Letter to the Editor

Eosinophil-degranulation products drive a proinflammatory fibroblast phenotype

To the Editor:

Severe asthma is characterized by persistent airflow obstruction and ongoing symptoms despite intense corticosteroid treatment. Asthma severity is associated with airway inflammation and increased numbers of neutrophils and eosinophils.[1,2] Although neutrophilia can be driven by type 17 immune responses, the mechanisms that lead to recruitment and persistence of neutrophils in asthmatic airways remain unknown. Eosinophils contribute to asthma pathobiology by releasing granule proteins and additional factors including cytokines and chemokines that can activate other cell types, such as bronchial fibroblasts.[3,4] Although bronchial fibroblasts contribute to airway remodeling in asthma by depositing extracellular matrix proteins, their interactions with eosinophils are not well characterized.[4] We recently performed RNA sequencing on parenchymal lung fibroblasts stimulated with activated-eosinophil–conditioned media.[5] Pathway analysis revealed a gene expression profile enriched in genes involved in neutrophil recruitment and activation.[5] In the present study, we sought to expand on these investigations using *ex vivo* cell culture models relevant to asthma pathogenesis. Specifically, our goals were to determine the effect of eosinophil-degranulation products on primary human bronchial fibroblasts (HBFs) and neutrophil recruitment.

A recent report found the presence of IgG complexed with eosinophil peroxidase in eosinophilic asthma,[6] suggesting that this can stimulate eosinophil degranulation. We have developed a model of aggregated IgG-induced eosinophil degranulation by priming human circulating eosinophils with IL-3 for 20 hours,

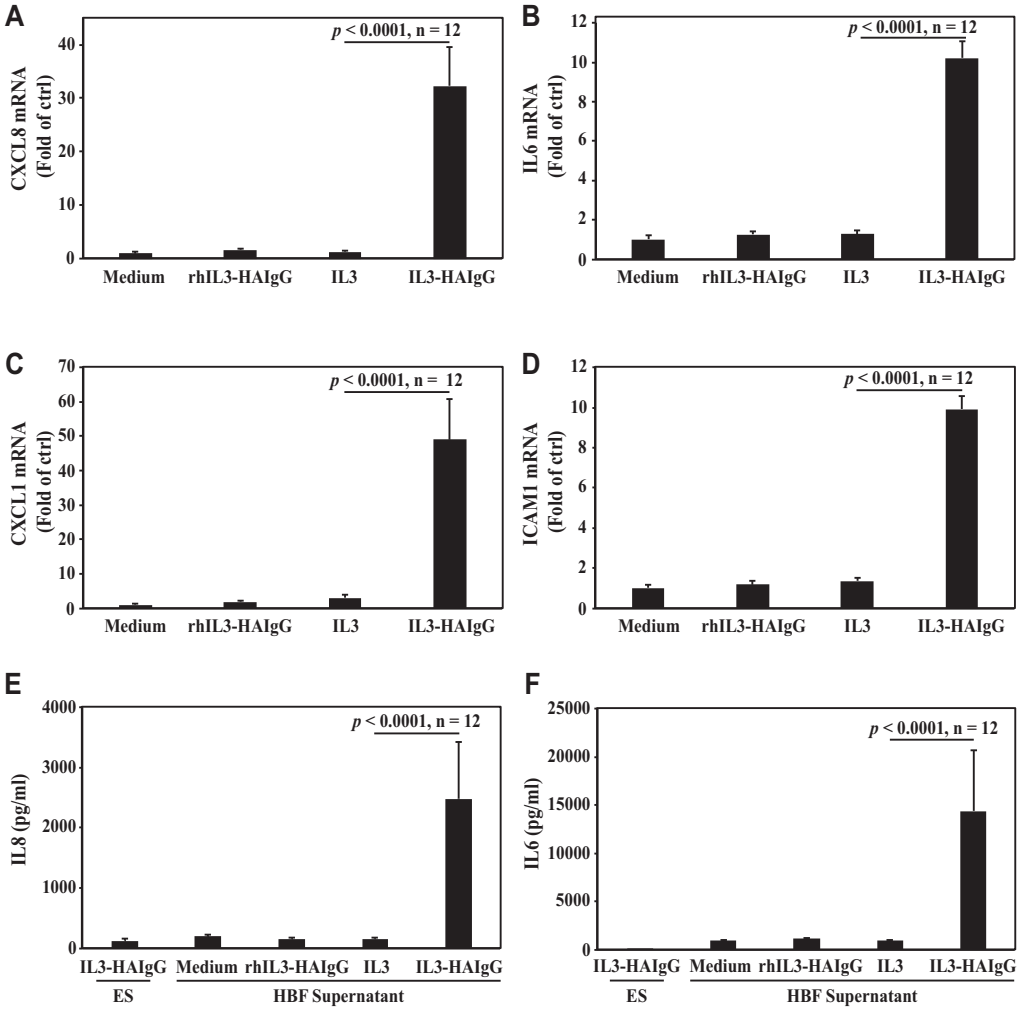

FIG 1. HBFs stimulated by IL-3-HAIgG–activated eosinophils express chemokines important for neutrophil migration. HBFs stimulated with IL-3-HAIgG ES, IL-3 ES, rhIL-3-HAIgG, or growth medium for 24 hours **(A-D)** or 72 hours **(E** and **F)**. mRNA levels of CXCL8 (Fig 1, *A*), IL-6 (Fig 1, *B*), CXCL1 (Fig 1, *C*), and ICAM1 (Fig 1, *D*) in HBFs. IL-8 (Fig 1, *E*) and IL-6 (Fig 1, *F*) ELISA of supernatant from HBFs or from IL-3-HAIgG ES alone. *Ctrl*, Control; *CXCL1*, chemokine (C-X-C motif) ligand 1; *CXCL8*, chemokine (C-X-C motif) ligand 8; *ICAM1*, intercellular adhesion molecule 1; *rhIL-3-HAIgG*, recombinant human IL-3 plus soluble HAIgG.

followed by plating on heat-aggregated human serum IgG (HAIgG) for 6 hours (IL-3-HAIgG condition) or cultured without IgG (IL-3 condition).[5,7] In the current study, we obtained circulating blood eosinophils from allergic subjects (see Table E1 in this article's Online Repository at www.jacionline.org), activated them using the above approach, and collected eosinophil supernatants (ESs) to measure the concentration of eosinophil-derived neurotoxin (EDN), a marker of degranulation (see Fig E1 in this article's Online Repository at www.jacionline.org). There was an approximate 10-fold increase in EDN released following IL-3-HAIgG treatment compared with IL-3 alone (Fig E1), indicating strong degranulation on complexed IgG. ES was then collected from activated eosinophils (IL-3-HAIgG ES), mixed 1:1 with serum-free media, and used to stimulate primary cultures of HBFs from donors without allergy or asthma.

In the first set of experiments, 4 different HBF lines were stimulated with IL-3-HAIgG ES from 4 different eosinophil donors. Eosinophil medium alone, medium with 1 ng/mL IL-3 and 0.5 μg/mL HAIgG (recombinant human IL-3 plus soluble HAIgG), and ESs from IL-3–treated eosinophils (IL-3 ESs) were used as controls.[5] After 24 hours of incubation, we observed a significant increase in the expression of 4 proneutrophilic genes, chemokine (C-X-C motif) ligand 8, *IL6*, chemokine (C-X-C motif) ligand 1, and intercellular adhesion molecule 1 by quantitative PCR in HBFs treated with IL-3-HAIgG ES, compared with controls (Fig 1, *A-D*).[5,8] This was consistent with our previous findings in parenchymal lung fibroblasts.[5] We then assessed HBF secretion of the proneutrophilic chemokines IL-8 and IL-6 after 72 hours by ELISA. In collected supernatants, there was a significant increase in the release of both IL-8 and IL-6 by HBFs stimulated with IL-3-HAIgG ES compared with control conditions (Fig 1, *E* and *F*). Notably, IL-8 and IL-6 concentrations in the IL-3-HAIgG ES alone were low (Fig 1, *E* and *F*), indicating that HBFs are the predominant source of IL-8 and IL-6 in this system. Interestingly, the induced production of IL-8 by fibroblasts was not strongly associated with levels of either EDN ($r = 0.37$; $P = .29$; n = 10) or IL-8 ($r = 0.36$; $P = .30$; n = 10) in the IL-3-HAIgG ES. This suggests that other eosinophilic mediator(s) besides granule proteins or IL-8 stimulate IL-8 production by HBFs.

Consequently, we sought to determine whether HBF-conditioned media would modify neutrophil chemotaxis. Again, we used IL-3-HAIgG ES from 4 different donors to activate HBFs from 4 different donors for 72 hours. HBF-conditioned media was then collected and used as a stimulus for a transwell-based neutrophil chemotaxis assay. Controls included HBF growth medium (negative control), IL-3-HAIgG ES alone, and HBF basal medium supplemented with recombinant IL-8 (positive control). There was a significant increase in neutrophil chemotaxis in response to conditioned media from IL-3-HAIgG ES–stimulated-HBFs compared with controls (Fig 2, *A*). Importantly, we found a clear correlation between IL-8 released in the HBF-conditioned media and neutrophil chemotaxis ($r = 0.94$; $P < .0001$; n = 12, Fig 2, *B*), indicating that IL-8 is a marker for chemotactic activity generated by these media. Importantly, IL-3-HAIgG ES alone did not induce neutrophil movement above baseline, suggesting that the fibroblast-derived soluble factors are critical for neutrophil recruitment. Altogether, these data show that HBF exposed to IL-3-HAIgG ES release *de novo* soluble factors that efficiently induce neutrophil chemotaxis.

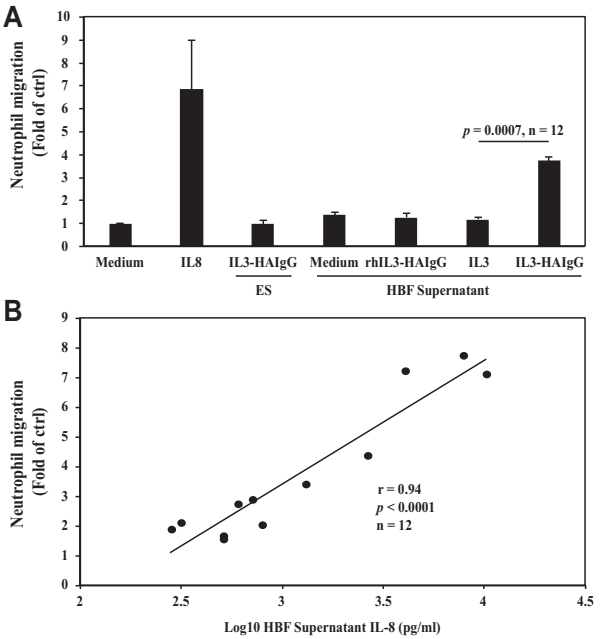

FIG 2. Supernatants from IL-3-HAIgG ES–stimulated HBFs induce increased neutrophil chemotaxis. **A,** Transwell neutrophil migration in response to indicated HBF supernatants, IL-3-HAIgG ES, 30 ng/mL IL-8, or growth medium. **B,** Correlation of transwell neutrophil migration to IL-8 content in IL-3-HAIgG ES–stimulated HBF supernatants. *Ctrl,* Control.

In summary, this study used a physiologically relevant *ex vivo* model of eosinophil degranulation to explore the role of eosinophil-degranulation products in the release of neutrophilic mediators by primary HBFs. We have observed an amplification of neutrophil recruitment by eosinophil-fibroblast interaction, which has not been reported previously. HBFs have a known role in matrix deposition in asthma, but there is little information on how fibroblasts modify airway inflammatory responses. Inflammatory signaling from parenchymal or vascular lung fibroblasts is not without precedent in pulmonary disorders,[9] and our work suggests that HBFs participate in the inflammatory response through neutrophil recruitment.

Although we used *ex vivo* cell culture models, a strength of this study is the use of primary cultures of human cells from physiologically relevant sources. Eosinophils were obtained from allergic donors, HBFs from airway biopsies, and neutrophils from human whole blood samples. Eosinophil priming and degranulation was modeled using prolonged IL-3-exposure followed by plating on HAIgG,[7] which is meant to recapitulate the increased IL-3 levels found in bronchoalveolar lavage fluid after an *in vivo* allergen challenge,[7] followed by exposure to high IgG levels that are present in airways of patients with asthma.[6] Eosinophil stimulation using this model results in stronger degranulation and amplified release of proinflammatory and profibrotic mediators compared with plating on HAIgG alone, IL-5 priming and plating on HAIgG, or IL-3 stimulation alone.[5,7] Thus, this experimental design appears to be relevant to the *in vivo* biology that drives asthma pathology.

In conclusion, using an *ex vivo* model of eosinophil degranulation, we have found that HBFs release IL-6 and IL-8, and promote neutrophil migration. These findings suggest that the interplay between eosinophil-derived soluble mediators and HBFs may be essential for recruiting neutrophils to the airway