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Endogenous Attenuation of Allergic Lung Inflammation by Syndecan-1[1]

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The airway plays a vital role in allergic lung diseases by responding to inhaled allergens and initiating allergic inflammation. Various proinflammatory functions of the airway epithelium have been identified, but, equally important, anti-inflammatory mechanisms must also exist. We show in this study that syndecan-1, the major heparan sulfate proteoglycan of epithelial cells, attenuates allergic lung inflammation. Our results show that syndecan-1-null mice instilled with allergens exhibit exaggerated airway hyperresponsiveness, glycoprotein hypersecretion, eosinophilia, and lung IL-4 responses. However, administration of purified syndecan-1 ectodomains, but not ectodomain core proteins devoid of heparan sulfate, significantly inhibits these inflammatory responses. Furthermore, syndecan-1 ectodomains are shed into the airway when wild-type mice are intranasally instilled with several biochemically distinct inducers of allergic lung inflammation. Our results also show that syndecan-1 ectodomains bind to the CC chemokines (CCL7, CCL11, and CCL17) implicated in allergic diseases, inhibit CC chemokine-mediated T cell migration, and suppress allergen-induced accumulation of Th2 cells in the lung through their heparan sulfate chains. Together, these findings uncover an endogenous anti-inflammatory mechanism of the airway epithelium where syndecan-1 ectodomains attenuate allergic lung inflammation via suppression of CC chemokine-mediated Th2 cell recruitment to the lung. ***The Journal of Immunology,*** **2005, 174: 5758–5765.**

A number of studies over the last several decades have identified many mediators of allergic lung inflammation, shedding light on key pathogenetic mechanisms. The central mediators include cellular effectors, such as T cells and eosinophils, and inflammatory mediators, such as chemokines/cytokines and proteases. Because all these mediators interact with other molecules to exert their biological functions, it is imperative to understand how they interact with their binding partners and how their activities are regulated during disease. However, the molecular mechanisms regulating these mediators have yet to be clearly defined.

Th cells activated by a variety of inhaled allergens drive allergic airway inflammation. Th2 cells that secrete cytokines such as IL-4, IL-5, and IL-13 (1, 2) are specifically implicated in airway obstruction that is the end result of allergic lung disease. The airway epithelium probably plays a vital role in this process by detecting allergenic adjuvants that initiate inflammation and by secreting chemokines that specifically recruit Th2 and other allergic effector cells to the airway (3, 4). These functions emphasize the proinflammatory role that the airway epithelium plays in allergic lung disease, but, equally important, anti-inflammatory functions of the airway epithelium that limit excessive tissue damage must also exist.

Heparan sulfate (HS)[3] and heparin are linear polysaccharides comprised of repeating disaccharide units of hexuronic acids alternating with an *N*-substituted glucosamine. Both glycosaminoglycans are found covalently conjugated to specific core proteins as HS proteoglycans (HSPGs) in vivo. Although heparin contains more sulfate content per disaccharide than HS, both can bind and regulate a wide variety of molecules that participate in allergic lung inflammation (5). Heparin has also been shown to inhibit asthmatic responses in humans (6–9). However, in vivo, heparin is found mainly in intracellular vesicles of connective tissue mast cells, whereas HS covalently attached to various HSPG core proteins is ubiquitously expressed. In addition, because HS contains all the structural motifs present in heparin, the physiological counterpart of pharmaceutical heparin in vivo is likely to be HSPGs, suggesting that HSPGs function as endogenous inhibitors of allergic lung inflammation.

Syndecan-1 is the major cell surface HSPG of epithelial cells, including the airway epithelium. Cell surface syndecan-1 can serve as a primary receptor for some ligands, but it often functions as a coreceptor by acting as a cell surface scaffold that catalyzes the encounter between ligands and their respective signaling receptors. Syndecan-1 also functions as a soluble HSPG, because it can be proteolytically cleaved in the juxtamembrane region and released into the extracellular environment in a process known as ectodomain shedding (5, 10, 11).

Available data indicate that the enzyme that cleaves syndecans is a metalloproteinase, and both soluble (12) and cell surface-associated (13, 14) matrix metalloproteinases (MMPs) have been shown to shed syndecan-1 ectodomains. Syndecan-1 shedding is

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[3] Abbreviations used in this paper used: HS, heparan sulfate; AHR, airway hyper-responsiveness; BAL, bronchoalveolar lavage; Ach, acetylcholine chloride; HSPG, HS proteoglycan; MMP, matrix metalloproteinase; R_L, lung resistance; WT, wild type.

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triggered by many inflammatory mediators in vitro (11, 14–16) and also by tissue injury and inflammatory conditions in vivo. In mice, airway epithelial syndecan-1 shedding is specifically activated during bacterial lung infection (17) and bleomycin-induced acute lung injury (12). Interestingly, in these lung injury models, basolateral syndecan-1 ectodomains are shed into the lumen, where they regulate various molecular interactions. Levels of syndecan-1 ectodomains have also been shown to be elevated in human tissue injury fluids, such as tracheal aspirates of intubated preterm infants and skin wound fluids (18, 19). These results indicate that activation of syndecan-1 shedding is one of the innate host responses to tissue injury and inflammation.

Why syndecan-1 shedding is activated by many tissue injury and inflammatory conditions is not fully understood. However, recent studies suggest that syndecan-1 ectodomains modulate inflammatory processes by regulating the activities of various inflammatory mediators. We have shown in mice that syndecan-1 ectodomains shed in response to lung infection by *Pseudomonas aeruginosa* bind and inhibit host defense factors (e.g., antimicrobials) to enhance bacterial virulence (17). In a mouse model of acute lung injury, shedding of syndecan-1 ectodomains regulates and confines inflammation to specific sites of epithelial injury by directing the generation of a CXC chemokine gradient that guides neutrophil migration into the alveolar space (12).

These data suggest that syndecan-1 shedding by the airway epithelium regulates both infectious and noninfectious lung inflammation by modulating key inflammatory mediators. We show in this study that airway syndecan-1 attenuates lung inflammation induced by intranasal administration of allergens. Deletion of syndecan-1 exacerbates allergic lung disease, whereas airway administration of purified syndecan-1 ectodomains attenuates disease parameters. These findings are physiologically relevant because ectodomain shedding of airway syndecan-1 is specifically activated upon allergen challenge. Furthermore, purified syndecan-1 ectodomains bind specifically to CC chemokines that function as key regulators of allergic diseases, such as CCL7 (MARC), CCL11 (eotaxin), and CCL17 (TARC); inhibits T cell migration stimulated by CCL11 and CCL17; and inhibits the recruitment of Th2 cells to the lung in an HS-dependent manner. These findings uncover a novel anti-inflammatory mechanism by which the airway epithelium limits excessive inflammatory damage by shedding syndecan-1 ectodomains that suppress CC chemokine-mediated accumulation of Th2 cells in the lung, a mechanism central to the development of allergic diseases such as asthma.

Materials and Methods

Mice

Syndecan-1-null (*sdc1*$^{-/-}$) mice were generated as previously described and backcrossed eight and six times onto the C57BL/6J and BALB/c genetic backgrounds, respectively (17, 20). MMP-2$^{-/-}$ and MMP-9$^{-/-}$ mice on the C57BL/6 background (21, 22) were provided by Dr. Z. Werb (University of California, San Francisco, CA). MMP-2$^{-/-}$/MMP-9$^{-/-}$ double-null mice were generated from F_2 and F_3 crosses of single-null mice. MMP-7$^{-/-}$ mice on the C57BL/6 background were provided by Drs. C. Wilson and W. Parks (Washington University School of Medicine, St. Louis, MO). Wild-type (WT) BALB/c mice and WT C57BL/6 mice were purchased from Harlan Sprague Dawley or The Jackson Laboratory or were propagated from WT littermates originating from crosses of mice heterozygous for the syndecan-1 gene. For these experiments, WT fully inbred and partially inbred mice produced equivalent responses. All mice were bred and housed at Baylor College of Medicine (Houston, TX) in an American Association for Accreditation of Laboratory Animal Care-accredited vivarium while maintained on OVA-free diets under pathogen-free conditions. All experimental protocols used in this study were approved by the institutional animal care and use committee of Baylor College of Medicine and followed federal guidelines.

Ags, Abs, and other reagents

Chicken egg OVA (Sigma-Aldrich; grade V) was precipitated in alum (OVA/alum) as previously described (2). *Aspergillus fumigatus* allergen was prepared from the culture filtrate of stationary phase *A. fumigatus* cultures (lot DC980809) as previously described and titrated according to proteinase activity to provoke robust allergic lung inflammation without inducing morphological lung injury (1, 23). Chicken egg OVA was reconstituted in sterile PBS and added to *A. fumigatus* allergen immediately before intranasal administration to give 25 μg/dose. For OVA-specific Ab isotypes, biotinylated anti-mouse IgE, IgG1, and IgG2a Abs were purchased from Caltag Laboratories. For detection of bronchoalveolar lavage (BAL) glycoproteins, including mucins, biotinylated jacalin lectin was purchased from Vector Laboratories and used as previously described (24). Rat anti-mouse syndecan-1 ectodomain (281-2) and rat anti-mouse syndecan-4 ectodomain (Ky8.2) mAbs (17) were purified from ascites fluids or conditioned media of hybridoma cultures by protein G affinity chromatography. Rabbit anti-mouse syndecan-1 cytoplasmic domain Ab was generated by immunizing rabbits with the synthetic peptide CNGGAYQKPT KQEEFYA. Affinity-purified Ab was prepared by protein A affinity chromatography, followed by synthetic peptide affinity chromatography. The affinity-purified Ab detects detergent-extracted transmembrane syndecan-1, but does not react with purified syndecan-1 ectodomains. Syndecan-1 ectodomains were purified from the conditioned medium of normal murine mammary gland epithelial cell cultures by strong anion exchange chromatography, CsCl density centrifugation, and 281-2 affinity chromatography as described previously (10). Recombinant mouse syndecan-1 core protein devoid of HS was expressed as a GST fusion protein in *Escherichia coli* and purified by glutathione affinity chromatography (17).

Ag immunization and intranasal challenge

Mice were sensitized with 25 μg of OVA precipitated in alum given once by i.p. inoculation on days 1, 7, and 14. Beginning on day 21, sensitized mice were given 25 μg of OVA intranasally daily for 5 consecutive days as described previously (2). Alternatively, mice were instilled intranasally with *A. fumigatus* allergen prepared with OVA on a schedule of every 4 days for a total of five challenges (25). Mice were killed ~18 h after the final allergen challenge.

Analysis of the asthma phenotype

Changes in airway physiology were assessed using a protocol modified from that described by Amdur and Meade (26) as previously reported (2). Briefly, mice were anesthetized with etomidate and ventilated with a rodent ventilator (Harvard Apparatus; model 687), and a 27-gauge needle was used to establish i.v. access in a tail vein. Animals were placed inside a custom-built plethysmograph coupled to a pressure transducer (Buxco) and constructed with ports for i.v. and ventilator access tubing. Lung resistance (R_L) was determined by continuously quantitating the quotient $\Delta Pt/\Delta V$ (where ΔPt is the change in tracheal pressure, and ΔV is the change in flow) at points of equal lung volume (70% tidal volume) using a pulmonary mechanics analyzer (model 6; Buxco). ΔPt was determined using a second pressure transducer (Buxco) connected to the tracheal cannula. To determine ΔV, plethysmograph pressure changes were calibrated to changes in volume over the physiologic range studied. The differential of this value over time is ΔV. After establishing a stable baseline R_L (<5% variation over 3 min), acetylcholine chloride (Ach; Sigma-Aldrich) was administered i.v. over 1 s in increasing doses of 0.5 log until at least a 200% increase in R_L was obtained. The provocative concentration of Ach (micrograms of Ach per gram) that caused a 200% increase in R_L (PC_{200}) was calculated from interpolation of the dose-response curves. BAL cytology, serum Ab isotypes, lung histopathology, and number of total lung IL-4- and IFN-γ-positive cells by ELISPOT assays were determined as described previously (1).

Syndecan-1 assays

For measurement of syndecan ectodomains, 10–200 μl of BAL fluids collected 24 h after the last allergen or saline challenge were acidified by addition of NaOAc, pH 4.5, to 50 mM, dot-blotted onto cationic nylon membranes (Immobilon NY$^+$; Millipore), and detected by immunoblotting using the 281-2, Ky8.2, or anti-syndecan-1 cytoplasmic domain Abs as previously described (27). Acidification of the samples allows only highly anionic molecules, such as syndecans, to be retained by the cationic membrane. The amount of shed syndecans in BAL fluid was quantified using purified syndecan-1 or -4 ectodomains as standards.

For ligand dot blotting, 0.1, 1, 5, 10, and 20 ng of CCL7, CCL11, CCL17, CXCL10, IL-4, and IL-13 and 20 ng of the 281-2 anti-syndecan-1

ectodomain Ab were dot-blotted onto a nitrocellulose membrane. The membrane was blocked overnight in TBS (50 mM Tris (pH 7.5) and 150 mM NaCl) containing 1% FCS at 4°C, then washed twice in binding buffer (TBS containing 1% FCS and 0.01% Tween 20) for 20 min at 25°C. Purified syndecan-1 ectodomain (3 μg) was radiolabeled with 500 μCi of $Na^{125}I$ by Iodogen (Pierce), and the radiolabeled ectodomain (650 ng) was incubated with the membrane in 20 ml of binding buffer for 2 h at 25°C. The blot was washed in binding buffer (three times) and in TBS (twice) and exposed on an x-ray film. The film was scanned and quantified by Image V1.62 (National Institutes of Health). The same blot was stripped of bound radiolabeled syndecan-1 ectodomains by incubation in stripping buffer (Pierce) and reprobed with ^{125}I-labeled syndecan-1 core proteins devoid of HS.

Cell migration assay

$CD4^+$ T cells were immunomagnetically positively selected from splenocytes (Miltenyi Biotec) isolated from C57BL/6 mice immunized with *A. fumigatus* allergen, and their chemotaxis in the absence or the presence of HS was determined using standard 48-well chemotaxis chambers (NeuroProbe) as previously described (28).

Histology

Lungs were perfused via the right ventricle, inflated, isolated, and fixed in 4% paraformaldehyde/PBS for 48 h at 4°C. Lung tissues were then embedded in paraffin, sectioned (5 μm), and stained with periodic acid-Schiff base. Metaplastic goblet cells appear bright red and are readily distinguished from other cells.

Statistics

Data are presented as the mean ± SEM and are representative of at least three independent experiments that used at least five mice in each group. Significant differences are expressed between two groups as indicated ($p \leq 0.05$), using Student's t test for logarithm-transformed PC_{200} data and Kruskal-Wallis test for all other data.

Results

Deletion of syndecan-1 exacerbates allergic lung disease

We reasoned that if syndecan-1 is important in regulating allergic lung inflammation, then hosts with altered syndecan-1 expression will respond differently to allergens. We challenged *sdc1*$^{-/-}$ mice on OVA-susceptible BALB/c and OVA-resistant C57BL/6 genetic backgrounds (2) with *A. fumigatus* allergen (combination of *A. fumigatus* culture filtrate and OVA).

For all end points, results for saline-challenged *sdc1*$^{-/-}$ mice were indistinguishable from those for saline-challenged WT mice. However, when instilled intranasally with the *A. fumigatus* allergen, *sdc1*$^{-/-}$ mice showed significantly enhanced disease features relative to WT mice regardless of background genotype (Fig. 1). The significant worsening of airway hyperresponsiveness (AHR), glycoprotein secretion, and eosinophilia in *sdc1*$^{-/-}$ mice was remarkable, because these allergen-induced obstructive features were already strongly induced in WT mice. Equivalent or slightly greater peribronchovascular accumulation of inflammatory cells and goblet cell metaplasia were also seen in lung sections of allergen-challenged *sdc1*$^{-/-}$ mice relative to WT mice (Fig. 2, *B* and *C*). Consistent with its protective effect in human asthma, intranasal administration of heparin significantly reduced peribronchovascular inflammation and goblet cell metaplasia (Fig. 2*D*) as well as AHR, glycoprotein secretion, and eosinophilia (data not shown). Other parameters of allergic lung disease, such as BAL levels of CCL7, CCL11, and CCL17, were also increased in allergen-challenged *sdc1*$^{-/-}$ mice relative to WT (1.3 ± 0.1 vs 1.0 ± 0.1, 2.2 ± 0.3 vs 1.0 ± 0.3, and 1.6 ± 0.2 vs 1.0 ± 0.1 ng/ml, respectively; $p < 0.05$ for each chemokine). These data suggest that airway syndecan-1 inhibits lung inflammation and thereby limits the degree of airway obstruction via its HS chains.

Enhanced Th2 activity in sdc1$^{-/-}$ *mice*

To gain insight into the molecular mechanism of how syndecan-1 attenuates allergic lung inflammation, we examined the immune response in *sdc1*$^{-/-}$ mice. Th2 cell homing to the lung after airway allergen challenge is required for the expression of allergic lung disease (1, 2, 29). We therefore examined the effect of syndecan-1

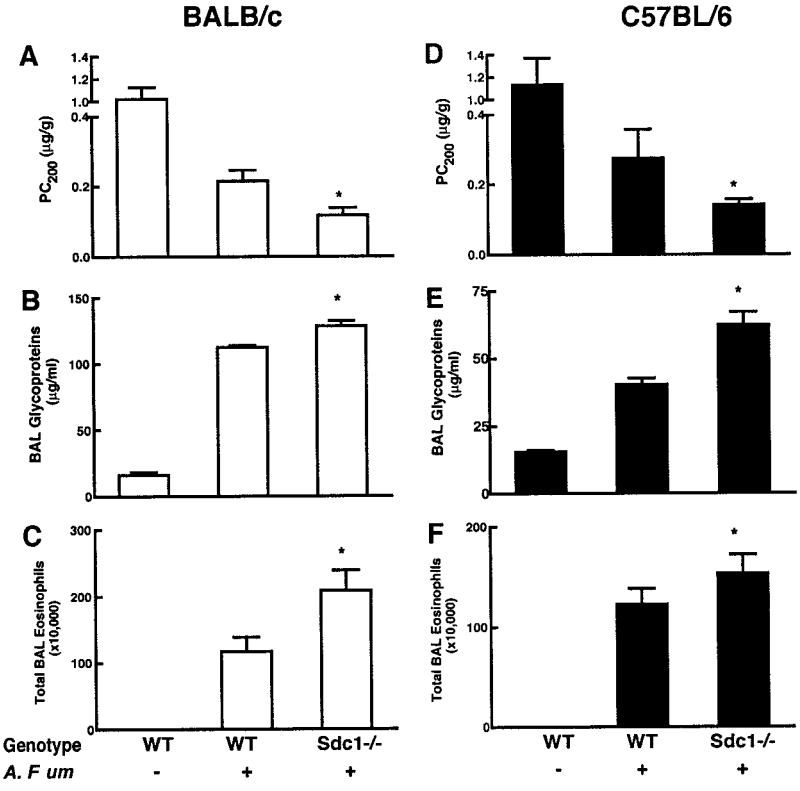

FIGURE 1. Allergic lung disease is exacerbated in the absence of syndecan-1. WT and *sdc1*$^{-/-}$ mice on either the BALB/c (*A–C*) or C57BL/6 (*D–F*) background were challenged five times intranasally with saline or *A. fumigatus* allergen and assessed 24 h after the final challenge for AHR by PC_{200}, BAL glycoproteins, and BAL eosinophils. *, $p < 0.05$ relative to WT mice receiving allergen.

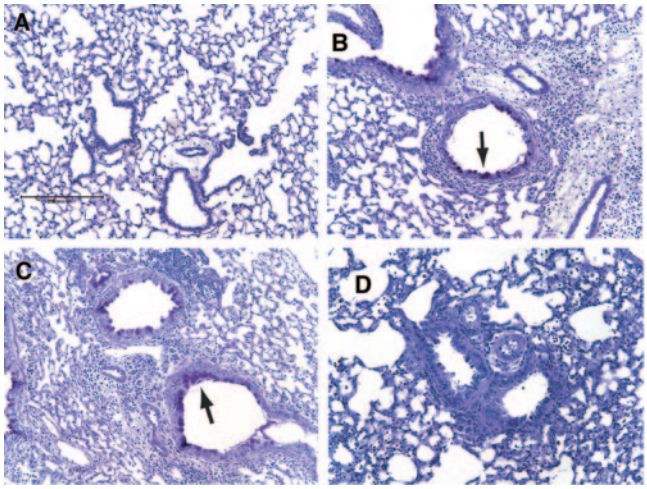

FIGURE 2. Histopathology of lungs from representative mice. Bronchovascular bundles are shown from naive (*A*), allergen-challenged WT (*B*), allergen-challenged *sdc1*$^{-/-}$ (*C*), and heparin- and allergen-challenged *sdc1*$^{-/-}$ (*D*) mice. Metaplastic goblet cells with mucin-containing vesicles are seen in *B* and *C* as red-staining cells within the airway epithelium (arrows). Relative size is indicated by the 200-μm bar in *A*. Periodic acid-Schiff base stain is used in all panels.

deletion on Th cell recruitment to the lung after the final allergen challenge by enumerating the number of total lung IL-4- and IFN-γ-expressing cells by ELISPOT assays to estimate Th2 and Th1 cell recruitment, respectively. Consistent with the exaggeration of other inflammatory indexes, IL-4$^+$ cell recruitment to the lung was increased by 100% in allergen-instilled *sdc1*$^{-/-}$ mice relative to WT mice (Fig. 3*A*). IFN-γ^+ cells, although 100-fold fewer in the lung relative to IL-4$^+$ cells, were also increased in allergen-challenged *sdc1*$^{-/-}$ mice (Fig. 3*B*).

The greater number of IL-4-producing cells in *sdc1*$^{-/-}$ lungs suggested that syndecan-1 primarily attenuates lung inflammation by inhibiting Th2 responses. We further examined this hypothesis by measuring serum levels of OVA-specific IgE in WT and *sdc1*$^{-/-}$ mice instilled intranasally with the *A. fumigatus* allergen. Ag-specific serum IgE responses are Th2 and IL-4 dependent (30–32). Furthermore, because IgE is synthesized in the lung by recruited plasma cells (33), serum IgE levels provide an indirect assessment of the number and activity of lung Th2 cells and/or IgE-secreting plasma cells. Relative to *A. fumigatus* allergen-challenged WT mice, serum OVA-specific IgE titers were ~2-fold greater in allergen-instilled *sdc1*$^{-/-}$ mice (Fig. 3*C*). In contrast, OVA-specific IgG2a titers, which are reflective of Th1 and IFN-γ responses (34, 35), were not significantly different between allergen-challenged *sdc1*$^{-/-}$ and WT mice (Fig. 3*D*). Together, these findings demonstrate that deletion of syndecan-1 promotes Th2 cell recruitment to the lung and Ag-specific IgE responses and provide additional evidence that endogenous syndecan-1 attenuates allergic lung disease by inhibiting Th2 cell responses.

Allergen instillation activates syndecan-1 ectodomain shedding in vivo

To understand how syndecan-1 attenuates allergic lung inflammation, we determined whether syndecan-1 ectodomains are shed into the airway in response to allergen challenge. Naive WT mice were instilled intranasally with the *A. fumigatus* allergen, and OVA-sensitized WT mice were instilled with OVA, then the concentrations of syndecan-1 and -4 ectodomains in BAL fluids were measured (Fig. 4). Syndecan-1 is the major HSPG of airway epithelia, but syndecan-4 is also expressed, albeit at a lower level. Mice challenged with both allergens showed ~8-fold more syndecan-1 ectodomains in their BAL fluids relative to saline-instilled mice, with no differences observed in levels of syndecan-4. Activation of syndecan-1 shedding was localized to the airway, because total lung levels of syndecan-1 ectodomains did not differ between saline- and allergen-instilled mice (data not shown). BAL syndecan-1 ectodomains were not detected when probed with an Ab directed against the syndecan-1 cytoplasmic domain (27), verifying that the ectodomains were shed, and that syndecan-1 was not released from damaged cells (data not shown). These results indicate that biochemically distinct experimental allergens similarly trigger syndecan-1 shedding in the airway and suggest that syndecan-1 inhibits allergic lung inflammation via generation of soluble ectodomains.

Several MMPs have been shown to shed syndecan-1 ectodomains. To determine whether syndecan-1 ectodomains are also shed by MMPs during allergic lung inflammation, we examined whether MMP-2, MMP-7, and MMP-9 shed syndecan-1 ectodomains in vivo. MMP-2 and MMP-9 have been implicated in the pathogenesis of allergic lung diseases (28, 36, 37), and MMP-7

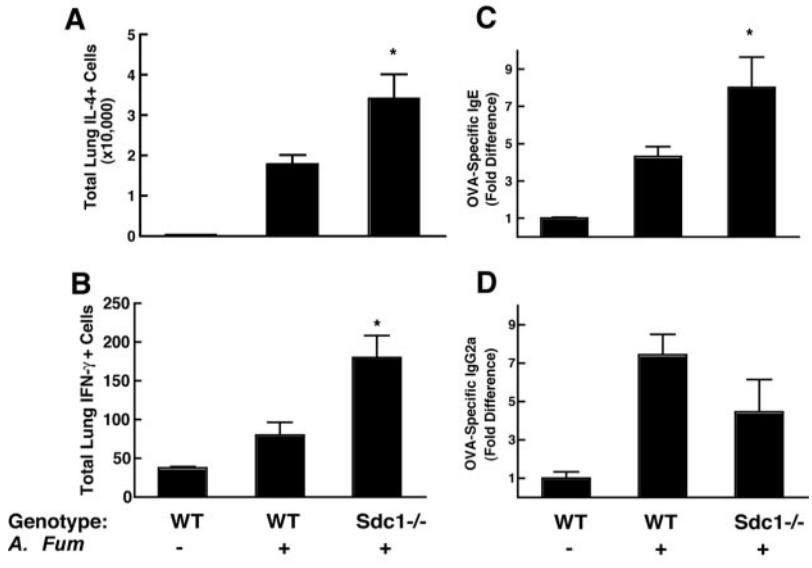

FIGURE 3. Enhanced Th2 cell and IgE responses in the absence of syndecan-1. WT and *sdc1*$^{-/-}$ mice on the C57BL/6 background were challenged with *A. fumigatus* allergen or saline as indicated, and total IL-4- and IFN-γ-secreting lung cells were enumerated by ELISPOT (*A* and *B*), and OVA-specific serum IgE and IgG2a levels were quantitated (*C* and *D*). *, $p < 0.05$ relative to mice treated with allergen alone.

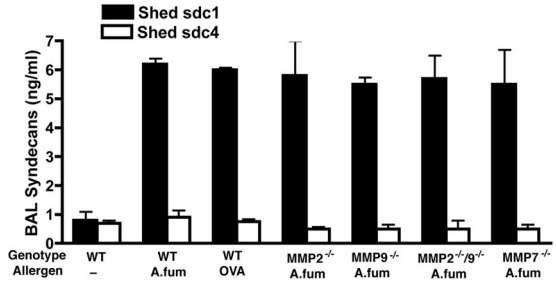

FIGURE 4. Syndecan-1 ectodomain shedding is activated by allergen challenge in vivo. WT and MMP-2$^{-/-}$, MMP-9$^{-/-}$, MMP-2$^{-/-}$/MMP-9$^{-/-}$, and MMP-7$^{-/-}$ mice on the C57BL/6 background were challenged intranasally with *A. fumigatus* allergen, and OVA-sensitized WT BALB/c mice were challenged with OVA as indicated. Naive mice were instilled with saline. BAL fluids were collected 24 h after the final airway challenge and assayed for syndecan-1 or -4 ectodomains as described. Data shown are the mean of triplicate measurements ± SEM.

sheds syndecan-1 ectodomains into the airway in bleomycin-induced acute lung injury in mice (12). To determine whether these MMPs function as syndecan-1 sheddases, mice null for these MMPs were challenged with *A. fumigatus* allergen, and BAL levels of syndecan-1 and -4 ectodomains were quantified. As shown, syndecan-1 ectodomain shedding was activated in MMP-null mice to a level similar to that in allergen-instilled WT mice, indicating that these MMPs are not essential for syndecan-1 shedding in response to allergen (Fig. 4).

Syndecan-1 ectodomains inhibit IL-4$^+$ cell recruitment to lung

If activation of syndecan-1 shedding is an important mechanism that attenuates allergic lung inflammation, then administering purified syndecan-1 ectodomains should inhibit allergen-induced lung inflammation. We tested this hypothesis by challenging WT mice with the *A. fumigatus* allergen with or without 640 ng of purified syndecan-1 ectodomains or recombinant syndecan-1 core proteins devoid of HS chains, and determined their effects on AHR, glycoprotein secretion, and eosinophilia. The dose of 640 ng was chosen based on our observation that both 300 and 800 ng of heparin inhibit allergic lung disease responses (data not shown). We did not observe a significant difference in glycoprotein secretion (data not shown). However, compared with mice challenged with allergen alone, instillation of syndecan-1 ectodomains, but not HS-free core proteins, significantly reduced AHR and eosinophilia (Fig. 5), indicating that syndecan-1 ectodomains suppress airway inflammation and obstruction through their HS chains.

Results from our studies suggested that features of allergic lung disease are exacerbated in *sdc1*$^{-/-}$ mice because of increased Th2 cell accumulation. The effect on IL-4$^+$ cells was particularly significant given the requirement of Th2 cells for disease expression in this model (1). We tested whether syndecan-1 ectodomains suppress IL-4$^+$ cell accumulation by determining the effects of exogenously administered syndecan-1 ectodomains on IL-4$^+$ and IFN-γ^+ cell recruitment in vivo. As shown in Fig. 6, coinstillation of purified syndecan-1 ectodomains, but not core proteins, reduced the number of lung IL-4-positive cells by ~70%. The number of IFN-γ-positive cells did not differ between mice instilled with syndecan-1 ectodomains or core proteins (Fig. 6). These results indicate that syndecan-1 ectodomains specifically suppress Th2 cell accumulation in the lung via their HS chains, uncovering a major endogenous mechanism by which the airway epithelium limits allergic airway inflammation and obstruction.

Syndecan-1 ectodomains bind Th2 chemokines and HS inhibits CC chemokine-mediated T cell migration

To determine how syndecan-1 ectodomains attenuate IL-4$^+$ cell accumulation in the lung, we examined whether syndecan-1 ectodomains bind and regulate chemokines and cytokines implicated in the homing of Th2 and Th1 cells in vitro. We previously determined (28) that the *A. fumigatus* allergen induced secretion into mouse BAL fluid of CCL11, CCL7, and CCL17, ligands for the Th2-specific chemokine receptors CCR3 and CCR4 (38). Ligand dot-blotting analyses demonstrated that radiolabeled syndecan-1 ectodomains bind to these chemokines and also to 281-2 anti-syndecan-1 ectodomain Abs (Fig. 7*A*). Syndecan-1 ectodomains did not bind to the Th1 chemokine CXCL10 (IFN-inducible protein-10) (39) or to the Th2 cytokines IL-4 and IL-13. Furthermore, radiolabeled syndecan-1 core protein devoid of HS did not bind to ligands other than 281-2 Abs, indicating that syndecan-1 ectodomains bind to CC chemokines through their HS chains (Fig. 7*A*). These results are consistent with those reported by Culley et al. (40), who demonstrated that HS and heparin specifically bind to CCL7 and CCL11 and inhibit eosinophil activation and chemotaxis induced by these CC chemokines.

We next examined whether HS inhibits CC chemokine activities. Migration of splenocytes enriched for CD4-positive T cells to CCL11, CCL17, or CXCL10 was determined in the absence or the presence of HS (2 μg). Consistent with the ability of syndecan-1 HS to preferentially bind Th2 chemokines, HS inhibited CCL11- and CCL17-mediated T cell migration by ~4- and 5-fold, respectively (Fig. 7*B*). HS did not affect baseline T cell migration and also did not inhibit CXCL10-mediated migration. These results indicate that one of the major mechanisms by which syndecan-1 ectodomains attenuate allergic lung inflammation is by inhibiting CC chemokine-mediated accumulation of Th2 cells in the lung.

Discussion

In this study we describe a novel endogenous mechanism that protects the host from allergic lung inflammation in vivo. Our results showed that deletion of syndecan-1 results in exacerbated allergic lung disease after airway allergen challenge. Relative to allergen-challenged WT mice, *sdc1*$^{-/-}$ mice showed exaggerated AHR,

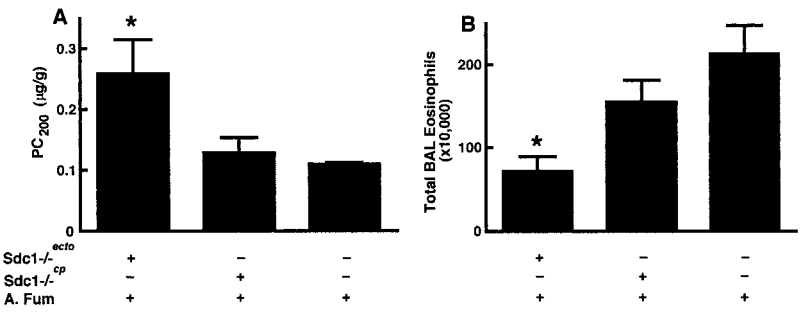

FIGURE 5. Syndecan-1 ectodomains suppress allergic lung inflammation via their HS chains. WT C57BL/6 mice were challenged five times intranasally with saline or *A. fumigatus* allergen along with either 640 ng of purified syndecan-1 ectodomains or recombinant syndecan-1 core proteins as indicated. Twenty-four hours after the final challenge, mice were assessed for AHR (*A*) and BAL eosinophils (*B*). *, $p < 0.05$ relative to syndecan-1 core protein- and allergen-treated mice.

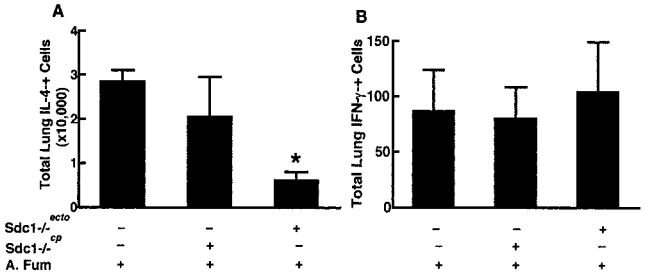

FIGURE 6. Instillation of syndecan-1 ectodomains inhibits Th2 cell recruitment to the lung. WT C57BL/6 mice were instilled with saline or *A. fumigatus* allergen with or without 640 ng of purified syndecan-1 ectodomains or core proteins, and total lung IL-4-positive (*A*) and IFN-γ-positive (*B*) cells were quantified by ELISPOT assays 24 h after the last allergen challenge. ∗, $p < 0.05$ relative to mice treated with allergen alone.

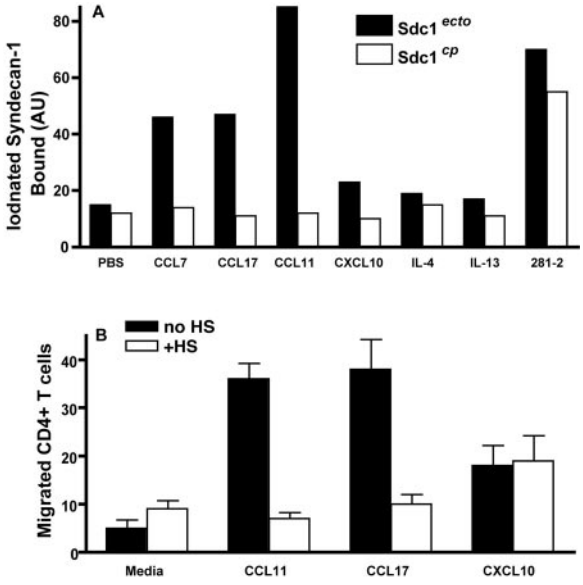

FIGURE 7. Syndecan-1 ectodomains bind and inhibit T cell migration through their HS chains. *A*, Binding of purified syndecan-1 ectodomains or core proteins radiolabeled with $Na^{125}I$ to 20 ng of CCL7, CCL11, CCL17, CXCL10, IL-4, IL-13, and 281-2 anti-syndecan-1 ectodomain Ab dot-blotted onto nitrocellulose membranes was assessed by ligand dot blotting. Results shown are the means of scanned absorbance units (AU) of duplicate measurements. *B*, Migration of $CD4^+$ T cells to CCL11, CCL17, or CXCL10 in the absence or the presence or 2 μg of HS was determined in a neuroprobe migration chamber assay. Results shown are the mean ± SEM ($n = 4$).

eosinophilia, glycoprotein hypersecretion, elevated serum IgE levels, and enhanced recruitment of $IL\text{-}4^+$ cells to the lung. However, when purified syndecan-1 ectodomains were coinstilled with allergens, these major facets of allergic lung disease were significantly suppressed, indicating that the lack of syndecan-1 ectodomains makes mice more susceptible to allergic disease. Our results also showed that airway syndecan-1, but not syndecan-4, ectodomains were shed by intranasal challenge with biochemically distinct allergens. Syndecan-1 ectodomains provided protection through their HS chains because only intact ectodomains and heparin, the pharmaceutical functional mimic of HS, reduced allergic lung disease, whereas syndecan-1 core proteins lacking HS had no effect. Our results also identified one of the major molecular mechanisms by which syndecan-1 ectodomains attenuate allergic lung inflammation. Syndecan-1 ectodomains bind and inhibit CC chemokines that potentiate accumulation of Th2 cells in the lung. We have therefore defined an endogenous mechanism that the airway epithelium has evolved to protect the host from tissue damage associated with excessive allergic inflammation through shedding of syndecan-1 ectodomains.

Syndecan-1 ectodomain shedding has been shown to regulate bleomycin-induced intrapulmonary trafficking of neutrophils by modulating chemokine gradients (12). However, in contrast to the anti-inflammatory function of syndecan-1 shedding in allergic lung inflammation, syndecan-1 shedding promotes airway inflammation in bleomycin-induced acute lung injury by directing the generation of a CXC chemokine gradient that guides the transepithelial migration of neutrophils. These observations indicate that syndecan-1 shedding has distinct regulatory functions in different inflammatory lung diseases. These findings are not unique to syndecan-1, because other inflammatory mediators have opposite roles in distinct inflammatory models. For example, MMP9-null mice are protected from endotoxic shock (41) and also from collagen-induced arthritis (42), but they show prolonged contact hypersensitivity, suggesting that MMP9 can function as a pro- or anti-inflammatory molecule in different inflammatory diseases. Similarly, IL-18 attenuates allergic lung disease (43, 44), but promotes infectious lung inflammation (45). These findings indicate that how a particular inflammatory mediator regulates inflammation is dictated by differences in the molecular mechanisms of the inflammatory disease.

HS chains of syndecan-1 ectodomains may possess additional anti-inflammatory properties, as suggested by the various anti-inflammatory effects described for HS and heparin (5, 46, 47). For example, heparin inhibits other key processes implicated in allergic lung disease, including airway smooth muscle cell proliferation (48) and eosinophil infiltration into the lungs (49). These data suggest that HS chains of syndecan-1 ectodomains function similarly and emphasize that the anti-inflammatory effects of syndecan-1 ectodomains are likely to be multifactorial. However, despite the redundancy and pleiotropy that characterize HSPG biology, several independent criteria indicate that syndecan-1 ectodomains derived from the airway are the major anti-inflammatory HSPGs in allergic lung disease. First, syndecan-1 is the predominant HSPG of airway epithelia. Second, our results show that activation of airway ectodomain shedding by biochemically distinct inducers of allergic lung disease is specific to syndecan-1. Third, inflammatory parameters are significantly reduced in allergen-challenged mice by administration of purified syndecan-1 ectodomains, but not core proteins. Furthermore, $sdc1^{-/-}$ mice show exaggerated allergic lung disease phenotypes relative to WT mice. These results indicate that HS chains of syndecan-1 ectodomains are the major airway epithelia-derived anti-inflammatory determinants in vivo.

Numerous studies have reported the beneficial effects of heparin in major inflammatory diseases, such as inflammatory bowel disease (50) and asthma (46, 51). In human asthma and animal models of experimental asthma (i.e., allergic lung inflammation), heparin inhibits allergen-induced early and late asthmatic responses (8) and exercise-induced bronchoconstriction (6, 7, 9). How heparin attenuates asthma has yet to be clearly defined, but because heparin is mainly found in intracellular vesicles of mast cells, the physiological counterpart of anti-inflammatory heparin in vivo is likely to be HSPGs (52). Our results showing that airway epithelia-derived syndecan-1 ectodomains attenuate major indexes of allergic lung disease via their HS chains are in agreement with this hypothesis. The available data indicate that the anti-inflammatory effects of heparin are not dependent on its anticoagulant activities (46, 49), which require the presence of 3-*O*-sulfated glucosamine residues (53). In HS, 3-*O*-sulfated glucosamines are extremely rare, typically comprising <0.5% of total sulfated moieties (54,

55), indicating that modifications other than 3-*O*-sulfation are essential for the anti-inflammatory activities of HS and heparin. Future studies will focus on defining the molecular structure of syndecan-1 ectodomain HS that enables this soluble HSPG to function as an endogenous anti-inflammatory agent in allergic lung diseases.

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Disclosures

The authors have no financial conflict of interest.

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