An imbalance between proteases and antiproteases is thought to play a role in the inflammatory injury that regulates wound healing. The activities of some proteases and antiproteases found in inflammatory fluids can be modified in vitro by heparin, a mast cell-derived glycosaminoglycan. Because syndecans, a family of cell surface heparan sulfate proteoglycans, are the major cellular source of heparin-like glycosaminoglycan, we asked whether syndecans modify protease activities in vivo.

Syndecan-1 and syndecan-4 ectodomains are shed into acute human dermal wound fluids (Subramanian, S. V., Fitzgerald, M. L., and Bernfield, M. (1997) J. Biol. Chem. 272, 14713–14720). Moreover, purified syndecan-1 ectodomain binds cathepsin G (K<sub>d</sub> = 56 nM) and elastase (K<sub>d</sub> = 35 nM) tightly and reduces the affinity of these proteases for their physiological inhibitors. Purified syndecan-1 ectodomain protects cathepsin G from inhibition by α<sub>1</sub>-antichymotrypsin and squamous cell carcinoma antigen 2 and elastase from inhibition by α<sub>1</sub>-proteinase inhibitor by decreasing second order rate constants for protease-antiprotease associations (k<sub>obs</sub>) by 3700-, 32-, and 60-fold, respectively. Both enzymatic degradation of heparan sulfate and immunodepletion of the syndecan-1 and -4 in wound fluid reduce these proteolytic activities in the fluid, indicating that the proteases in the wound environment are regulated by interactions with syndecan ectodomains. Thus, syndecans are shed into acute wound fluids, where they can modify the proteolytic balance of the fluid. This suggests a novel physiological role for these soluble heparan sulfate proteoglycans.

Multiple factors orchestrate the inflammatory response to tissue injury. These include proteases, antiproteases, cytokines, chemokines, and the growth factors derived from the plasma and cells associated with the injury, as well as from cells invading the injury site (1). Emigrating polymorphonuclear leukocytes release proteolytic enzymes into the injury site, including the most potent serine proteases, neutrophil elastase and cathepsin G (CatG).<sup>1</sup> These enzymes aid wound repair by digesting extracellular proteins, releasing growth factors from extracellular matrix, and remodeling the tissue (2–4). However, these enzymes can also destroy tissues when proteolysis is prolonged, inappropriate, or excessive (5, 6).

Enormous local concentrations of proteases, estimated to be in the millimolar range for elastase and cathepsin G, are released into extracellular spaces during the leukocyte activation associated with tissue injury (7). Serine protease inhibitors (serpins), provide efficient control mechanisms to prevent undesirable extracellular protein degradation at the injury site (8). These antiproteases, mostly derived from plasma, share three principal properties: (i) they form 1:1 covalent complex with proteases, (ii) complex formation results in both inactivation of the protease and proteolytic cleavage of the serpin, and (iii) inhibition is essentially irreversible (9). The balance of proteases and antiproteases at the site of injury can regulate the extent of the inflammatory response during the repair process (10, 11).

Dermal wound repair requires harmonious protease-antiprotease interactions or proteolytic balance. Excessive elastase action in the wound bed can account for endothelial damage (12), degradation of the epidermal/dermal junction (13), and the development of chronic skin ulcers (14). Physiological neutrophil elastase inhibitors include plasma-derived α<sub>2</sub>-macroglobulin and, most importantly, α<sub>1</sub>-proteinase inhibitor (also known as α<sub>1</sub>-PI, α<sub>1</sub>-antitrypsin, or α<sub>1</sub>-AT). The importance of α<sub>1</sub>-PI in regulating the response to tissue injury is emphasized by the extensive elastin and collagen fiber destruction leading to pulmonary emphysema in the lungs of individuals with congenital α<sub>1</sub>-PI deficiency (5). The major physiological cathepsin G inhibitor is α<sub>1</sub>-antichymotrypsin (α<sub>1</sub>-AChy), another plasma-derived serpin (15). Inherited α<sub>1</sub>-AChy deficiency is pleiomorphic, but it is often associated with chronic active hepatitis and increased residual lung volumes (16). Another serpin that inhibits cathepsin G is the squamous cell carcinoma antigen 2 (SCCA2), a newly described product of skin and respiratory tract epithelia (17).

Although the activity of one class of serpins is accelerated by binding to heparin or other glycosaminoglycans (GAGs) (9, 18), α<sub>1</sub>-PI and α<sub>1</sub>-AChy belong to the class of serpins that function independently of heparin and other GAGs. However, heparin can bind with high affinity to both neutrophil elastase and cathepsin G (19, 20). This binding inhibits the enzymatic activities, but most importantly, it reduces the ability of the enzymes to interact with serpins (19, 20). The heparin used clinically and in these studies is a pharmaceutical product derived from processing of the heparin proteoglycan within mast cells (21). The major physiological source of the heparin-cell carcinoma antigen 2; Suc-AAPF-pNA, succinyl-Ala-Ala-Pro-Phe-para-nitroanilide; ACE, affinity co-electrophoresis; HS, heparan sulfate; Hase, heparinase; HSase, heparitinase; GAG, glycosaminoglycan; serpin, serine protease inhibitor; CBZ, benzoyloxy carbonyl.

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‡ The abbreviations used are: CatG, neutrophil cathepsin G; α<sub>1</sub>-AChy, α<sub>1</sub>-antichymotrypsin; α<sub>1</sub>-PI, α<sub>1</sub>-proteinase inhibitor; SCCA2, squamous

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like GAG, heparan sulfate, is found in proteoglycans within cells, at the cell surface and in the extracellular matrix (22).

Most cellular heparan sulfate derives from the syndecan family of cell surface proteoglycans. This family (currently known as syndecan 1–4 in mammals) consists of single transmembrane proteins containing conserved cytoplasmic and transmembrane domains and less well conserved extracellular domains (ectodomains), which bear variable numbers of GAG chains. All syndecans bear heparan sulfate, although syndecan-1 and -3 can also bear chondroitin sulfate. Syndecans bind many of the factors that orchestrate the inflammatory response to tissue injury as well as a variety of extracellular matrix components and adhesion molecules via their heparan sulfate chains and are individually expressed in distinct cell-, tissue-, and development-specific patterns (23).

Syndecan expression is highly regulated during wound repair. During cutaneous wound repair, keratinocytes migrating from the wound edge show loss of cell surface syndecan-1 (24). Concomitantly, syndecan-1 expression increases in the endothelial cells, and syndecan-4 expression increases on the dermal fibroblasts that form the granulation tissue (24, 25), apparently due to inductive action of neutrophil-derived antimicrobial peptides (26). Syndecans on cell surfaces can be cleaved near the plasma membrane, which releases the now soluble intact proteoglycan ectodomains into the surrounding milieu (27). This shedding is accelerated by activation of protease (e.g. thrombin) and growth factor receptors (epidermal growth factor family members) and by the direct action of proteases (e.g. plasmin) involved in wound repair (27). Moreover, soluble syndecan-1 and -4 ectodomains are detected in acute dermal wound fluids (27). Although syndecan expression and shedding are highly regulated during the response to tissue injury, the role these processes play in this response is not clear.

A key aspect of the response to tissue injury is the establishment and maintenance of proteolytic balance at the wound site. The action of the major proteases, neutrophil elastase and cathepsin G, must be countered by their major inhibitors, α1-PI and α1-Achy, for normal wound repair to ensue. Loss of this balance can prevent normal repair, potentially leading to chronic wounds, which in the skin are difficult to treat satisfactorily (14). We postulated that because activities of the major proteases in acute wound fluids can be modified in vitro by heparin, soluble syndecan ectodomains could be involved in establishing and maintaining the proteolytic balance in wounds in vivo. We found syndecan-1 and -4 ectodomains in acute human dermal wound fluids. We also found that purified syndecan-1 ectodomain binds to both neutrophil elastase and cathepsin G, markedly reducing their affinity for serpins and thus protecting these enzymes from their physiological inhibitors. Moreover, both degradation of endogenous heparan sulfate and shedding of syndecan-1 and -4 from wound fluids reduce proteolytic activities in the fluid. Thus, syndecan ectodomains maintain the proteolytic balance in acute wound fluids, a novel physiological role for soluble heparan sulfate proteoglycans.

EXPERIMENTAL PROCEDURES

Materials—Flat bottomed, low binding 96-well microtiter plates were obtained from Costar (Cambridge, MA). Human trypsin and mouse IgG were from Sigma, human neutrophil cathepsin G and human plasma α1-PI were from Athens Research & Technology Inc. (Athens, GA), human neutral elastase was from Calbiochem (La Jolla, CA), and human plasma α1-Achy was from Biodiagnostics International (Kennebunk, ME). Purified glutathione S-transferase-SCCA2 fusion protein was a kind gift from Dr. Gary Silverman, Children's Hospital, Boston, MA (17). For enzyme substrates, succinyl-Ala-Ala-Pro-Pha-nitroanilide (Suc-AAPF-pNA) for cathepsin G and N-benzoyl-Pha-Val-Arg-pNA for trypsin were from Sigma, and (benzoyloxy carbonyl) (CBZ)-Ala-Ala-Ala-Ala-Thr110 for elastase was from Molecular Probes Inc. (Eugene, OR). Heparin (porcine intestinal mucosa) was from Hepar Laboratories Inc. (Franklin, OH), and chondroitin-6-sulfate, chondroitin sulfate ABC lyase (chondroitinase ABC, EC 4.2.2.4), heparan sulfate lyase III (heparitinase III, EC 4.2.2.8), and human-sulfate ABC lyase (EC 4.2.2.7) were from Seikagaku America Inc. (Rockville, MD). Human syndecan-1 ectodomain was purified to homogeneity from the conditioned medium of NMU-MG mouse mammary epithelial cells (28). One mg of this syndecan-1 core protein contains 5 mg of HS.2 cDNA for ectodomains of human syndecan-1 and -4 in glutathione S-transferase expres- sion vector pGEX-2T (Amersham Pharmacia Biotech) were expressed as fusion proteins in Escherichia coli, induced by 0.2 mM isopropyl-1-thio-β-D-galactopyranoside for 6 h at 37 °C, solubilized with 1% Triton X-100, and centrifuged 12,000 × g for 10 min. Supernatants were purified on glutathione-agarose beads (Sigma); washed with PBS; eluted with 50 mM Tris, pH 8, and 5 mM reduced glutathione (Janssen Chimica, New Brunswick, NJ); subjected to 10% SDS-polyacrylamide gel electrophoresis; and detected with Coomassie Blue. Antibodies used were polyclonal antiserum HSE-1 against the recombinant human syndecan-1 ectodomain (25); monoclonal antibodies MCA-681 from Serotec (Oxford, United Kingdom) and m-101 against human syndecan-1, 5G9 and SC7 (25) against human syndecan-4, and horseradish peroxidase (HRP)-conjugated anti-rabbit IgG and horseradish peroxidase-conju- gated anti-mouse IgG were from Jackson ImmunoResearch Laboratories Inc. (West Grove, PA).

For production of monoclonal antibodies specific to human syndecan-1, recombinant syndecan-1 was used for immunization of mice, and production of monoclonal antibody was by Maine Biotechnology Services, Inc. (Portland, ME). Mice were immunized and boosted with 100 μg of recombinant syndecan-1. Out of 12 hybridoma clones, which produced antibodies reacting with human syndecan-1 fusion protein, only 1 (m-101) reacted specifically with native human syndecan-1 ectodomain purified from conditioned media of human A431 cells. This was assessed as reactivity on Western blotting with a 300-kDa proteoglycan smear, which reduced to a 70-kDa core protein after treatment with heparitinase and chondroitinase ABC as described previously (30). Affinity Co-electrophoresis (ACE) Analyses—NMU-MG cells were labeled with iodine and used to estimate the apparent Kd values as described earlier (32).

Assays for Enzyme Inhibition—The amounts of proteases and serpins were calibrated by the method of Chase and Shaw (33). Trypsin was calibrated by using p-nitrophenyl-p'-guanidinobenzene (Sigma), except that 100 mM Tris-HCl, pH 8.3, was used in place of sodium barbiturate buffer. The concentration of α1-PI was standardized against calibrated trypsin. Elastase and cathepsin G were calibrated against the standardized α1-PI. α1-Achy was calibrated against the standardized cathepsin G. Reaction buffers were 50 mM Hepes, 150 mM NaCl, 5 mM N,N-dimethylformamide, pH 7.4, for cathepsin G, and 50 mM Tris, 150 mM NaCl, 0.1 mg/ml bovine serum albumin, pH 7.4, for elastase. Enzyme inhibition was determined by mixing enzyme with increasing concentrations of syndecan-1 in the appropriate reaction buffer and incubating for 15 min at 25 °C. The inhibitor was added, and residual enzyme activity was determined by adding the appropriate substrate and measuring hydrolysis at 405 nm with a UVmax microplate reader (Molecular Devices) or at 488 nm with a FluorImager 575 (Molecular Dynamics). For cathepsin G, 8 μg of recombinant cathepsin G, 34 μg of α1-Achy or SCCA2, and 3 μg Suc-AAPF-pNA. The concentrations for elastase assays were 34 nM elastase, 34 nM α1-PI, and 5 μM (CBZ-Ala-Ala-Ala-Ala)-R110. In some experiments, syndecan-1

Syndecan Ectodomains Modify Proteolytic Balance

Syndecan-1 ectodomain binds cathepsin G and elastase via its heparan sulfate chains. ACE of the soluble syndecan-1 ectodomain with cathepsin G (A and B) and elastase (C and D) is shown. \([^{35}\text{S}]\)Sulfate-labeled syndecan-1 ectodomain was electrophoresed through agarose gels containing various concentrations of cathepsin G (A) or elastase (C) as described under “Experimental Procedures.” The effects of chondroitin 6-sulfate (1 mg/ml) and heparin (1 mg/ml) on the \([^{35}\text{S}]\)sulfate-labeled syndecan-1 ectodomain binding to cathepsin G (B) and elastase (D) are shown. Equal amounts of \([^{35}\text{S}]\)sulfate-labeled syndecan-1 ectodomain were introduced into each slot, the electrophoresis was run, and distribution of radioactivity on the gel was visualized by autoradiography. The concentrations of proteases are indicated below each lane.

**RESULTS**

**Syndecan-1 Ectodomain Binds Elastase and Cathepsin G**—Because heparin can protect elastase and cathepsin G against inhibition by certain plasma-derived serpins (19, 20), we speculated that the soluble syndecan ectodomains in wound fluid might act similarly. \([^{35}\text{S}]\)Sulfate-labeled syndecan-1 ectodomain purified from the conditioned media of NMuMG cells was incubated with nitrocellulose filters containing dots of purified human neutrophil cathepsin G and elastase, and serum-derived \(\alpha_1\)-PI and \(\alpha_1\)-Ach. The syndecan-1 ectodomain bound to cathepsin G and elastase at picomolar levels of protease, whereas no binding to the antiproteases was detected at 10-fold higher concentrations (data not shown). ACE (32) of \([^{35}\text{S}]\)sulfate-labeled syndecan-1 ectodomain with cathepsin G and elastase confirmed this binding and yielded apparent \(K_d\) values of 56 nM for cathepsin G and 35 nM for elastase (Fig. 1, A and C). Heparin (1 mg/ml) completely abrogated binding to the proteases, whereas chondroitin sulfate (1 mg/ml) had little or no effect, indicating that the binding is mainly due to the heparan sulfate chains on syndecan-1 (Fig. 1, B and D). The ACE profiles with both enzymes showed heterogeneity in syndecan-1 ectodomain binding at concentrations near the \(K_d\) values (80 nM), suggesting that there are subfractions of the ectodomain that differ in their avidity for the proteases (data not shown).

**Binding of Syndecan-1 Ectodomain to the Protease Reduces the Effect of Antiprotease**—To determine whether the binding of the syndecan-1 ectodomain to the proteases affects their rate of interaction with a serpin, rate constants \((k_{\text{obs}})\) for these interactions were measured in the presence and absence of soluble syndecan-1 ectodomain (Table 1). The protease and serpin form a 1:1 complex. Because neither heparin or the syndecan-1 ectodomain alters this stoichiometry (Fig. 2), the \(k_{\text{obs}}\) were determined under second order conditions (15). Equimolar amounts (34 nM) of protease and serpin were incubated in the presence or absence of the syndecan-1 ectodomain at concentrations indicated in Table I. After various times,
complex formation was quenched by adding substrate, and the remaining free enzyme activity was measured as described under “Experimental Procedures.” The $k_{\text{obs}}$ for the interaction was calculated from linear regressions (Equation 1). The $k_{\text{obs}}$ for cathepsin G with $\alpha_1$-antichymotrypsin decreased over 3700-fold and with SCCA2 over 32-fold in the presence of the syndecan-1 ectodomain (Table I). The $k_{\text{obs}}$ for elastase with $\alpha_1$-proteinase inhibitor was decreased 60-fold by the syndecan-1 ectodomain (Table I). For comparison, second order rate constants were also measured for protease-serpin complex formation in the presence of a heparin concentration equivalent to that of the syndecan-1 ectodomain HS (Table I). At the concentrations tested, the soluble syndecan-1 ectodomain decreased the rates of protease-serpin association at least as effectively as authentic heparin. The association rate for cathepsin G and elastase-serpin interactions: the rates of protease-serpin association at least as effectively as that of the syndecan-1 ectodomain HS, 16.5 μg/ml heparin; B, [CatG] = 34 nm, [Succ-AAPF-pNA] = 3 nm, 10 μg/ml syndecan-1 ectodomain HS, 10 μg/ml heparin; C, [neutrophil elastase] (NE) = 34 nm, [CBZ-Ala-Ala-Ala-Ala]2-R110 = 5 μM, 7.5 μg/ml syndecan-1 ectodomain HS, 7.5 μg/ml heparin. Relative activity is the rate relative to the rate observed in the absence of serpin. The lines are drawn by the least squares method from data obtained without GAG. Addition of GAG did not statistically affect the 1:1 stoichiometry.

Soluble Syndecan-1 Ectodomain Modifies Proteolytic Balance—The effect of the purified syndecan-1 ectodomain on protease activity was assessed in the presence and absence of serpin. The ectodomain was preincubated with cathepsin G or elastase for 15 min and assayed for protease activity with or without equimolar concentrations of serpins (Fig. 3). Syndecan-1 ectodomain alone reduced cathepsin G activity in a concentration-dependent manner, reaching maximal inhibition (35%) at 2 μg/ml as core protein (Fig. 3A). However, the syndecan-1 ectodomain markedly decreased the ability of both $\alpha_1$-Ach and SCCA2 to inhibit cathepsin G activity (Fig. 3A). In the absence of ectodomain, these serpins completely inhibit the protease, but with increasing concentrations of ectodomain, their inhibitory activity is reduced and ultimately abolished (Fig. 3A). The syndecan-1 ectodomain was more effective in reducing cathepsin G inhib-
heparitinase-treated (●) syndecan-1 ectodomain. B, progress curves for the inhibition of cathepsin G by SCCA2 in the absence (○) or presence of 0.5 μg/ml untreated (●), heparinase-treated (△), or heparinase and heparitinase-treated (▲) syndecan-1 ectodomain. C, progress curves for the inhibition of elastase by α1-PI in the absence (○) or presence of 1.5 μg/ml untreated (●), heparinase-treated (△), or heparitinase-treated (▲) syndecan-1 ectodomain. Final concentrations: A, [CatG] = 34 nM, [α1-Achy] = 34 nM, [Suc-AAPF-pNA] = 3 mM; B, [CatG] = 34 nM, [SCCA2] = 34 nM, [Suc-AAPF-pNA] = 3 mM; C, [neutrophil elastase] = 34 nM, [α1-PI] = 34 nM, |CBZ-Ala-Ala-Ala-Ala-R110| = 5 μM.

Syndecan-1 and -4 ectodomains are in acute wound fluids. Acute human dermal wound fluids (WF, days 1–3) were made cell-free by centrifugation, and samples (20 μl) were blotted on Immobilon N membrane. Human syndecan-1 ectodomain (0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ng) and syndecan-4 ectodomain (0, 10, 20, 40, and 80 ng) glutathione S-transferase fusion proteins were blotted as standards. The syndecan-1 and -4 ectodomains were detected by ECL using antibodies HSE-1 and 5G9, respectively.

DISCUSSION

In this study, we provide new insights into the regulation of protease-antiprotease balance during tissue injury. We show that syndecan-1 and -4, cell surface heparan sulfate proteoglycans, play a role in balancing proteolytic activity in wound fluids. The soluble syndecan ectodomains maintain the proteolytic balance of wound fluids but do not complex with all proteases and their inhibitors. The addition of heparin to wound fluids before and after de-
Wound repair requires precise temporal and spatial regulation of a panoply of effectors, including chemo-
kines, growth factors, extracellular components, cell adhesion proteins, proteases, and antiproteases. Many of these proteins bind heparin and heparan sulfate under physiological condi-
tions and with high affinities (35). During repair of skin injury, cellular expression of syndecan-1 and -4 is altered (24, 25), and cell surface syndecan-1 and -4 are converted to soluble mole-
cules by juxtamembrane cleavage of their extracellular do-
mains (ectodomains), a process known as shedding (27). Recent
studies have shown that syndecan shedding is a highly regu-
lated process that is stimulated by certain agents released at
the site of tissue injury (27). Shedding instantly converts a cell
surface proteoglycan into a soluble effector.

The functions of these soluble ectodomains are not clear. Syndecans on cell surfaces can act as co-receptors for heparin-
binding growth factors; notably, the action of FGF-2 requires a
growth factor-heparan sulfate proteoglycan-FGFR1 complex
(36). However, because the soluble ectodomains retain all their
HS, they can bind the same ligands as the cell surface synde-
cans, enabling them to be potential inhibitors of these ligand
interactions. On the other hand, the soluble ectodomains place
HS chains containing heparin-like domains into the wound
environment. These chains can interact with heparin-binding
proteins and peptides involved in the repair.

The inflammatory phase of tissue repair is characterized by
plasma exudation and the involvement of neutrophils that
produce and secrete the matrix remodeling enzymes elastase
and cathepsin G. Although heparin binds and accelerates ac-
tivity of some serpins (9), heparin does not interact with the
serpins that regulate these enzymes. Rather, the enzymes
themselves bind heparin, which reduces their affinity for the
serpin and protects them from inhibition (19, 20).

Our results indicate that the HS chains on the soluble syn-
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wound environment (Fig. 4). Indeed, the syndecan-1 ectodo-
main HS chains are at least as effective in decreasing the
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ectodomain for the protease approximate that for heparin (Fig.
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Proteolytic Balance in Wound Repair—Proteolysis is impor-
tant for fibrinolysis, growth factor mobilization and activation,
cell migration into the wound site, reepithelialization, angio-
genesis, and extracellular matrix degradation (37). An imbal-
ance of proteolytic activity disrupts normal wound repair and
capillary morphogenesis (38, 39). If the soluble ectodomains
also act like heparin to accelerate the activity of heparin-acti-
vatable serpins (viz. antithrombin III, protease nixin I, plas-
minogen activator inhibitor-1, and others), the ectodomain
could regulate several aspects of proteolysis during wound
repair.

Proteases in wounds co-exist with their physiological inhib-
itors, and thus their activity is finely regulated to provide
optimal activity for repair. This activity results from a balance,
involving enzyme production and activation counterpoised by
enzyme degradation and inhibition. The involvement of syn-
decan ectodomains in regulating proteolytic balance could explain
several observations, including the variability of elastase ac-
tivity and the inconsistency of fibronectin degradation in
wound fluids (11). Our finding that both HS degradation and
immunodepletion of syndecan ectodomains reduce the proteo-
lytic activity of acute wound fluids (Figs. 6 and 7) indicates that

![Image](50x289) human syndecan-1 (MCA-681 and DL-101) and syndecan-4 (5G9 and
wound fluid was incubated with purified monoclonal antibodies against
addition of heparin (●). Human wound fluids were treated with 150 milliunits/ml
of Hase and HSase for 3 h at 37 °C in 50 mM Hepes, 150 mM NaCl, pH 7.4,
with buffer alone. Elastolytic activity was measured in untreated (□) and Hase/HSase-treated fluids before (▾) and 5 min after (●), the
addition of 5 μg/ml heparin. The Hase/HSase mixture itself contained
no detectable elastolytic activity, nor did it directly effect the activity of
proteases in test reactions. Each of three acute (day 1) wound fluids
presented to cathepsin G and elastase. This interaction reduces the
balance results from binding of the HS chains on the synde-
cans, enabling them to be potential inhibitors of these ligand
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![Image](107x326)
these soluble proteoglycans contribute to balanced proteolytic activity in the wound environment. Alterations in proteolytic balance are thought to be one reason why acute wounds do not heal properly and become chronic (11, 40). The high levels of proteolytic activity in chronic wound fluid have led to the proposal that misregulated proteases contribute to the inability of chronic wounds to heal even when treated with exogenous matrix and growth factors (41, 42). Whether alterations in the levels of syndecan ectodomains could lead to loss of proteolytic balance and thus to development of chronic wounds needs investigation.

Abnormalities in Proteolytic Balance—Optimal proteolytic activity is needed for normal wound repair. Formation of the fibrin-rich provisional matrix produced after tissue injury is an initial step in the repair process. Once the fibrin clot has formed, migrating keratinocytes at the wound edge and emigrating neutrophils produce a variety of serine proteases and matrix metalloproteases to degrade this matrix and close the wound. An imbalance of proteases and serpins contributes to chronic inflammatory conditions, such as rheumatoid arthritis, pulmonary fibrosis, emphysema, and the development of vascular plaques of atherosclerosis and amyloid plaques in the central nervous system in Alzheimer’s disease (9). Whether or not syndecans have a role in regulating proteolytic activities in these events is not known, but in light of our data, this possibility seems worth investigating.

We have found that syndecan-1 and -4 ectodomains act within human acute wound fluids to maintain proteolytic balance. Although no evidence so far exists, the ectodomains of other heparan sulfate proteoglycans that can be shed, such as glypicancin and CD-44 (Refs. 43 and 29, respectively), might act similarly. Altered proteolytic balance in the wound environment has the potential to interfere with therapeutic procedures ranging from growth factor application to skin grafting. Thus, syndecan expression and shedding should be considered in clinical practice.

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