

Syndecans, cell surface heparan sulfate proteoglycans, are induced by a proline-rich antimicrobial peptide from wounds

(epithelial-mesenchymal interactions/growth factor receptors/matrix receptors)

RICHARD L. GALLO*^{†‡}, MINORU ONO[§], THOMAS POVSIC[†], CURTIS PAGE[¶], ELOF ERIKSSON[¶],
MICHAEL KLAGSBRUN[§], AND MERTON BERNFIELD[†]

*Department of Dermatology, [†]Joint Program in Neonatology, [§]Department of Surgery, Boston Children's Hospital, [¶]Department of Plastic Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115

Communicated by Jerome Gross, July 18, 1994

ABSTRACT Cell surface heparan sulfate proteoglycans, such as the syndecans, are required for cellular responses to heparin-binding growth factors and extracellular matrix components. Expression of syndecan-1 and -4 is induced in mesenchymal cells during wound repair in the mouse, consistent with a role for syndecans in regulating cell proliferation and migration in response to these effectors. Here we show that wound fluid contains inductive activity that mimics the *in vivo* induction in time of appearance, specificity for mesenchymal cells, and selectivity for syndecan-1 and -4. We have purified and synthesized a 4.8-kDa proline-rich protein from wound fluid that reproduces this induction of syndecan-1 and -4 in cultured cells. This peptide, identical to the antibacterial peptide PR-39, is released into the wound by the cellular infiltrate and induces syndecan expression at the same peptide concentrations that lyse bacteria. These results indicate that wounds contain a multifunctional protein that induces mammalian cells to express cell surface heparan sulfate proteoglycans as part of the wound repair process and that kills bacteria as part of a nonimmune defense mechanism.

Complex cellular behaviors such as those during morphogenesis and wound repair are influenced by a large variety of diffusible peptides and insoluble matrix components. These behaviors are highly integrated, enabling the cells to act in a coordinated manner. Although a successful response by cellular tissues to the large mix of effector molecules depends on this integration of cellular behaviors, it is not clear how cells distinguish between multiple cellular effectors in the microenvironment.

To exert their effects, many of the effector molecules must bind to the heparan sulfate chains that are at the cell surface (1). For example, interaction with cell surface heparan sulfate is required for cells to respond to fibroblast growth factor 2 (2, 3) and to the extracellular matrix component fibronectin (4, 5). Indeed, we (6), and others (7–9), have proposed that cell surface heparan sulfate and specific signaling receptors act together as coreceptors, and that the response to the effector depends on the level of cell surface heparan sulfate. This heparan sulfate is derived mostly from the four members of the syndecan family of transmembrane proteoglycans (10).

In mature tissues, syndecan-1 is expressed on the cell surface of epithelial cells but not on the surface of mesenchymal cells (11). However, during cutaneous wound repair in the mouse, syndecan-1 is lost from the surface of the epithelial cells migrating into the wound and is induced on the dermal endothelial cells and fibroblasts of the forming granulation tissue (12). These changes in syndecan expression resemble the changes in syndecan-1 expression occurring

during embryonic tissue interactions: syndecan-1 is lost from epithelia undergoing changes in shape while it is induced on their associated mesenchymal cells (13). Thus, in both wound repair and morphogenesis, mesenchymal cells are induced to increase syndecan-1 at their surfaces, enabling interaction with heparin-binding effector molecules.

To explore the mechanism by which cell surface syndecan is induced, we have used a cell culture system to assess a variety of biological fluids for activity that induces cell surface syndecan-1. We find this activity in the fluid accumulating in cutaneous wounds undergoing repair. The induction of syndecan-1 and -4 mRNA occurs in mesenchymal cells, but not in epithelial or neural cells. It occurs with minimal changes in other cell surface proteins and with kinetics that mimic syndecan-1 and -4 induction during skin wound repair. Purification and synthesis of the inducing activity show that it resides in PR-39 (14), a proline-rich peptide previously identified as an antibacterial peptide in intestine, but found here in cells within a repairing wound. Finding this molecule with both antimicrobial activity and the ability to induce syndecan indicates the presence of a potential class of agents that can simultaneously reduce infection and influence the action of growth factors, matrix components, and other cellular effectors involved in wound repair.

MATERIALS AND METHODS

Cell Culture. Cultures of mouse embryo fibroblasts prepared from minced C57BL/6 embryos or dermis at embryonic day 15–17, BALB/c 3T3 endothelioid cells, and NMuLi liver epithelial cells (American Type Culture Collection) were grown in Dulbecco's modified Eagle's medium containing glucose at 1 g/liter (Mediatech, Washington, DC) and 10% fetal calf serum (TC Biologicals, Tulare, CA). NIH 3T3 and Swiss 3T3 fibroblastic cells (American Type Culture Collection) were in Dulbecco's modified Eagle's medium containing glucose at 4.5 g/liter and 10% defined bovine serum (HyClone). C3H 10T½ fibroblastic cells were in Eagle's basal medium (GIBCO) with 10% fetal calf serum. Bovine adrenal microvascular endothelial cells, NMuMG mammary epithelial cells (passage 13–22), BK-1, PAM 212, and BALB/c MK keratinocytes were maintained as described previously (15–17). Mouse keratinocytes were prepared and cultured as previously described (18). The C17 mouse cerebellar cell line was maintained in Dulbecco's modified Eagle's medium containing glucose at 4.5 g/liter and 10% fetal calf serum (19).

For use in experiments, primary cells and cell lines were cultured in their respective media on 96-well flat-bottomed tissue culture plates (Becton Dickinson). When indicated,

Abbreviations: IL, interleukin; RPC, reversed-phase chromatography.

[‡]To whom reprint requests should be addressed at: Joint Program in Neonatology, Harvard Medical School, 300 Longwood Avenue, Boston, MA 02115.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

cells were grown to postconfluence by maintaining cultures for 72 h after they became a confluent monolayer.

Wound Fluid. Vinyl chambers (P. A. Medical, Columbia, TN) were attached aseptically over 1.2-mm-deep partial thickness skin wounds on 3- to 4-month-old Yorkshire pigs. Each chamber received 1 ml of sterile 0.9% NaCl containing penicillin G and streptomycin as previously described (20). At 24-h intervals the fluid within the chamber was removed, centrifuged for 10 min at $300 \times g$ to remove the cellular infiltrate, and passed through a 0.45- μm sterile filter to yield wound fluid designated day 1–10 depending on the day of collection. Each day, after collecting the fluid, the chamber was replaced and fresh saline solution was added to it. In some experiments the wound fluid was applied to a heparin-Sepharose column (30 \times 1 cm, Pharmacia) in 10 mM Tris-HCl, 200 mM NaCl, pH 7.4, and the flow-through fraction was collected and filtered through a 0.45- μm sterile filter.

Growth Factors. Insulin-like growth factors I and II, recombinant human interleukin (IL) 1 α , transforming growth factor α , epidermal growth factor, and recombinant human interferon γ were from Collaborative Biomedical Products (Bedford, MA). Recombinant human tumor necrosis factor α and porcine platelet transforming growth factor β were from R & D Systems. Human platelet-derived growth factor AB heterodimer and anti-human platelet-derived growth factor AB were from Upstate Biotechnology. Recombinant mouse granulocyte-macrophage colony stimulating factor, IL-3, IL-4, and IL-6 were from Genzyme. Mouse embryo extract was prepared from homogenates of 15 day C57BL/6 embryos. Chick embryo extract was purchased from GIBCO.

Detection of Cell-Surface Syndecan-1. The amount of syndecan-1 at the cell surface was determined on cells in 96-well plates by means of an ELISA. Cells were washed one time in Tris-buffered saline (TBS; 10 mM Tris-HCl/150 mM NaCl, pH 7.4), fixed for 30 min in 2% Hepes-buffered paraformaldehyde, blocked with 5% goat serum, and incubated for 1 h with 0.15 $\mu\text{g}/\text{ml}$ monoclonal antibody (mAb) 281-2 specific for syndecan-1 (21) or rat IgG2a (Zymed). Cells were then rinsed three times for 5 min each with TBS, incubated with alkaline phosphatase-conjugated goat anti-rat antibody (Caltag, San Francisco) for 30 min, rinsed three times for 5 min each with TBS, and phosphatase activity was determined by measuring the absorbance of a 1 mg/ml solution of *p*-nitrophenylphosphate (Sigma) at 405 nm in 10 mM Tris-HCl/100 mM NaCl/5 mM MgCl, pH 9.5. Rates of change in absorbance were determined by microplate reader (Molecular Devices). This ELISA provided an accurate quantitative assessment of cell surface syndecan-1, because the amount of cell surface syndecan-1 quantitatively removed with trypsin (22) from cells in duplicate wells and measured by radioimmunoassay (18) correlated highly over the range of syndecan-1 detected in this study ($r = 0.96$).

SDS/PAGE and Western Blotting. The extracellular domain of syndecan-1 released from cell surfaces by trypsin treatment (22) was partially purified on DEAE-cellulose (18, 20), separated electrophoretically on a 3–15% gradient SDS/polyacrylamide gel, and transferred to Immobilon-N membranes (Millipore). Syndecan-1 was detected with mAb 281-2, horseradish-peroxidase-conjugated goat anti-rat IgG, and the ECL detection system (Amersham). Where indicated, glycosaminoglycan side chains were removed by treatment with heparin-sulfate lyase (heparitinase, EC 4.2.2.8) and chondroitin ABC lyase (chondroitinase ABC, EC 4.2.2.4, Seikagaku America, Rockville, MD) (18).

RNA Isolation and Northern Blot Analysis. Approximately 10 μg of total RNA extracted from NIH 3T3 cells (23) was separated by electrophoresis through a 1% agarose/formaldehyde gel and transferred to a GeneScreenPlus membrane (DuPont/New England Nuclear). Hybridization was

carried out at 65°C in QuikHyb Solution (Stratagene) according to the manufacturer's instructions using random-primer-labeled mouse full-length syndecan-1 cDNA (24), the cDNA coding for the extracellular domain of syndecan-2, -3, and -4 (25), or the 800-bp *Pst* I fragment of murine β -actin RNA as hybridization probes. Filters were washed in $2 \times$ SSPE/0.1% SDS (1 \times SSPE is 150 mM sodium chloride/10 mM sodium phosphate/1 mM EDTA, pH 7.4) two times for 15 min each at room temperature, and in $0.2 \times$ SSPE/0.1% SDS, two times for 15 min each at 55°C.

Purification of Syndecan Inductive Activity. The supernatant from an overnight 4°C incubation of 20 ml of wound fluid at pH 4.0 was dialyzed against 20 mM Tris-HCl/100 mM NaCl, pH 7.4, and applied to a 16×150 mm Toyopearl cation-exchange SP650S column (Toso Haas, Philadelphia). After washing the column with 0.2 M NaCl, the syndecan-1 inductive activity was eluted with a 300-ml linear gradient of 0.2–1 M NaCl. Syndecan-1 inductive activity eluted from the column in fractions containing 0.65–0.8 M NaCl and was applied to a C_4 reversed-phase chromatography (RPC) column (4.6 \times 250 mm, Vydac, Hesperia, CA) and eluted with a 60-ml linear gradient of 20–80% acetonitrile in 0.1% trifluoroacetic acid (vol/vol). Eluted fractions (1 ml) were lyophilized, redissolved in 25 μl of phosphate-buffered saline (PBS; 150 mM NaCl/10 mM sodium phosphate, pH 7.4), and analyzed for syndecan inductive activity.

Analysis of Purified and Synthetic PR-39. Aliquots of the syndecan inductive activity eluting from the RPC column at 39% acetonitrile (approximately 12 μg protein) were initially evaluated by SDS/10% PAGE and silver stain. Aliquots were subsequently analyzed by laser desorption mass spectrometry (externally calibrated Finnigan Lasermat), and by automated amino acid sequencing (HP-G100A sequencer). Synthetic PR-39 (Chiron Mimotopes Peptide Systems) was 96% pure by RPC and had a molecular weight of 4721.1 (4719.7 expected).

Detection of PR-39 in Wound Fluid Cells. The cellular infiltrate of day 3 wound fluid was washed once in TBS, fixed for 10 min in 2% Hepes-buffered paraformaldehyde, washed three times for 5 min each time in TBS, and then permeabilized for 2 min with 0.2% Triton X-100 in PBS. PR-39 was detected by specific interaction with the mAb PR-485-4 (MABTECH, Stockholm) at a final concentration of 10 $\mu\text{g}/\text{ml}$, and Cy3-conjugated goat anti-mouse IgG (2 $\mu\text{g}/\text{ml}$; Cy3 = cyanine 3.18; Caltag). An unrelated mouse IgG-1a (10 $\mu\text{g}/\text{ml}$) served as a control.

RESULTS

Wound Fluid Induces Cell Surface Syndecan. To explore the mechanism by which cell surface syndecan-1 is induced, NIH 3T3 cells were grown to confluence in serum-containing medium, exposed to potential inducers, then evaluated by ELISA for syndecan-1 expression. A wound fluid preparation, obtained by passing the fluid through a heparin-Sepharose column to remove heparin-binding factors such as platelet-derived growth factor, fibroblast growth factor 2 and heparin-binding epidermal growth factor (26), induced cell-surface syndecan-1 approximately 3-fold but caused no detectable change in cell number, total protein, or cell morphology. Inductive activity was detected in wound fluid collected from day 1 through day 6 after injury, but not after day 7. This activity was not duplicated by several other biological fluids: (3–10%, vol/vol) fetal calf, calf, and pig sera, human amniotic fluid, conditioned medium from U937 macrophages, primary murine keratinocytes, human umbilical vein endothelial cells, TA3 mammary carcinoma cells, chick or mouse embryo extracts (200–500 μg of protein per ml), or a variety of growth factors released during wound repair (transforming growth factors α and β 1, tumor necrosis factor α , epidermal growth factor, insulin-like growth factors 1 and 2, granulocyte-macrophage colony stimulating factor,

IL-1, -3, -4, and -6, interferon γ and vascular endothelial growth factor) each tested for 48 h at greater than physiological concentrations. In contrast, induction of cell-surface syndecan-1 was detected in wound fluid collected from other species. Inductive activity was observed with wound fluids collected for up to 7 days from wire mesh chambers implanted subcutaneously in sheep and from liquid-tight chambers affixed for 1 day over newly prepared human split-thickness skin grafts (data not shown).

Syndecan-1 induction during organ development occurs transiently in condensed mesenchyme (6), and during wound repair occurs within 12 h after injury in dermal mesenchymal cells (12). In culture, only postconfluent cells responded to the wound fluid preparation (Fig. 1A). Cells at 50% confluence did not respond, regardless of whether they were proliferating or they were growth-arrested by either exposure to 12,500 rads (1 rad = 0.01 Gy) of irradiation or treatment with mitomycin C (data not shown). Cell surface syndecan-1 increases nearly linearly for 60 h during exposure to the wound fluid preparation, but this increase is rapidly reversible (Fig. 1B). When medium containing the wound fluid preparation was replaced with fresh medium lacking wound fluid, cell surface syndecan-1 was lost with a half-life of about 1.5 h.

The induction is selective for mesenchymal cells. All postconfluent cells of mesenchymal origin tested, including five independently prepared cultures of primary mouse dermal and embryonic fibroblasts, NIH 3T3, Swiss 3T3, and C3H 10T $\frac{1}{2}$ fibroblasts, BALB/c 3T3 endothelioid cells, and capillary endothelial cells, responded to the wound fluid preparation with an increase in cell surface syndecan-1. All nonmesenchymal cells tested, including the cerebellar cell line C17 and the epithelial cell lines NMuMG (mammary), NMuLi (hepatic), BALB/c MK, BK-1, PAM 212 (keratinocytes), and primary mouse keratinocytes failed to respond (data not shown).

Cell surface syndecan-1 induced by wound fluid is a proteoglycan with reduced glycosylation. On Western blots the extracellular domain of syndecan-1 released from cells after induction is detected as the characteristic broad proteoglycan smear but the smear has a lower modal molecular mass than syndecan-1 constitutively present at the cell sur-

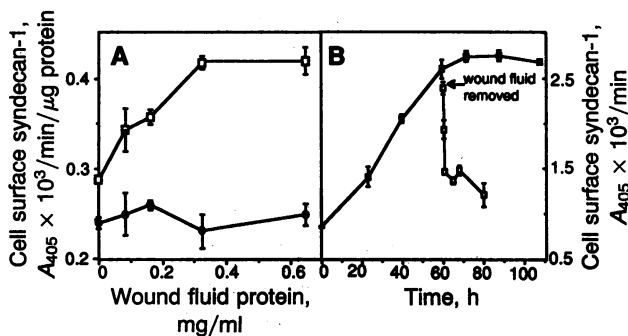


FIG. 1. Induction of syndecan-1 expression by the wound fluid preparation. (A) Induction of cell surface syndecan-1 is dependent on wound fluid concentration and cell density. Postconfluent (□) or 50% confluent (●) NIH 3T3 cells were treated for 24 h with a wound fluid preparation obtained by passing collected fluid through heparin-Sepharose, and then assayed for cell surface syndecan-1. Data are normalized for total cellular protein per well (7 μ g per well for confluent and 4 μ g per well for subconfluent cells). (B) Wound fluid induces and stabilizes syndecan-1 at the cell surface. Postconfluent NIH 3T3 cells were cultured as in A with 400 μ g of protein per ml of wound fluid preparation. At 60 h some wells (□) were rinsed once with medium and fresh medium without the wound fluid preparation was added. Cultures were assayed for cell surface syndecan-1 at intervals over the next 40 h. Data represent the mean \pm SD of triplicate syndecan-1 determinations from a single experiment representative of two experiments.

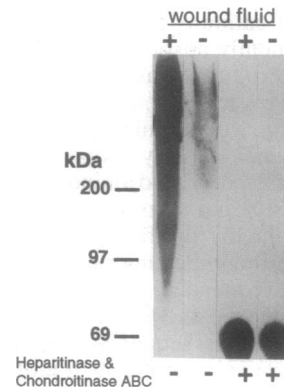


FIG. 2. Induced cell surface syndecan-1 has reduced glycosylation. Postconfluent NIH 3T3 cells were treated for 48 h with medium containing 400 μ g of protein per ml of the wound fluid preparation (+) or with medium alone (-). Trypsin-releasable materials (22) were partially purified on DEAE-cellulose, separated by PAGE through a 3–15% gradient gel, and analyzed by Western blot with mAb 281-2 (21). Some samples were treated with heparin-sulfate lyase and chondroitin ABC lyase. Molecular sizes were determined by comparison with known molecular mass standards.

face (Fig. 2). Enzymatic removal of both heparan sulfate and chondroitin sulfate yielded the same size core proteins.

Syndecan-1 induction in repairing murine skin wounds is accompanied by the induction of syndecan-4, but there is no change in the expression of either syndecan-2 or -3 (R.L.G. and M.B., unpublished data). The wound fluid preparation duplicates this selectivity. Transcripts for both syndecan-1 and -4 increase as early as 6 h after exposure to the wound fluid preparation, reaching a maximum induction of about 3-fold for syndecan-1 mRNA and about 14-fold for syndecan-4 mRNA (Fig. 3). No change in syndecan-2 mRNA levels was seen and no syndecan-3 mRNA was detected.

Purification of Syndecan Inductive Activity. The syndecan inductive activity in wound fluid was abolished by trypsin (20 μ g/ml, 37°C for 30 min) or heat (56°C for 30 min) (data not shown), suggesting that the activity is associated with protein. To purify this activity, wound fluid was first incubated at pH 4.0, precipitating 75% of the total protein. The soluble fraction contained 90% of the original inductive activity and

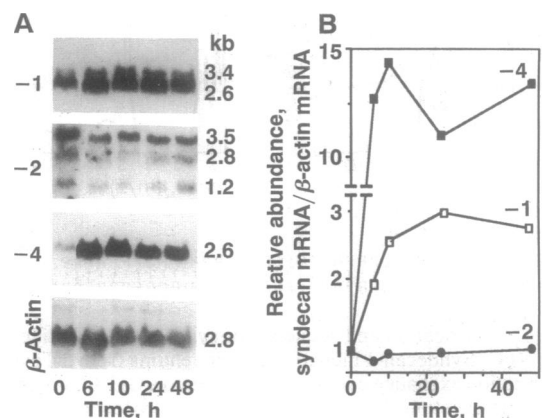


FIG. 3. Wound fluid preparation induces syndecan-1 and -4 transcripts. (A) Total RNA was extracted from postconfluent cultures of NIH 3T3 cells at the indicated times after culture in the wound fluid preparation (400 μ g of protein per ml). Equal amounts of RNA (about 10 μ g) were analyzed by Northern blots with probes specific for syndecan-1, -2, -3, -4, or for β -actin. No syndecan-3 transcript was detected. (B) The relative abundance of each syndecan mRNA was determined densitometrically by comparison with β -actin mRNA levels. The relative abundance is the increment from the abundance of each syndecan transcript determined at time zero.

was retained on a cation-exchange column. The eluted activity was subsequently adsorbed on an RPC column, from which it eluted reproducibly as a single peak at 39% acetonitrile (Fig. 4A). This fraction, representing a 700–1000-fold purification from the wound fluid preparation, with a yield of 1–2% of the original activity, was analyzed directly by PAGE, mass spectrometry, and amino acid sequencing. Both PAGE (Fig. 4A *Inset*) and mass spectrometry (Fig. 4B) were consistent with a highly purified peptide with a molecular mass of about 4708 Da, indicating a peptide of about 39 amino acids. Amino acid sequencing established unequivocally the N-terminal 36 amino acids without detection of minor sequences (Fig. 4C). This sequence was identical with the N-terminal 36 amino acids of PR-39, a proline- and arginine-rich 39-amino acid peptide found in pig intestine but hitherto undetected in skin or repairing wounds (14).

PR-39 Induces Syndecan-1 and -4 and Is Present in the Cellular Wound Infiltrate. Chemically synthesized PR-39 possesses the inductive activity found in wound fluid. Syndecan-1 was induced in cell cultures by synthetic PR-39 at the identical concentrations (and kinetics, data not shown) as the chromatographically purified PR-39 from wound fluid (Fig. 5A). Moreover, synthetic PR-39 induced syndecan-1 and -4 mRNA with a similar magnitude and selectivity as the wound fluid preparation (Fig. 5B).

To determine the source of PR-39 in wound fluid, fresh fluid collected on day 3 after wounding was centrifuged at $300 \times g$ to obtain the cellular infiltrate. The majority of cells in this infiltrate (>80%) were granulocytes. A monoclonal antibody directed against PR-39 detected antigen within the granules in these cells (Fig. 6) although all cells demonstrated some staining. No staining was seen when this antibody was used with unpermeabilized cells, or when an unrelated mouse IgG was used (data not shown).

DISCUSSION

Cell surface expression of heparan sulfate can enable cells to become more responsive to a variety of components of the

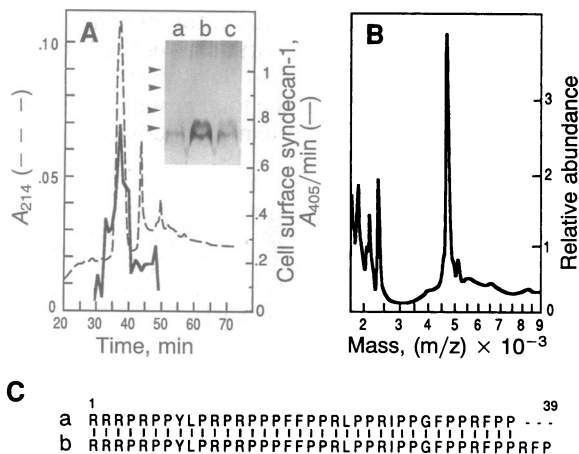


FIG. 4. Purification and sequence of syndecan-1 inductive activity. (A) RPC. Syndecan-1 inductive activity eluting from a Toyopearl SP650S cation exchange column was applied to a C₄ RPC column and eluted with an acetonitrile gradient in 0.1% trifluoroacetic acid. A peak of protein and activity eluted reproducibly at 36 min (39% acetonitrile). (*Inset*) Silver stain of SDS/10% PAGE of fractions eluting at 35 min (lane a), 36 min (lane b), and 37 min (lane c). Arrows indicate migration of molecular mass standards of 46, 30, 14.3, and 6.5 kDa, respectively. (B) Laser desorption mass spectrometry of the active fraction eluting at 39% acetonitrile indicating a mass of about 4708 Da. (C) Amino acid sequences. (a) Sequence of the active fraction eluting at 39% acetonitrile. Thirty-six amino acids were established unequivocally. (b) Sequence of intestinal PR-39 (14). Amino acids are designated by their one-letter code. Vertical lines extending between amino acids in a and b connect identical sequences.

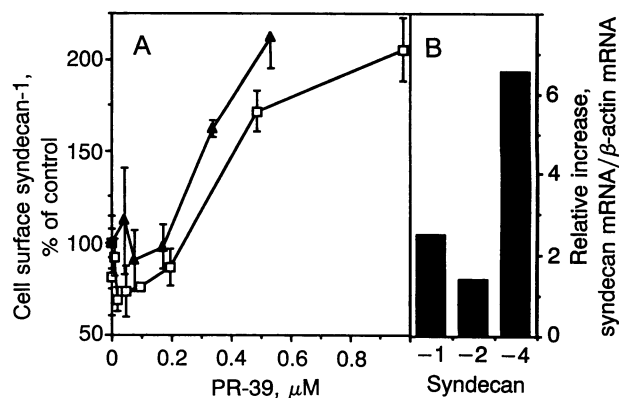


FIG. 5. Both chemically synthesized and purified PR-39 show identical syndecan inductive activity. (A) Inductive activity of synthetic (\square) or purified PR-39 (\blacktriangle) assayed after 60 h in culture as in Fig. 1. (B) Messenger RNAs for syndecan-1, -2, and -4 were detected by Northern blot after 24 h in culture in the presence of 1 μM synthetic PR-39 and quantitated as in Fig. 3B. The relative increase was determined by comparison to identical cells cultured for 24 h in medium without synthetic PR-39.

cellular microenvironment involved in wound repair. The results presented here demonstrate that a protein in the fluid that accumulates in repairing skin wounds enhances the expression of the syndecans, the major cell surface heparan sulfate proteoglycans. This induction occurs in cultured fibroblasts and endothelia and reproduces the induction of the syndecans in repairing skin wounds. The inducing protein, PR-39, is a proline-rich, antimicrobial peptide found in inflammatory cells within the wound. PR-39 was previously thought to function in a nonimmune defense of intestinal mucosal surfaces. It was not known to exist in the wound environment and not known to influence the selective expression of cell surface proteoglycans. The data indicate that inflammatory cells entering skin wounds release PR-39, which induces mesenchymal cells to express specific cell surface heparan sulfate proteoglycans and to kill bacteria to prevent further tissue injury, both activities aiding the repair process.

The syndecan-inducing activity described here is unique. Testing various biological fluids, growth factors and cytokines revealed that only wound fluid and PR-39 were able to induce syndecans in the absence of changes in cell shape or cell proliferation. The inducing activity correlates temporally with active wound repair and spatially with the cells that are induced *in vivo*: activity was detected in early-phase wounds

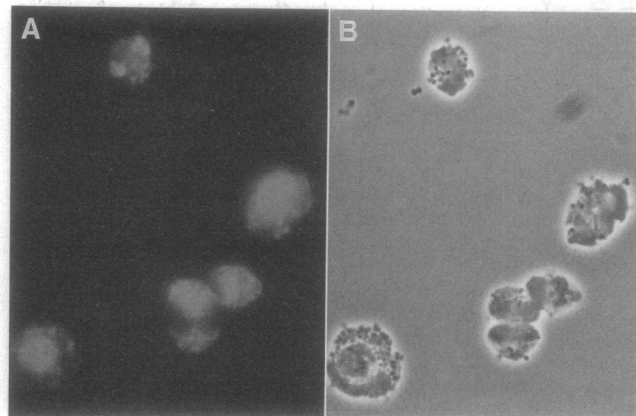


FIG. 6. Detection of PR-39 within cells isolated from wound fluid. (A) Cells from wound fluid collected 3 days after wounding were stained with monoclonal antibody PR-485-4 directed against intestinal PR-39. Intense staining is seen within cytoplasmic granules. (B) Phase-contrast image of same field as in A. ($\times 340$.)

(prior to day 6) and before reepithelialization, and only mesenchymal cells, both endothelial and fibroblastic, were induced. Finally, the activity induces syndecan-1 and -4 mRNA, the same gene selectivity seen in repairing wounds. This activity does not affect syndecan expression exclusively; it also causes minor differences in the proteins secreted by NIH 3T3 cells (R.L.G. and M.B., unpublished data).

PR-39 is an inducer of cell surface syndecans in wounds. The inducing activity in wound fluid did not bind to heparin-Sepharose but bound to and eluted from a cation-exchange column as a broad peak, and from a C₄ RPC column as a highly purified protein. The size and sequence of the purified protein corresponded exactly with that of PR-39, a protein previously isolated from pig intestine and known for its antibacterial activity against both Gram-negative and Gram-positive strains of bacteria (14). Moreover, PR-39 obtained either by chemical synthesis or by chromatographic purification had identical syndecan inductive activities. The same concentration of PR-39 induced syndecans with the same kinetics and selectivity regardless of the source. These are the same concentrations (0.5–1 μ M) that exert the antibacterial effect of PR-39 (14). PR-39 was localized by immunofluorescence within leukocytes, presumably granulocytes, isolated from wound fluid and is likely secreted into the fluid. Consistent with this finding, a cDNA corresponding to the predicted sequence of the PR-39 precursor was recently cloned from bone marrow cells (27).

The discovery of PR-39 as an inducer of cell surface syndecans in wound fluid was wholly unexpected because this peptide was not known to be present in wounds and not known to influence the expression of cell surface heparan sulfate. PR-39 is a proline- and arginine-rich peptide that shares antimicrobial activity but not sequence similarity with several small cationic peptides that appear to play a role in the host defense of a variety of species. PR-39 is related to Bac5 and Bac7 found in bovine neutrophils by the proline-arginine repeats in its 39-amino acid mature form, its N-terminal pro-sequence, and its antimicrobial spectrum (27). Functionally analogous peptides include the cecropins in insect cuticle, magainins in amphibian skin, squalamine in shark stomach, and defensins and β -defensins in mammalian neutrophils (28). The antimicrobial action of these molecules has been ascribed to their amphipathic character, which enables them to permeate bacterial membranes, causing lysis. Our finding of a broad spectrum antimicrobial peptide in skin wounds suggests that the peptides assist in maintaining sterility in the wound, thus reducing the resultant inflammatory response.

The antimicrobial and syndecan-inductive activities of PR-39 are complementary in enhancing wound repair. Induction of cell surface heparan sulfate can trigger cellular behaviors such as proliferation and migration due to its ability to bind and thus augment the action of heparin-binding growth factors, including fibroblast growth factor 2, heparin-binding epidermal growth factor-like growth factor and platelet-derived growth factor AB heterodimer, found in repairing wounds (26, 29). The induction of syndecans can also contribute to the effects of extracellular matrix components involved in wound repair because syndecan-1 binds fibronectin, thrombospondin, tenascin, and the fibrillar collagens (reviewed in ref. 6). Finally, the induction can contribute to the antibacterial action of PR-39, since bacterial pathogens bind to heparan sulfate (30), and killing may be facilitated by immobilization. Thus, syndecan induction by PR-39 may mediate growth-factor responsiveness, augment antimicrobial activity, and permit the changes in cell proliferation, migration, and adhesion that must take place for wound repair to proceed. While PR-39 is an inducer of cell surface syndecans that duplicates many of the activities of wound

fluid, there may be other inducers in wounds, in other biologic fluids, or within the extracellular matrix.

This newly recognized activity for PR-39 indicates that the antimicrobial peptides may have multiple physiological functions and that regulation of cell surface heparan sulfate is a normal response to injury. The mechanism by which PR-39 exerts its selective induction is unknown, but the data suggest that expression of the individual syndecans can be controlled specifically and that antimicrobial proline-rich peptides, such as PR-39, may function as signaling molecules in complex cellular behaviors. Taken together, the current data lead to a previously unrecognized role for these molecules and to a better understanding of the physiological response to injury.

We thank Lynda Herrera for secretarial assistance and Dr. William S. Lane at the Harvard microchemistry facility for mass spectrometry and amino acid sequencing. This work was supported by National Institutes of Health Grants K08AR01874 (to R.L.G.) and R01 CA28735, R37 HD06763, and P30 HL46491 (to M.B.), and by the Lucille P. Markey Charitable Trust.

- Ruoslahti, E. & Yamaguchi, Y. (1991) *Cell* **64**, 867–869.
- Rapraeger, A. C., Krufka, A. & Olwin, B. B. (1991) *Science* **252**, 1705–1708.
- Yayon, A., Klagsbrun, M., Esko, J. D., Leder, P. & Ornitz, D. M. (1991) *Cell* **64**, 841–848.
- Guan, J. L., Treuithick, J. E. & Hynes, R. O. (1991) *Cell Regul.* **2**, 951–964.
- Woods, A., McCarthy, J. B., Furcht, L. T. & Couchman, J. R. (1993) *Mol. Biol. Cell* **4**, 605–613.
- Bernfield, M., Kokenyesi, R., Kato, M., Hinkes, M. T., Spring, J., Gallo, R. L. & Lose, E. (1992) *Annu. Rev. Cell Biol.* **8**, 365–394.
- Jalkanen, M., Elenius, K. & Rapraeger, A. (1993) *Trends Glycosci. Glycotechnol.* **5**, 107–120.
- David, G. (1993) *FASEB J.* **7**, 1023–1030.
- Rapraeger, A. C. (1993) *Curr. Opin. Cell Biol.* **5**, 844–853.
- David, G., Lories, V., Decock, B., Marynen, P., Cassiman, J. J. & Van den Berghe, H. (1990) *J. Cell Biol.* **111**, 3165–3176.
- Hayashi, K., Hayashi, M., Jalkanen, M., Firestone, J. H., Trelstad, R. L. & Bernfield, M. (1987) *J. Histochem. Cytochem.* **35**, 1079–1088.
- Elenius, K., Vainio, S., Laato, M., Salmivirta, M., Thesleff, I. & Jalkanen, M. (1991) *J. Cell Biol.* **114**, 585–595.
- Trautman, M. S., Kimelman, J. & Bernfield, M. (1991) *Development (Cambridge, U.K.)* **111**, 213–220.
- Agerberth, B., Lee, J.-Y., Bergman, T., Carlquist, M., Boman, H. G., Mutt, V. & Jornvall, H. (1991) *Eur. J. Biochem.* **202**, 849–854.
- David, G. & Bernfield, M. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 786–790.
- Yuspa, S. H., Koehler, B., Kulesz-Martin, M. & Hennings, H. (1981) *J. Invest. Dermatol.* **76**, 144–146.
- Gallo, R. L., Staszewski, R., Sauder, D. N., Knisely, T. L. & Granstein, R. D. (1991) *J. Invest. Dermatol.* **97**, 203–209.
- Sanderson, R. D., Hinkes, M. T. & Bernfield, M. (1992) *J. Invest. Dermatol.* **99**, 390–396.
- Snyder, E. Y., Deitcher, D. L., Walsh, C., Arnold-Aldege, S., Hartwig, E. A. & Cepko, C. L. (1992) *Cell* **68**, 33–51.
- Breuing, K., Eriksson, E., Liu, P. & Miller, D. R. (1992) *J. Surg. Res.* **52**, 50–58.
- Jalkanen, M., Nguyen, H., Rapraeger, A., Kurn, N. & Bernfield, M. (1985) *J. Cell Biol.* **101**, 976–984.
- Rapraeger, A. & Bernfield, M. (1985) *J. Biol. Chem.* **260**, 4103–4109.
- Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159.
- Saunders, S., Jalkanen, M., O'Farrell, S. & Bernfield, M. (1989) *J. Cell Biol.* **108**, 1547–1556.
- Kim, C., Goldberger, O., Gallo, R. & Bernfield, M. (1994) *Mol. Biol. Cell* **5**, 797–805.
- Marikovskiy, M., Breuing, K., Liu, P. Y., Eriksson, E., Higashiyama, S., Farber, P., Abraham, J. & Klagsbrun, M. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 3889–3893.
- Storici, P. & Zanetti, M. (1993) *Biochem. Biophys. Res. Commun.* **196**, 1058–1065.
- Boman, H. G. (1991) *Cell* **65**, 205–207.
- Clark, R. A. F. & Henson, P. M. (1988) *The Molecular and Cellular Biology of Wound Repair* (Plenum, New York), pp. 243–271.
- Liang, O. D., Ascencio, F., Fransson, L.-A. & Wadstrom, T. (1992) *Infect. Immun.* **60**, 899–906.