Role of Syndecan-1 in Leukocyte–Endothelial Interactions in the Ocular Vasculature

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PURPOSE. Leukocyte endothelial interactions are a key feature of ocular angiogenesis but also play a role in nonproliferative vascular alterations as are found in early diabetes or uveitis. The adhesion of leukocytes to endothelial cells during inflammation is a multistep process that involves leukocyte rolling, adhesion, and extravasation mediated by selectins, cell adhesion molecules (CAMs), integrins, and chemokines. Heparan sulfate (HS) is known to bind to and modify the function of these molecules under physiological conditions. In this study, the role of the HS proteoglycan syndecan-1 in mediating leukocyte–endothelial interactions in the ocular vasculature was investigated.

METHODS. Mice carrying a deletion in the gene encoding the cell surface HS proteoglycan syndecan-1 (*sdc1*) were used to study the interactions of leukocytes and endothelial cells in vivo, using a perfusion technique with FITC-coupled ConA and intravital microscopy.

RESULTS. In a retina perfusion model, $Sdc1^{-/-}$ mice showed increased leukocyte adhesion that was largely attributable to the leukocytes. Intravital microscopy studies revealed a dramatic increase in adhesion after tumor necrosis factor (TNF)-α treatment of $sdc1^{-/-}$ mice compared with similarly treated wild-type mice. The higher degree of leukocyte adhesion may account for the increase in inflammation-mediated corneal angiogenesis observed in $sdc1^{-/-}$ mice.

CONCLUSIONS. The results indicate a role for syndecan-1 as a negative regulator of leukocyte-mediated inflammatory responses. Thus, syndecan-1 could have use as a target for prevention of pathologic leukocyte–endothelial interactions in angiogenesis and inflammation. (*Invest Ophthalmol Vis Sci.* 2002;43:1135–1141)

A common feature of inflammation and neovascularization is increased leukocyte–endothelial interaction.[1] Even diseases that were originally not suggested to be associated with inflammation, such as diabetic retinopathy, have now been demonstrated to have altered leukocyte involvement.[2,3] Therefore, the understanding of common regulatory mechanisms of the leukocyte–endothelial interactions in different conditions is important for the development of better treatment approaches.

The syndecans are a family of cell surface heparan sulfate proteoglycans that are expressed in a developmental and cell-type–specific pattern.[4,5] Their major functional domains, the heparin-related heparan sulfate chains, bind specifically to a large number of extracellular ligands that are active in processes as diverse as morphogenesis and tissue repair, host defense, and energy metabolism.[5,6] The interaction of leukocytes and endothelial cells is a process involving multiple steps: Proteins of the selectin and integrin family as well as chemokines are involved in a cascade of sequential molecular interactions.[7,8] After endothelial cell activation, leukocytes bind to selectins on the endothelium in a low-affinity interaction known as rolling. The next step is a tight leukocyte–endothelial cell interaction mediated by integrins, which is ultimately followed by transmigration of the leukocytes. Heparan sulfate specifically binds to and modifies the action of several of these molecules, such as the C-C and CXC-chemokines,[9,10] the integrin Mac-1,[11,12] and L-selectin, which are expressed on leukocytes, and P-selectin, which is expressed on platelets and endothelial cells.[13–17] Syndecan-1, which in the adult is predominant in epithelial tissues, is expressed on pre–B-cells and plasma cells in their attached (noncirculating) states, and syndecan-1 expression can be induced on macrophages.[18,19] Furthermore, the expression of syndecan-1 can be induced on the surface of endothelial cells in the context of wound healing and inflammation,[20,21] suggesting a role for syndecan-1 in the interaction of leukocytes with endothelial cells. We used the $sdc1^{-/-}$ mouse[22] to elucidate the role of syndecan-1 in this process. $Sdc1^{-/-}$ mice develop normally and are fertile.[22] In this study, we demonstrate that the absence of syndecan-1 leads to enhanced leukocyte–endothelial cell interactions, increased angiogenesis, and increased inflammatory responses.

MATERIALS AND METHODS

Animals

All animal experiments followed the guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Animal Care and Use Committees of the Children's Hospital and the Center for Blood Research. *Sdc1* knockout and wild-type control mice of a mixed background (129 Sv/Jae: C57/Bl6J) were back-crossed to C57/Bl6J mice for two generations and to BALB/c mice for six generations, respectively.[22] The mice were fed on standard

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laboratory chow and were allowed free access to water in an air-conditioned room with a 12-hour light–12-hour dark cycle, until they were used for experiments.

Analysis of Leukocyte Adhesion

Leukocyte adhesion to vascular endothelium was measured as previously described.[3] With mice under deep anesthesia with 50 mg/kg pentobarbital sodium, the chest cavity was carefully opened. The left heart ventricle was then entered with a 20-gauge perfusion cannula, which was fixed by a vessel clamp, avoiding ventricle obstruction. The right atrium was opened by a 16-gauge needle to achieve an outflow. The animals are first perfused with a total volume of 250 mL phosphate-buffered saline (PBS) per kg body weight (BW) to wash out erythrocytes and nonsticking leukocytes. Physiological pressure of 90 mm Hg was maintained while the heart was pumping. Then fixation was achieved by perfusion with 1% paraformaldehyde and 0.5% glutaraldehyde, maintaining the above pressure using a total volume of 200 mL/kg BW over approximately 3 minutes. After inhibition of nonspecific binding with 1% albumin in PBS (total volume 100 mL/kg BW), the perfusion was continued with FITC-coupled ConA lectin (20 μg/mL in PBS, [pH 7.4], total concentration 5 mg/kg BW; Vector Laboratories, Burlingame, CA) to stain adherent leukocytes and vascular endothelial cells. Endothelial cell staining with the lectin was followed by PBS-bovine serum albumin (BSA) for 1 minute and PBS alone for 4 minutes to remove surplus FITC-ConA. With this technique, adherent leukocytes were stained as well as endothelial cells of arterial and venous vessels and capillaries. Previous experiments with *Lycopersicum esculentum* lectin as well as with *Ricinus communis* lectin showed that ConA lectin gave the most homogenous staining, independent of vessel origin or inflammation.[3] The retinas were carefully dissected and flatmounted in fluorescence anti-fading medium (Southern Biotechnology, Birmingham, AL). Observation was by fluorescence microscopy (Axiovert, FITC filter; Carl Zeiss, Oberkochen, Germany). Only retinas in which peripheral collecting vessels of the ora serrata were visible and intact were used for analysis. The location of the leukocytes was scored as being in the arteries, precapillary arteries, veins, precapillary veins, or capillaries. Precapillary venules and precapillary arterioles were grouped together with venules or arterioles, respectively. Total numbers of leukocytes per retina were counted. Twelve retinas in six animals (C57/Bl6) or six retinas in three animals (BALB/c), were analyzed for each genotype.

Bone Marrow Transplantation

Bone marrow transplantation between $sdc1^{+/+}$ and $sdc1^{-/-}$ mice of the BALB/c background was performed using standard techniques. In brief, bone marrow was flushed out of the hindlimbs using DMEM with 10% fetal calf serum (FCS), and red blood cells were lysed. Mononuclear cells (5×10^6 cells/mouse) were injected into recipient mice that had been lethally irradiated with two doses of 600 rad at a 3-hour interval with an irradiator (Gammacell 40 Cesium; Nordion, Kanata, Ontario, Canada). To reduce peritransplant morbidity, the drinking water was acidified during the 2 weeks after the transplantation. Animals were used for leukocyte adhesion assays after at least 6 weeks of recovery in autoclaved microisolator cages. Complete hematopoietic chimerism was confirmed by staining lipopolysaccharide (LPS)-stimulated splenocytes with the anti-syndecan-1 mAb 281-2 (Research Diagnostics, Inc., Flanders, NJ) and subsequent flow cytometry. The following sample numbers (retinas/animals) were analyzed for each transplantation group (donor/recipient): wild-type/wild-type, 5/3; wild-type/knockout, 17/9; knockout/wild-type, 12/6; and knockout/knockout, 8/4.

Growth Factor–Independent Corneal Angiogenesis

In mice under intramuscular general anesthesia with 10 mg/kg xylazine (Bayer, Leverkusen, Germany) and 150 mg/kg ketamine hydrochloride (Phoenix Pharmaceuticals, St. Joseph, MO) and additional

topical application of lidocaine (Alcon, Fort Worth, TX), inflammatory neovascularization was induced by application of 2 μL of 0.15 mM NaOH to the central cornea as previously described.[1] Corneal epithelium was subsequently scraped off with a blunt von Graefe knife. The limbal areas were gently massaged over 360° for 3 minutes. To prevent infection, eyes were treated with antibiotic ointment (neomycinsulfate 3.5 IE/mg, bacitracin 0.3 IE/mg, and polymyxin B sulfate 7.5 IE/mg; Polyspectran; Alcon) after surgery. Eight retinas from four animals were analyzed for each genotype.

Visualization and Quantitation of Corneal Neovascularization

Corneal neovascularization was visualized with a FITC-coupled lectin that binds to endothelial cells when injected into the vascular system. To achieve homogeneous staining, mice were perfused systemically as described. After perfusion, the corneas were carefully dissected at the sclera 1 mm behind the corneoscleral limbus. Lens and iris were removed. The tissue was further fixed in 10% formaldehyde for 1 hour. For measurement of vessel length, the limbus was determined during fluorescence microscopy as the line between iris pigment epithelium and clear cornea. Measurements of vessel length were taken at two distinct points in each quadrant. The full length of all retinal vessels was measured by following the main vessels and their branches under the microscope. Quantification of the vascularized area was determined using the following formula: $x = r^2 - (r^2 - L)$, an ellipse formula, where the radius (r) is 1.6 mm, and L is the measured length of vessel.[23] All corneas were photographed with a standardized technique to compare vascular density. The grading was performed in a masked manner by the same observer.

Detection of Rolling Leukocytes by Intravital Microscopy

Mice were anesthetized using tribromoethanol (0.3 mg/mL), a midline abdominal incision was made, and the mesentery was gently exteriorized. A minimum of three venules (100–200 μm diameter, shear rate ~100 sec[24]) were observed by inverted microscope (objective ×32, 0.4 numeric aperture; Axiovert 135; Carl Zeiss) connected to a video recorder (Panasonic SVHS AG-6720A; Matsushita Electric, Osaka, Japan) using a charge-coupled device (CCD) video camera (Hamamatsu Photonic Systems, Hamamatsu City, Japan). Exposed tissue was kept moist by periodic superfusion using PBS (without additional Ca^{2+} or Mg^{2+}) warmed to 37°C. Mice were injected intraperitoneally with murine recombinant TNFα (0.5 mg/mL in PBS; Genzyme Corp., Boston, MA) 3 hours before intravital microscopy analysis. Three $sdc1^{+/+}$ or four $sdc1^{-/-}$ animals were used for each group, with a minimum of two veins studied per animal.

Image Analysis

Centerline erythrocyte velocity (V_{rbc}) was measured using an optical Doppler velocimeter (Microcirculation Research Institute, Texas A&M College of Medicine, College Station, TX). The venular shear rate (τ) was calculated based on the Poiseuille law for a Newtonian fluid: $\tau = 8 (V_{mean}/D_v)$, where D_v is the diameter of the venule and V_{mean} is estimated from the measured (V_{rbc}) using the empiric correlation $V_{mean} = V_{rbc}/1.6$. The critical velocity (V_{crit}) for each venule was calculated using the equation: $V_{crit} = D_p/D_v \times (2 - D_p/D_v)$, where D_p is the diameter of a leukocyte. Leukocytes traveling a distance of at least 30 μm at a velocity less than V_{crit} were scored as rolling. Any leukocyte interacting with the endothelium at a velocity less than V_{crit} but not fulfilling the criteria for rolling was labeled as captured. The average number of rolling or captured leukocytes per minute over a venular segment of 250 μm was determined by taking 10 counts of 1 minute. Rolling was determined on a minimum of three vessels for a period of 5 minutes, and the number of rolling leukocytes was quantitated by counting the number of cells passing a given plane perpendicular to the vessel axis in 1 minute.
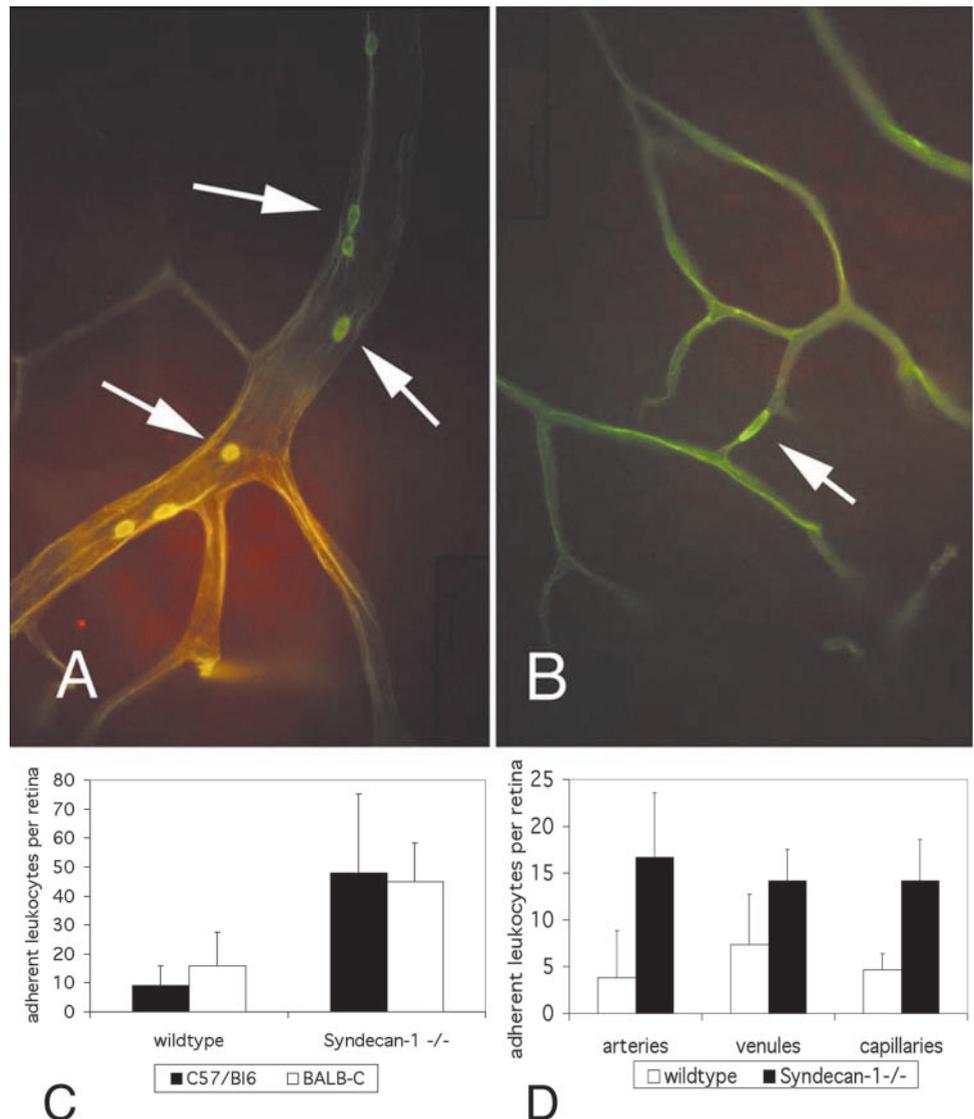
FIGURE 1. Leukocyte adhesion to retina blood vessels. $Sdc1^{+/+}$ and $sdc1^{-/-}$ mice of two genetic backgrounds were perfused with fluorescently labeled lectin. Adhering leukocytes (*arrows*) are visualized in a retinal venule (**A**) and a capillary (**B**) of $sdc1^{-/-}$ mice. (**C**) Leukocytes adhering to the blood vessels of whole retinas of $sdc1^{+/+}$ and $sdc1^{-/-}$ mice were counted. Twelve retinas were used for each genotype. (**D**) Vessel distribution of leukocyte adhesion in the retinas of $sdc1^{+/+}$ and $sdc1^{-/-}$ mice (BALB/c background). Six retinas were analyzed for each genotype.

Statistical Analysis

All results are expressed as the mean $\pm$ SD. The data were compared by analysis of variance (ANOVA). Differences were considered statistically significant at $P < 0.05$.

RESULTS

Leukocyte Adhesion to the Retinal Vasculature in $Sdc1^{-/-}$ Mice

Syndecan-1 is induced in endothelial cells during skin wound healing and in a subgroup of tumor blood vessels[20,21] (Götte and Bernfield, unpublished data, 2000). To test whether absence of syndecan-1 leads to an alteration of endothelial cell–leukocyte interactions, adhesion of leukocytes to blood vessels in the retinas of $sdc1^{-/-}$ and $sdc1^{+/+}$ mice was measured in a perfusion-based assay.[3] Leukocytes adhering to the vascular endothelium were visualized after in situ labeling with FITC-linked ConA (Figs. 1A, 1B), and adhesion was quantified by counting all leukocytes per retina. A threefold (BALB/c) to fivefold (C57Bl6/129) increase in leukocyte adhesion to the retina blood vessels was observed in $sdc1^{-/-}$ mice ($P < 0.01$; Fig. 1C). This increase was largely independent of the vessel type (Fig. 1D). In the peripheral blood of $sdc1^{-/-}$ and $sdc1^{+/+}$

mice, no differences in the number of leukocytes was found (results not shown).

To determine whether the increase of leukocyte adhesion was due to the absence of syndecan-1 on leukocytes or on the endothelium, a bone marrow transplantation between γ-irradiated $sdc1^{-/-}$ and $sdc1^{+/+}$ mice was performed, and the adhesion of leukocytes to retina blood vessels was determined. As expected, a large increase of leukocyte adhesion was observed in the retinas of $sdc1^{-/-}$ mice which had received $sdc1^{-/-}$ bone marrow when compared with wild-type mice that received wild-type bone marrow (Fig. 2). Wild-type mice that had received $sdc1^{-/-}$ bone marrow showed an increase in leukocyte adhesion identical with $sdc1^{-/-}$ mice. Thus, leukocytes from $sdc1^{-/-}$ mice show an increased adhesion to the endothelium of the retina. Statistically relevant differences were achieved in leukocyte adhesion depending on the leukocytes transplanted ($P < 0.01$). However, there was no significant difference in leukostasis, depending on the recipient ($P > 0.05$). Thus, we cannot rule out an endothelial component at present.

Intravital Microscopy of $Sdc1^{-/-}$ Mice

The interaction of leukocytes and endothelial cells in an inflammatory context is a multistep process.[7,8] To distinguish
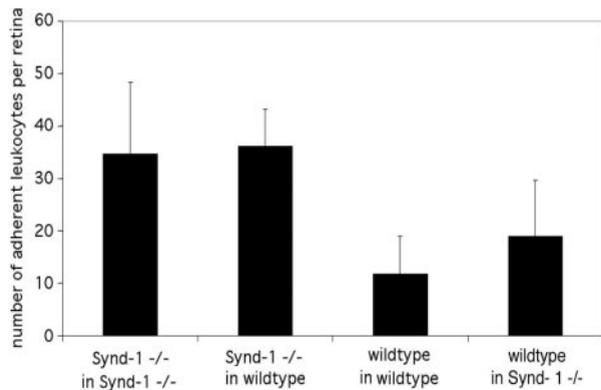
FIGURE 2. Leukocyte adhesion to retina blood vessels in hematopoietic chimeras. $Sdc1^{+/+}$ and $sdc1^{-/-}$ recipient mice received bone marrow transplants from $sdc1^{+/+}$ and $sdc1^{-/-}$ donor mice, respectively. On reconstitution of the hematopoietic system, the animals were perfused with fluorescently labeled lectin and the number of leukocytes adhering to the retina blood vessels was determined. Five to 15 retinas were analyzed for each genotype.

between leukocyte rolling, integrin-mediated adhesion, and transmigration, we used intravital microscopy of the mesentery venules.[25-27] The interaction of platelets with an activated endothelium depends on the exocytosis of both P-selectin[28] and von Willebrand factor[29] from their endothelial storage granules (Weibel-Palade body). Both rolling of leukocytes and platelets after stimulation with a $Ca2^+$ ionophore (A23187), which depend on P-selectin and von Willebrand factor expression on the surface of activated endothelial cells,[24,25,27,28] were normal in $sdc1^{-/-}$ mice (data not shown).

However, $sdc1^{-/-}$ venules treated for 3 hours with TNFα (which is known to induce both P- and E-selectin synthesis) showed a dramatic increase of leukocyte adhesion to the vessel wall compared with wild-type mice (Figs. 3A, 3B). This increase was reflected by a threefold reduction in the number of the remaining leukocytes rolling in the $sdc1^{-/-}$ mice (Fig. 3C) and a reduction in their average velocity (Fig. 3D). In contrast, the $sdc1^{+/+}$ mice showed increased rolling but no pronounced adhesion of leukocytes to the vessel wall. It is of interest to note that in two of three experimental series performed under these conditions, an extravasation of leukocytes from the mesentery vessels was observed in the $sdc1^{-/-}$ mice that was not observed in $sdc1^{+/+}$ mice. Neutrophils, monocytes, and lym-
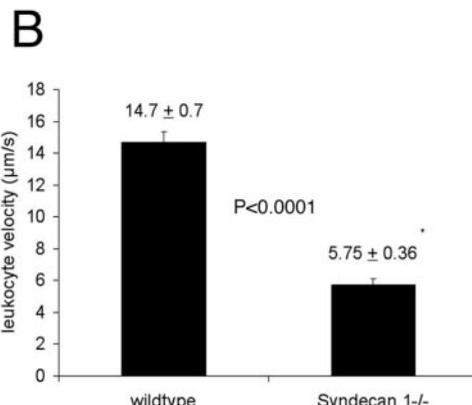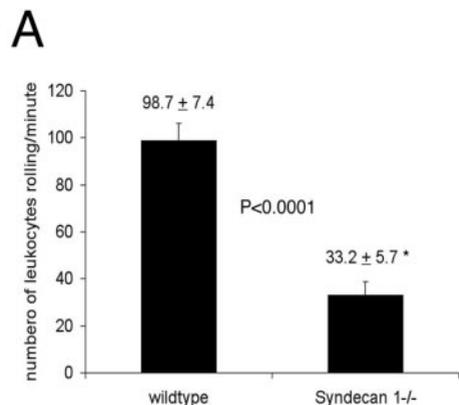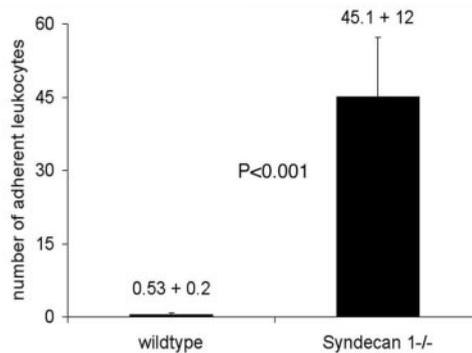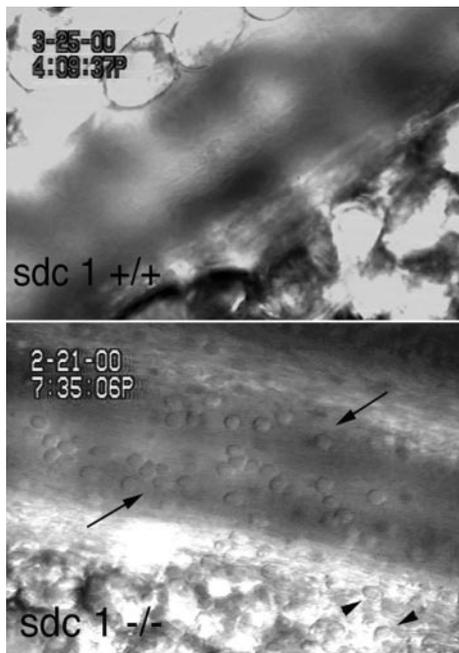

FIGURE 3. Leukocyte adhesion and rolling in TNF-α–stimulated venules. Mice were prepared for intravital microscopy of mesentery venules 3 hours after administration of TNF-α. Venular blood flow and defractive rolling leukocytes were videotaped for 20 minutes. Averages were obtained from at least three animals per genotype. (**A**) Video frames of large mesentery venules of $sdc1^{+/+}$ and $sdc1^{-/-}$ animals. A large number of leukocytes adhering to the vessel walls were seen in $sdc1^{-/-}$ mice (*arrows*). Extravasated leukocytes could be seen in the $sdc1^{-/-}$ mice (*arrowheads*). (**B**) Number of adherent leukocytes per 250-μm vessel length; (**C**) average number of rolling leukocytes per minute; (**D**) average leukocyte velocity.

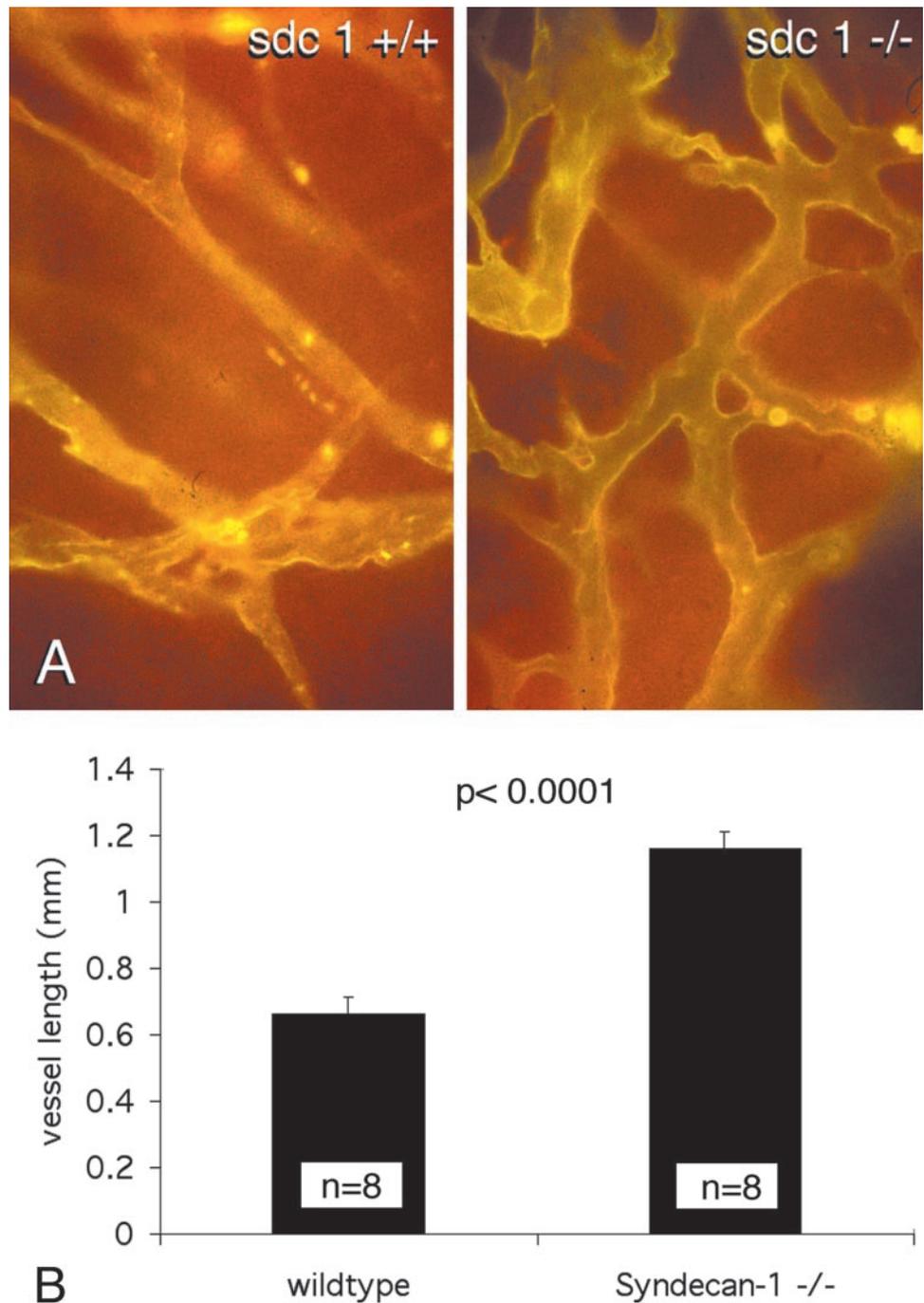FIGURE 4. Growth factor–independent angiogenesis assay. The average length of blood vessels growing toward an experimentally induced cornea lesion was determined 7 days after cornea wounding and removal of the corneal limbus. (**A**) FITC-ConA-perfused blood vessels of *sdc1*$^{+/+}$ and *sdc1*$^{-/-}$ mice. Sectors of the corneae are shown. (**B**) Quantification of vessel length of *sdc1*$^{+/+}$ and *sdc1*$^{-/-}$ mice. Eight corneae of four mice of each genotype were analyzed.

phocytes, but no erythrocytes, were seen among the extravasated cells, arguing against the idea of a specific subset of leukocytes being involved in the increased interaction with the endothelium.

Angiogenesis in *Sdc1*$^{-/-}$ Mice

Increased local accumulation of leukocytes facilitates angiogenesis.[1,29,30] Through secretion of heparin-binding angiogenic factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), neutrophils and macrophages stimulate the proliferation of fibroblasts, vascular endothelium, and neural and smooth muscle cells.[31-33] Therefore, we hypothesized that angiogenesis would be increased in the *sdc1*$^{-/-}$ animals. To test our

hypothesis, a growth factor–independent corneal angiogenesis assay was used (Fig. 4A). The average length of newly formed blood vessels in the cornea of *sdc1*$^{-/-}$ mice increased 1.75-fold compared with wild-type mice, supporting the idea of increased leukocyte-mediated angiogenesis (Fig. 4B). Increased angiogenesis in *sdc1*$^{-/-}$ was also noted in a bFGF-mediated synthetic basement membrane (Matrigel; BD Biosciences, Lincoln Park, NJ) angiogenesis assay[34] (Götte and Bernfield, unpublished data, 2000); however, the cornea assay yielded less variable results.

Apart from the angiogenic effect of increased leukocyte adhesion, it is important to note that heparin has antiangiogenic effects that are partly due to inhibition of angiogenic chemokines, such as macrophage-derived IL-8.[35-37]

CONCLUSIONS

Our data emphasize the physiological role of syndecan-1 in inflammation and angiogenesis in vivo. The absence of the cell surface heparan sulfate proteoglycan syndecan-1 leads to an increased adhesion of leukocytes to endothelial cells which in turn results in increased corneal angiogenesis. This phenotype is similar to the one found in early diabetic retinopathy[3] or uveitis.

Syndecan-1 therefore acts as a negative modulator in the adhesion process. The major functional domains of syndecan-1, its heparan sulfate chains, bind to many molecules that are important in the leukocyte-adhesion process.[5] The extravasation of lymphocytes appears to depend on heparin sulfate–degrading enzymes such as endo-β-glucuronidase, which is released by activated platelets, neutrophils, and T cells.[38,39] Enzymatic removal of HS reduces binding of monocytes to the subendothelial matrix.[40] Heparin sulfate is known to bind to and inhibit E-, L-, and P-selectin[13–17,41]; leukocyte integrins; intercellular adhesion molecule (ICAM)-1; and chemokines, under physiological conditions. Because all these molecules are key players in the adhesion process, the absence of syndecan-1 is very likely to produce multiple effects. Our intravital microscopy data reveal that the absence of syndecan-1 did not affect the interaction of leukocytes with P-selectin expressed on the surface of activated endothelium. However, the slow (E-selectin–dependent) rolling of leukocytes and the adhesion process (β2-integrin–dependent) were affected by the absence of syndecan-1. Whether syndecan-1 affects E-selectin, integrins, or both, the affinity for their leukocyte ligand remains to be determined, and double-deficient mice (syndecan-1/E-selectin$^{-/-}$) are currently being bred to answer that question.

In the intravital microscopy experiments, a dramatic increase of leukocyte adhesion to mesentery blood vessels of $sdc1^{-/-}$ mice was observed after TNFα stimulation, whereas the increased adhesion in retinal blood vessels could already be observed in unstimulated animals. This difference could be attributed to the fact that leukocyte adhesion is influenced by the vascular environment. Endothelial cells derived from the retinal microvasculature express a different pattern of adhesion molecules than, for example, aortic endothelial cells,[42] resulting in different adhesive properties. In addition, the different methodological approaches may contribute to the observation, because the perfusion system allows for a more accurate determination of total leukocyte counts in a situation where only a small number of adhering leukocytes (an average of nine per retina in C57/Bl6 mice; Fig. 1B) can be determined in the wild-type control group.

Although leukocyte rolling was not affected in the $sdc1^{-/-}$ mouse in vivo, a strong adhesion to the retinal vascular endothelium was observed in the $sdc1^{-/-}$ mouse. The bone marrow transplantation experiments show that this effect is largely leukocyte dependent, although we cannot completely rule out an endothelial component, because of a high SE in the group in which wild-type leukocytes were transplanted into knockout animals. Most leukocytes did not express syndecan-1 on their surfaces in an unstimulated state; however, in some instances, syndecan-1 expression could be induced by inflammatory stimuli, as it is the case in macrophages[19] and LPS-stimulated B lymphocytes (Klein, unpublished data, 2000). In fact, a temporally restricted and highly regulated expression is a hallmark of syndecan-1's function during embryonic development and wound healing.[4,5] In addition, it has to be noted that the intact ectodomain of syndecan-1 can be proteolytically cleaved from the cell surface in a regulated manner.[43] The syndecan-1 ectodomain has been shown to act as a soluble effector, both in a physiological and pathologic context.[44,45] One could envisage that syndecan-1, shed from the surface of activated endothelial cells, binds to chemokines and attenuates their function. In the $sdc1^{-/-}$ mouse, the absence of soluble syndecan-1 could lead to a dysregulation and potentiation of chemokine action. Because chemokines induce or activate a variety of adhesion molecules on leukocyte surfaces, this would have a profound effect on leukocyte properties and their function. In addition, soluble syndecan-1 derived from noncirculating cells could bind to and block heparin-binding sites of adhesion molecules on the leukocyte surface of $sdc1^{+/+}$ mice. The absence of syndecan-1 in $sdc1^{-/-}$ mice would lead to an exposure of these sites and to a more efficient binding to endothelial receptor molecules. In the retinal vasculature, increased adhesion is mediated through integrins or CAMs rather than selectins (Adamis, unpublished data, 1999) in conditions such as experimental diabetic retinopathy. Similar mechanisms seem to contribute to the leukostasis in the $sdc1^{-/-}$ mouse. This effect could be due to a more efficient activation of integrins through chemokines. It has recently been shown that syndecans can indeed influence integrin signaling.[46] The absence of syndecan-1 leads to a phenotype resembling early changes in diabetic retinopathy in the retina. For diabetic retinopathy, we have previously shown that inhibition of leukocyte–endothelial interaction through specific blockade of ICAM-1 or CD-18 leads to a subsequent reduction of increased leukostasis as well as the associated endothelial cell death and vascular leakage.[2,3] The concept of an inhibitory role of syndecan-1 on leukocyte adhesion is supported by our data on corneal angiogenesis. Absence of syndecan-1 leads to an increased angiogenic response; however, absence of ICAM or CD-18 reduces the inflammatory response.[1]

Apart from the possible mode of syndecan's action just described, a developmental role during leukocyte maturation could be envisaged as an aspect in the complex phenotype. It could be speculated that the contact of a leukocyte with a syndecan-1–expressing cell is a requirement for the proper development of the leukocyte (which does not express syndecan-1 by itself). In the $sdc1^{-/-}$ mouse, this interaction could not occur, resulting in an aberrant development and modification of leukocyte properties, which in turn could influence the complex interplay of different cell types and chemokines and lead to leukocyte adhesion. Although more has to be learned about the role of cell surface heparan sulfate proteoglycans in general, we can establish a role for syndecan-1 in the process of leukocyte–endothelial cell interactions. The significantly increased adhesiveness of leukocytes of the $sdc1^{-/-}$ mouse will stimulate thoughts on new therapeutic applications of heparan sulfate and heparin in a clinical context of ocular angiogenesis and inflammation.

References

1. Moromizato Y, Stechschulte S, Miyamoto K, et al. CD18 and ICAM-1-dependent corneal neovascularization and inflammation after limbal injury. *Am J Pathol.* 2000;157:1277–1281.
2. Miyamoto K, Khosrof S, Bursell SE, et al. Prevention of leukostasis and vascular leakage in streptozotocin-induced diabetic retinopathy via intercellular adhesion molecule-1 inhibition. *Proc Natl Acad Sci USA.* 1999;96:10836–10841.
3. Joussen AM, Murata T, Tsujikawa A, Kirchhof B, Bursell SE, Adamis AP. Leukocyte-mediated endothelial cell injury and death in the diabetic retina. *Am J Pathol.* 2001;158:147–152.
4. Bernfield M, Kokenyesi R, Kato M, et al. Biology of the syndecans: a family of transmembrane heparan sulfate proteoglycans. *Annu Rev Cell Biol.* 1992;8:365–393.
5. Bernfield M, Götte M, Park PW, et al. Functions of cell surface heparan sulfate proteoglycans. *Annu Rev Biochem.* 1999;68:729–777.
6. Perrimon N, Bernfield M. Specificities of heparan sulphate proteoglycans in developmental processes. *Nature.* 2000;404:725–728.

7. Springer TA. Traffic signals on endothelium for lymphocyte recirculation and leukocyte emigration. *Annu Rev Physiol.* 1995;57: 827–872.

8. Vestweber D, Blanks J. Mechanisms that regulate the function of the selectins and their ligands. *Physiol Rev.* 1999;79:181–213.

9. Hoogewerf AJ, Kuschert GS, Proudfoot AE, et al. Glycosaminoglycans mediate cell surface oligomerization of chemokines. *Biochemistry.* 1997;36:13570–13578.

10. Webb LM, Ehrengruber MU, Clark-Lewis I. Binding to heparan sulfate or heparin enhances neutrophil responses to interleukin 8. *Proc Natl Acad Sci USA.* 1993;90:7158–7162.

11. Peter K, Schwarz M, Conradt C, Nordt T, Moser M, Kubler W, Bode C. Heparin inhibits ligand binding to the leukocyte integrin Mac-1 (CD11b/CD18). *Circulation.* 1999;100:1533–1539.

12. Diamond MS, Alon R, Parkos CA, Quinn MT, Springer TA. Heparin is an adhesive ligand for the leukocyte integrin Mac-1 (CD11b/CD1). *J Cell Biol.* 1995;130:1473–1482.

13. Giuffre L, Cordey AS, Monai N, Tardy Y, Schapira M, Spertini O. Monocyte adhesion to activated aortic endothelium: role of L-selectin and heparan sulfate proteoglycans. *J Cell Biol.* 1997;136: 945–956.

14. Handa K, Nudelman ED, Stroud MR, Shiozawa T, Hakomori S. Selectin GMP-140 (CD62; PADGEM) binds to sialosyl-Le(a) and sialosyl-Le(x), and sulfated glycans modulate this binding. *Biochem Biophys Res Commun.* 1991;181:1223–1230.

15. Nelson RM, Cecconi O, Roberts WG, Aruffo A, Linhardt RJ, Bevilacqua MP. Heparin oligosaccharides bind L- and P-selectin and inhibit acute inflammation. *Blood.* 1993;82:3253–3258.

16. Norgard-Sumnicht KE, Varki NM, Varki A. Calcium-dependent heparin-like ligands for L-selectin in nonlymphoid endothelial cells. *Science.* 1993;261:480–483.

17. Norgard-Sumnicht KE, Varki A. Endothelial heparan sulfate proteoglycans that bind to L-selectin have glucosamine residues with unsubstituted amino groups. *J Biol Chem.* 1995;270:12012–12024.

18. Sanderson RD, Lalor P, Bernfield M. B lymphocytes express and lose syndecan at specific stages of differentiation. *Cell Regul.* 1989;1:27–35.

19. Yeaman C, Rapraeger AC. Post-transcriptional regulation of syndecan-1 expression by cAMP in peritoneal macrophages. *J Cell Biol.* 1993;122:941–950.

20. Elenius K, Vainio S, Laato M, Salmivirta M, Thesleff I, Jalkanen M. Induced expression of syndecan in healing wounds. *J Cell Biol.* 1991;114:585–595.

21. Gallo R, Kim C, Kokenyesi R, Adzick NS, Bernfield M. Syndecans-1 and -4 are induced during wound repair of neonatal but not fetal skin. *J Invest Dermatol.* 1996;107:676–683.

22. Alexander CM, Reichsman F, Hinkes MT, et al. Syndecan-1 is required for Wnt-1-induced mammary tumorigenesis in mice. *Nat Genet.* 2000;25:329–332.

23. Joussen AM, Becken WD, Moromizato Y, Schwartz A, Kirchhof B, Poulaki V. Inhibition of inflammatory corneal angiogenesis by TNP-470. *Invest Ophthalmol Vis Sci.* 2001;42:2510–2516.

24. Andre P, Denis CV, Ware J, et al. Platelets adhere to and translocate on von Willebrand factor presented by endothelium in stimulated veins. *Blood.* 2000;96:3322–3328.

25. Mayadas TN, Johnson RC, Rayburn H, Hynes RO, Wagner DD. Leukocyte rolling and extravasation are severely compromised in P selectin-deficient mice. *Cell.* 1993;74:541–554.

26. Kubes P, Ward PA. Leukocyte recruitment and the acute inflammatory response. *Brain Pathol.* 2000;10:127–135.

27. Denis CV, Andre P, Saffaripour S, Wagner DD. Defect in regulated secretion of P-selectin affects leukocyte recruitment in von Willebrand factor-deficient mice. *Proc Natl Acad Sci USA.* 2001;98: 4072–4077.

28. Frenette PS, Johnson RC, Hynes RO, Wagner DD. Platelets roll on stimulated endothelium in vivo: an interaction mediated by endothelial P-selectin. *Proc Natl Acad Sci USA.* 1995;92:7450–7454.

29. Koch AE, Kunkel SL, Chensue SW. Expression of interleukin-1 and interleukin-1 receptor antagonist by human rheumatoid synovial tissue macrophages. *Clin Immunol Immunopathol.* 1992;65:23–29.

30. Ribatti D, Loria MP, Tursi A. Lymphocyte-induced angiogenesis: a morphometric study in the chick embryo chorioallantoic membrane. *Acta Anat (Basel).* 1991;142:334–338.

31. Burgess WH, Maciag T. The heparin-binding (fibroblast) growth factor family of proteins. *Annu Rev Biochem.* 1989;58:575–606.

32. Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science.* 1989;246:1306–1309.

33. Besner GE, Higashiyama S, Klagsbrun M. Isolation and characterization of a macrophage-derived heparin-binding growth factor. *Cell Regul.* 1990;1:811–819.

34. Passaniti A, Taylor RM, Pili R, et al. A simple, quantitative method for assessing angiogenesis and antiangiogenic agents using reconstituted basement membrane, heparin, and fibroblast growth factor. *Lab Invest.* 1992;67:519–528.

35. Koch AE, Polverini PJ, Kunkel SL, et al. Interleukin-8 as a macrophage-derived mediator of angiogenesis. *Science.* 1992;258:1798–1801.

36. Miller MD, Krangel KS. Biology and biochemistry of the chemokines: a family of chemotactic and inflammatory cytokines. *Crit Rev Immunol.* 1992;12:17–46.

37. Weinstat-Saslow D, Steeg PS. Angiogenesis and colonization in the tumor metastatic process: basic and applied advances. *FASEB J.* 1994;8:401–407.

38. Lider O, Mekori YA, Miller T, et al. Inhibition of T lymphocyte heparinase by heparin prevents T cell migration and T cell-mediated immunity. *Eur J Immunol.* 1990;20:493–499.

39. Nelson RM, Venot A, Bevilacqua MP, Linhardt RJ, Stamenkovic I. Carbohydrate-protein interactions in vascular biology. *Annu Rev Cell Dev Biol.* 1995;11:601–631.

40. Pillarisetti S, Obunike JC, Goldberg IJ. Lysolecithin-induced alteration of subendothelial heparan sulfate proteoglycans increases monocyte binding to matrix. *J Biol Chem.* 1995;270:29760–29765.

41. Luo J, Kato M, Wang H, Bernfield M, Bischoff J. Heparan sulfate and chondroitin sulfate proteoglycans inhibit E-selectin binding to endothelial cells. *J Cell Biochem.* 2001;80:522–531.

42. Suzuma K, Takagi H, Otani A, Honda Y. Hypoxia and vascular endothelial growth factor stimulate angiogenic integrin expression in bovine retinal microvascular endothelial cells. *Invest Ophthalmol Vis Sci.* 1998;39:1028–1035.

43. Fitzgerald ML, Wang Z, Park PW, Murphy G, Bernfield M. Shedding of syndecan-1 and -4 ectodomains is regulated by multiple signaling pathways and mediated by a TIMP-3-sensitive metalloproteinase. *J Cell Biol.* 2000;148:811–824.

44. Park PW, Pier GB, Hinkes MT, Bernfield M. Exploitation of syndecan-1 shedding by *Pseudomonas aeruginosa* enhances virulence. *Nature.* 2001;411:98–102.

45. Kato M, Wang H, Kainulainen V, et al. Physiological degradation converts the soluble syndecan-1 ectodomain from an inhibitor to a potent activator of FGF-2. *Nat Med.* 1998;4:691–697.

46. Rapraeger A. Syndecan-regulated receptor signaling. *J Cell Biol.* 2000;149:995–997.