0.625 U/mL Dispase (BD); for Sdc-1 detection, dispase was excluded. The isolated cells were passed through a 70-μm cell strainer to remove debris and then counted, followed by staining using a standardized protocol. The cell antibodies used included anti-Gr1 (RB6-8C5), anti-Mac1 (M1/70), anti-CD3 (145-2C11), anti-CD45 (30-F11), and anti-CD138 (281-2), all from BD. Cell suspensions were analyzed by flow cytometry (FACSort, BD) and type-specific numbers of cells present in each sample were quantified and recorded.

**Gelatin Zymography**

Aortic tissue extract was prepared in 100 μL of tissue homogenizing buffer (30 mmol/L Tris-Cl, pH 7.5, 150 mmol/L NaCl, 10 mmol/L CaCl₂, 10 μmol/L E-64, 0.05% Brij35, 0.02% NaN₃, and 100 mmol/L phenylmethylsulfonyl fluoride). Protein concentration was quantified using the BCA protein assay kit (Pierce). Five micrograms of aortic tissue extract was run in 10% polyacrylamide containing 10% gelatin (Bio-Rad) under nonreducing conditions. The gel was developed (37°C, 3 days) and stained with 0.125% Coomassie Blue. Gelatinolytic activity was quantified by densitometry (National Institutes of Health ImageJ software).

**Quantitative (Real-Time) Reverse Transcription–PCR**

Messenger RNA levels within the aortic wall were analyzed with reverse transcription–PCR using 18S rRNA as the internal control. All primers were obtained from Applied Biosystems. Four or 5 samples, each containing up to 3 pooled aortas, were obtained from each experimental time point. All PCRs were performed in triplicate with 10 to 25 ng of cDNA using the TaqMan PCR system (Applied Biosystems). Results were analyzed by comparing RNA level of samples with RNA obtained from untreated aortas using the comparative Ct method.

**Antibody Depletion**

Mice were made T lymphocytopenic by intraperitoneal injection of anti-CD3 antibody (50 μg, clone 17A2, Biolegend) at days −1, 4, and 9 after elastase perfusion. Time course of depletion protocol was verified with Thy-1\textsuperscript{1−/−} (GT, Southern Biotech) staining in the spleen. Splenocytes were harvested and finely minced in RPMI 1640 medium supplemented with 5% fetal calf serum, tissue was passed through a 100 μm cell strainer, red blood cells were lysed, and total cell count was recorded. Flow cytometry was used to analyze spleenic T-cell population.

**Bone Marrow Transplantation**

Sdc-1\textsuperscript{1−/−} and Sdc-1\textsuperscript{1−/−} recipients underwent lethal gamma irradiation (11 Gy) to eliminate endogenous bone marrow (BM) stem cells and circulating leukocytes. Using sterile procedures, BM cells were obtained by flushing femoral and tibial bones from Sdc-1\textsuperscript{1−/−} and Sdc-1\textsuperscript{1−/−} mice. Each irradiated mouse was injected intraorbitally with 10⁶ BM cells from designated donors. Three groups (8–10 mice/group) were studied: (1) Sdc-1\textsuperscript{1−/−} BM cells transplanted to...
Sdc-1+/− recipients, (2) Sdc-1−/− BM cells transplanted to Sdc-1+/+ recipients, and (3) Sdc-1−/−/ BM cells transplanted to Sdc-1−/− recipients. Mice were enrolled in the elastase perfusion model 8 weeks after transplantation.

Chemotaxis
CD4+ T cells were positively selected from splenocytes (Miltenyi Biotec), harvested 8 to 10 days after *Listeria monocytogenes* (*10⁶ CFU/mouse*) infection to produce the desired Th1 polarized response. Chemotaxis assays were performed using 4×10⁵ cells in 5-μm Transwell plates (Corning) with RPMI, 0.1% bovine serum albumin base media with or without chemokine. In some cases, HS (50 μg/mL) was included in the lower well. The migration response was quantified after 4 hours at 37°C in a humidified incubator.

Chemokine Profile
Day 4 thioglycollate elicited peritoneal macrophages were collected from C57BL/6J mice and plated in Dulbecco’s modified Eagle’s medium (10% fetal bovine serum) at 20×10⁶ per 100×20-mm tissue culture dish. Classically activated M1 macrophage were generated through interferon (IFN)-γ (100 U/mL)/lipopolysaccharide (10 ng/mL) stimulation and Sdc-1 expression was induced with 6-Bnz-cAMP (100 μM/mL). Sdc-1 expression was verified with flow cytometry (anti-CD138, 281–2). Serum-free Dulbecco’s modified Eagle’s medium was added after initial stimulation, and conditioned medium was collected after 12 hours. Adhered cells were exposed to a 1 mol/L NaCl wash to disrupt any surface electrostatic binding interactions. Conditioned media and wash fractions were combined, filtered (0.2 μm), subjected to dialysis and concentration 4-fold (Amicon Ultra 3000 nominal molecular weight limit), and analyzed for regulated upon activation, normal T expressed and secreted; monokine induced by interferon gamma; macrophage-1a; macrophage inflammatory protein-1b; interferon gamma-induced for regulated upon activation, normal T expressed and secreted; interleukin 8; monokine induced by interferon gamma; macrophage-derived chemokine; and keratinocyte-derived chemokine using the Mouse Common Chemokine ELISAArray (SA Biosciences). Three independent experiments were conducted.

Statistical Analysis
Mean and SEM were calculated for each parameter. All data were analyzed via 2-tailed Student t test, with the exception of frequency and mortality contingency tables in the angiotensin II AAA model (Fisher exact test). Values of *P*<0.05 were considered statistically significant.

Results

**Macrophage-Associated Sdc-1 Expression Attenuates Experimental Aneurysm Formation**
Transient intraaortic perfusion with elastase is a common experimental model of AAA with consistent development of an infrarenal aortic aneurysm 14 days after initial perfusion. Immunohistochemistry was used to characterize Sdc-1 expression in concert with the neutrophil and macrophage infiltrate over the 14-day time course of aneurysm development. We observed discrete regions of neutrophil infiltration at 1, 4, and 7 days after elastase infusion; particularly, evident in both the intima and periadventitia (Supplemental Figure IA–ID, available online at http://atvb.ahajournals.org). The 7- and 14-day inflammatory response was dominated by transmural infiltration of macrophages (Supplemental Figure IE–IH). Sdc-1 expression was accentuated during the 14-day time course, with most Sdc-1-positive cells localized to the adventitia (Supplemental Figure II–IL). Little to no expression of Sdc-1 was detectable in the native aorta (data not shown). Similar patterns of neutrophil and macrophage recruitment were observed in Sdc-1-deficient mice (Supplemental Figure IM–IT). Immunohistochemical analysis revealed an association between macrophage and Sdc-1-positive staining, which was confirmed by double immunofluorescence staining. As illustrated, Sdc-1 expression colocalized with Mac-3-positive cells, consistent with the notion that Sdc-1 expression is specific to infiltrating macrophages (Figure 1A).

To study the functional significance of Sdc-1 in the pathogenesis of AAA, we enrolled Sdc-1-deficient mice (Sdc-1−/−, C57BL/6 background) in the elastase perfusion model and compared the results with those of wild-type C57BL/6 mice (Sdc-1+/+). Sdc-1-deficient mice have been previously characterized as healthy, with normal growth, reproduction, tissue morphology, hematologic profile, and serum chemistry parameters. There was moderate aortic dilatation in both Sdc-1+/+ and Sdc-1−/− mice up to 4 days after elastase perfusion. Increased dilatation was noted by day 7, with AAA (Δaortic diameter >100%) in 34% of Sdc-1−/− mice and 81% of Sdc-1−/− mice. All mice formed aneurysms by day 14; however, the extent of aortic dilatation was significantly greater among the Sdc-1−/− group (*P*<0.01; Figure 1B and Supplemental Table I), suggesting that endogenous Sdc-1 expression exerts a protective role in elastase-induced AAA. BM transplantation experiments confirmed that the protective source of Sdc-1 expression originates from the circulating leukocyte population during the development of AAA (*P*<0.01; Figure 1C and 1D). Finally, to further characterize Sdc-1 expression in AAA tissue, we used flow cytometry (Mac-1/Sdc-1) to examine 14-day aortic tissue digests. Sdc-1+ cells were observed as a subset of Mac-1+ cells (Figure 1E); significantly, collective observations from double immunofluorescent staining, BM transplantation, and flow cytometry all suggest that macrophages provide a significant source of Sdc-1 expression in AAA.

To further test the hypothesis that macrophage Sdc-1 expression is protective in AAA, we used a second model of experimental aneurysm formation. The murine model of angiotensin-associated aortic aneurysm is produced by subcutaneous administration of angiotensin II into ApoE−/− mice. Standard crossbreeding was used to generate ApoE−/− Sdc-1−/− double knockout mice, which were enrolled in this model and responses compared with those of ApoE−/− Sdc-1+/+ mice. Animals were euthanized at 3, 7, and 14 days, and aortic tissue was harvested for immunohistochemical examination. Sdc-1/macroage colocalization was confirmed by double immunofluorescence staining (Supplemental Figure IIA–IIP). Despite equivalent blood pressure responses and serum cholesterol levels (Supplemental Table II), significantly higher rates of AAA formation (>2-fold) and rupture (>6-fold) were observed in ApoE−/− Sdc-1−/− mice (*P*<0.05; Figure 1F and 1G). These data suggest that the protective role of macrophage-associated Sdc-1 in AAA is independent of experimental animal model.

**Sdc-1 Deficiency Increases Proteolytic Activity in the Aortic Wall**
Destruction of medial elastin is a hallmark of AAA pathogenesis. We stained aortic sections for elastic fibers and used a
grading scheme to compare degradation of the medial elastic lamellae in Sdc-1+/+ and Sdc-1−/− mice 7 and 14 days after elastase infusion (Figure 2A–2D).24 We observed increased degradation in Sdc-1−/− mice at both time points (P<0.05; Figure 2E). Matrix metalloproteinase (MMP) activity is abundant in AAA tissue and MMP-2 and MMP-9 have been causally linked to damage of structural matrix components in experimental models.25 Gelatin zymography revealed increased MMP-2 and -9 activity in aortic tissues harvested from Sdc-1−/− mice 7 days after elastase infusion (P<0.05; Figure 2F and 2G). These results suggest that Sdc-1 protects against AAA formation by dampening proteolytic activity.

**Sdc-1 Deficiency Promotes Increased Inflammatory Cell Recruitment Into the Aortic Wall**

Given the difficulty in quantifying the inflammatory response with standard immunohistochemistry, flow cytometry...
was used to compare neutrophil, macrophage, and T-cell recruitment into the aortic tissue of Sdc-1 \(^+\/+\) and Sdc-1 \(^-/-\) mice. To broadly characterize leukocyte infiltration, total CD45\(^+\) cell count per aorta was analyzed 7 and 14 days after elastase infusion. A trend toward a greater inflammatory response was identified in Sdc-1 \(^-/-\) mice at day 7; by day 14, a significant increase \((P<0.05)\) in CD45\(^+\) cell count was observed in Sdc-1 \(^-/-\) AAA (Figure 3A–3D). Macrophage, neutrophil, and T-cell populations were analyzed after normalizing the data to total CD45\(^+\) cell count within the harvested aortic tissue. As expected, the proportion of macrophages within the inflammatory infiltrate increased from day 7 to day 14 with a decline in the neutrophil population (Figure 3E, 3F, 3H, 3I, and Supplemental Figure III), consistent with a shift from an acute to a chronic inflammatory response. The T-cell population represented a smaller fraction of the overall inflammatory cell population, but the relative proportion of T-cells was significantly higher in Sdc-1 \(^-/-\) aortas at both time points \((P<0.01; \) Figure 3G and 3J). Given the increased severity of AAA in Sdc-1 \(^-/-\) mice, the observation of an augmented inflammatory response was not surprising. However, the enhanced T-cell response was unanticipated and provides an additional mechanistic link in understanding the protective role of Sdc-1 in AAA.

**T Cells Mediate Enhanced Severity of AAA in Sdc-1-Deficient Mice**

To confirm increased T-cell localization in Sdc-1 \(^-/-\) AAA, CD4\(^+\) and CD8\(^+\) T-cell populations were characterized using immunohistochemical staining. CD4\(^+\) and CD8\(^+\) T cells were readily detectable in the adventitia of Sdc-1 \(^+\/+\) and Sdc-1 \(^-/-\) aneurysms at 14 days after elastase infusion (Figure 4A). Quantitative analysis confirmed a dominant CD4\(^+\) response in both Sdc-1 \(^+\/+\) and Sdc-1 \(^-/-\) AAA; in addition, there was a significant increase in CD4\(^+\) localized T cells in Sdc-1 \(^-/-\) over Sdc-1 \(^+\/+\) aneurysm tissue \((P<0.05; \) Figure 4B). To explore the functional role of T cells in the enhanced inflammatory environment of Sdc-1 \(^-/-\) AAA, T-cell depletion was performed. \(^6\) No detectable difference was observed in the T-cell population (CD90\(^+\)) assessed by flow cytometry in spleens harvested from Sdc-1 \(^+\/+\) and Sdc-1 \(^-/-\) mice (Figure 4C). Anti-CD3 treatment afforded 75% depletion in T cells at day 14 and resulted in a statistically significant reduction in final aortic diameter of Sdc-1 \(^-/-\) mice \((P<0.01; \) Figure 4D and 4E), confirming the active role of T cells in aneurysmal dilatation.

**Characterization of Inflammatory and Anti-inflammatory Mediators in Sdc-1 \(^+\/+\) and Sdc-1 \(^-/-\) AAA**

To provide further insight into the inflammatory signaling cascade, quantitative real-time reverse transcription–PCR was used to measure mRNA levels of IFN-\(\gamma\), interleukin (IL)-4, IL-10, and Foxp3 (forkhead/winged helix transcription factor). IFN-\(\gamma\) and IL-4 have both been implicated in the inflammatory environment of Sdc-1 \(^+\/+\) AAA; moreover, IFN-\(\gamma\)/IL-4 ratio is often characterized to describe the dominant T-cell cytokine profile. \(^26,27\) IL-10 and Foxp3 were chosen as representative anti-inflammatory markers. \(^28,29\) In both Sdc-1 \(^+\/+\) and Sdc-1 \(^-/-\) AAA, IFN-\(\gamma\) was markedly upregulated (40-fold), whereas IL-4 expression was low (2-fold), with no significant difference between groups (Figure 5A and 5B). IL-10

**Figure 2.** Syndecan-1 (Sdc-1) deficiency increases medial elastin degradation and matrix metalloproteinase (MMP) activity in the aortic wall during abdominal aortic aneurysm (AAA) formation. A to D, Medial elastin degradation during the course of elastase-induced aneurysm formation. Elastin degradation-grading keys were as follows: A, grade I; B, grade II; C, grade III; D, grade IV. Scale bar=100 \(\mu\)m. E, Histological sections were scored for the integrity of aortic elastin on a 4-point scale according to the elastin degradation-grading keys \((n=3)\). *\(P<0.05\). F, Gelatin substrate zymogram analysis of MMP-2 and MMP-9 in the aortic wall during elastase-induced AAA formation \((n=3 \) for Sdc-1 \(^+\/+\) and Sdc-1 \(^-/-\) mice; 5 \(\mu\)g/well). G, Densitometry was used to quantify gel zymogram; results are presented as relative density units. *\(P<0.05\). Data represent mean±SEM.
expression was significantly upregulated (4-fold) in the Sdc-1/+/+ mice, but not in Sdc-1−/− mice (Figure 5C). Although Foxp3 expression was upregulated in both wild-type and knockout mice, levels were significantly higher in Sdc-1+/+ mice (34-fold versus 16-fold; Figure 5D). We sought to confirm the presence of regulatory T cells in the aortic wall, given the increased levels of Foxp3 in Sdc-1+/+ mice. Regulatory T cells were readily detectable in the adventitia at both 7 and 14 days by immunohistochemical staining for Foxp3 (Figure 5E-5H). Quantitative analysis confirmed a greater number of regulatory T cells in the AAA tissue from Sdc-1+/+ mice (Figure 5I). Collectively, these results illustrate the reduced capacity of Sdc-1−/− mice to limit vessel wall inflammation in AAA.
Based on our findings that Sdc-1 plays an active role in restricting the localized T-cell population during aneurysm formation, we elected to investigate the potential for Sdc-1 to modulate AAA-driven T-cell chemotaxis. Chemokines are small HS-binding proteins that direct infiltration of leukocytes into inflamed tissue and are recognized as critical components in sustaining chronic inflammation. Glycosaminoglycan/chemokine binding interactions are well established and thought to be mediated, at least in part, by electrostatic interactions of basic chemokines with negatively charged HS. Such interactions provided motivation to test the capacity of HS, as a syndecan-1 mimetic, to competitively inhibit T-cell chemotaxis to AAA relevant chemokines. In light of the CD4+/IFN-γ-dominant response that was characterized during the formation of AAA, we specifically investigated chemotaxis in Th1 polarized CD4+ T cells from Sdc-1−/− mice. Quantitative real-time reverse transcription–PCR was used to identify chemokines of interest (potential to elicit Th1 localization) in elastase-induced aneurysm formation. In both Sdc-1+/+ and Sdc-1−/− AAA, there was significant expression of CCL2 (monocyte chemotactic protein-1), CCL3 (macrophage inflammatory protein-1α), CCL5 (regulated upon activation, normal T cell expressed and secreted), and CXCL9 (MIG); however, no difference in expression was noted between genotypes (data not shown). We tested the ability of HS to limit Th1 polarized CD4+ T-cell chemotaxis to CCL2, CCL3, CCL5, and CXCL9 in a standard Transwell migration assay. HS effectively limited chemotaxis to CCL2, CCL3, and CXCL9 without influencing basal migration, and we did not observe an impact on CCL5-driven chemotaxis (*P<0.05; Figure 6). Of note, initial studies were performed to ensure equivalent chemotactic response in Sdc-1+/+ and Sdc-1−/− CD4+ T cells, and no inherent differences were observed; in addition, both naïve and activated CD4+ T cells from Sdc-1+/+ mice were examined for Sdc-1 expression using flow cytometry, and there was no evidence of Sdc-1 expression on CD4+ T cells (data not shown). These studies suggest that macrophage Sdc-1 may modulate the T-cell inflammatory response through HS-chemokine binding interactions. Alternatively, Sdc-1-expressing macrophages may represent a regulatory or M2 macrophage population displaying a
unique chemokine profile with dampened potential to incite inflammatory cell chemotaxis. We examined the chemokine profile of Sdc-1-expressing macrophages against classically activated (M1) inflammatory macrophages. Sdc-1 induction was achieved using cAMP, the only reported stimulant to drive expression, whereas IFN-γ/lipopolysaccharide induction was used to induce classically activated macrophages. Significantly, we observed a dramatic difference in chemokine profile between Sdc-1-expressing and M1 macrophages, with dampened detection of regulated upon activation, normal T cell expressed and secreted, monocyte chemotactic protein-1, macrophage inflammatory protein-1α, interferon gamma-induced protein 10; monokine induced by interferon gamma; macrophage-derived chemokine; and keratinocyte-derived chemokine in the Sdc-1 induced population (Supplemental Figure IVA). Notably, we confirmed absence of Sdc-1 expression on M1 macrophages, with dampened detection of regulated upon activation, normal T cell expressed and secreted, monocyte chemotactic protein-1, macrophage inflammatory protein-1α, interferon gamma-induced protein 10; monokine induced by interferon gamma; macrophage-derived chemokine; and keratinocyte-derived chemokine in the Sdc-1 induced population (Supplemental Figure IVA). Notably, we confirmed absence of Sdc-1 expression on M1 macrophages (Supplemental Figure IVB and IVC), lending plausibility to the idea that Sdc-1-expressing macrophages represent an alternatively activated regulatory population in the vessel wall.

Discussion

Abdominal aortic aneurysm (AAA) is a complex multifactorial disease. Little is understood about the mechanisms that initiate AAA; however, chronic inflammation plays a key role in driving the progressive and pathological remodeling of the aorta. This dysregulated process of inflammation arises from an imbalance of pro- and anti-inflammatory mediators in the vascular wall. Our investigations identify macrophage-expressed Sdc-1 as an endogenous modulator of the inflammatory response in AAA. Significantly, we provide evidence for the presence of a macrophage counterbalance to T-cell–driven inflammation in the vascular wall.

We used Sdc-1−/− mice, in concert with both the elastase perfusion and angiotensin II–induced experimental models of AAA, to explore the functional relevance of Sdc-1 in aneurysm pathology. During the course of aneurysm formation in both experimental models, the expression of macrophage-associated Sdc-1 became increasingly prominent within the aortic wall. As evident by the significant increase in aneurysm diameter in the elastase perfusion model and an increase in the incidence of aneurysm formation and frequency of aortic rupture in the angiotensin II infusion model, macrophage expressed Sdc-1 appears to play a protective role. BM transplantation studies confirmed that deficiency of Sdc-1 in marrow-derived cells significantly contributed to AAA severity. Medial elastin degradation and MMP analysis revealed greater proteolytic activity within the aortic wall in Sdc-1−/− mice. Intriguingly, we observed a dramatic difference in chemokine profile between Sdc-1-expressing and M1 macrophages, with dampened detection of regulated upon activation, normal T cell expressed and secreted, monocyte chemotactic protein-1, macrophage inflammatory protein-1α, interferon gamma-induced protein 10; monokine induced by interferon gamma; macrophage-derived chemokine; and keratinocyte-derived chemokine in the Sdc-1 induced population (Supplemental Figure IVA). Notably, we confirmed absence of Sdc-1 expression on M1 macrophages (Supplemental Figure IVB and IVC), lending plausibility to the idea that Sdc-1-expressing macrophages represent an alternatively activated regulatory population in the vessel wall.

Discussion

Abdominal aortic aneurysm (AAA) is a complex multifactorial disease. Little is understood about the mechanisms that
mice. Gel zymography alone does not allow us to attribute the increase in Sdc-1‒/‒ proteolytic activity to a specific cell type within the aortic wall. However, several reports have now demonstrated that within aortic aneurysm tissue, macrophages and smooth muscle cells are the primary source of MMP-9 and MMP-2, respectively.17,25 Flow cytometry of digested AAA tissue confirmed increased inflammatory cell recruitment in Sdc-1‒/‒ samples; accordingly, by absolute number, Sdc-1‒/‒ aneurysms contained a greater number of neutrophils, macrophages, and T cells. We chose to focus further efforts on T-cell infiltration after subsequent analysis of individual neutrophil, macrophage, and T-cell populations as a proportion of total CD45+ signal revealed a significantly higher percentage of T cells in Sdc-1‒/‒ mice at both 7 and 14 days. We characterized a CD44+ /IFN-γ dominant response in our elastase-induced model of AAA and used depletion studies to confirm the role of T cells in aneurysmal dilation.

Furthermore, in reporting dampened expression of IL-10 and Foxp3, we provide evidence that Sdc-1‒/‒ mice display a reduced capacity to limit inflammation within the aortic wall and, in vitro, we demonstrate the capacity of Sdc-1-expressing macrophages to control the local chemokine environment. Thus, we conclude that Sdc-1-expressing macrophages serve as a critical component in the vessel wall anti-inflammatory response. Notably, regulatory macrophages would exert influence through multiple pathways within aneurysm tissue; however, the current report directs focus toward the specific modulation of the T-cell response.

T lymphocytes have long been recognized as a significant infiltrate in AAA, yet the specificity of adaptive immunity in aneurysm pathogenesis remains controversial and undefined.33,34 T-cell receptor gene expression has been probed in human tissue to provide evidence for selective, antigen specific activation. Yen et al35 analyzed 5 human explants, reporting polyclonal or nonrestricted diversity, and Platsoucas et al36 examined 10 human explants and reported evidence for oligoclonal T-cell populations, suggesting antigen specific expansion. Many immunologic triggers have been considered, including microbial infection, molecular mimicry, and products of vessel wall proteolysis.37 Infiltrating T cells provide a powerful vehicle for mediating proinflammatory cytokine release by macrophages. Thus, many studies have sought to characterize the dominant cytokine profile during the course of AAA formation. Xiong et al reported the critical role of CD4+ T cells and IFN-γ in a mouse model of CaCl2-induced aneurysm formation.27 The predominant presence of CD4+ T cells with high levels of IFN-γ or Th1-type transcripts have also been reported in human AAA.38 In contrast, using a murine model of aortic transplantation, Shimizu et al26 reported that an IL-4 or Th2 dominant response was critical to aneurysm formation, and King et al39 reported a protective role for IFN-γ in angiotensin-II induced AAA; both reports suggest that a Th1 response may limit aneurysm formation. In effect, the literature addressing the role of T lymphocytes in AAA is conflicting and highlights the need for continued investigation. Moreover, the current paradigm broadens the realm of T-cell subsets and cautions against simple definitions that rely on terminal commitment of Th1 or Th2 responses.40,41

HS-chemokine binding interactions are well established. Thus, it is reasonable to hypothesize that Sdc-1 interferes with T-cell infiltration through HS-mediated sequestration of T-cell-specific chemokines. Furthermore, augmented T-cell responses have been previously reported in Sdc-1‒/‒ mice, and Sdc-1 sequestration of T-cell‒specific chemokines has been shown to inhibit T-cell migration.9,10 We tested the ability of HS to interfere with effector CD4+ T-cell migration to chemokines specifically upregulated in our elastase-induced model of aneurysm formation. HS significantly inhibited chemotaxis to CCL2, CCL3, and CXCL9, all potentially important chemokines in driving T-cell migration in AAA formation.42–44 We did not observe a significant impact on CCL5 migration; however, regulated upon activation, normal T cell expressed and secreted has been reported to be more discriminatory in glycosaminoglycan binding.45 Adventitial localization and sequential infiltration of macrophage and T-cell populations is presumed to be critical in the specific inhibition of the T-cell population by Sdc-1. Macrophage Sdc-1 expression is firmly established in the adventitia, as the T-cell population emerges during aneurysm formation; such spatiotemporal expression is believed to impart some specificity to HS proteoglycan‒chemokine binding interactions.6 In addition to chemokine sequestration, it is also plausible that Sdc-1-expressing macrophages modulate the chemokine environment in a more direct manner; we chose to characterize the chemokine expression profile of Sdc-1 macrophages against classically activated M1 inflammatory macrophages. Sdc-1-expressing macrophages display a significantly dampened chemokine profile compared with M1 macrophages, such reduced expression of inflammatory chemokines supports the notion that Sdc-1-expressing macrophages function to regulate the inflammatory response during AAA. Notably, we observed no evidence of Sdc-1 expression on macrophages driven to an M1 phenotype; continued investigations within our laboratory are directed at characterizing the specific subtype of Sdc-1-expressing macrophages. Collectively, these findings define a protective role for Sdc-1 expression during the formation
of AAA. By modulating the chemokine environment, Sdc-1-expressing macrophages can influence the participation of T lymphocytes and dampen the magnitude of the inflammatory response. However, an important caveat of our study is that T lymphocyte participation in AAA may be governed through multiple pathways, including trafficking, survival, and proliferation. Although many reports have documented the significance of chemokine driven lymphocyte trafficking to chronically inflamed aortic tissue, alternate hypotheses warrant further study.

Our in vivo data support the notion that Sdc-1 expression may be an important component in a series of programmed events designed to downregulate the inflammatory response within the aortic wall. Indeed, IL-10 was upregulated only in Sdc-1+/− mice, and expression of the regulatory T-cell marker Foxp3 was much lower in the absence of Sdc-1. Standard immunohistochemistry confirmed the localization of regulatory T cells to aneurysm tissue, and subsequent quantification revealed a significantly greater presence of regulatory T cells in Sdc-1+/− AAA. Regulatory T cells can specifically home to inflamed tissue and effectively suppress both innate and adaptive immune responses. Significantly, regulatory T cells orchestrate suppression of innate immunity through release of anti-inflammatory cytokines, such as IL-10 and transforming growth factor-β, and they have been implicated in dampening the inflammatory potential of neighboring macrophages.

Indeed, regulatory T cells may contribute to elevated IL-10 transcripts observed in Sdc-1+/− AAA. Given reported macrophage/regulatory T-cell cross-talk, it is tempting to speculate that polarized macrophage populations influence localization of regulatory T cells to sites of inflammation. However, further study is warranted to assess the mediators, which govern both trafficking and suppressor function of regulatory T cells in aneurysm tissue.

Our study was designed to examine the role of macrophage-expressed Sdc-1 in AAA. Because syndecans have been reported to both augment and inhibit inflammatory signaling events, predicting the role of Sdc-1 in AAA was not intuitive. Moreover, studying the functional significance of Sdc-1 in macrophages, a cell population fundamentally regarded as inflammatory in chronic disease, complicated interpretation of results. The significance of our findings lies in the seemingly contradictory observation that an endogenous anti-inflammatory counterbalance emanates from an AAA localized population of macrophages. However, the presence of a counterbalance is expected within the current paradigm of the inflammatory cascade, and macrophages are recognized as key components in the switch to inflammatory resolution. Microenvironmentally derived signals give rise to macrophages that are polarized with specific functional properties, including: classically activated, proinflammatory M1 macrophages, and alternatively activated, potentially anti-inflammatory M2 macrophages. Inherent negative feedback control in macrophage activation and functional plasticity would be expected to result in an overlapping M1/M2 presence in chronic inflammation. In both human and mouse AAA, Sdc-1 expression is augmented in a subset of localized macrophages; herein, we present evidence that Sdc-1 is induced on regulatory macrophages in the context of a programmed response to dampen inflammation. Indeed, it has been suggested that chemokine modulation is a critical functional attribute in alternatively activated macrophages. However, the effectiveness of this negative regulation is most likely limited in a pathological setting. By the very nature of chronic inflammation, the native processes that serve to restore homeostasis can be overpowered by the exaggerated degree of the inflammatory insult. Within this paradox lie new targets for therapeutic intervention. Characterizing the molecular mechanisms that regulate the intrinsic counterbalance is an important step toward the development of new approaches to promote the resolution of chronic inflammation in the vascular wall.

Sources of Funding
This work was funded by National Institutes of Health Grant HL069093 (to E.L.C.) and fellowships from the American College of Surgeons (to S.V.S.) and the American Heart Association (to J.A.).

Disclosures
None.

References


Syndecan-1 Displays a Protective Role in Aortic Aneurysm Formation by Modulating T Cell–Mediated Responses
Jiantao Xiao, Julianty Aangsana, Jing Wen, Sumona V. Smith, Pyong Woo Park, Mandy L. Ford, Carolyn A. Haller and Elliot L. Chaikof

Arterioscler Thromb Vasc Biol. 2012;32:386-396; originally published online December 15, 2011;
doi: 10.1161/ATVBAHA.111.242198
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2011 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/32/2/386

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2011/12/14/ATVBAHA.111.242198.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/
**Supplementary Table I** Effect of Sdc-1 Deficiency on Aortic Dilation

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>AD Pre (mm)</th>
<th>AD Post (mm)</th>
<th>AD Final (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sdc-1**+**</td>
<td>32</td>
<td>0.50 ± 0.01</td>
<td>0.91 ± 0.01</td>
<td>1.29 ± 0.02</td>
</tr>
<tr>
<td>Sdc-1**-/-**</td>
<td>32</td>
<td>0.49 ± 0.01</td>
<td>0.90 ± 0.01</td>
<td>1.61 ± 0.03</td>
</tr>
<tr>
<td>t-test</td>
<td></td>
<td>p=0.12</td>
<td>p=0.13</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>

Aortic diameter (AD) and extent of aortic dilatation at day 0 and 14. AD were obtained before (Pre) and immediately after (Post) elastase perfusion, with final measurement obtained at day 14 (Final). Data represent mean ± SEM.
### Supplementary Table II  Mean Blood Pressure and Mean Total Cholesterol

<table>
<thead>
<tr>
<th></th>
<th>ApoE&lt;sup&gt;−/−&lt;/sup&gt; Sdc-1&lt;sup&gt;+/+&lt;/sup&gt;</th>
<th>ApoE&lt;sup&gt;−/−&lt;/sup&gt; Sdc-1&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure pre-ang II (mmHg)</td>
<td>105 ± 7</td>
<td>112 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>Blood pressure post-ang II (mmHg)</td>
<td>151 ± 23</td>
<td>162 ± 19</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol on an atherogenic diet (mg/dL)</td>
<td>1149 ± 150</td>
<td>1365 ± 240</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, no significant difference. Results are mean ± SD.
Data Supplement Figure I
Data Supplement Figure II
Data Supplement Figure III
Data Supplement Figure IV
Supplement Figure I. Immunohistochemical staining of the abdominal aorta during the course of elastase-induced AAA formation. A-D, Neutrophil staining in Sdc-1\(^{+/+}\) mice at day 1, 4, 7, and 14 respectively. E-H, Macrophage staining for Sdc-1\(^{+/+}\) mice at day 1, 4, 7, and 14 respectively. I-L, Sdc-1 staining in Sdc-1\(^{+/+}\) mice at day 1, 4, 7, and 14 respectively. M-P, Neutrophil staining for Sdc-1\(^{-/-}\) mice at day 1, 4, 7, and 14 respectively. Q-T, Macrophage staining for Sdc-1\(^{-/-}\) mice at day 1, 4, 7, and 14 respectively. Scale bars, 100 µm.
Supplement Figure II. Immunohistochemical and immunofluorescent staining of the abdominal aorta during the course of angiotensin II-induced aneurysm formation. A-D, Neutrophil staining for ApoE^+/Sdc-1^+/ mice at day 3, 7, and 14 respectively, and ApoE^−/−Sdc-1^+/ mice at day 14. E-H, Macrophage staining for ApoE^+/Sdc-1^+/ mice at day 3, 7, and 14 respectively, and ApoE^+/Sdc-1^−/− mice at day 14. I-L, Sdc-1 staining for ApoE^+/Sdc-1^+/ mice at day 3, 7, and 14 respectively, and ApoE^+/Sdc-1^−/− mice at day 14. M, Immunofluorescent staining of the abdominal aorta at 14 days after angiotensin II infusion, representative merged image; N, macrophage staining, Alexa 594, red; O, Sdc-1 staining, fluorescein, green; dapi nuclear stain, blue; P, macrophage and Sdc-1 merge. Scale bars, 100 µm.
**Supplement Figure III.** Inflammatory cell recruitment into aneurysmal tissue. A-D, Representative FACS plots of aortic tissue digests at 14 days post elastase perfusion. A and B, Aortic tissue extracts were analyzed for macrophages (Mac1⁺, Gr1⁻) and neutrophils (Mac1⁺, Gr1⁺), plots gated on Mac1⁺; boxed data represents neutrophil population contributing to Mac1 signal. C and D, Aortic tissue extracts were analyzed for T cells (CD45⁺, CD3⁺), plots gated on CD45⁺, boxed data represents T cell population.
**Supplement Figure IV.** Chemokine profile in classically activated (M1) and syndecan-1 expressing macrophages. A, Chemokine profile measured with ELISAArray, n=3, *P<0.05. B, Representative FACS plot, M1 induced macrophages stained for syndecan-1 (281-2 APC) and macrophage specific marker (F4/80 PE). C, Representative FACS plot, syndecan-1 induced macrophages stained for syndecan-1 (281-2 APC) and macrophage specific marker (F4/80 PE).