Eosinophil-fibroblast interactions induce fibroblast IL-6 secretion and extracellular matrix gene expression: Implications in fibrogenesis

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Background: Eosinophils are frequently associated with tissue remodeling and fibrosis in allergic and other diseases and animal models. Their close physical proximity to fibroblasts at sites of tissue remodeling strongly implicates them in fibrogenesis, including subepithelial fibrosis and airway remodeling characteristic of asthma.

Objective: To identify the mediators and characterize the mechanisms underlying the fibrogenic activities of eosinophils.

Methods: A coculture system of blood eosinophils or eosinophil cell lines with normal fibroblasts was used to assess their ability to induce a fibrogenic fibroblast phenotype, including IL-6 secretion and mRNA expression, and induction of genes involved in extracellular matrix production and homeostasis. The mediators of these responses were identified by using transwell barrier cocultures, eosinophil-conditioned media, and cytokine-specific antibody neutralization.

Results: Eosinophil-fibroblast coculture induced potent fibroblast IL-6 secretion and mRNA expression, responses further enhanced by IL-5. The soluble nature of the eosinophil-derived mediators was demonstrated by using eosinophil-fibroblast coculture in the presence of permeable transwell barriers, and fibroblast culture in eosinophil-conditioned media, indicating that cell contact was not required. Induction of fibroblast IL-6 expression was accompanied by increased expression of fibronectin and the extracellular matrix regulatory genes plasminogen activator inhibitor 1 and tissue inhibitor of metalloproteinase 1. Antibody neutralization identified the principal eosinophil-derived mediator of fibroblast IL-6 expression as IL-1β (>60%), with lesser contributions from IL-1α, IL-4, and TGF-β (10% to 20%).

Conclusion: Eosinophils express at least 2 potent mediators (IL-1β and TGF-β) that induce a fibrogenic fibroblast phenotype, strongly supporting a role for the eosinophil in the dysregulation of extracellular matrix homeostasis and consequent tissue remodeling and fibrosis in eosinophil-associated diseases. (J Allergy Clin Immunol 2005;116: 796-804.)

Key words: *Eosinophil, fibrosis, IL-1α, IL-1β, IL-5, IL-6, TGF-β, fibronectin, PAI-1, TIMP-1*

Tissue fibrosis is characterized by the pathologic accumulation of extracellular matrix (ECM) in response to tissue damage, a variety of infectious processes, and dysregulated host inflammatory responses. Eosinophils are hypothesized to play a significant role in mediating tissue remodeling and fibrosis in eosinophil-associated diseases that involve pathological tissue fibrosis. For example, eosinophil-associated diseases such as asthma,[1] eosinophil myalgia syndrome,[2] eosinophilic endomyocardial fibrosis,[3] idiopathic pulmonary fibrosis,[4] scleroderma,[2] and eosinophilic esophagitis[5] all involve tissue remodeling and fibrosis. Flood-Page et al[6] have shown that a reduction in bronchial mucosal eosinophils induced by anti–IL-5 antibody (mepolizumab; GlaxoSmithKline, Middlesex, United Kingdom) significantly decreased the expression of the ECM proteins tenascin, lumican, and type III collagen in the bronchial reticular basement membrane of patients with asthma, and recent studies by Humbles et al[7] using the murine asthma model in eosinophil-deficient mice further support a role for the eosinophil in allergic airways remodeling. However, the mechanisms by which eosinophils and their cytokine and granule protein mediators of inflammation alter normal fibroblast function and phenotype leading to tissue fibrosis have not been fully elucidated.

We previously reported that eosinophil granule major basic protein (MBP) synergizes with TGF-β or IL-1 primed human lung fibroblasts to induce significant increases in gene transcription and secretion of members of the IL-6 family of inflammatory cytokines including IL-6, IL-11, and leukemia inhibitory factor (LIF).[8] Evidence strongly implicating IL-6 in tissue remodeling and fibrogenesis includes findings that its expression is highly

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Abbreviations used
ECM: Extracellular matrix
ECP: Eosinophil cationic protein
EDN: Eosinophil-derived neurotoxin
MBP: Eosinophil granule major basic protein
NF-κB: Nuclear factor-κB
PAI-1: Plasminogen activator inhibitor-1
TIMP: Tissue inhibitor of metalloproteinase

correlated with fibrosis in tissues such as the liver,[9] skin,[10] and lung,[11] the development of subepithelial fibrosis in transgenic mice with targeted overexpression of IL-6 in the lung,[12] and reduction of fibrosis in IL-6 null (knockout) mice.[13] TGF-β–induced fibroblast secretion of IL-6, in what is described as an autocrine loop, is implicated in the production of collagens, tissue inhibitor of metalloproteinases (TIMPs), and glycosaminoglycans in fibrogenesis.[14] Growth factors and cytokines such as platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), nerve growth factor (NGF), and TNF-α, and cytokines including IL-1β, IL-4, IL-11, and IL-13, have also been implicated in studies of airway remodeling, or fibrogenesis in various tissues including lung, skin, kidney, and liver.[15-17] Although many of these cytokines are expressed by eosinophils,[18] their participation in eosinophil-mediated fibrogenesis has not been thoroughly investigated.

In the current work, we have characterized eosinophil-fibroblast interactions in terms of the eosinophil-derived mediators responsible for fibroblast expression and secretion of the profibrogenic cytokine IL-6, and increased expression of fibronectin and inhibitors of matrix metalloproteinases, responses that can lead to the pathologic dysregulation of ECM homeostasis and tissue fibrosis. We have identified IL-1β, IL-1α, TGF-β, and possibly IL-4 as eosinophil-secreted mediators that may be responsible for the tissue remodeling and fibrosis seen in many eosinophil-associated diseases.

METHODS

Purification of blood eosinophils

Eosinophils were purified by magnetic-activated cell sorting as described previously[19] from the blood of normal, nonallergic, healthy donors with informed consent according to guidelines established by the Institutional Review Board of the University of Illinois at Chicago. The purity and viability of the eosinophils was routinely >98%.

Eosinophil-fibroblast cocultures, culture supernatants, and eosinophil-conditioned media

Two different human fibroblast sources (skin and lung) were used to confirm eosinophil coculture findings in more than 1 tissue type. Primary foreskin fibroblasts, isolated as described previously,[20] and the lung-derived fibroblast cell line CCL-202 (ATCC) were grown in Eagle's Minimum Essential Medium supplemented with 10% FBS, 1 mmol/L L-glutamine, 1% vitamins (Gibco, Carlsbad, Calif), and 1% penicillin/streptomycin (Gibco). Purified blood eosinophils, the eosinophil-committed myeloblast cell line AML14, and the differentiated eosinophil myelocyte cell line AML14.3D10[21] were cultured in RPMI 1640 media supplemented with 8% FBS, 2 mmol/L sodium pyruvate, 1 mmol/L L-glutamine, 50 μmol/L β-mercaptoethanol, and 1% penicillin/streptomycin. Unless otherwise stated, all coculture experiments were performed as follows: AML14, AML14.3D10 (0.5×10^6/mL), or purified blood eosinophils (1×10^6/mL) were added to confluent cultures of lung or skin fibroblasts and cocultured with or without IL-5 (2.5 ng/mL) for 24 hours. Culture supernatants were harvested and centrifuged at 300*g* for 5 minutes to remove any residual eosinophils, and the cell-free fibroblast culture supernatants were assayed for IL-6 by using an IL-6 ELISA kit (R & D Systems, Minneapolis, Minn). Eosinophil conditioned media were prepared from purified blood eosinophils (1×10^6/mL), AML14 myeloblasts, or AML14.3D10 eosinophils (5×10^5/mL) cultured for 24 hours at 37°C in the presence or absence of IL-5 (2.5 ng/mL).

Transwell barrier cultures

Experiments were performed by using Transwell plates (Costar; Corning Inc., Corning, NY) with 0.4-μm pores. Fibroblasts were seeded and cultured in EMEM complete media without Transwell inserts in 6-well tissue culture plates to confluence. Transwell inserts containing 5×10^5 AML14.3D10 eosinophils/mL in RPMI complete media were cocultured with the confluent fibroblasts at 37°C for 24 hours, and the supernatant below the transwell plate was collected and assayed for fibroblast IL-6 secretion by ELISA.

Antibody neutralization of eosinophil-conditioned media

Antibodies were mixed with eosinophil-conditioned media and incubated at room temperature for 1 hour before adding the conditioned media to confluent fibroblast cultures. The antibodies included neutralizing antigen affinity purified antibodies (R & D Systems; αFGF [basic], αIL-1α, αGM-CSF, αNGF), neutralizing protein G-affinity purified IgG (R & D Systems; αTGF-β, αIL-1β, αTNF-α, αGM-CSF, αIL-4); Western blotting protein G purified antibodies (generated in-house; αMBP, α eosinophil-derived neurotoxin [EDN; rabbit], α eosinophil cationic protein [ECP]), and αEDN (mouse monoclonal; kindly provided by Drs Gerald Gleich and Hirohito Kita). Fibroblast culture supernatants were harvested after 24 hours and assayed for IL-6 secretion by ELISA.

RT-PCR analyses

Total fibroblast RNA was isolated for standardized semiquantitative RT-PCR by using Ultraspec RNA Isolation kits (Biotex, Houston, Tex). cDNA synthesis was performed by using a First Strand cDNA Synthesis Kit (Pharmacia, Piscataway, NJ). The linear range for amplification conditions (both cycle number and cDNA input) for each set of primer pairs was first determined to establish linear relationships between RNA (cDNA) input and amplified PCR product (see Table E1 in the Online Repository in the online version of this article at www.jacionline.org). The composition of the PCR reaction mixtures and amplification protocols for each primer set are available in the Online Repository (see Methods in the online version of this article at www.jacionline.org). The expression levels for all genes of interest were normalized to the glyceraldehyde-3-phosphate dehydrogenase signal for data analysis and presentation (see Methods in the Online Repository in the online version of this article at www.jacionline.org).

Statistical analyses

Results are expressed as means ± SDs unless otherwise indicated. The differences between means were assessed for statistical significance by using 1-way ANOVA, followed by post hoc analyses using