CONCISE REPORT

Eosinophils Stimulate Fibroblast DNA Synthesis

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Fibrosis complicates a number of chronic inflammatory diseases. This observation has motivated investigations of the relationship between inflammatory cells and fibroblasts in fibrogenesis. Some soluble products of chronic inflammatory cells stimulate in vitro a variety of fibroblast functions that are considered important factors in scar formation. Mononuclear phagocytes and lymphocytes, when appropriately activated, secrete factors that can promote fibroblast growth as well as synthesis and secretion of collagen, fibronectin, collagenase, and prostaglandins. 1

Tissue fibrosis has also been observed in selected conditions following chronic hypersinusophilic syndromes in which peripheral blood eosinophilia and tissue eosinophilia occur. 2 However, the relationship between eosinophilia and fibrosis has not been explored. Accordingly, we assessed whether eosinophils, like macrophages and lymphocytes, might be a source of fibrogenic factors. We now report the presence of eosinophil-derived factor(s) that stimulate fibroblast DNA synthesis in vitro. This finding draws attention to a previously unappreciated source of fibroblast stimulating factors that may participate in fibrogenesis. Our findings also suggest an additional potential role for the eosinophil, a cell whose functions are still incompletely understood.

MATERIALS AND METHODS

Preparation of leukocytes. Human eosinophils were purified on discontinuous metrizamide gradients (Accurate Chemical, Westbury, NY) as previously described. 3 In brief, heparinized (10 U/mL of preservative-free heparin, A.H. Robbins, Richmond, VA) blood drawn from healthy normal volunteers was mixed with 4.5% Dextran (T500, Pharmacia Laboratories, Piscataway, NJ, or Dextran 150,000, Sigma Chemical, St Louis) in 0.15 mol/L of phosphate-buffered saline (PBS, pH 7.4) and was allowed to sediment for 30 minutes at 37°C. The leukocyte-rich plasma was removed and washed twice with Eagle’s minimal essential medium (MEM, GIBCO, Grand Island, NY) containing 100 U/mL of penicillin, 100 μg/mL of streptomycin, 10% heat-inactivated fetal calf serum (FCS, Flow Laboratories, Rockville, MD) and 2 mmol/L of l-glutamine. The cells were resuspended to 1 x 10^6/mL and layered on discontinuous gradients. After centrifugation, the fractions were counted and pooled. Preparations contained >85% eosinophils; 15% were exclusively neutrophils. For comparison studies between eosinophils and neutrophils, neutrophils were harvested from 23% hypereosinophilic syndromes in animals receiving 1 mg of polymyxin B intraperitoneally every week, as previously described. 4 Peritoneal exudates were collected, washed in PBS, and layered on discontinuous metrizamide gradients. Preparations contained >95% eosinophils. Trypan blue dye exclusion revealed >95% viability of both human and guinea pig cells. Preparations of cells extracts. Leukocytes were washed three times and suspended in Hanks’ balanced salt solution (HBSS). Cells were subjected to six freeze–thaw cycles, and the cellular debris was removed by centrifugation at 100 g for 15 minutes. Supernatants were dialyzed against MEM prior to addition to fibroblast cultures. For studies of granular contents, eosinophils suspended at 5 x 10^6/mL were homogenized in 0.34 mol/L of sucrose. Cellular debris was removed by centrifugation at 1,000 g for 10 minutes. The granules were then pelleted by centrifugation, resuspended in PBS, and ruptured by repeat freeze–thaw cycles. Experiments using cell-free supernatants were prepared from resting eosinophils, as previously described. 3 In brief, eosinophils suspended in HBSS to a concentration of 2 x 10^6/mL were incubated for 1 hour at 37°C. Cells were removed by centrifugation, and the supernatants were dialyzed at 4°C overnight against RPMI 1640 medium (nominal exclusion 1,000 kd). Cell viability was >98% by trypan blue dye exclusion.

Fibroblast cultures and proliferation assay. Human fibroblast cultures were obtained from newborn foreskins and processed by our previously published methods. 2 Confluent cultures were passaged following treatment with trypsin and EDTA. Fibroblasts were used in assays between passages 4 and 12 according to our published methods. 5 In brief, cells suspended in RPMI 1640 medium supplemented with antibiotics and 10% heated FCS were plated in 24-well polystyrene plates (Costar, Cambridge, MA) at a density of 5 x 10^4/well and incubated overnight at 37°C. Cells were washed, and cultures were replenished with serum-free medium. Twenty-four hours later, stimuli were added for an additional 24 hours, the last four with 1.0 μCi/well (final vol 1.0 mL) of [3H]thymidine (specific activity 6.7 Ci/mmol, New England Nuclear). Cells were harvested on glass fiber filters and counted in a scintillation counter in the presence of scintillation cocktail. Means ± SEM of triplicate samples were determined. Data are expressed as the percentage of the untreated control to minimize variation due to differences in the control recorded in different experiments.

RESULTS

Cell-free extracts of human and guinea pig cell populations highly enriched for eosinophils were prepared by

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freeze–thaw and centrifugation and contained substances which stimulated [³H]thymidine incorporation by human fibroblasts (Fig 1A). Fibroblast response was dose dependent. Maximum stimulation was observed with dilutions of 1:5 of human eosinophil extract and 1:10 of guinea pig eosinophil extract. We also observed that eosinophils can spontaneously release fibroblast-stimulating activity. Supernatants derived from resting eosinophils stimulated fibroblast proliferation (Fig 1B).

In related studies, we prepared extracts of isolated eosinophil and neutrophil granules and assessed their effects on human fibroblasts (Fig 1C). Eosinophil granule extracts stimulated fibroblast [³H]thymidine uptake at all concentrations tested. In contrast, neutrophil granule extracts were inhibitory to fibroblasts. In the presence of neutrophil granule extracts, the magnitude of [³H]thymidine incorporation was below that observed in fibroblasts grown in medium alone. This inhibitory effect was not observed with more dilute (1:500) extracts, and no significant stimulation was observed.

**DISCUSSION**

Our results indicate that eosinophils contain factor(s) that can stimulate fibroblast [³H]thymidine incorporation which, under the conditions we use, is a measure of fibroblast DNA synthesis and proliferation. Fibroblast-stimulating activity may be specifically localized to the granule compartment (Fig 1C). This does not exclude the possibility that cytoplasmic fibroblast-stimulating factors may also exist. Nonetheless, that isolated neutrophil-derived granules lack this activity indicates a distinct difference between these granulocyte populations. Furthermore, our ability to detect fibroblast stimulating activity in supernatants of apparently viable eosinophils (Fig 1B) suggests that these cells may spontaneously elaborate low levels of such factors, possibly by degranulation. Promotion of eosinophil degranulation by physiological agents such as interleukin 1 or antibody-mediated adherence to helminths may also result in elaboration of the fibroblast-stimulating factor(s).

Fibrosis is a frequent complication of certain tissue helminthic infections in which direct interaction of eosinophils and parasites occurs. In schistosomiasis, eosinophils can participate in killing of an infective larval stage and are also present in large numbers in the granulomas that surround the eggs deposited in tissue. These granulomas secrete fibroblast-stimulating factors that are believed to be responsible for subsequent hepatic fibrosis. The findings we report here suggest that eosinophils may be a source of the granuloma-derived factor. In lymphatic filariasis, lymphatic scarring occurs following infiltration of eosinophils around the parasites. On the basis of our findings, we propose that one potential mechanism of lymphatic scarring is release of eosinophil-derived fibroblast-stimulating factor(s). Another pathologic condition in which eosinophils may play a role in fibrosis is endomyocardial fibrosis, a model for which we have previously described in polymyxin B-treated guinea pigs.

We have not yet characterized the eosinophil-derived
fibrogenic factor(s). Eosinophil granules contain major basic protein, cationic protein, and protein X. Because these factors can be cytotoxic and because we have observed no evidence of toxic effects of eosinophil extracts on fibroblasts, these extracts are unlikely candidates for growth factors.

This report adds eosinophils to the increasing list of cells that produce fibroblast growth factors. The histopathologic association between tissue eosinophils and subsequent scar formation in certain parasitic infections and various other diseases suggests a causal relationship. Our findings also suggest a new potential role for eosinophils, cells whose functions remain largely ill-defined.

REFERENCES

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