NIH Public Access
Author Manuscript
Arterioscler Thromb Vasc Biol. Author manuscript; available in PMC 2016 February 01.

Published in final edited form as:
Arterioscler Thromb Vasc Biol. 2015 February ; 35(2): 332–340. doi:10.1161/ATVBAHA.114.304720.

Syndecan-1 Modulates the Motility and Resolution Responses of Macrophages

Julianty Angsana[1], **Jiaxuan Chen**[2], **Sumona Smith**[3], **Jiantao Xiao**[3], **Jing Wen**[3], **Liying Liu**[2], **Carolyn A. Haller**[2,*], and **Elliot L. Chaikof**[2,4,*]

[1]Department of Bioengineering, Georgia Institute of Technology, Atlanta, GA

[2]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA

[3]Department of Surgery, Emory University, Atlanta, GA

[4]Wyss Institute of Biologically Inspired Engineering, Harvard University, Boston, MA

Abstract

Objective—Syndecan-1 (Sdc-1) is a member of a family of cell surface proteoglycans that has been reported to participate in the regulation of events relevant to tissue repair and chronic injury responses, including cell-substrate interactions, matrix remodeling and cell migration. In this study, we report the functional significance of Sdc-1 in polarized macrophage populations and their role in adhesion and motility events relevant to resolution of the inflammatory program.

Approach and Results—Macrophage Sdc-1 expression is associated with differentiated M2 macrophages with high intrinsic motility and Sdc-1 deficiency is characterized by impaired migration and enhanced adhesion. Leukocyte infiltration and emigration were examined in a thioglycollate-induced model of peritonitis in Sdc-1$^{+/+}$ and Sdc-1$^{-/-}$ mice. Although the infiltration of inflammatory cells was similar in both cohorts, a significant delay in the lymphatic clearance of Sdc-1$^{-/-}$ macrophages was observed. Moreover, we observed enhanced inflammation and greater burden of atherosclerotic plaque in ApoE$^{-/-}$Sdc-1$^{-/-}$ mice maintained on a Western diet.

Conclusions—These results demonstrate that defective motility in Sdc-1$^{-/-}$ macrophages promotes a persistent inflammatory state with relevance to the pathogenesis of atherosclerosis.

Keywords

macrophage; migration; Sdc-1; inflammation; resolution

A persistent population of activated lesional macrophages has been linked to the progression of atherosclerosis. Unrestrained macrophage activation plays a vital role in pathogenic remodeling and plaque destabilization that can culminate in rupture and acute coronary syndromes. Intensive research efforts are focused on understanding the regulatory

*Address correspondence to: Elliot L. Chaikof, M.D., Ph.D. or Carolyn A. Haller, Ph.D., BIDMC, 110 Francis St, Suite 9F, Boston, MA 02215, Tel: (617) 632-9581, Fax: (617)-632-9701, echaikof@bidmc.harvard.edu or challer@bidmc.harvard.edu.

DISCLOSURES
None.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

mechanisms that signal resolution programs characteristic of a healthy inflammatory response and a return to tissue macrophage homeostasis. Nonphlogistic clearance of apoptotic cells has been suggested as a key event that drives macrophage resolution via anti-inflammatory signaling and enhanced egress of macrophages from inflammatory foci.[1, 2] Indeed, secondary necrosis observed in advanced atherosclerotic plaques may be a signature of failed resolution programs in sessile lesional macrophages that do not efficiently clear apoptotic debris and likewise do not propagate signals to silence inflammation and exit the vascular wall.[3–6] Such observations have renewed interest to understand macrophage motility dynamics, as they relate to a persistent inflammatory state within the vessel wall with the hope of developing new strategies to limit macrophage burden in atherosclerosis.

Plaque regression has been documented in numerous animal studies and remains the ultimate therapeutic goal in the treatment of human atherosclerosis.[7–10] Recent investigations using a mouse surgical transplant model of plaque regression have suggested that dramatic improvements in plasma lipoprotein profiles can induce monocyte-derived cells to emigrate out of the inflamed vascular wall and that this process correlates with reduced plaque burden.[11, 12] Cell tracking studies identified egress depots in the iliac and hepatic lymph nodes, suggesting dissemination through draining lymph nodes, as well as reentry into the blood stream.[13] Consistent with this hypothesis of failed macrophage clearance in chronically inflamed vascular lesions, Moore and associates have reported the expression of inhibitory guidance cues, netrin-1 and semaphorin 3E, in atheroma macrophages.[14, 15] Moore offers a paradigm of competing trafficking signals to explain macrophage retention, where neuronal guidance molecules secreted by activated macrophages may serve as a stop signal to inhibit activation of the actin cytoskeleton and override chemokine-mediated macrophage emigration from the atherosclerotic wall. Conversely, data has been reported in an apoE complementation model of regression, where reduced macrophage burden is strongly correlated with decreased monocyte recruitment and apoptotic turnover rather than migratory egress.[16] Collectively, these reports emphasize the complexity in dissecting the macrophage resolution response and provide motivation for continued investigations of inflammatory effectors that govern macrophage retention in atherosclerosis.

In this study, we report the functional significance of the cell surface heparan sulfate proteoglycan syndecan-1 (Sdc-1) on macrophage adhesion and motility events relevant to inflammatory resolution. We have previously reported macrophage Sdc-1 as an endogenous modulator of the inflammatory response in abdominal aortic aneurysm (AAA) and we speculated that Sdc-1 expressing macrophages represented an alternatively activated regulatory population in the chronically inflamed wall.[17] Anti-inflammatory functions of Sdc-1 are often attributed to the sequestration of inflammatory mediators by pendant glycosaminoglycans,[18] however, Sdc-1 has also been reported to regulate cell adhesion and migration.[19–21] Macrophage Sdc-1 protein expression is governed through the cAMP/protein kinase A signaling cascade, which is a signaling program reported to inhibit inflammation in macrophages.[22–25] However, there have been no previous reports examining Sdc-1 expression as a function of macrophage polarization. Moreover, predicting the anticipated role of Sdc-1 in macrophage motility is not intuitive because Sdc-1 has been reported to both inhibit and augment motility in a cell and tissue dependent context.[21, 26–28]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

We demonstrate that Sdc-1 expression is specific to differentiated M2 macrophages with high intrinsic motility and that Sdc-1 deficiency is characterized by impaired migration and enhanced extracellular matrix adhesion. In a self-resolving model of inflammation, we examined both infiltration and emigration of macrophages in wild type (Sdc-1$^{+/+}$) and Sdc-1 deficient (Sdc-1$^{-/-}$) mice. Significantly, we observed no difference in the kinetics of leukocyte influx or the magnitude of the inflammatory response; however, a significant difference in the resolution response was observed with delayed lymphatic clearance of Sdc-1$^{-/-}$ macrophages. We further examined the relevance of macrophage Sdc-1 in apolipoprotein E (ApoE$^{-/-}$) deficient mice maintained on a Western diet. Sdc-1$^{-/-}$ApoE$^{-/-}$ mice were characterized by the presence of an enhanced population of inflammatory macrophages and a greater burden of atherosclerotic plaque. Collectively, these results support the notion that defective motility of mature macrophage populations may promote a persistent inflammatory state and suggests that Sdc-1 expression is part of a broad program to regulate trafficking of alternatively activated macrophages.

METHODS

Materials and methods are available in the supplement.

RESULTS

Sdc-1 Expression on Polarized Macrophage Populations

Macrophage activation has been operationally divided into two subsets: classically activated, pro-inflammatory M1 and alternatively activated M2. M2 polarization encompasses a broad spectrum of macrophage activation and additional subdivisions have been suggested to clarify functional phenotypes, such as M2a to distinguish traditional Th2-type (IL-4, IL-13) polarization and M2c to distinguish anti-inflammatory (IL-10, TGF-β, glucocorticoid) polarization.[29] Cell surface marker expression, biochemical signature, and phagocytotic capacity of Sdc-1 positive macrophages were characterized for M1, M2a, and M2c standard populations generated from elicited peritoneal macrophages. Flow cytometry was used to measure cell surface expression of MHCII, CD86, and PD-L2, three markers that have been previously reported to associate with M1 or M2 polarization.[30, 31] M1 polarized cells displayed characteristically high expression of the antigen presenting molecule, MHCII, and the co-activating molecule, CD86 (Fig. 1A). M2a polarized cells were most easily characterized as MHCII^{+} with high expression of the programmed death ligand 2 (PD-L2), a Th2 stimulated molecule that inhibits T-cell proliferation.[32] M2c does not positively correlate with MHCII, CD86, or PD-L2 consistent with reports that IL-10 deactivates macrophages.[33, 34] Sdc-1 expressing macrophages, which can be enriched after treatment with a membrane-permeant cAMP analogue or adenosine-induced activation of endogenous adenyl cyclase (Fig. 1B, media control presented in Supplemental Fig. I), displayed the strongest similarity to M2c with a MHCII^{low}, CD86^{low}, PD-L2^{-} signature.

Macrophage populations were further assayed for soluble mediators; with elevated nitrite levels observed in the M1 population (Fig. 1C), while arginase activity was enhanced in M2a macrophages (Fig. 1D). A significant increase in IL-10 production was observed for Sdc-1 expressing macrophages, as compared to M1 or M2a polarized populations. However,

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

M2c polarized macrophages secreted the highest level of IL-10 (Fig. 1E). Finally, bead phagocytosis, which has been reported to be upregulated in alternatively activated M2 macrophages,[35] was found to be significantly elevated in all populations as compared to M1 (Fig. 1F). Characterization of Sdc-1 expression in polarized standard populations suggests that Sdc-1 protein expression is not induced within the context of a primary Th1 (IFN-γ) or Th2 (IL-4) inflammatory response (Fig. 1G), but instead may be displayed in those macrophages that have received a deactivation response, typified by decreased antigen presentation ($MHCII^{low}$) and increased immunosuppression ($IL10^{+}$). Induction of Sdc-1 was not observed with IL-10 treatment (Fig. 1G), however, additional protocols commonly reported to suppress or deactivate macrophages were examined, including exposure to TGFβ or glucocorticoids, as well as efferocytosis mediated deactivation.[36, 37] Significantly, up-regulation of Sdc-1 protein and mRNA was observed following efferocytosis of apoptotic macrophages (Fig. 1H-I and Supplemental Fig. II), suggesting a physiologic program for expression that is consistent with deactivation and inflammatory resolution.

Leukocyte Chemoattractive Potential of Sdc-1 Macrophages

In vitro phenotypic analysis suggests that macrophage Sdc-1 expression is not consistent with a classically activated M1 subtype and subsequent quantitative real-time reverse transcription PCR studies confirmed this distinction. A higher level of pro-inflammatory genes and chemokines was observed in M1 verses Sdc-1 enriched macrophages (Tables I and II). Given the impact that leukocyte recruitment has on inflammatory outcome, we examined the chemoattractive potential of conditioned media generated from M1 or Sdc-1 enriched populations toward purified circulating monocytes or activated CD4 T cells. Significantly, conditioned media from Sdc-1 macrophages elicited significantly reduced chemotaxis of monocytes and CD4 T cells (Fig. 2A,B; controls presented in Supplemental Fig. III). These results suggest that while M1 macrophages actively contribute to the progression of inflammation, Sdc-1 expressing macrophages should correlate with reduced leukocyte influx in vivo.

Distinct Motility Behavior in Polarized Macrophage Populations

Decreased migratory capability may contribute to macrophage retention in chronic inflammatory environments. Given that Sdc-1 has been traditionally studied in the context of cell adhesion and migration, we examined the functional consequence of macrophage Sdc-1 deficiency on motility and adhesion in polarized populations. Motility was first investigated by characterizing the migration of $Sdc\text{-}1^{+/+}$ and $Sdc\text{-}1^{-/-}$ macrophages toward FBS using a Boyden chamber assay. Cells were specifically induced toward a M1 or $Sdc\text{-}1^{+}$ phenotype. We observed dramatically dampened motility in classically activated $Sdc\text{-}1^{+/+}$ and $Sdc\text{-}1^{-/-}$ M1 macrophages, with no significant difference noted between genotypes (Fig. 3A). However, when the migration of $Sdc\text{-}1^{+/+}$ and $Sdc\text{-}1^{-/-}$ macrophages was examined under conditions to induce a $Sdc\text{-}1^{+}$ phenotype through the addition of adenosine to basal media, we observed a significant reduction in the motility of $Sdc\text{-}1^{-/-}$ macrophages (Fig. 3A and Supplemental Fig. IV). Given our previous results suggesting the absence of Sdc-1 expression on M1 polarized macrophages, the lack of a functional role for Sdc-1 in M1 macrophage motility is not surprising. Instead, our results suggest that Sdc-1 functionally contributes to the motility of specifically induced M2 macrophage populations. $Sdc\text{-}1^{+/+}$ and

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Sdc-1$^{-/-}$ motility experiments were repeated using conditioned media generated from apoptotic macrophages as a surrogate to chemotactic signals generated in the atherosclerotic lesion environment.[38–41] A significant reduction in the motility of Sdc-1$^{-/-}$ macrophages was noted toward apoptotic conditioned media suggesting that Sdc-1 deficiency may limit the efficiency of efferocytosis processes in chronic inflammatory environments by restricting movement toward apoptotic cells (Fig. 3B and controls in Supplemental Fig. V). We examined motility-dependent efferocytosis using a Boyden chamber assay, violet-labeled Sdc-1$^{+/+}$ or Sdc-1$^{-/-}$ macrophages were seeded in the upper chamber and CFSE green-labeled apoptotic macrophages were provided as the chemotactic stimulant in the bottom chamber, we quantified total number of migrated cells and total number of migrated cells with engulfed green apoptotic cells. As we observed in our migration experiments to apoptotic conditioned media, a significant reduction in the migration events was observed for Sdc-1$^{-/-}$ macrophages, consequently, we also observed a significant reduction in efferocytosis events for Sdc-1$^{-/-}$ macrophages (Fig. 3C). When efferocytosis efficiency is examined under static conditions, we observe no difference between Sdc-1$^{+/+}$ and Sdc-1$^{-/-}$ macrophages, this is consistent with our hypothesis that defective motility in Sdc-1$^{-/-}$ macrophages may promote a persistent inflammatory state.

The adhesion profiles of Sdc-1$^{+/+}$ and Sdc-1$^{-/-}$ macrophages were investigated in comparison to WT M1-induced macrophages. M1-induced macrophages displayed the greatest adhesion; with increased adhesion noted for Sdc-1$^{-/-}$ as compared to Sdc-1$^{+/+}$ macrophages (Fig. 3D). Subsequent staining of the actin cytoskeleton revealed impaired ability of Sdc-1$^{-/-}$ macrophages to form cell protrusions (Fig. 3E,F), consistent with previous reports that Sdc-1 can regulate cell protrusions that may be functionally important in migration by mediating outward extension of cell edges.[42, 43] We confirmed that Sdc-1$^{-/-}$ macrophages polarize to the expected M1 or M2 phenotype when incubated with Th1 or Th2 cytokines, thus the only phenotypic defect observed in Sdc-1$^{-/-}$ macrophages was reduced motility and enhanced adhesion (Supplemental Fig. VI). Collectively, these results suggest that Sdc-1 expression modulates motility in mature polarized macrophage populations and may limit macrophage retention in chronic inflammatory environments.

Sdc-1 Contributes to the Resolution of Inflammatory Responses

We utilized a thioglycollate induced murine model of peritonitis, as an example of self-resolving inflammation to investigate the role of Sdc-1 in leukocyte infiltration and resolution. Cell counts, neutrophil and macrophage populations, as well as Sdc-1 expression were recorded over a 12 day time frame. At the induction of peritonitis, Sdc-1 expression was not detected on peritoneal macrophages. However, 24 to 48 h after onset of peritonitis, Sdc-1 macrophages were abundant and then decreased over the 12 day time course (Fig. 4A). Analysis of total cell counts revealed no difference in the kinetics or magnitude of the initial inflammatory response between Sdc-1$^{+/+}$ and Sdc-1$^{-/-}$ mice (Fig. 4B, time 0-2d). Indeed the evolution and resolution of the neutrophil response was identical in Sdc-1$^{+/+}$ and Sdc-1$^{-/-}$ mice (Fig. 4C). Significantly, the resolution of the late inflammatory response was delayed in Sdc-1$^{-/-}$ mice, with a greater number of macrophages detected at 8-12 days (Fig. 4D). There was no difference in the magnitude or kinetics of macrophage influx between Sdc-1$^{+/+}$ and Sdc-1$^{-/-}$ mice; however, the time interval required for a 50% to 25% reduction

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

in the number of peritoneal macrophages was significantly prolonged in Sdc-1$^{-/-}$ mice (1.7 vs 4.7 d, $p < 0.05$; Fig. 4E and Supplemental Fig. VII).[44] This result is consistent with a delayed clearance of macrophages from the peritoneal cavity in the absence of Sdc-1 expression. Adoptive transfer was used to examine macrophage emigration into the lymphatic system during the resolution phase of peritonitis. Fluorescently labeled Sdc-1$^{+/+}$ and Sdc-1$^{-/-}$ macrophages were introduced into the peritoneal cavity at day 3 and mesenteric lymph or spleen were harvested 18 h post adoptive transfer. Significantly, an increase in lymphatic trafficking in Sdc-1$^{+/+}$ macrophages was noted (Fig. 3F). Thus, in vitro and in vivo migration studies suggest that Sdc-1 expression may be an important modulator of macrophage motility and, specifically, may be an important functional component of macrophage emigration and resolution.

Sdc-1 Limits Atherosclerotic Lesion Formation

To examine the role of Sdc-1 in atherosclerosis, Apoe$^{-/-}$ Sdc-1$^{-/-}$ mice were generated[17] and the presentation of atherosclerosis compared with that observed in Apoe$^{-/-}$ mice when both cohorts were maintained on a Western diet. At 8 weeks, brachiocephalic lesion area was quantified in consecutive tissue sections and the inflammatory profile examined using standard immunohistochemistry.[45–48] We did not observe Sdc-1 staining in native brachiocephalic sections (data not shown), but by 8 weeks Sdc-1 expression was clearly evident in macrophage positive areas around lesional plaques and within the adventitial tissue (Fig. 5A,B; staining control in Supplemental Fig. VIII). Although serum cholesterol levels were identical in both cohorts, a significant increase in atherosclerotic lesion area was measured in Apoe$^{-/-}$ Sdc-1$^{-/-}$ mice as compared to Apoe$^{-/-}$ mice (88.03 ± 8.66 vs. 57.40 ± 8.08, $P < 0.05$; Fig. 5C and Supplemental Fig. IX). Consistent with enhanced lesion formation, we observed enhanced expression of the macrophage M1 marker, iNOS, and greater necrotic area within Apoe$^{-/-}$Sdc-1$^{-/-}$ lesions (Fig. 5D,E).

To explore the relevance of our findings in human atherosclerosis, we examined human carotid plaque sections for macrophage Sdc-1 staining. Atherosclerotic plaques were harvested during the course of carotid endarterectomy from symptomatic patients having reported a recent stroke or transient ischemic attack. Immunohistochemical staining revealed colocalization of macrophages and Sdc-1 positive staining within identical regions of the lesion (Fig. 5F). To further investigate Sdc-1 expression in human macrophages, we examined polarization and motility in THP1 macrophages in vitro. Notably, Sdc-1 expression was consistent with M2 polarization and an enhanced motility profile (Supplemental Fig. X).

DISCUSSION

Recent findings suggest that the resolution of inflammation involves both efferocytosis and emigration of monocyte-derived cells out of sites of inflammation through nearby lymphatic vessels.[1, 49] However, both processes may be impaired in atherosclerosis, which accounts for macrophage accumulation within plaques.[4, 50] In this regard, the mechanism for impeded efferocytosis and emigration from plaques remains a topic of intense interest for potential therapeutic intervention. We have determined that macrophage Sdc-1 expression is

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

associated with anti-inflammatory M2 polarization and enhanced motility in both murine and human macrophages. Sdc-1$^{-/-}$ macrophages displayed a significant reduction in motility and increased adhesion and in vivo, Sdc-1 macrophage deficiency resulted in delayed egress of macrophages in a model of self-resolving peritonitis and enhanced atherosclerotic lesion formation in ApoE$^{-/-}$Sdc-1$^{-/-}$ mice. These data highlight a functional role for Sdc-1 in the motility of alternatively activated macrophage populations and reinforce the significant relationship of the dynamics of macrophage motility to responses that underlie the resolution of inflammation.

In adult tissue, Sdc-1 expression is restricted to non-circulating plasma cells and epithelial cells. Expression can be induced in additional cell types, such as endothelial cells, smooth muscle cells, fibroblasts, and macrophages.[17, 18] There are limited studies documenting in vivo expression and functional relevance of Sdc-1 expression in macrophages. Indeed, Sdc-1 expression is not observed in circulating monocytes or resident macrophages, however, Sdc-1 mRNA is upregulated upon macrophage elicitation.[51] Protein expression is governed via cAMP/prtotein kinase A as a post-transcriptionally regulated event, which has also been reported in keratinocytes, mesenchymal cells, and epithelial cells.[52–54] The molecular mechanisms governing this non-traditional regulation of expression have yet to be elucidated, but presumably on-demand expression would allow macrophages to rapidly tailor cell surface properties to meet specific functional requirements. Agents that elevate intracellular cAMP, such as E-type prostaglandins, membrane permeable cAMP analogues, or adenosine, promote Sdc-1 protein expression in macrophages. Nonphlogistic clearance of apoptotic cells, or efferocytosis, is a key process regulating macrophage M2 inflammatory polarization and resolution. Indeed, there are numerous reports detailing macrophage immune silencing as consequence of efferocytosis. Macrophage engulfment of apoptotic cells can trigger the secretion of soluble anti-inflammatory mediators, such as IL-10, TGF-β, adenosine, and prostaglandin E_2. [55, 56] Moreover, efferocytosis has been suggested to modulate macrophage motility and promote emigration[1] and our studies confirm that efferocytosis induces Sdc-1 protein expression. Given that the only phenotypic defect in Sdc-1$^{-/-}$ macrophages was reduced motility and enhanced adhesion, it is plausible that efferocytosis-induced Sdc-1 expression contributes to the emigration program of macrophages and resolution of inflammation.

Traditionally, Sdc-1 has been studied in the context of cell adhesion and migration in mesenchymal and epithelial cell types. As a membrane anchored proteoglycan expressed on the basolateral surface of epithelial cells, Sdc-1 supports cell contact with extracellular matrix proteins and modulates cytoskeletal organization leading to changes in cell shape and adhesion. Sdc-1 has been reported to augment epithelial migration in skin and cornea but inhibit migration in the lung by slowing focal adhesion disassembly.[21, 27, 57] Both overexpression and gene knockout of Sdc-1 reduces migration in a wound-healing assay, suggesting that an optimal balance of surface expression is required to support the locomotion of mesenchymal and epithelial cells.[28, 58] There are limited reports documenting a functional role of Sdc-1 in adhesion or motility of leukocytes, which can utilize amoeboid migration without adherence to matrix components.[59, 60] Macrophages display intermediate migration speed and are capable of both amoeboid and mesenchymal migration. They do not exhibit large focal complexes consistent with their requirement for rapid migration

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

responses. Sdc-1 has been linked to the formation of fascin-dependent cell protrusions, which can guide migration events.[43] Fascin spikes, which have been reported in dendritic cells, are dynamic extensions of the plasma membrane that can extend and retract quickly and assemble in the absence of focal contacts.[42] The morphological absence of cell protrusions is evident in Sdc-1$^{-/-}$ macrophages treated with agents that induce high cell surface Sdc-1 expression, such as membrane permeable cAMP analogues or adenosine. This morphological distinction coupled with a motility defect that is observed in the absence of Sdc-1 expression, suggests that expression is critical in modulating cytoskeletal dynamics for this specific subset of M2 macrophages. Given the capacity of macrophages to engage in both integrin-mediated adhesion-dependant motility and adhesion-independent interstitial migration, future efforts will focus on examining the contribution of Sdc-1 within the context of both motility paradigms.

Recent investigations have highlighted the potential significance of heterogeneous populations of specialized macrophages in the initiation and resolution of inflammatory responses. One of the defining features of chronic inflammation is the persistence of activated macrophages. In atherosclerotic plaque, activated lesional macrophages possess a potent repertoire of pro-inflammatory mediators to sustain a sessile M1 profile and hinder the evolution of resolution programs. We have demonstrated that Sdc-1 expression on macrophages is associated with an anti-inflammatory M2 polarization and enhanced motility in both murine and human macrophages. We further demonstrated a role in macrophage resolution and in limiting atherosclerotic macrophage burden. Given the pathologic role of macrophage trapping in chronic inflammation, it is critical to define the endogenous modulators of macrophage resolution. The macrophage Sdc-1 expression program represents a bridge between efferocytosis, polarization, and motility and may be fundamental to the clearance of inflammatory macrophages.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

None.

SOURCES OF FUNDING

This work was funded by NIH grant HL060903 (E.L. Chaikof) and a fellowships from the American College of Surgeons (S.V. Smith) and the American Heart Association (J. Angsana).

REFERENCES

1. Ariel A, Serhan CN. New lives given by cell death: Macrophage differentiation following their encounter with apoptotic leukocytes during the resolution of inflammation. Front Immunol. 2012; 3:4. [PubMed: 22566890]
2. Schif-Zuck S, Gross N, Assi S, Rostoker R, Serhan CN, Ariel A. Saturated-efferocytosis generates pro-resolving cd11b low macrophages: Modulation by resolvins and glucocorticoids. Eur J Immunol. 2011; 41:366–379. [PubMed: 21268007]
3. Ait-Oufella H, Pouresmail V, Simon T, Blanc-Brude O, Kinugawa K, Merval R, Offenstadt G, Leseche G, Cohen PL, Tedgui A, Mallat Z. Defective mer receptor tyrosine kinase signaling in bone

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

marrow cells promotes apoptotic cell accumulation and accelerates atherosclerosis. Arterioscler Thromb Vasc Biol. 2008; 28:1429–1431. [PubMed: 18467644]

4. Randolph GJ. Emigration of monocyte-derived cells to lymph nodes during resolution of inflammation and its failure in atherosclerosis. Curr Opin Lipidol. 2008; 19:462–468. [PubMed: 18769227]
5. Tabas I. Apoptosis and efferocytosis in mouse models of atherosclerosis. Curr Drug Targets. 2007; 8:1288–1296. [PubMed: 18220705]
6. Thorp E, Cui D, Schrijvers DM, Kuriakose G, Tabas I. Mertk receptor mutation reduces efferocytosis efficiency and promotes apoptotic cell accumulation and plaque necrosis in atherosclerotic lesions of apoe −/− mice. Arterioscler Thromb Vasc Biol. 2008; 28:1421–1428. [PubMed: 18451332]
7. 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]
8. Feig JE, Parathath S, Rong JX, Mick SL, Vengrenyuk Y, Grauer L, Young SG, Fisher EA. Reversal of hyperlipidemia with a genetic switch favorably affects the content and inflammatory state of macrophages in atherosclerotic plaques. Circulation. 2011; 123:989–998. [PubMed: 21339485]
9. Feig JE, Pineda-Torra I, Sanson M, et al. Lxr promotes the maximal egress of monocyte-derived cells from mouse aortic plaques during atherosclerosis regression. J Clin Invest. 2010; 120:4415–4424. [PubMed: 21041949]
10. Feig JE, Rong JX, Shamir R, Sanson M, Vengrenyuk Y, Liu J, Rayner K, Moore K, Garabedian M, Fisher EA. Hdl promotes rapid atherosclerosis regression in mice and alters inflammatory properties of plaque monocyte-derived cells. Proc Natl Acad Sci U S A. 2011; 108:7166–7171. [PubMed: 21482781]
11. Trogan E, Fayad ZA, Itskovich VV, Aguinaldo JG, Mani V, Fallon JT, Chereshnev 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]
12. 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]
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. van Gils JM, Derby MC, Fernandes LR, et al. The neuroimmune guidance cue netrin-1 promotes atherosclerosis by inhibiting the emigration of macrophages from plaques. Nat Immunol. 2012; 13:136–143. [PubMed: 22231519]
15. Wanschel A, Seibert T, Hewing B, Ramkhelawon B, Ray TD, van Gils JM, Rayner KJ, Feig JE, O'Brien ER, Fisher EA, Moore KJ. Neuroimmune guidance cue semaphorin 3e is expressed in atherosclerotic plaques and regulates macrophage retention. Arterioscler Thromb Vasc Biol. 2013; 33:886–893. [PubMed: 23430613]
16. Potteaux S, Gautier EL, Hutchison SB, van Rooijen N, Rader DJ, Thomas MJ, Sorci-Thomas MG, Randolph GJ. Suppressed monocyte recruitment drives macrophage removal from atherosclerotic plaques of apoe −/− mice during disease regression. J Clin Invest. 2011; 121:2025–2036. [PubMed: 21505265]
17. Xiao J, Angsana J, Wen J, Smith SV, Park PW, Ford ML, Haller CA, Chaikof EL. Syndecan-1 displays a protective role in aortic aneurysm formation by modulating t cell-mediated responses. Arterioscler Thromb Vasc Biol. 2012; 32:386–396. [PubMed: 22173227]
18. Teng YH, Aquino RS, Park PW. Molecular functions of syndecan-1 in disease. Matrix Biol. 2012; 31:3–16. [PubMed: 22033227]
19. Inki P, Jalkanen M. The role of syndecan-1 in malignancies. Ann Med. 1996; 28:63–67. [PubMed: 8932508]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

20. Liu W, Litwack ED, Stanley MJ, Langford JK, Lander AD, Sanderson RD. Heparan sulfate proteoglycans as adhesive and anti-invasive molecules. Syndecans and glypican have distinct functions. J Biol Chem. 1998; 273:22825–22832. [PubMed: 9712917]
21. Stepp MA, Liu Y, Pal-Ghosh S, Jurjus RA, Tadvalkar G, Sekaran A, Losicco K, Jiang L, Larsen M, Li L, Yuspa SH. Reduced migration, altered matrix and enhanced tgfbeta1 signaling are signatures of mouse keratinocytes lacking sdc1. J Cell Sci. 2007; 120:2851–2863. [PubMed: 17666434]
22. Aronoff DM, Canetti C, Serezani CH, Luo M, Peters-Golden M. Cutting edge: Macrophage inhibition by cyclic amp (camp): Differential roles of protein kinase a and exchange protein directly activated by camp-1. J Immunol. 2005; 174:595–599. [PubMed: 15634874]
23. Peters-Golden M. Putting on the brakes: Cyclic amp as a multipronged controller of macrophage function. Sci Signal. 2009; 2:pe37. [PubMed: 19531801]
24. Wall EA, Zavzavadjian JR, Chang MS, Randhawa B, Zhu X, Hsueh RC, Liu J, Driver A, Bao XR, Sternweis PC, Simon MI, Fraser ID. Suppression of lps-induced tnf-alpha production in macrophages by camp is mediated by pka-akap95-p105. Sci Signal. 2009; 2:ra28. [PubMed: 19531803]
25. Kim C, Wilcox-Adelman S, Sano Y, Tang WJ, Collier RJ, Park JM. Antiinflammatory camp signaling and cell migration genes co-opted by the anthrax bacillus. Proc Natl Acad Sci U S A. 2008; 105:6150–6155. [PubMed: 18427110]
26. Kliment CR, Englert JM, Gochuico BR, Yu G, Kaminski N, Rosas I, Oury TD. Oxidative stress alters syndecan-1 distribution in lungs with pulmonary fibrosis. J Biol Chem. 2009; 284:3537–3545. [PubMed: 19073610]
27. Pal-Ghosh S, Tadvalkar G, Jurjus RA, Zieske JD, Stepp MA. Balb/c and c57bl6 mouse strains vary in their ability to heal corneal epithelial debridement wounds. Exp Eye Res. 2008; 87:478–486. [PubMed: 18809399]
28. Stepp MA, Gibson HE, Gala PH, Iglesia DD, Pajoohesh-Ganji A, Pal-Ghosh S, Brown M, Aquino C, Schwartz AM, Goldberger O, Hinkes MT, Bernfield M. Defects in keratinocyte activation during wound healing in the syndecan-1-deficient mouse. J Cell Sci. 2002; 115:4517–4531. [PubMed: 12414997]
29. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol. 2004; 25:677–686. [PubMed: 15530839]
30. Huber S, Hoffmann R, Muskens F, Voehringer D. Alternatively activated macrophages inhibit t-cell proliferation by stat6-dependent expression of pd-l2. Blood. 2010; 116:3311–3320. [PubMed: 20625006]
31. Lolmede K, Campana L, Vezzoli M, Bosurgi L, Tonlorenzi R, Clementi E, Bianchi ME, Cossu G, Manfredi AA, Brunelli S, Rovere-Querini P. Inflammatory and alternatively activated human macrophages attract vessel-associated stem cells, relying on separate hmgb1- and mmp-9-dependent pathways. J Leukoc Biol. 2009; 85:779–787. [PubMed: 19197071]
32. Latchman Y, Wood CR, Chernova T, et al. Pd-l2 is a second ligand for pd-1 and inhibits t cell activation. Nat Immunol. 2001; 2:261–268. [PubMed: 11224527]
33. Chawla A. Control of macrophage activation and function by ppars. Circ Res. 2010; 106:1559–1569. [PubMed: 20508200]
34. Gordon S. Alternative activation of macrophages. Nat Rev Immunol. 2003; 3:23–35. [PubMed: 12511873]
35. Tiemessen MM, Jagger AL, Evans HG, van Herwijnen MJ, John S, Taams LS. Cd4+cd25+foxp3+ regulatory t cells induce alternative activation of human monocytes/macrophages. Proc Natl Acad Sci U S A. 2007; 104:19446–19451. [PubMed: 18042719]
36. Savill J, Dransfield I, Gregory C, Haslett C. A blast from the past: Clearance of apoptotic cells regulates immune responses. Nat Rev Immunol. 2002; 2:965–975. [PubMed: 12461569]
37. Voll RE, Herrmann M, Roth EA, Stach C, Kalden JR, Girkontaite I. Immunosuppressive effects of apoptotic cells. Nature. 1997; 390:350–351. [PubMed: 9389474]
38. Truman LA, Ford CA, Pasikowska M, Pound JD, Wilkinson SJ, Dumitriu IE, Melville L, Melrose LA, Ogden CA, Nibbs R, Graham G, Combadiere C, Gregory CD. Cx3cl1/fractalkine is released

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

from apoptotic lymphocytes to stimulate macrophage chemotaxis. Blood. 2008; 112:5026–5036. [PubMed: 18799722]

39. Lauber K, Bohn E, Krober SM, et al. Apoptotic cells induce migration of phagocytes via caspase-3-mediated release of a lipid attraction signal. Cell. 2003; 113:717–730. [PubMed: 12809603]
40. Hochreiter-Hufford A, Ravichandran KS. Clearing the dead: Apoptotic cell sensing, recognition, engulfment, and digestion. Cold Spring Harbor perspectives in biology. 2013; 5:a008748. [PubMed: 23284042]
41. Cullen SP, Henry CM, Kearney CJ, Logue SE, Feoktistova M, Tynan GA, Lavelle EC, Leverkus M, Martin SJ. Fas/cd95-induced chemokines can serve as "find-me" signals for apoptotic cells. Molecular cell. 2013; 49:1034–1048. [PubMed: 23434371]
42. Adams JC. Fascin protrusions in cell interactions. Trends Cardiovasc Med. 2004; 14:221–226. [PubMed: 15451513]
43. Chakravarti R, Sapountzi V, Adams JC. Functional role of syndecan-1 cytoplasmic v region in lamellipodial spreading, actin bundling, and cell migration. Mol Biol Cell. 2005; 16:3678–3691. [PubMed: 15930135]
44. Bannenberg GL, Chiang N, Ariel A, Arita M, Tjonahen E, Gotlinger KH, Hong S, Serhan CN. Molecular circuits of resolution: Formation and actions of resolvins and protectins. J Immunol. 2005; 174:4345–4355. [PubMed: 15778399]
45. Johnson J, Carson K, Williams H, Karanam S, Newby A, Angelini G, George S, Jackson C. Plaque rupture after short periods of fat feeding in the apolipoprotein e-knockout mouse: Model characterization and effects of pravastatin treatment. Circulation. 2005; 111:1422–1430. [PubMed: 15781753]
46. Jackson CL, Bennett MR, Biessen EA, Johnson JL, Krams R. Assessment of unstable atherosclerosis in mice. Arterioscler Thromb Vasc Biol. 2007; 27:714–720. [PubMed: 17332492]
47. Williams H, Johnson JL, Carson KG, Jackson CL. Characteristics of intact and ruptured atherosclerotic plaques in brachiocephalic arteries of apolipoprotein e knockout mice. Arterioscler Thromb Vasc Biol. 2002; 22:788–792. [PubMed: 12006391]
48. Rosenfeld ME, Polinsky P, Virmani R, Kauser K, Rubanyi G, Schwartz SM. Advanced atherosclerotic lesions in the innominate artery of the apoe knockout mouse. Arterioscler Thromb Vasc Biol. 2000; 20:2587–2592. [PubMed: 11116057]
49. Bellingan GJ, Caldwell H, Howie SE, Dransfield I, Haslett C. In vivo fate of the inflammatory macrophage during the resolution of inflammation: Inflammatory macrophages do not die locally, but emigrate to the draining lymph nodes. J Immunol. 1996; 157:2577–2585. [PubMed: 8805660]
50. Thorp E, Subramanian M, Tabas I. The role of macrophages and dendritic cells in the clearance of apoptotic cells in advanced atherosclerosis. Eur J Immunol. 2011; 41:2515–2518. [PubMed: 21952808]
51. Yeaman C, Rapraeger AC. Post-transcriptional regulation of syndecan-1 expression by camp in peritoneal macrophages. J Cell Biol. 1993; 122:941–950. [PubMed: 8394371]
52. Hayashida K, Johnston DR, Goldberger O, Park PW. Syndecan-1 expression in epithelial cells is induced by transforming growth factor beta through a pka-dependent pathway. J Biol Chem. 2006; 281:24365–24374. [PubMed: 16807246]
53. Sanderson RD, Hinkes MT, Bernfield M. Syndecan-1, a cell-surface proteoglycan, changes in size and abundance when keratinocytes stratify. J Invest Dermatol. 1992; 99:390–396. [PubMed: 1401995]
54. Vainio S, Jalkanen M, Bernfield M, Saxen L. Transient expression of syndecan in mesenchymal cell aggregates of the embryonic kidney. Dev Biol. 1992; 152:221–232. [PubMed: 1644217]
55. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving tgf-beta, pge2, and paf. J Clin Invest. 1998; 101:890–898. [PubMed: 9466984]
56. Freire-de-Lima CG, Xiao YQ, Gardai SJ, Bratton DL, Schiemann WP, Henson PM. Apoptotic cells, through transforming growth factor-beta, coordinately induce anti-inflammatory and

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

suppress pro-inflammatory eicosanoid and no synthesis in murine macrophages. J Biol Chem. 2006; 281:38376–38384. [PubMed: 17056601]

57. Altemeier WA, Schlesinger SY, Buell CA, Brauer R, Rapraeger AC, Parks WC, Chen P. Transmembrane and extracellular domains of syndecan-1 have distinct functions in regulating lung epithelial migration and adhesion. J Biol Chem. 2012; 287:34927–34935. [PubMed: 22936802]
58. Elenius V, Gotte M, Reizes O, Elenius K, Bernfield M. Inhibition by the soluble syndecan-1 ectodomains delays wound repair in mice overexpressing syndecan-1. J Biol Chem. 2004; 279:41928–41935. [PubMed: 15220342]
59. Pixley FJ. Macrophage migration and its regulation by csf-1. Int J Cell Biol. 2012; 2012:501962. [PubMed: 22505929]
60. Lammermann T, Bader BL, Monkley SJ, Worbs T, Wedlich-Soldner R, Hirsch K, Keller M, Forster R, Critchley DR, Fassler R, Sixt M. Rapid leukocyte migration by integrin-independent flowing and squeezing. Nature. 2008; 453:51–55. [PubMed: 18451854]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

SIGNIFICANCE

Atherosclerosis is a chronic inflammatory disease that is distinguished by the persistence of inflammatory macrophages in vascular lesions. There is intense interest in understanding why the macrophage population fails to resolve in atherosclerotic plaques, recent findings suggest that macrophage migration out of the site of inflammation may be an important process to trigger the return to tissue homeostasis. Our data show that the cell surface heparan sulfate proteoglycan, syndecan-1, modulates motility and adhesion in mature polarized macrophage populations. In vivo, we observed that this motility defect translated to reduced macrophage trafficking during inflammatory resolution and enhanced atherosclerotic plaque formation. Collectively these results support the notion that defective motility of mature macrophage populations may promote a persistent inflammatory state and suggest that syndecan-1 is part of a broad program to regulate resolution phase trafficking.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

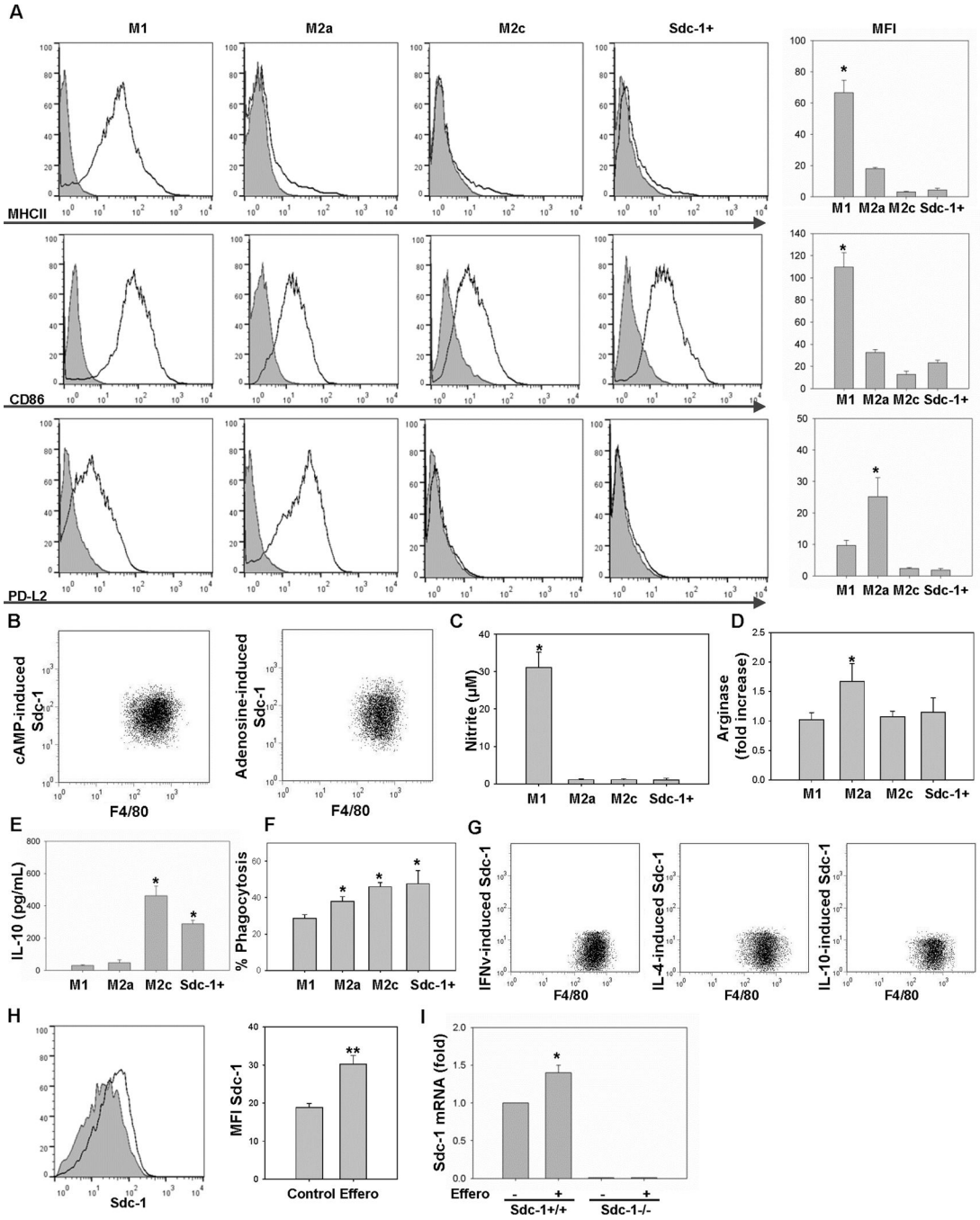

Figure 1.

Sdc-1 expression on polarized macrophage populations. A, Sdc-1 expressing macrophages were examined against standard populations for surface expression of M1 (MHCII, CD86) and M2a (PD-L2) markers, data is displayed in histogram format against the IgG control (grey) and graphed as mean fluorescent intensity (MFI). B, Sdc-1 expression can be induced on F4/80 positive macrophages following exposure to 6-bnz-cAMP or adenosine. C, Elevated M1-induced iNOS activity was characterized via Griess reaction. D, Arginase activity was measured in cell lysates and reported as fold increase over un-stimulated control

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

macrophages. E, IL-10 was measured via conditioned media ELISA. F, % phagocytosis was characterized in polarized populations by fluorescent bead uptake. G, Sdc-1 expression is not induced under standard M1, M2a, or M2c conditions. H-I, Sdc-1 expression is induced after efferocytosis of apoptotic macrophages. H, data plotted as histogram (Sdc-1, white) against IgG control (grey) and as mean fluorescent intensity (MFI). I, quantitative PCR analysis of Sdc-1 mRNA in Sdc-1 wild type and knockout macrophages +/− efferocytosis. Data are representative of triplicate sample mean ± SEM, $*P < 0.05$, $**P < 0.01$.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

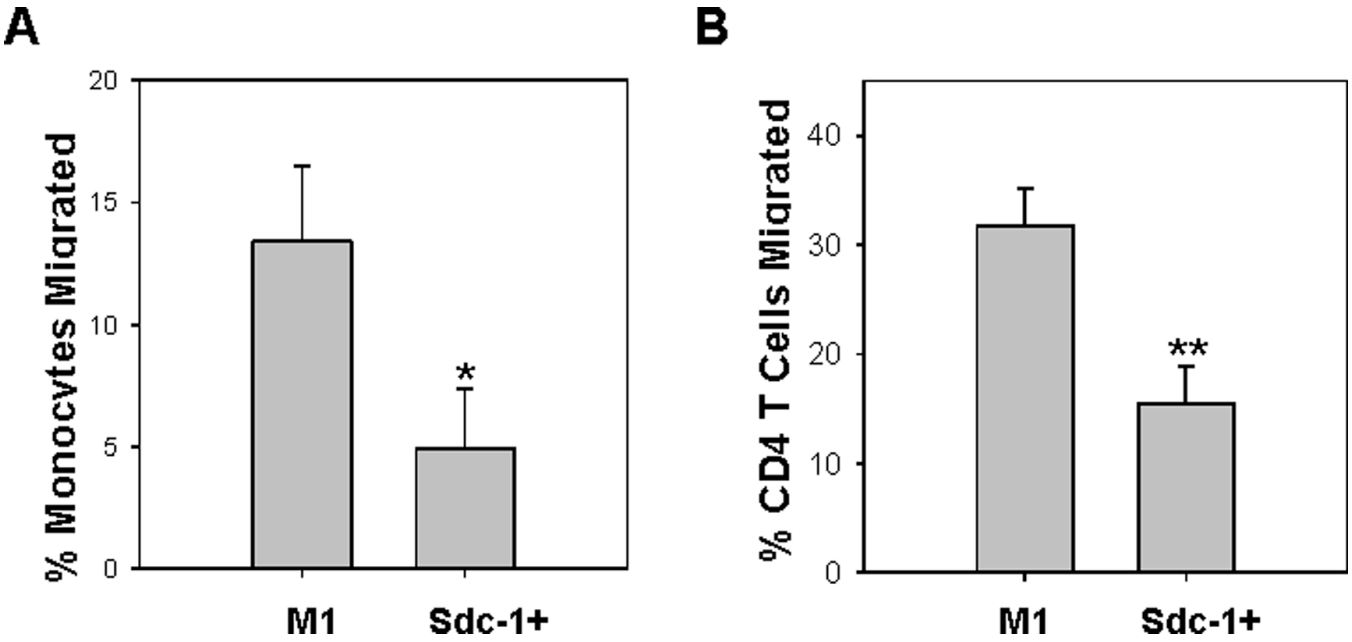

Figure 2.

Sdc-1 macrophages display reduced leukocyte chemoattractive potential. Leukocyte chemoattractive potential was measured by transwell chemotaxis of murine circulating monocytes (A) or murine activated CD4+ T cells (B) to conditioned media generated from M1 or Sdc-1 macrophages. Cells that migrated to the lower chamber where counted and reported as % migrated, N=5, mean ± SEM, *$P < 0.05$. **$P < 0.01$.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

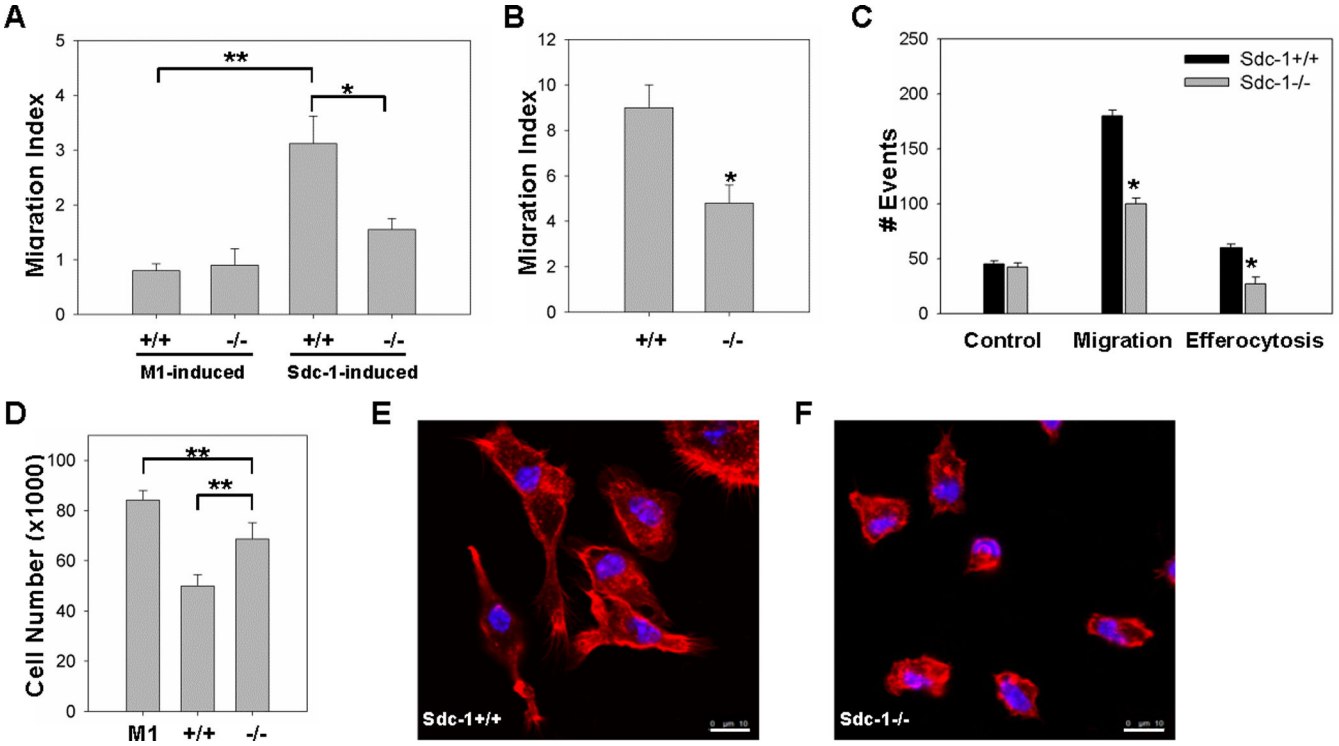

Figure 3.

Role of Sdc-1 in the motility of polarized macrophages. A, Migration index to serum was calculated for M1-induced Sdc-$1^{+/+}$ and Sdc-$1^{-/-}$ and for Sdc-1-induced (+ adenosine) Sdc-$1^{+/+}$ and Sdc-$1^{-/-}$ macrophages. Sdc-$1^{+/+}$ macrophages display significantly enhanced motility compared to Sdc-$1^{-/-}$ and M1 polarized cells. B, Sdc-$1^{-/-}$ show a significantly reduced motility response to apoptotic cell conditioned media. C, In an assay of migration-dependent efferocytosis, Sdc-$1^{-/-}$ show significantly reduced efferocytosis events, control bars represent migration events with basal media on top and bottom. D, Increased adhesion was observed in Sdc-$1^{-/-}$ and M1 macrophages compared to Sdc-$1^{+/+}$. Actin cytoskeletal staining (red) reveals increased formation of cell protrusions in Sdc-$1^{+/+}$ (E) vs. Sdc-$1^{-/-}$ (F) macrophages, nucleus stained with DAPI (blue), scale bars, 10 μm. Data are representative of triplicate samples, mean ± SEM, *$P < 0.05$, **$P < 0.01$.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

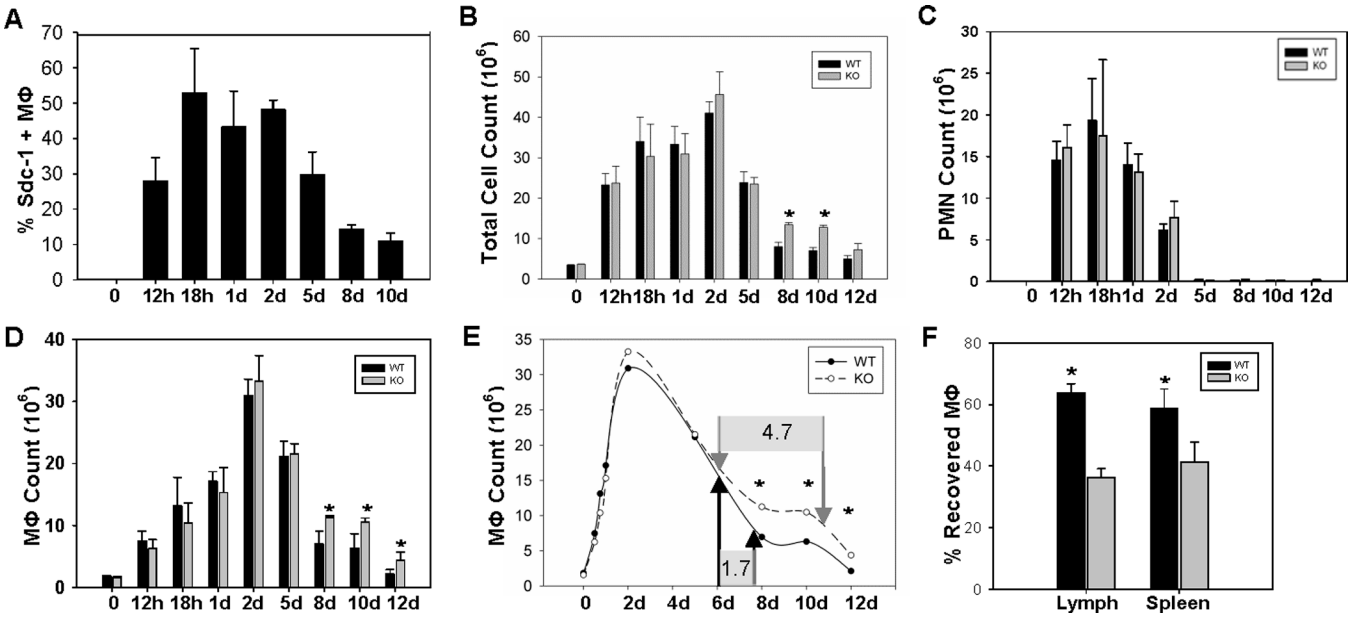

Figure 4.

Sdc-1 contributes to macrophage resolution in thioglycollate-induced peritonitis. A, Macrophage Sdc-1 expression was monitored using flow cytometry over the time course of peritonitis. Total cell count (B), PMN count (C), and macrophage count (D) reveals an altered macrophage resolution response in Sdc-1$^{-/-}$ (KO) peritonitis vs. Sdc-1$^{+/+}$ (WT). E, Resolution curve in WT vs. KO peritonitis, time interval for a 50% to 25% reduction (designated with black arrows for WT and grey arrows for KO) was significantly prolonged in KO (1.7 vs. 4.7 d, analysis curve detailed in Supplemental Figure I). F, Adoptively transferred Sdc-1$^{+/+}$ (WT) cells traffic more efficiently from the peritoneal cavity to the lymph and spleen compared to Sdc-1$^{-/-}$ (KO). N=4-5 mice/time point, data are mean ± SEM, * $P < 0.05$.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

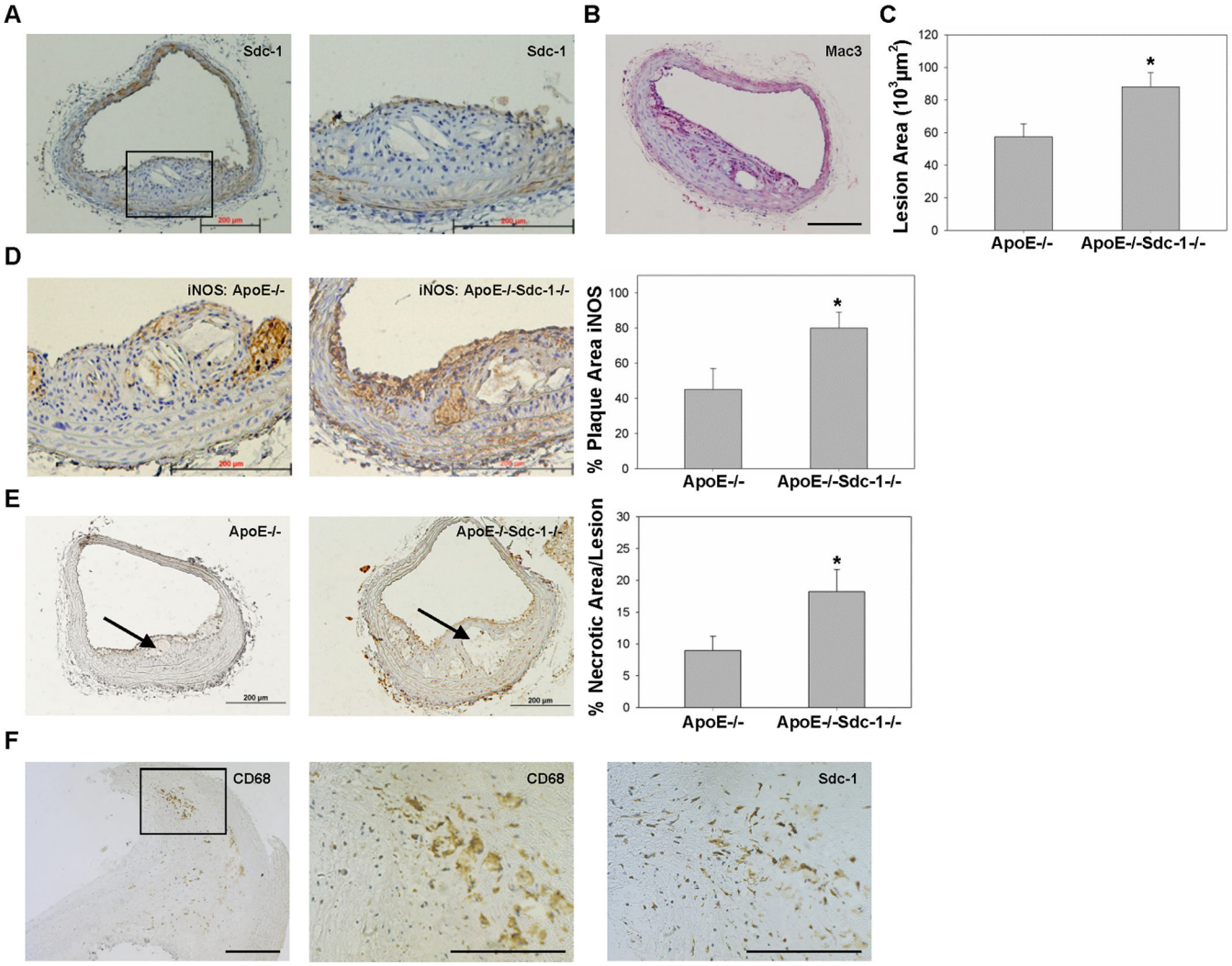

Figure 5.

Sdc-1 is protective in atherosclerosis. A, Immunohistochemical staining of Sdc-1 in 8 week Western diet-induced lesions. B, Macrophage positive staining observed in regions of Sdc-1 staining. C, A significant increase in lesion area was measured at 8 weeks in ApoE$^{-/-}$Sdc-1$^{-/-}$ mice vs. ApoE$^{-/-}$, N=19/genotype. D, Enhanced iNOS staining was observed in ApoE$^{-/-}$Sdc-1$^{-/-}$ lesions, representative of 3 mice/genotype. E, A significant increase in necrotic area (arrow) was observed in ApoE$^{-/-}$Sdc-1$^{-/-}$ lesions, representative of N=10/genotype. F, Sdc-1 positive staining was observed in macrophage positive zones of human plaque samples. Scale bars, 200 μm. Data are mean ± SEM, * $P < 0.05$.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 1

mRNA expression profile of pro-inflammatory genes in M1 vs. Sdc-1+

Gene	M1:Sdc-1*	Gene Information
M-CSF	7	Monocyte/macrophage differentiation factor
GM-CSF	29	Granulocyte differentiation factor
IL1α	14	Proinflammatory cytokine
NFκβ	6	Transcription factor for many inflammatory genes
MyD88	5	Adapter protein that participates in the activation of NFκβ
TNFα	103	Proinflammatory cytokine

*Genes that displayed at least a five-fold difference in expression with $P \leq 0.05$ are reported.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 2

mRNA chemokine expression profile in M1 vs. Sdc-1+

Gene	M1:Sdc-1*	Gene Information
CCL2/MCP1	78	Monocyte chemoattractant
CCL4/MIP1β	60	Induce neutrophil superoxide production
CCL5/RANTES	354	TNFα and IL1β inducible, T cell chemoattractant
CL7/MCP-3	100	Monocyte chemoattractant
CCL8/MCP-2	27	Monocyte, T cell and NK cell chemoattractant
CCL12/MCP-5	834	Monocyte chemoattractant
CXCL1	32	Neutrophil and monocyte recruitment
CXCL2	67	Neutrophil recruitment
CXCL9	99,768	IFN-γ inducible, T cell and monocyte recruitment
CXCL10	5,019	IFN-γ inducible, T cell and NK cells recruitment
CXCL11	211	IFN-γ inducible, T cell recruitment

*Genes that displayed at least a five-fold difference in expression with $P \leq 0.05$ are reported.