Subglottic Stenosis Examined as a Fibrotic Response to Airway Injury Characterized by Altered Mucosal Fibroblast Activity

Tripti Singh, MD; Vlad C. Sandulache, MD, PhD; Todd D. Otteson, MD; Mark Barsic, BS; Edwin C. Klein, VMD; Joseph E. Dohar, MD, MS; Patricia A. Hebda, PhD

Objective: To investigate the association between mucosal fibroblast activity and subglottic stenosis (SGS) development.

Design: Prospective study of an animal model of SGS.

Setting: Academic research laboratory.

Subjects: New Zealand white rabbits were assigned to either the cricothyroidotomy and carbon dioxide laser injury group or the cricothyroidotomy and silver nitrate injury group. Airways were excised for histologic analysis and the establishment of primary fibroblast cultures. Lesions from surgical excision of established SGS and subglottic tissue were used to analyze SGS recurrence.

Interventions: The subglottis was approached via cricothyroidotomy and was subjected to either carbon dioxide laser or silver nitrate injury before closure. The SGS lesions were excised at 8 to 10 weeks and were used to establish explants for fibroblast culture. The animals underwent recovery for an additional 14 days to follow recurrence of SGS. After 14 days, all the animals were killed humanely, and subglottic tissue was harvested for histologic evaluation. Rates of migration and contraction of SGS and normal airway fibroblasts were assayed using established in vitro methods under basal conditions and with prostaglandin E2 treatment.

Main Outcome Measures: For in vivo studies, injury, healing, and scarring of the mucosa and cartilage were the primary measures. For cultured fibroblast experiments, cellular responses of fibroblasts from normal and stenosed mucosa were compared and contrasted.

Results: Mucosal injury resulted in acute fibroplasia and chronic SGS, surgical excision of mature SGS at 8 weeks resulted in rapid recurrence of stenosis, and SGS-derived fibroblasts were relatively refractory to the effects of prostaglandin E2 on migration and contraction.

Conclusions: Subglottic stenosis represents a fibrotic airway repair process that involves fibroblasts that produce recurrent, excessive scar formation. We suggest that SGS development and recurrence may be partially dictated by altered fibroblast responsiveness to antifibroplastic signals during mucosal repair.

Critical SGS development is an example of a relatively common phenomenon wherein injury to a tissue results in aberrant and fibrotic wound healing. This phenomenon is also exemplified by pathologic conditions in the skin, specifically, hypertrophic scars and keloids. In fact, virtually every connective tissue in the body is vulnerable to anatomical and functional compromise resulting from excessive scarring as a part of wound repair. In contrast to wound healing in the skin, to our knowledge, healing of airway mucosa has not been extensively studied. The mechanisms that underlie the development of fibrotic dermal healing phenotypes have been largely elucidated and can be organized into 3 general categories. First, there seems to be an altered inflammatory response to injury primarily with respect to the soluble component of inflammation that leads to the development of keloids and hypertrophic scar. Second, there is substantial evidence that hypertrophic scars and keloids are, to a large degree, the result of abnormal fibroblast activity in the dermal wound bed. More specifically, our group and other researchers have demonstrated that keloid fibroblasts possess intrinsically altered rates of migration, contraction, and extracellular matrix synthesis, processes essential to the development of fibroplasia. Third, there exists an imbalance skewed in favor of profibrotic agents and against antifibrotic agents that further exacerbates intrinsically altered fibroblast activity, leading to a suboptimal wound-healing response. Prostaglandin E2 (PGE2) has been shown to play an important role in the regulation of scar formation after injury. Activation of 4 G-coupled protein receptors (EP1-EP4) by PGE2 triggers downstream intracellular signaling cascades that can regulate target cell migration, contraction, protein synthesis, and gene expression. Activation of intracellular cyclic adenosine monophosphate, whether by PGE2 itself or more directly by specific pharmacologic agonists (forskolin), has been demonstrated to be key to the antifibroplastic role that PGE2 may play during wound healing. Herein, we plan to extend this line of analysis to scar formation in the rabbit model of SGS.

Our analysis of SGS development revealed many parallels between it and its dermal counterparts. First, we confirmed that SGS development is accompanied by a robust inflammatory component. Second, we showed that the histologic and morphologic characteristics of SGS closely parallel those of hypertrophic scars and keloids (except for extension outside the boundaries of initial injury in the case of keloids). Third, we demonstrated an aberrant SGS fibroblast phenotype in human pathologic specimens regarding basal activities and in response to putative antifibrotic agents, including PGE2.

This study was designed to further extend previous work in the context of a well-established animal model of SGS. We hypothesize that SGS development constitutes an acute fibroplastic response to injury that matures into a chronic mucosal scar. We further propose that the initial mucosal injury triggers a shift in the cellular and molecular components of the healing and healed wound in favor of profibrotic forces that cannot be surgically corrected and, therefore, results in SGS recurrence after excision. These findings demonstrate that these tissue characteristics include an intrinsically distinct SGS fibroblast phenotype having altered responses to antifibrotic agents and mediators.

AIRWAY WOUNDING AND VISUALIZATION

All animal experiments were performed using approved protocols in compliance with Institutional Animal Care and Use Committee regulations. Briefly, the subglottis was entered using an anterior cricothyroidotomy, and the posterior quadrant was injured using a laser or chemical cautery. A carbon dioxide (CO2) laser was used to deliver 1-second continuous exposure, with a beam diameter of 2 mm and power settings of 12 W delivered in 4 pulses spaced in an arc across the posterior subglottic region in each airway, as previously described. Chemical injury was produced using a silver nitrate (AgNO3) cautery stick applied to the posterior subglottis using a single rolling pass for a total of 1 second, as described in detail in a previous publication. The presence of SGS was assessed and documented endoscopically. Images were video captured and were converted into digital movie files and images (Pinnacle Systems Software; Mountain View, California).

ANIMALS

The adult New Zealand white rabbit model has been used extensively in the study of subglottic airway repair. For this study, animals were used as follows. Three animals were used to provide histologic evidence of short-term (14 days) and long-term (84 days) mucosal scarring after CO2 laser or AgNO3 subglottic airway injury. These animals were included in a previous study and are used herein solely to provide an in vivo context for subsequent data. Six animals were used to produce subglottic airway explants for establishing primary cultures of normal airway fibroblasts (NAFs) (n=3) and SGS-derived fibroblasts (SGSFs) (n=3). Six animals were used to characterize restenosis of the subglottic airway after surgical removal of CO2 laser–induced (n=3) or AgNO3–induced (n=3) SGS. Two animals that underwent anterior cricothyroidotomy without posterior wounding were used as sham controls for mucosal healing.

RESTENOSIS

Airways that contained documented chronic SGS were accessed by making a surgical incision through the previously created (and healed) anterior cricothyroidotomy. The posterior subglottis was exposed, and the SGS was excised using a cold steel scalpel. As detailed in previous studies, the posterior SGS covered approximately 60% to 70% of the airway circumference, with a cranio-caudal height of 3 to 4 mm. Using a No. 11 scalpel and fine forceps, the SGS scar was excised from underlying cartilage, with care taken to not injure the cartilage layer. Hemostasis was achieved using pressure applied with sterile cotton-tipped applicators. The mucosal defect was left to heal by secondary intention for 14 days, at which point endoscopic examination determined that stenosis had recurred. The animals were euthanized, and the subglottic airways were excised and processed for subsequent histologic analysis. Two previously uninjured animals also underwent full-thickness mucosal excision as controls for normal healing.
TISSUE COLLECTION AND ANALYSIS

Animals were euthanized under deep anesthesia using a ketamine hydrochloride and xylazine hydrochloride mixture (35 and 5 mg/kg, respectively), followed by intracardiac administration of pentobarbital sodium (50 mg/kg). The larynx and proximal tracheal regions were excised and processed for microscopic analysis.

Tissue specimens were fixed in 10% buffered formalin, bisected through the site of injury or healing, processed, paraffin embedded, and cut into 5-µm-thick sections. Sections were stained with hematoxylin-eosin or Masson trichrome stain using standard methods.

CELL CULTURE

The subglottis was injured as described in the “Airway Wounding and Visualization” subsection of this section and was allowed to develop into mature stenosis. Animals were killed humanely, fibrotic tissue was removed, and SGSFs were grown out of the explants of tissue harvested 56 to 70 days after injury, time points that correlate with what is considered to be mature chronic SGS.7,9,13 Two cultures were established from SGS associated with each injury modality (CO2 laser and AgNO3 cautery). The NAF cultures were established from 2 uninjured upper airway explants. Cells were cultured in Dulbecco modified eagle medium (Invitrogen, Grand Island, New York) supplemented with 10% fetal bovine serum (Invitrogen) and penicillin, 10 U/mL; streptomycin sulfate, 10 U/mL; and amphotericin B, 0.025 µg/mL (antibiotic-antimycotic) (Life Technologies, Rockville, Maryland) to inhibit fibroblast migration and contraction. Effective doses of these chemicals on dermal fibroblasts based on growth characteristics and morphologic features in culture.

CHEMICALS

We used PGE2 (1 µM), butaprost (1 µM) (Cayman Chemical, Ann Arbor, Michigan), and forskolin (25 µM) (Calbiochem, San Diego, California) to inhibit fibroblast migration and contraction. Effective doses of these chemicals on dermal fibroblast migration and contraction were established in previous studies.7,13

DIRECTIONAL MIGRATION ASSAY

All directional migration assays were performed using a 2-dimensional “scratch” assay as previously described.13 Briefly, airway fibroblasts were plated in 24-well plates in a monolayer and were allowed to reach confluence. A sterile 200-µL pipette tip was used to scratch the confluent monolayer of cells in the middle of each well perpendicular to the previously drawn line on the underside of the 24-well plates. Cells were rinsed with phosphate-buffered saline twice to remove any cellular debris. The wells were then filled with the desired experimental medium. Images of each well were taken at 0, 2, 4, and 8 hours at the same point using an inverted microscope equipped with a phase contrast objective (Nikon Inc, Melville, New York) connected to a digital camera (Diagnostic Instruments, Sterling Heights, Michigan). Changes in the scratched area across time were measured using Metamorph software (Universal Imaging, Downingtown, Pennsylvania). This change in area was then converted to the speed of fibroblast migration for the various conditions tested.13

COLLAGEN GEL CONTRACTION

A modification of the standard fibroblast-populated collagen lattice method was used to measure in vitro fibroblast collagen gel contraction. Briefly, under sterile conditions, collagen solution was prepared from type I collagen (Vitrogen; Angiotech Biomaterials, Palo Alto, California) in a humidified atmosphere containing 5% CO2 with 0.025 µg/mL (antibiotic-antimycotic) (Life Technologies, Rockville, Maryland) and supramolecular modulations with 10% fetal bovine serum (Invitrogen) and penicillin, 10 U/mL; streptomycin sulfate, 10 U/mL; and amphotericin B, 0.025 µg/mL (antibiotic-antimycotic) (Life Technologies, Rockville, Maryland). The collagen solution (200 µL per well) was cast in 0.5-in Teflon cylindrical rings (Seastorm Mfg Co, Twin Falls, Idaho) in 60×15-mm Petri dishes (Cardinal Health, Warren, Pennsylvania). The Petri dishes were placed in a 37°C water bath for 1 hour for collagen gelation. Fibroblasts were dispensed evenly on top of the collagen gels inside the rings in a 200-µL volume containing 2.88×104 cells in medium supplemented with 10% fetal bovine serum. The dishes were incubated for 30 minutes to ensure fibroblast adhesion to the collagen gels. Medium (7 mL) supplemented with 2% fetal bovine serum (control) with and without pharmacologic agents was then added. The Teflon rings were then removed, and the collagen gels were loosened from the surface of the Petri dish using a pipette tip. Images of the collagen gels were taken at 0, 2, 4, and 24 hours using a digital camera connected to a dissecting microscope (Olympus, Melville, New York). Changes in the collagen gel area were measured using Metamorph software. Percentage of contraction was calculated as the change in area from the initial area at time 0. Images of the collagen gels were taken with a grid underneath for calibration.9

STATISTICAL ANALYSIS

Statistical significance throughout the study was determined using the t test, with significance set at P < .05. Carbon dioxide laser and AgNO3 mucosal injuries trigger an acute fibroplastic wound-healing response that results in mature scar formation (SGS). Previous studies6,7,14,15 demonstrated that CO2 laser and AgNO3 injuries can trigger an acute inflammatory response, followed by the normal sequence of tissue repair, including fibroplasia, scar formation, and maturation. Herein, we used airways harvested as part of a previous study to offer histologic evidence of an acute fibroplastic response to mucosal injury and, more important, the development of chronic mature SGS in the rabbit airway. Histologic changes have previously been documented using qualitative and quantitative approaches. In this study, a detailed qualitative histologic examination of the injured mucosa was performed by a blinded veterinary pathologist (E.C.K.) and was used as the baseline for histologic changes that accompany restenosis. In contrast to the sham-injured airways, which displayed an essentially normal posterior subglottic mucosa, CO2 laser–injured and AgNO3–injured airways exhibited profound architectural changes in the short-term (day 14) and long-term (day 84) phases of mucosal healing and SGS formation (Figure 1). Fourteen days after either injury, epithelialization was incomplete; the epithelium was primarily proliferative and mildly dysplastic, in contrast to the pseudostratified columnar epithelium found in sham-injured airways. Inflammatory cells (primarily granulocytes) were present throughout the injured mucosa, as were areas of residual exudative crust superficial to the

©2010 American Medical Association. All rights reserved.
epithelium. The lamina propria displayed early fibroplasia and granulation tissue formation. The underlying perichondrium was found to be disrupted, and a region of apparent newly forming cartilage had developed in the region of perichondrial disruption. In the case of AgNO3 cautery–injured airways, residual AgNO3 grains were found throughout the healing mucosa associated with localized phagocytosing cells.

Eighty-four days after injury, reepithelialization was complete and significant fibroplasia was present, associated with mature, thick collagen fibers. New areas of focal cartilage growth were observed in no immediate relationship with the initial cartilaginous structure and original perichondrium. Prominent blood vessels were observed exhibiting a dilated, congested appearance generally associated with cavernous vessel structures. Minimal patchy inflammation was noted, consisting primarily of scanty granulocytic infiltrates.

Both NAFs and SGSFs display differential basal rates of migration and contraction. The SGSFs used in this study were derived from pathologic specimens of chronic SGS collected at 8 to 10 weeks. As such, they reflect the properties of the chronic pathologic condition and represent the fibroblast phenotypes present in the SGS tissue at the time of reinjury. Both types of SGSF cell strains exhibited greater speeds of 2-dimensional migration compared with NAF cells, although this difference achieved statistical significance only in the case of AgNO3 cautery–generated SGSFs.

**Figure 2A.** Mean (SEM) speeds under basal conditions were as follows: NAF, 4.5 (0.2) µm/h; SGSF (CO2 laser), 4.7 (0.3) µm/h; and SGSF (AgNO3), 6.7 (0.3) µm/h. These results show a trend comparable with that observed with normal adult skin and keloid fibroblasts, respectively: 4.4 (0.1) µm/h and 4.9 (0.2) µm/h.9 Contraction rates varied, with NAF exhibiting greater rates at 2 and 4 hours than either of the SGSF cell strains and CO2 laser–generated SGSFs displaying the maximal rate of contraction at 24 hours (Figure 2B).

**PGE2 EFFECTS ON SGSF MIGRATION AND CONTRACTION**

Previous research9,13 examined the effects of a putative antifibroplastic agent, PGE2, on fibroblast migration and contraction. The SGSFs used in this study were derived from pathologic specimens of chronic SGS collected at 8 to 10 weeks. As such, they reflect the properties of the chronic pathologic condition and represent the fibroblast phenotypes present in the SGS tissue at the time of reinjury. Both types of SGSF cell strains exhibited greater speeds of 2-dimensional migration compared with NAF cells, although this difference achieved statistical significance only in the case of AgNO3 cautery–generated SGSFs.

**Figure 2A.** Mean (SEM) speeds under basal conditions were as follows: NAF, 4.5 (0.2) µm/h; SGSF (CO2 laser), 4.7 (0.3) µm/h; and SGSF (AgNO3), 6.7 (0.3) µm/h. These results show a trend comparable with that observed with normal adult skin and keloid fibroblasts, respectively: 4.4 (0.1) µm/h and 4.9 (0.2) µm/h.9 Contraction rates varied, with NAF exhibiting greater rates at 2 and 4 hours than either of the SGSF cell strains and CO2 laser–generated SGSFs displaying the maximal rate of contraction at 24 hours (Figure 2B).
CO\textsubscript{2} laser or AgNO\textsubscript{3} cautery, were partially refractory to the effects of exogenous PGE\textsubscript{2} on cell migration (Figure 3) and contraction (Figure 4). The differential inhibitory effect was also observed in the presence of a specific EP2 agonist (butaprost) or a direct adenylate cyclase activator (forskolin), which stimulates the same signaling pathway as PGE\textsubscript{2}. Figure 4B presents only the 2-hour time point values for the purpose of simplicity but are consistent with data collected at the 4- and 24-hour time points (data not shown). The chosen agents and concentrations are consistent with a previous work\textsuperscript{15} investigating the mechanisms by which PGE\textsubscript{2} differentially regulates the migratory and contractile properties of different fibroblast phenotypes. To determine whether SGSFs are completely refractory to PGE\textsubscript{2} effects, additional experiments, performed at higher PGE\textsubscript{2} concentrations (10 µM), revealed that SG SF migration and contraction can be inhibited, albeit at higher concentrations than needed for NAFs. This is consistent with a previous study\textsuperscript{15} using dermal fibroblasts that identified comparable fibroblast phenotypes as partially, but not completely, refractory to PGE\textsubscript{2} effects.

**EXCISION OF ESTABLISHED SGS AND ACUTE RECURRENCE**

Established mature SGS was surgically excised using cold steel, and the airways were monitored during the post-operative period. Injured airways restenosed to a substantial degree in the first 14 days after excision (Figure 5). For both types of primary injury, the pattern of restenosis approximated the extent of the original SGS and, in a span of only 14 days, recapitulated a substantial degree of the original airway obstruction. In sharp contrast, cold steel excision of previously uninjured airways produced little evidence of stenosis, as observed in numerous previous studies.\textsuperscript{3,6,7}

Detailed histologic analysis was used to correlate these findings to the initial mucosal repair process. In general, the healing tissue seemed to be more mature than would be expected 14 days after injury and was clearly more abundant than comparable healing tissue of normal mucosa (Figure 6). The SGS mucosa exhibited a minimal inflammatory component; mature-appearing, spindle-shaped fibroblasts; and cavernous, dilated blood vessels. Both CO\textsubscript{2} laser–injured and AgNO\textsubscript{3}–injured airways exhibited a similar degree of tissue maturity and comparable degrees of reepithelialization, fibroplasia, and revascularization. This stenotic response was in sharp contrast to that of the control group of full-thickness excisional wounds made in previously uninjured airways (Figure 6, bottom row). The control wounds presented with some focal areas of collagen deposition (mild fibroplasias), uniform vascular ectasia, and minimal residual inflammation with some scattered granulocytes in the area of injury. Compared with the reinjured wounds, the control wound mucosa was much less thick, with little evidence of stenosis.

**COMMENT**

Scarring is an essentially ubiquitous process that occurs in postnatal tissue repair in a variety of tissue types. It is consistently accompanied by loss of original tissue structure and function and, depending on the tissue type, various degrees of morbidity and mortality. Acquired SGS is a well-described phenomenon that recapitulates the essential components of connective tissue scarring and fibrosis and continues to represent an important clinical

![Figure 3](image1.png)

**Figure 3.** Subglottic stenosis–derived fibroblast (SGSF) migration is relatively refractory to prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) inhibition. All migration assays were performed using confluent cell monolayers in regular fibroblast medium (Dulbecco modified eagle medium) in the presence or absence of PGE\textsubscript{2} (1 µM), butaprost (EP2 agonist) (BUTA (1 µM), and forskolin (adenylate cyclase activator) (FSK) (25 µM). Data are presented as means, with error bars representing SEM. *P<.05 vs control values, using the t test. AgNO\textsubscript{3} indicates silver nitrate; CO\textsubscript{2}, carbon dioxide; NAF, normal airway fibroblast.

![Figure 4](image2.png)

**Figure 4.** Subglottic stenosis–derived fibroblast (SGSF) contraction is relatively refractory to prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) inhibition. All contraction assays were performed in regular fibroblast media (Dulbecco modified eagle medium) supplemented with 2% fetal bovine serum in the presence or absence of PGE\textsubscript{2} or butaprost (EP2 agonist) (1 µM). A, Normal airway fibroblast (NAF) contraction was significantly inhibited in the presence of PGE\textsubscript{2} or butaprost at 2 hours. B, Contraction of SGSF was not inhibited by PGE\textsubscript{2} or butaprost at 2 hours. Control indicates the absence of PGE\textsubscript{2} in media. Data are presented as means, with error bars representing SEM. *P<.05 vs control values, using the t test. AgNO\textsubscript{3} indicates silver nitrate; CO\textsubscript{2}, carbon dioxide.
concern. Our laboratory has attempted a multifaceted approach aimed at elucidating the precise molecular and cellular mechanisms that lead to SGS formation. Specifically, previous research demonstrated that the early inflammatory reaction to injury of the mucosa is proportional to the extent of injury and the degree of long-term scarring. More recently, it was shown that, as in the skin, the fibroplastic response to mucosal injury encountered during SGS formation is characterized by abnormal fibroblast activity. This study was designed to further expand on our previous work by continuing to establish a link between tissuewide fibroplasia and specific fibroblast activity.

The results shown in Figures 3 and 4 demonstrate different responses to mediators of fibroblasts cultured from SGS mucosa compared with normal subglottic airway fibroblasts. This suggests that the fibroblast population in the tissue has changed to a different phenotype in the course of healing. This concept has been well established for skin fibroblasts, for which it has been shown, for example, that the phenotypes of normal and hypertrophic scar fibroblasts differ from each other and from uninjured dermis. We characterized fibroblasts derived from mature SGS specimens associated with 2 different injury modalities. The data indicate that, as in the skin, there exist distinct fibroblast phenotypes. Rabbit NAFs and the 2 types of SGSFs (laser and chemical cautery) exhibit varying rates of basal in vitro migration and contraction. Interestingly, SGSF associated with chemical cautery–induced injury displayed a markedly elevated migratory rate, although both SGSF strains exhibited lower fibroblast-populated collagen lattice contractile rates than did NAFs. Taken together, these data suggest that the mechanics of fibrosis in the airway mucosa may be different from those encountered in the dermis. Additional work using in vivo and in vitro approaches is required to establish whether mucosal fibrosis is as dependent as is skin fibrosis on fibroblast contraction of the extracellular matrix for scar formation and maturation. Based solely on our findings to date, we conclude that, as in the case of keloid fibroblasts, basal differences between NAF and SGSF migration and contraction will be small and variable, although suggestive of an altered phenotype. Furthermore, the transition in phenotype seems to correlate with the severity of injury and the degree of stenosis. One explanation for this is that greater injury causes the recruitment of additional fibroblastic cells from the sur-

Figure 5. Surgical excision of chronic subglottic stenosis (SGS) fails to establish permanent normal airway patency. Chronic SGS was established using either carbon dioxide (CO2) laser or silver nitrate (AgNO3) cautery (preoperative). Surgical excision of the SGS scar was performed to remove all soft stenosis to the perichondrium (postoperative), and airways were allowed to heal. As shown in representative images, on day 4, there is early evidence of the onset of restenosis; at 14 days after surgical excision, CO2 laser–injured and AgNO3–injured airways displayed significant restenosis.

Figure 6. Subglottic restenosis exhibited histologic patterns similar to the initial acute fibroplastic response to injury. Rabbits with mature subglottic stenosis (SGS) underwent surgery to excise the stenosis by means of full-thickness excision using a scalpel. Restenosis occurred rapidly in the SGS animals but not in the control animals with normal airways that underwent sharp, cold steel excision alone. Carbon dioxide (CO2) laser–injured and silver nitrate (AgNO3)–injured airways displayed thickened lamina propria (LP), abundant collagen deposition, and aberrant underlying cartilage (Cart) morphology 14 days after scar excision. H&E indicates hematoxylin-eosin; n-Cart, new cartilage; TRI, Masson trichrome stain.
rounding tissue, for example, pericytes, and from circulating blood, for example, fibrocytes. These cells are recruited into the wound bed and then contribute to an altered healing response and greater stenosis. Another explanation is that higher levels of inflammatory mediators released during more extensive injury may induce greater changes in the phenotypic expression of the responding fibroblasts. Both these events may, in fact, be contributing factors to the resulting wound fibroblast phenotype.

Fibroblast activity in the wound bed depends on the intrinsic phenotype and exogenous signals. It is the precise environmental milieu, the balance of profibrotic and antifibrotic signals, that has been shown to guide the scarring process and to dictate, to a large degree, the ultimate extent of fibroplasia.9,12,16-22 We focused attention in the past on a putative antifibroplastic agent, PGE2, and demonstrated, along with other researchers, that PGE2 signaling is altered during dermal fibrosis.9,15,21 Specifically, we showed an imbalance tilted against PGE2 during keloid formation and postulated that when PGE2 levels are insufficient to control fibroblast migration, contraction, and collagen synthesis, excessive scarring occurs.9 We are currently applying the same approach to the study of SGS. Previous work15 showed that SGSFs derived from human pathologic specimens maintain normal PGE2 synthetic capabilities and appropriate underlying signaling mechanisms as related to this pathway. This study represents the companion to previous work by analyzing the response of SGSFs to exogenous PGE2 signaling. As in the dermis, these data reveal an imbalance tilted against PGE2 activity in the mucosal wound bed. The relatively refractory response of SGSFs to PGE2 inhibition of migration and contraction strongly suggests an altered dynamic during mucosal repair, with PGE2 signaling being insufficient to overcome the profibrotic signals present in the healing tissue bed.

An important characteristic of keloids is the high rate of recurrence after simple excision. To date, a variety of combinatorial approaches have been developed to improve clinical outcomes associated with keloid removal, but recurrence remains a clinically important sequela.23-25 These empirical data, combined with a wealth of in vitro studies, strongly suggest an important dynamic associated with keloid formation. Specifically, they suggest that keloid formation and recurrence result from altered cellular dynamics and that there likely exists a population of cells in the scar margin capable of regenerating the aberrant tissue after excision. Clinicians have observed that SGS possesses similar characteristics and that simple excision of SGS is associated with recurrence. We believe that although SGS is not a precise airway correlate to keloid (insofar as it lacks the ability to extend outside the margins of the original injury), there are some relevant parallels between the 2 aberrant healing processes. The presence of an altered SGSF phenotype, particularly as it relates to responsiveness to anti-fibroplastic agents (PGE2), strengthens this hypothesis. This study demonstrates, using a well-described animal model, a correlation between an altered fibroblast phenotype and a recurrent, aberrant scarring phenomenon. The recurrence of excised SGS with the characteristics of the initial injury and the histologic characteristics of mature scar strongly suggest that the initial injury triggers a fundamental tissue imbalance that, unless corrected, directs the course of airway mucosal healing toward an iterative process of scarring and stenosis.

In conclusion, the results of this study demonstrate that differential degrees of injury and resulting stenosis in the subglottic airway involve mucosal fibroblasts with distinct phenotypes that contribute to the outcome of healing. This differential phenotype seems to persist and lead to subsequent aberrant healing. Many aspects of SGS formation have been characterized using clinical, empirical approaches. We are developing a comprehensive animal model for this significant clinical problem. In the confines of this model, we are attempting to elucidate the complex mechanisms that accompany fibrotic mucosal healing to develop therapeutic approaches aimed at ameliorating changes in tissue structure and function. This study provides additional strong evidence that without such manipulation, surgical correction of airway stenosis is unlikely to yield optimal outcomes. It also directs our attention to the interaction between inflammation and fibroplasia, and the PGE2 signaling pathway in particular, as a possible target for pharmacologic manipulation aimed at controlling excessive fibroblast activity in the mucosal wound bed.

Submitted for Publication: October 3, 2008; final revision received March 9, 2009; accepted April 22, 2009.

Correspondence: Patricia A. Hebda, PhD, Department of Pediatric Otolaryngology, Children’s Hospital of Pittsburgh, 4401 Penn Ave, Rangos Research Center, Room 5120, Pittsburgh, PA 15224 (hebda@pitt.edu).

Author Contributions: Drs Singh, Sandulache, and Hebda and Mr Barsic had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Singh, Sandulache, Dohar, and Hebda. Acquisition of data: Singh, Sandulache, Otteson, Barsic, and Hebda. Analysis and interpretation of data: Singh, Sandulache, Klein, Dohar, and Hebda. Drafting of the manuscript: Singh, Sandulache, Hebda. Critical revision of the manuscript for important intellectual content: Singh, Sandulache, Otteson, Barsic, Klein, Dohar, and Hebda. Statistical analysis: Singh. Obtained funding: Dohar and Hebda. Administrative, technical, and material support: Sandulache, Dohar, and Hebda. Study supervision: Dohar and Hebda.

Financial Disclosure: None reported.

Funding/Support: This work was supported by grant R01 DC007437 from the National Institutes of Health (Dr Hebda) and by the Children’s Hospital of Pittsburgh.

Additional Contributions: Ryan Branski, PhD, provided extensive discussion and interpretation of results.

REFERENCES


