


Pathologic Fibroblasts in Idiopathic Subglottic Stenosis Amplify Local Inflammatory Signals

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Abstract

Objective. To characterize the phenotype and function of fibroblasts derived from airway scar in idiopathic subglottic stenosis (iSGS) and to explore scar fibroblast response to interleukin 17A (IL-17A).

Study Design. Basic science.

Setting. Laboratory.

Subjects and Methods. Primary fibroblast cell lines from iSGS subjects, idiopathic pulmonary fibrosis subjects, and normal control airways were utilized for analysis. Protein, molecular, and flow cytometric techniques were applied in vitro to assess the phenotype and functional response of disease fibroblasts to IL-17A.

Results. Mechanistically, IL-17A drives iSGS scar fibroblast proliferation ($P < .01$), synergizes with transforming growth factor $\beta 1$ to promote extracellular matrix production (collagen and fibronectin; $P = .04$), and directly stimulates scar fibroblasts to produce chemokines (chemokine ligand 2) and cytokines (IL-6 and granulocyte-macrophage colony-stimulating factor) critical to the recruitment and differentiation of myeloid cells ($P < .01$). Glucocorticoids abrogated IL-17A-dependent iSGS scar fibroblast production of granulocyte-macrophage colony-stimulating factor ($P = .02$).

Conclusion. IL-17A directly drives iSGS scar fibroblast proliferation, synergizes with transforming growth factor $\beta 1$ to promote extracellular matrix production, and amplifies local inflammatory signaling. Glucocorticoids appear to partially abrogate fibroblast-dependent inflammatory signaling. These results offer mechanistic support for future translational study of clinical reagents for manipulation of the IL-17A pathway in iSGS patients.

Keywords

IL-17A, IL-17, idiopathic subglottis stenosis, fibroblast, tracheal stenosis, laryngotracheal stenosis, iSGS

Idiopathic subglottis stenosis (iSGS) is a rare disease of localized fibroinflammatory scar leading to progressive upper airway obstruction.¹⁻³ Natural history studies showed striking similarity in the demographics, histopathology, and physiologic impairment of affected patients.^{2,4,5} Recent interrogation of iSGS mucosal scar demonstrated abundant fibroblasts, disordered subepithelial extracellular matrix (ECM) deposition, and activation of the interleukin 17A (IL-17A) inflammatory pathway.^{6,7} However, the mechanistic link among iSGS scar fibroblasts, upregulated IL-17A, and pathogenic fibrosis has not yet been determined.

Fibrosis is defined by the excessive accumulation of ECM in inflamed tissue.⁸ A defining characteristic of all fibrotic diseases is the presence of ECM-producing activated fibroblasts, which are the key mediators of tissue remodeling.⁹ During equilibrium, tissue-resident fibroblasts are in a quiescent state yet remain metabolically active and

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provide dynamic biomechanical support to the surrounding tissues. To repair and restore homeostasis after injury, fibroblasts are activated, leading to proliferation and increased ECM synthesis. While activated fibroblasts were initially thought to derive exclusively from tissue-resident fibroblasts, recent studies in parallel human fibrotic diseases indicated that they can also differentiate from recruited epithelial cells via a molecular program of epithelial-mesenchymal transition (EMT).¹⁰ EMT has been implicated as a primary mechanism of fibrosis in chronic kidney disease, pulmonary fibrosis, and liver disease. Alternatively, activated fibroblasts can originate from circulating fibrocyte precursor cells trafficking into inflamed tissue via chemotaxis.⁴ Circulating fibrocytes have been implicated in the process of proinflammatory and fibrosing cascades of cardiac fibrosis, asthma, and several autoimmune conditions, including scleroderma, Graves's disease, rheumatoid arthritis, and granulomatosis with polyangiitis. Characterizing the cellular source of scar fibroblasts is critical to rationally designed antifibrotic therapies.

Prior work showed elevated expression of IL-17A in the subglottic scar of iSGS, and parallel human studies provided mechanistic support for a direct role for IL-17A in the pathogenesis of fibrotic airway remodeling.⁶ Despite these findings, a direct link between iSGS fibroblast phenotype and local immunologic mediators such as IL-17A has not yet been established. We sought to define the phenotype and function of local fibroblasts in iSGS scar to increase our understanding of iSGS disease biology and elucidate new approaches to therapy. In this study, we applied protein, molecular, and flow cytometric techniques to characterize the functional response of iSGS airway scar fibroblasts to IL-17A. Our results highlight an immunologic circuit linking IL-17A with resident local fibroblasts to drive local tissue remodeling in iSGS.

Methods

This study was performed in accordance with the Declaration of Helsinki and good clinical practice and was approved by the Institutional Review Board at Vanderbilt University Medical Center, Nashville, Tennessee (140429).

Fibroblast Acquisition

Airway fibroblasts derived from iSGS subjects, idiopathic pulmonary fibrosis (IPF) subjects, and normal airway subjects were utilized for experiments. Each iSGS and IPF diagnosis was confirmed with previously described clinical and serologic criteria.³ All scar fibroblasts from iSGS subjects were derived from biopsies taken from the subglottis. Each iSGS subject also had a matched healthy airway specimen obtained from mucosal biopsies at the level of the fourth-fifth tracheal ring. All scar fibroblasts from IPF subjects were derived from biopsies of explanted lung tissue.¹¹ All normal control fibroblasts were derived from mucosal biopsies from the fourth-fifth tracheal ring from lung transplant donors.

Cell Isolation and Culture

Fibroblasts from biopsies of normal control, IPF, and iSGS subjects were isolated and seeded onto 24-well culture plates (Falcon, Tewksbury, Massachusetts) at 100,000 cells/well and cultured in standard media as previously described.¹² As indicated, recombinant cytokines (IL-17A; PeproTech, Rocky Hill, New Jersey), IL-4 (PeproTech), IL-13 (PeproTech), β 2-estradiol (E2; Cayman Chemical Company, Ann Arbor, Michigan), and transforming growth factor β 1 (TGF- β 1; PeproTech) or antagonist antibodies (human IL-17A receptor antagonist antibody [IL17rAb]; Novus Biologicals, Littleton, Colorado) were added to culture media at initial seeding at the specified concentration. Cells were trypsinized and counted with an automated hemocytometer at the indicated time points. All proliferation experiments were conducted in triplicate for each biological replicate.

Immunohistochemistry

Paraffin-embedded sections (3 μ m) of subglottic scar from 10 additional iSGS subjects or 10 matched postintubation subglottic stenosis controls were utilized as previously described.⁶ For E-cadherin (CDH1) staining, slides were incubated with anti-CDH1 antibody (R&D Systems, Minneapolis, Minnesota) for 1 hour at a 1:500 dilution, followed by a biotinylated anti-goat IgG (Vector Laboratories Inc, Burlingame, California) for 30 minutes at a 1:200 dilution. The Bond Polymer Refine Detection System (Leica Biosystems Inc, Buffalo Grove, Illinois) was used for visualization. Immunostained slides were imaged on a Leica SCN400 Slide Scanner (Leica Biosystems Inc). Slides were imaged at 20 \times magnification to a resolution of 0.5 μ m/pixel. Cells were identified with standard Ariol analysis scripts (Leica Biosystems Inc). Upper and lower thresholds for color, saturation, intensity, size, roundness, and axis length were set for blue hematoxylin staining of nuclei and brown 3,3'-diaminobenzidine reaction products.

Flow Cytometry

To quantify circulating fibrocytes, peripheral blood from 15 additional iSGS subjects or 15 matched healthy controls were rested in RPMI medium supplemented with 10% fetal calf serum for 2 hours. Cells were suspended in FACS buffer (BD Biosciences, Franklin Lakes, New Jersey) and stained with CD45 PerCP (BioLegend, San Diego, California). Cells were washed in PermWash Buffer (BD Biosciences) and stained with intracellular antibodies to type 1 collagen (Col-1) fluorescein isothiocyanate (Millipore, Burlington, Massachusetts) or isotype control (Millipore). Cells were then washed and analyzed by flow cytometry. For the gating strategy, doublets were excluded and dead cells excluded by size. CD45+ cells were analyzed for their intracellular Col-1 expression relative to the isotype control. To measure intracellular Col-1 and fibronectin, cells were fixed with Cytofix (BD Biosciences) for 20 minutes at 4°C, washed, and stained with Col-1-fluorescein isothiocyanate (Millipore) or fibronectin-APC (R&D

systems) for 30 minutes on ice. All flow cytometry experiments were acquired with a LSR II flow cytometer (BD Biosciences). Analysis was performed with FlowJo LLC software (FlowJo, LLC, Ashland, Oregon). A minimum of 200,000 events were acquired for each sample.

Polymerase Chain Reaction

Following mechanical digestion of surgical biopsies, mRNA was extracted as previously described.⁶ Complementary DNA was transcribed with established reagents according to the manufacturer's instructions. Commercial primers (Qiagen, Valencia, California) were utilized for TGF- β 1, CDH1, and housekeeping genes (GAPDH and HPRT1) in a StepOnePlus instrument (Applied Biosystems, Foster City, California). Expression analysis was performed via the 2-DDCt method (Qiagen).¹³

Enzyme-Linked Immunosorbent Assay

Enzyme-linked immunosorbent assays for IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), and chemokine ligand 2 (CCL2; R&D Systems) were performed on cell culture supernatant per the manufacturer's instructions. Values were normalized to obtain the protein production per cell.

Statistical Analysis

Statistical significance was set to 5% ($\alpha = 0.05$) in all analyses. Normal distribution of variables was tested with the Shapiro-Wilk test. Differences between x and y groups were determined with the Kruskal-Wallis and Mann-Whitney tests for normal and nonnormal distributions, respectively. All statistical analyses were performed with Prism 7.0 software (GraphPad Software Inc, La Jolla, California).

Results

Airway Scar in iSGS Does Not Show Evidence of EMT

Loss of CDH1 is a defining event in EMT. Comparison of mRNA expression for CDH1 between 10 iSGS subjects and 10 controls showed no statistically significant difference (**Figure 1A**). These results were confirmed with immunohistochemistry demonstrating similar CDH1 expression between iSGS samples and controls (**Figure 1B**). We also investigated the co-localization of epithelial and mesenchymal markers with immunohistochemistry. Our results showed no co-localization of epithelial (red, thyroid transcription factor 1) and mesenchymal (brown, α -smooth muscle actin) markers in iSGS airway scar (**Figure 1C**), indicating no evidence of EMT.

iSGS Patients Do Not Have Elevated Levels of Circulating Fibrocytes

Given recent results in human fibrotic diseases indicating that activated fibroblasts can originate from circulating precursor cells,^{4,14-16} we sought to examine if this phenomenon contributed to the fibroblasts found in iSGS airway scar. Utilizing flow cytometry of peripheral blood mononuclear

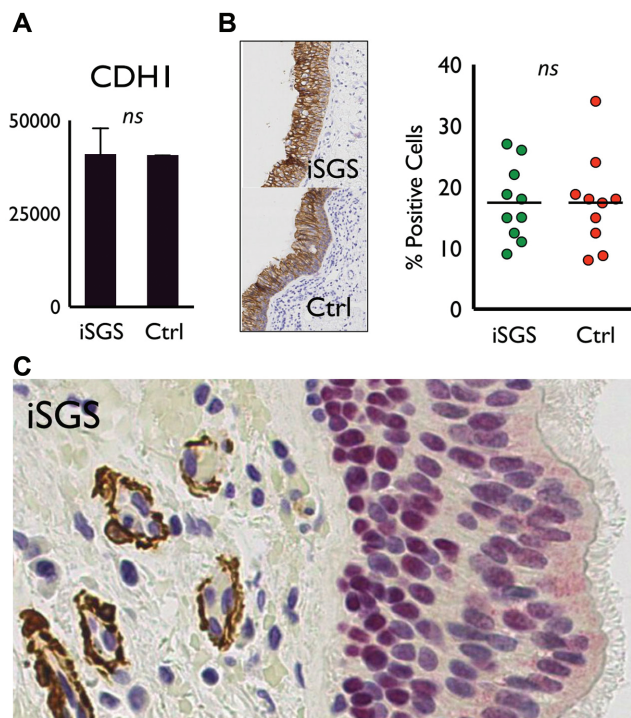


Figure 1. Airway scar in iSGS does not show evidence of EMT. (A) E-cadherin mRNA expression of iSGS and controls (mean \pm SD). (B) E-cadherin immunohistochemistry of iSGS and controls. (C) No co-localization of epithelial and mesenchymal markers. Ctrl, control; EMT, epithelial-to-mesenchymal transition; iSGS, idiopathic subglottic stenosis; ns, not significant; CDH1, E-cadherin.

cells from 15 iSGS subjects and 15 controls, we examined the levels of circulating fibrocytes (CD45⁺Col-I⁺ cells) and found no significant difference (**Figure 2**). The lack of EMT or elevated peripheral fibrocyte counts suggests that local tissue fibroblasts have a central role in the local tissue remodeling seen in iSGS.

Fibroblasts Derived from Airway Scar in iSGS Proliferate in Response to IL-17A

Given prior results demonstrating local elevation of IL-17A in iSGS scar,⁶ we sought to probe the direct effect of IL-17A on iSGS fibroblasts. First, we assessed if primary fibroblasts derived from 3 controls or 3 IPF subjects proliferated in response to IL-17A. Despite 5 days of IL-17A exposure, neither group showed increased proliferation (**Figure 3A**). In contrast, fibroblasts derived from 3 unique iSGS donors were cultured for 5 days in the presence of varying concentrations of IL-17A, and all showed a dose-dependent increase in proliferation ($P = .03$; **Figure 3B**). Matched fibroblasts taken from normal distal trachea in the same iSGS subjects did not demonstrate a dose-dependent response to IL-17A (**Figure 3B**). Primary fibroblasts derived from unique iSGS donors were exposed to IL-17A, IL17rAb, nothing (control), or the combination of IL-17A and IL17rAb for the specified period. In all biologic replicates, the addition of IL17rAb abrogated IL-17A-dependent

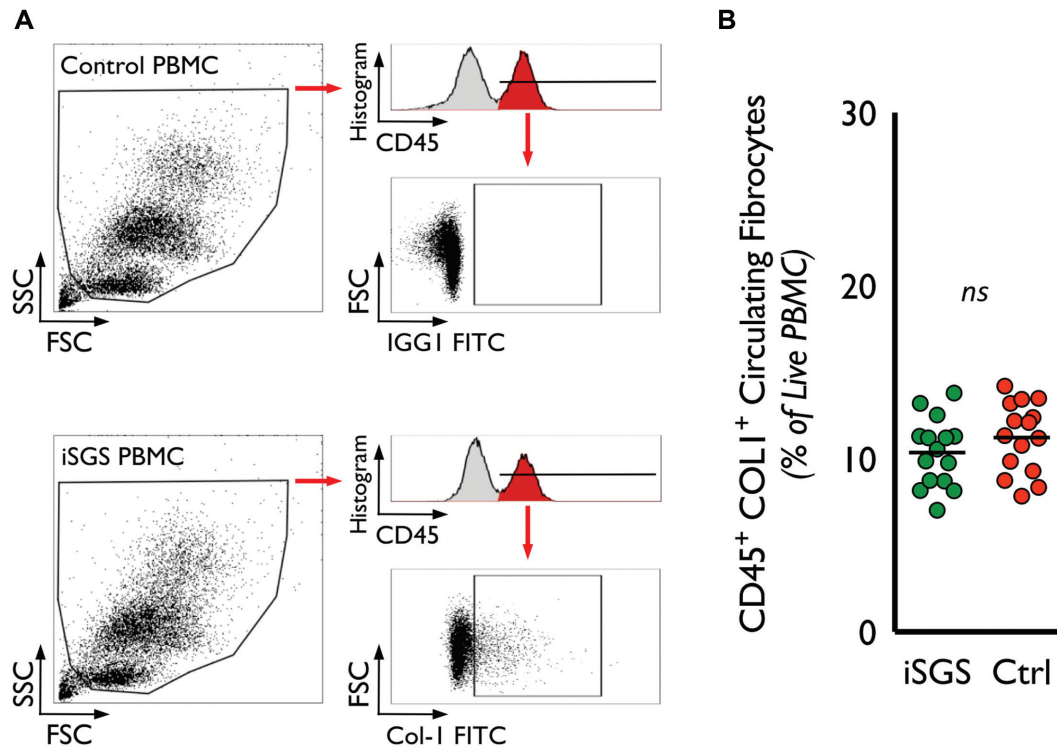


Figure 2. iSGS patients do not show elevation in circulating fibrocytes. (A) Flow cytometry of PBMCs for circulating fibrocytes. (B) No significant difference in level of circulating fibrocytes between iSGS and control PBMCs. FSC, forward scatter; FITC, fluorescein isothiocyanate; IGG1, immunoglobulin G1; iSGS, idiopathic subglottic stenosis; PBMC, peripheral blood mononuclear cell; SSC, side scatter; Col-1, Type I collagen.

proliferation ($P < .05$; **Figure 3C**). These results demonstrate a unique IL-17A-responsive phenotype in iSGS scar fibroblasts and show that IL-17A signals through its cognate receptor to achieve this effect.

TGF- β 1 Present in iSGS Airway Scar Synergizes with IL-17A to Drive Fibroblast Collagen Production

We sought to explore the role of IL-17A in driving ECM production in iSGS scar fibroblasts. After 5 days of culture with varying concentrations of IL-17A, the intracellular levels of Col-1 and fibronectin protein were analyzed via flow cytometry. At 20 ng/mL, IL-17A did not increase iSGS scar fibroblast ECM production alone (**Figure 4A**). We next sought to assess for the presence of TGF- β 1 in the airway scars from iSGS patients. Polymerase chain reaction showed significantly higher TGF- β 1 mRNA levels in iSGS airway scar as compared with normal airway samples ($P = .01$; **Figure 4B**). We subsequently explored the cooperative roles of TGF- β 1 and IL-17A^{17,18} on iSGS fibroblast ECM production. Primary iSGS fibroblasts were cultured with control media, IL-17A, TGF- β 1, or the combination of IL-17A and TGF- β 1 for 5 days, and intracellular levels of Col-1 were analyzed. IL-17A alone did not affect Col-1 protein expression (**Figure 4C**). TGF- β 1 promoted Col-1 production. In 2 of the 3 iSGS subjects, the combination of IL-17A and TGF- β 1 produced significantly more Col-1 than TGF- β 1 alone on a per-cell level ($P = .04$ and $P = .02$). The third subject had a result that approached but did not reach statistical significance ($P = .07$).

IL-17A Induces Inflammatory Chemokine and Cytokine Production from iSGS Airway Scar Fibroblasts

Emerging evidence in alternate disease models suggested a direct role in inflammatory cytokine production by tissue fibroblasts¹⁸⁻²² via recruitment of monocytes and macrophages.²³⁻²⁵ We sought to examine if iSGS scar fibroblasts responded similarly to IL-17A. Primary fibroblasts from iSGS airway scar were cultured with media alone (control), IL-17A, glucocorticoids, or a combination of IL-17A and glucocorticoids for 48 hours. Enzyme-linked immunosorbent assay of the supernatants showed significant elevations in IL-6, GM-CSF, and CCL2 protein levels following IL-17A exposure ($P = .001$; **Figure 5**). Glucocorticoid itself decreased baseline iSGS scar fibroblast GM-CSF protein ($P = .01$) and was able to block IL-17A-dependent GM-CSF production ($P = .02$; **Figure 5B**). These results show that iSGS scar fibroblasts respond to IL-17A by producing chemokines and cytokines known to recruit and instruct myeloid-cell in situ differentiation toward an inflammatory phenotype.

Discussion

This study provides a mechanistic link among scar fibroblasts, upregulated IL-17A, and pathologic fibrosis in iSGS. With prior work showing a local upregulation of IL-17A in iSGS mucosal scar,⁶ the results of this study implicate that IL-17A directly drives iSGS scar fibroblast proliferation and synergizes with TGF- β 1 to promote ECM production.

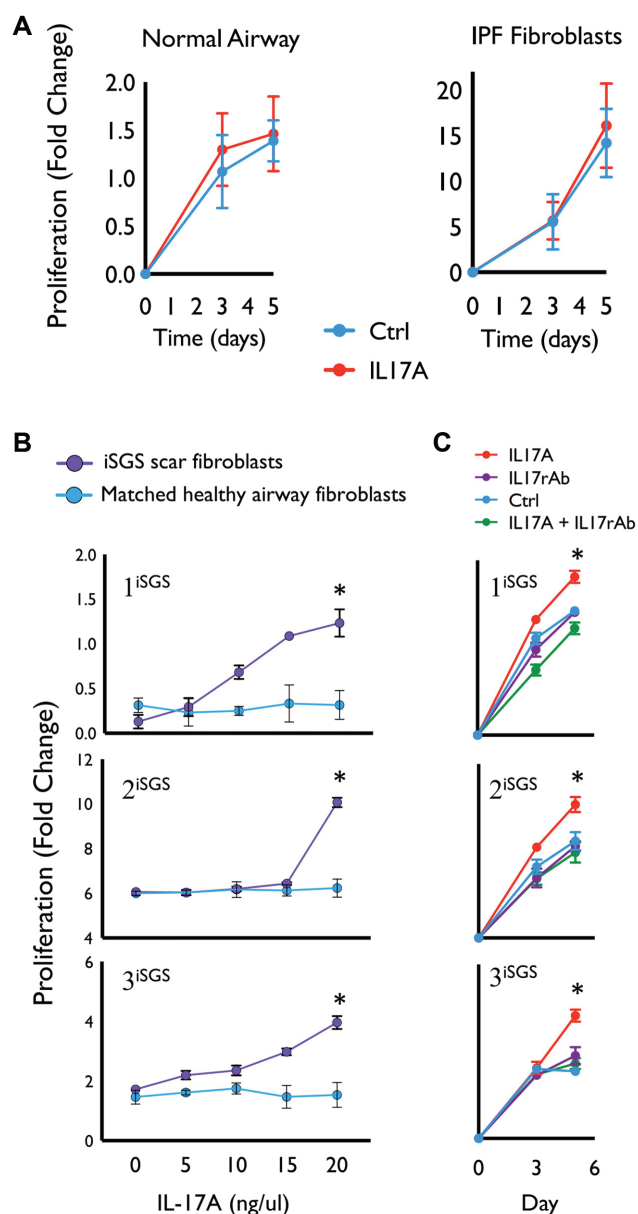


Figure 3. Fibroblasts derived from airway scar in iSGS proliferate in response to IL-17A. Values are presented as mean \pm SD. * $P < .05$. (A) Fibroblasts from controls (left) or IPF (right) cultured with IL-17A. iSGS fibroblasts from 3 unique donors: (B) iSGS fibroblasts cultured with and without IL-17A; (C) iSGS fibroblasts cultured with IL-17A, IL-17rAb, nothing, or IL-17A and IL-17rAb. Ctrl, control; iSGS, idiopathic subglottic stenosis; IPF, intrapulmonary fibrosis; IL-17A, interleukin 17A; IL17rAb, interleukin 17A receptor antibody.

Excitingly, the results also suggest that rather than acting simply as end effectors of inflammation, iSGS scar fibroblasts shape and amplify the local inflammatory response via their own production of critical chemokines and cytokines.

Given the lack of evidence for EMT or elevated circulating fibrocytes in iSGS, our results suggest that resident fibroblasts play a dominant role in iSGS pathogenesis. This

supports findings in alternate airway fibrotic diseases where resident fibroblasts are responsible for the structural and functional remodeling of airway epithelium.^{26,27} Despite their unique microenvironment, the phenotype of iSGS scar fibroblasts resembles that of fibroblasts isolated from airway scar after endotracheal tube injury. Hillel et al showed that these cells proliferate faster, express more collagen, and possess an altered metabolic profile²⁸ when compared with matched controls.²⁹ Our results parallel these findings while providing the additional insight that increased proliferation and enhanced collagen production occur in response to IL-17A in iSGS. Taken together, the data likely suggest a shared molecular program driving a “reprogrammed” fibroblast phenotype in response to divergent external inflammatory cues within airway scar.

A critical question that emerges from this “reprogrammed” fibroblast hypothesis is whether it is possible to reverse the observed phenotype. Future work will need to define the intracellular and molecular mechanisms responsible for the divergent behavior of scar fibroblasts in iSGS. A similar “reprogrammed” phenomenon was reported in resident synovial fibroblasts exposed to the chronic inflammatory stimuli of rheumatoid arthritis.⁸ Rheumatoid arthritis synovial fibroblasts are key players in the development of rheumatoid arthritis and actively contribute to joint remodeling and destruction.³⁰ Driven by the inflammatory synovial cytokine milieu produced by autoimmunity in rheumatoid arthritis, epigenetic modifications lead to altered rheumatoid arthritis synovial fibroblast gene expression characterized by increased antiapoptotic molecules and reduced tumor suppressor genes.^{31,32} Carcinoma-associated fibroblasts generate tensile forces within the ECM that support favorable cues for tumor invasion. Albregues et al demonstrated that sustained carcinoma-associated fibroblast activation appears driven by epigenetic modification of the *STAT3* gene with constitutive activation of the JAK1/STAT3 signaling pathway.³³ Interestingly, 7-day stimulation with inflammatory cytokines was sufficient to confer a long-term activated phenotype to normal fibroblasts even after withdrawal of cytokines. This activated phenotype could be abrogated by treatment combining a reagent targeting the JAK1/STAT3 pathway (ruxolitinib) with a reagent inhibiting epigenetic modification (DNA methylation inhibitor 5-Aza). Results in both alternate disease models support the role of the local inflammatory milieu in the reprogramming of resident tissue fibroblasts, as well as the potential for the reprogrammed fibroblasts to be restored to their native phenotype via targeted medical therapies.³²

One unique finding of our study is that iSGS scar fibroblasts themselves amplify the initial inflammatory IL-17A signal via production of myeloid-recruiting chemokines and cytokines. Myeloid cells are key effector cells during inflammatory processes,³⁴⁻³⁷ infiltrating sites of inflammation in response to chemokines and then differentiating into inflammatory subtypes guided by local cytokine signals.^{34,38} Myeloid cell migration was implicated in fibrosis in a murine model of laryngotracheal stenosis,²⁵ and studies of

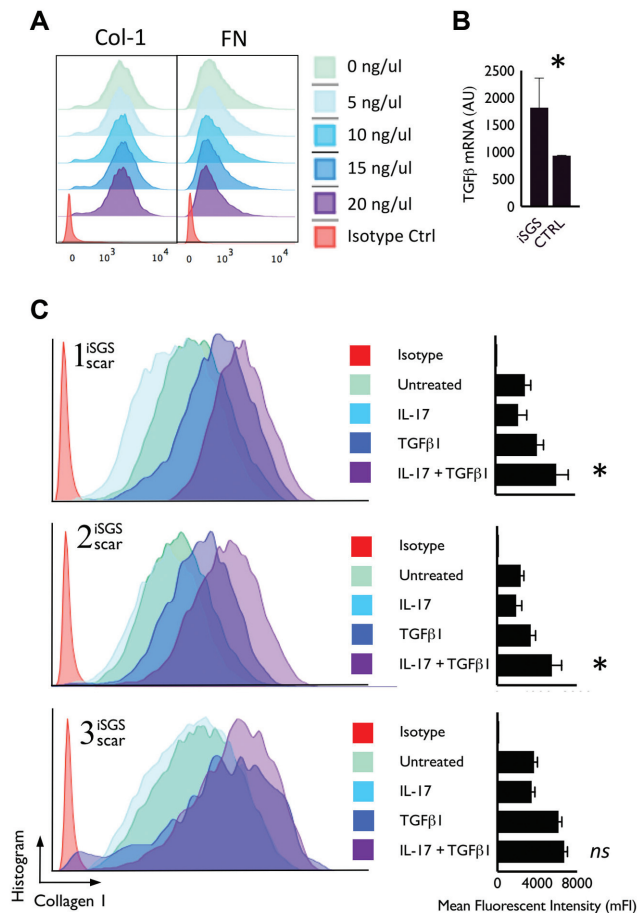


Figure 4. TGF- β synergizes with IL-17A to drive fibroblast collagen production in iSGS. (A) Flow cytometry levels of intracellular Col-1 and fibronectin iSGS of fibroblasts cultured with IL-17A. (B) qPCR levels of TGF- β mRNA in iSGS airway scar and controls. (C) Flow cytometry levels of Col-1 in iSGS fibroblasts from 3 unique donors, cultured with control media, IL-17A, TGF- β , or IL-17A and TGF- β . Values are presented as mean \pm SD. * P < .05. Col-1, type I collagen; Ctrl, control; FN, fibronectin; IL-17A, interleukin 17A; iSGS, idiopathic subglottic stenosis; qPCR, quantitative polymerase chain reaction; TGF- β , transforming growth factor β .

laryngeal stenosis demonstrated that interactions between activated myeloid cells and fibroblasts promote inflammation associated with mucosal scarring.³⁹ In a murine model of myocarditis, IL-17A induced GM-CSF production from cardiac fibroblasts, which then directed the proinflammatory differentiation of monocytes, resulting in cardiac fibrosis.²² Mechanistically, GM-CSF was responsible for the initial histologic inflammation, but IL-17A and GM-CSF were both necessary for tissue fibrosis.

Our results suggest cooperative effects of IL-17A and TGF- β 1 on ECM deposition. These findings are consistent with animal studies delineating cooperative and amplifying roles of IL-17A and TGF- β 1 in the development of airway fibrosis,¹⁷ as well as human studies supporting a critical role of IL-17A in the pathogenesis of obliterative bronchiolitis.¹⁹ Our findings are also consistent with the known role of

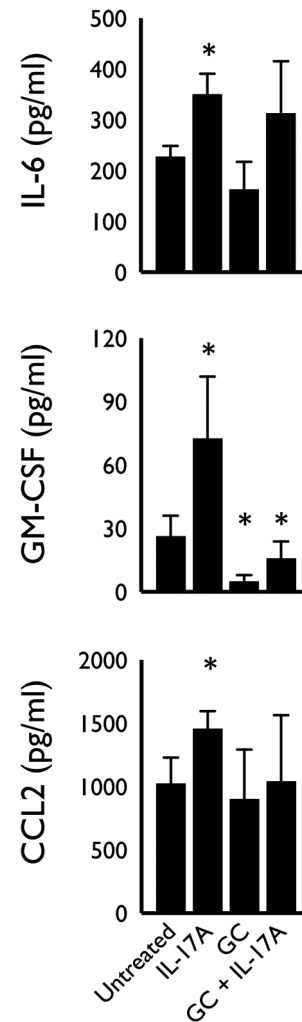


Figure 5. IL-17A induces myeloid chemokines and cytokine production from iSGS fibroblasts. ELISA analysis of IL-1, GM-CSF, and CCL2 levels of iSGS fibroblasts cultured with IL-17A, GC, or IL-17A and GC. Values are presented as mean \pm SD. * P < .05. CCL2, chemokine ligand 2; ELISA, enzyme-linked immunosorbent assay; GC, glucocorticoid; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; iSGS, idiopathic subglottic stenosis.

TGF- β 1 in airway remodeling seen in animal models^{40,41} and clinical studies of human subglottic stenosis.⁴² With previous findings, our results support the role of resident tissue fibroblasts in integrating complex inflammatory signals within the local tissue environment.

Scar modulation occurs over 3 months, and ECM turnover also continues in the longer term. Given the longer time course involved with *in vivo* scar remodeling, 1 potential limitation of our *in vitro* experiments is that the effects of TGF- β 1 and IL-17A over 5 days in culture may be different from responses in human tissue over longer time frames. Exploration of phenotypic and molecular changes in iSGS scar fibroblasts over longer periods of cytokine exposure is a question requiring future study.

Given the rarity of iSGS as a disease entity, our results are based on a small number of individuals. The use of distinct biologic replicates and the use of healthy and alternative disease controls help to mitigate this bias. Ultimately, our results will require confirmation among larger cohorts of subjects with iSGS pooled from multiple institutions. Ongoing study within the North American Airway Collaborative in a 1000-patient cohort (iSGS¹⁰⁰⁰) will facilitate this endeavor. Another important limitation is that this study does not illuminate the inciting event behind the dramatic increase in IL-17A in the subglottic scar of patients with iSGS. Mechanistic understanding is currently limited by the lack of an animal model replicating the unique features of iSGS. Our prior work suggests that $\gamma\delta$ T cells are a source of IL-17A in subglottic scar among iSGS subjects.⁶ However, the primary stimulus for $\gamma\delta$ T-cell recruitment and activation remains a fundamental question demanding further investigation. Despite the female preponderance seen in iSGS, estradiol did not appear to drive ECM production alone or in combination with IL-17A (Supplemental Figure S1, in the online version of the article). As such, why iSGS is a disease nearly universally restricted to adult Caucasian women remains opaque. The role of genetics and the influence of estrogen on host inflammation and fibrosis in iSGS are important questions outside the narrow scope of this work, but they demand future study.

Several implications arise when the results of our study are examined in concert with prior work on the pathologic fibroblast phenotype in postintubation airway scar. Our results help conceptualize (1) therapies aimed at “quieting” the inflammatory milieu that promotes fibroblast reprogramming, (2) treatments seeking to limit the downstream effects of the reprogrammed fibroblasts, (3) therapies attempting to restore native fibroblast phenotype, and (4) treatments that remove the offending reprogrammed fibroblasts in their entirety. Interestingly, our data do show transient abrogation of GM-CSF production after exposure to glucocorticoids. This finding may account for the reported clinical benefit derived from serial local glucocorticoid application in iSGS.⁴³⁻⁴⁵ Importantly, our results also explain the durability seen in surgical therapies for iSGS, such as the established cricotracheal resection⁴⁶ or the investigational “Maddern” procedure (personal communication, Robert R. Lorenz, MD and Guri Sandhu, MBBS, MD, July 2018), which remove the affected subglottic scar, thereby extirpating diseased fibroblasts responsible for amplifying pathologic inflammation.

Conclusion

This study offers evidence supporting a mechanistic role for IL-17A in the pathogenesis of airway fibrosis in iSGS. Coupled with prior work showing a unique local upregulation of IL-17A in iSGS mucosal scar, the results confirm that IL-17A directly drives iSGS scar fibroblast proliferation and synergizes with TFG- β 1 to promote ECM production in vitro. Excitingly, the data also suggest that rather than acting simply as end effectors of inflammation, iSGS scar fibroblasts appear to shape and amplify the local inflammatory

response via their own production of chemokines and cytokines. Future work to delineate the precise molecular alterations occurring in airway scar fibroblasts and to define therapies that reverse these changes has the potential to transform our treatment of airway fibrosis.

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Author Contributions

Robert J. Morrison, performed data analysis, drafted the initial manuscript, critically revised manuscript, and approved the final manuscript as submitted; **Nicolas-George Katsantonis**, carried out the project methods, performed data analysis, critically revised the manuscript, and approved the final manuscript as submitted; **Kevin M. Motz**, carried out the project methods, performed data analysis, critically revised the manuscript, and approved the final manuscript as submitted; **Alexander T. Hillel**, conceptualized and designed the project, critically revised the manuscript, and approved the final manuscript as submitted; **C. Gaelyn Garrett**, conceptualized and designed the project, critically revised the manuscript, and approved the final manuscript as submitted; **James L. Netterville**, conceptualized and designed the project, critically revised the manuscript, and approved the final manuscript as submitted; **Christopher T. Wootten**, conceptualized and designed the project, critically revised the manuscript, and approved the final manuscript as submitted; **Susan M. Majka**, conceptualized and designed the project, performed data analysis, critically revised the manuscript, and approved the final manuscript as submitted; **Timothy S. Blackwell**, conceptualized and designed the project, performed data analysis, critically revised the manuscript, and approved the final manuscript as submitted; **Wunder P. Drake**, conceptualized and designed the project, performed data analysis, critically revised the manuscript, and approved the final manuscript as submitted; **Alexander Gelbard**, conceptualized and designed the project, carried out the project methods, performed data analysis, critically revised the manuscript, and approved the final manuscript as submitted.

Disclosures

Competing interests: Alexander T. Hillel, Olympus, USA—consultant.

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Supplemental Material

Additional supporting information is available in the online version of the article.

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