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Research Article

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Eosinophil Hematopoietins Antagonize the Programmed Cell Death of Eosinophils

Cytokine and Glucocorticoid Effects on Eosinophils Maintained by Endothelial Cell-conditioned Medium

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Abstract

Granulocyte-macrophage colony-stimulating factor (GM-CSF) was established as the constitutive and elicited human umbilical vein endothelial cell-derived eosinophil viability-sustaining factor. Stimulation of endothelium cell monolayers with IL-1 α (5 U/ml) increased the 48-h elaboration of GM-CSF from a mean of 3.2 to a mean of 8.2 pM ($P < 0.05$). Dexamethasone (100 nM) decreased the constitutive GM-CSF elaboration by 49% ($P < 0.001$) but did not diminish production by IL-1 α -stimulated endothelium. However, eosinophil viability decreased by 21% in dexamethasone-pretreated IL-1 α -stimulated endothelial cell-conditioned medium ($P < 0.05$), which suggested viability antagonism by glucocorticoids. After 24 h of culture, eosinophil viability for replicate cells in enriched medium alone or with 1 pM GM-CSF decreased from means of 43 and 75% to means of 21 and 54%, respectively, when dexamethasone was included ($P < 0.05$). However, 10 pM GM-CSF, IL-3, or IL-5 protected the cells against dexamethasone and against endonuclease-specific DNA fragmentation. In this model system of eosinophil-tissue interactions, dexamethasone prevents the endothelial cells from inducing a pathobiologic phenotypic change in the eosinophil by suppression of GM-CSF elaboration to concentrations that are not cytoprotective. Cytokine priming by GM-CSF, IL-3, or IL-5 may account for the differential responsiveness of select eosinophilic disorders to glucocorticoids. (*J. Clin. Invest.* 1991; 88:1982-1987.) Key words: apoptosis • cytokine • cell culture • DNA • interleukin

Introduction

The survival in vitro of normodense human peripheral blood eosinophils for at least 14 d requires their continued exposure to one of three hematopoietins whose genes are located on the short arm of the fifth chromosome: granulocyte-macrophage colony-stimulating factor (GM-CSF),¹ IL-3, or IL-5 (1-4). During the interval of cytokine exposure, the eosinophils undergo an alteration in their physical and functional properties characterized by a decrement in their relative centrifugation density in discontinuous gradients of metrizamide (hypodense), augmented antibody-dependent cytotoxicity against the *Schistosoma mansoni* schistosomula, enhanced elaboration of LTC₄ and generation of superoxide when stimulated with N-formyl-L-methionyl-L-leucyl-L-phenylalanine, and increased size of the cell-associated proteoglycans (1-3, 5, 6). Previously, we have reported that both bovine pulmonary artery or human umbilical vein endothelial cells constitutively elaborate a soluble activity that, in a manner comparable to that of the three eosinophil hematopoietins, maintains the ex vivo viability of normodense eosinophils and primes them for enhanced LTC₄ generation in response to stimulation with calcium ionophore A23187 (1-3, 7). We now report that both the constitutively elaborated eosinophil viability-sustaining activity and the activity from IL-1 α -stimulated endothelial cells are GM-CSF, and that this eosinophil hematopoietin along with IL-3 and IL-5 maintains eosinophil viability by preventing endonuclease-specific DNA degradation.

Although the constitutive elaboration of GM-CSF was attenuated by pretreatment of the endothelium with dexamethasone, such pretreatment had no effect on the IL-1 α -stimulated endothelium. Dexamethasone had an antagonistic effect on the viability of normodense eosinophils cultured in the presence of suboptimal concentrations of GM-CSF, IL-3, and IL-5. In contrast, 10 pM GM-CSF, IL-3, and IL-5, concentrations that are optimal for the 7-d survival of eosinophils in vitro and for converting them to the functionally primed hypodense phenotype (1-3), fully protected the eosinophils from the effect of dexamethasone. Thus, the previous findings of enhanced eosinophil viability and augmentation of effector function during 7 d of culture with GM-CSF, IL-3, and IL-5 (1-3) are extended by the demonstration of a rapid onset cytoprotective action against programmed cell death and against dexamethasone-mediated cytotoxicity. Furthermore, the therapeutic role of dexamethasone in reducing or eliminating the hypodense eosinophil phenotype, which has been associated with certain human eosinophilic disorders, could involve both decreased production of GM-CSF and the consequent diminution in GM-CSF-mediated cytoprotection of eosinophils.

Methods

Culture and stimulation of endothelial cells. Human umbilical vein endothelial cells (passage 13, CRL-1730; American Type Culture Collection, Rockville, MD) were plated onto 75-ml plastic flasks (CoStar, Cambridge, MA) and were cultured in enriched medium (MCDB 110 [Sigma Chemical Co., St. Louis, MO] supplemented with 25 mM Hepes, 0.1% sodium bicarbonate, 2 mM L-glutamine, 100 U/ml penicillin, 100 μ g/ml streptomycin, 100 μ M minimal essential amino acids, 10% FBS [Gibco, Grand Island, NY], 30 μ g/ml endothelial cell growth factor [Boehringer-Mannheim Biochemicals, Indianapolis, IN], and 100 μ g/ml pork heparin) (7). The medium was changed every other day. Just before the cells achieved confluence, they were passaged by incubating the monolayer with 0.05% trypsin containing 0.53 mM

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1. Abbreviation used in this paper: GM-CSF, granulocyte-macrophage colony-stimulating factor.

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EDTA in HBSS without calcium or magnesium for 5 min at 37°C. The cells were washed, resuspended in enriched medium, and plated onto 35-mm plastic tissue culture dishes ($\sim 2.5 \times 10^5$ cells/dish). All experiments were performed after contact-inhibited endothelial cell monolayers had become confluent. For all experiments, passages 14–19 were used. Endothelial cell-conditioned medium was harvested after a 48-h incubation with or without recombinant human IL-1 α (5 U/ml; Genetics Institute, Cambridge, MA) (8) in the presence or absence of dexamethasone (100 nM to 10 μ M dexamethasone 21-phosphate; Sigma Chemical Co., St. Louis, MO). Conditioned medium was filtered in a sterile manner through a 0.45- μ m filter and stored at -70°C until tested.

Isolation of eosinophils and maintenance in culture. Normodense human peripheral blood eosinophils were isolated by the discontinuous metrizamide gradient technique (1–3, 7). The peripheral blood was obtained from nine donors, none of whom was ingesting aspirin, other nonsteroidal antiinflammatory drugs, or corticosteroids. Normodense eosinophils of > 80% purity, as determined by Wright's and Giemsa staining, were used for each experiment. Residual contaminating erythrocytes were eliminated by hypotonic lysis, and neutrophils were the sole contaminating leukocyte. The initial cell viability was > 98%, and the viability of the eosinophils in culture was again determined by their ability to exclude trypan blue (1–3, 7). Freshly isolated eosinophils were suspended in enriched medium (10 6 cells/ml), and 100- μ l cell suspensions were placed into 96-well flat-bottomed microtiter plates (CoStar) containing 100 μ l of enriched medium alone, enriched medium supplemented with defined concentrations of human recombinant GM-CSF, IL-3, or IL-5 (all provided by Genetics Institute), or various concentrations of endothelial cell-conditioned medium. In some experiments, 100 nM dexamethasone, prednisolone, hydrocortisone, and testosterone (all from Sigma Chemical Co.) were included in the eosinophil cultures. The plates were maintained at 37°C in a 5% CO₂ atmosphere.

Antibody neutralization of cytokine activity. For some experiments, various dilutions of a rabbit neutralizing polyclonal antibody against human GM-CSF (Genzyme Corp., Boston, MA) or against human IL-3 (Genetics Institute), or a rat monoclonal antibody against mouse IL-5 (purified from clone TRFK-5 and provided by R. L. Coffman of DNAX Research Institute, Palo Alto, CA) conjugated to cyanogen bromide-activated Sepharose CL-4B beads (Sigma Chemical Co.) were preincubated for 1–2 h at 37°C with conditioned medium or recombinant cytokine before the eosinophils were added (9, 10). In experiments with anti-IL-5, the beads were sedimented by centrifugation of the incubation mixture at 250 g for 5 min at 4°C, and the supernatant was used for subsequent experiments.

Quantitation of GM-CSF levels in conditioned medium. Conditioned medium was prepared from the endothelial cells alone or after stimulation with IL-1 α in the presence or absence of dexamethasone. GM-CSF concentrations in these media were determined by a solid phase ELISA (Genzyme Corp.), which uses the multiple antibody sandwich technique. GM-CSF was detectable in the linear portion of the binding curve at concentrations from 7 to 65 pM using biosynthetic (recombinant) standards. No immunologic cross-reactivity was noted for IL-3 or IL-5 at concentrations of 67 and 77 pM, respectively.

Analysis of DNA fragmentation by eosinophils. Freshly isolated normodense eosinophils ($1.0\text{--}1.5 \times 10^6$ cells) were incubated for various intervals in enriched medium alone, or in enriched medium containing 10 pM GM-CSF, 10 pM IL-3, 10 pM IL-5, 1,000 U/ml tumor necrosis factor- α (TNF α), 100 nM dexamethasone, or combinations of cytokine and glucocorticoid, at 37°C in a 5% CO₂ atmosphere. The eosinophils were counted, pelleted at 4°C, resuspended in 20 μ l of 10 mM EDTA and 50 mM Tris-HCl, pH 8.0, containing 0.5% sodium lauryl sarkosinate and 0.5 mg/ml proteinase K, and incubated for 60 min at 50°C (11). A 10- μ l portion of 0.5 mg/ml ribonuclease A was added to each sample, and the samples were incubated for 60 min more at 50°C. Then, 10 μ l of 10 mM EDTA, pH 8.0, containing 0.25% bromophenol blue and 40% sucrose were mixed with each DNA extract. The individual DNA extracts were loaded into the wells of a 2%

agarose gel containing 3 μ g/ml of ethidium bromide. As a DNA size standard, 2 μ l of PhiX174 RF DNA-Hae III digest (New England Biolabs, Beverly, MA) was loaded into adjacent wells. Electrophoresis was performed at 4°C in 90 mM Tris-HCl, pH 8.3, containing 90 mM boric acid and 2.5 mM EDTA until the marker dye had migrated 6–8 cm. The gel was visualized and photographed in UV light with Polaroid type 57 high-speed film, and a negative image was obtained from the positive.

Statistical analysis. The statistical significance of differences between sample means for each set of eosinophils was based upon comparisons as determined by the two-tailed Student's *t* test.

Results

Effect of endothelial cell-conditioned medium on eosinophil viability. The 3-d survival of normodense peripheral blood eosinophils increased in a dose-dependent manner from 21±6% (mean±SEM, $n = 5$) for cells maintained in enriched medium alone to a maximum of 52±5% for eosinophils maintained in 50% constitutive endothelial cell-conditioned medium ($P < 0.001$). The 3-d survival of eosinophils maintained in 1–50% IL-1 α -stimulated endothelial cell-conditioned medium was significantly increased ($P < 0.05$) at all concentrations above their survival in constitutive endothelial cell-conditioned medium alone. The 3-d viability of eosinophils maintained in 50% IL-1 α -stimulated endothelial cell-conditioned medium was increased from 25±2% to a maximum of 73±0% ($n = 5$). An ED₅₀ of 5 and 3% conditioned medium was observed for replicate eosinophils cultured for 3 d in constitutive-derived endothelial cell-conditioned medium and IL-1 α -stimulated endothelial cell-conditioned medium, respectively. The supplementation of enriched medium with an equivalent concentration of IL-1 α alone did not increase eosinophil viability above that observed with enriched medium alone (25±2%, $n = 5$, and 21±6%, $n = 5$, respectively) ($P > 0.2$).

Identification of the constitutive and IL-1 α -stimulated endothelial cell-derived eosinophil viability-sustaining activity. In preliminary experiments, it was determined that anti-GM-CSF at a concentration of 100 μ g/ml completely neutralized the eosinophil viability-sustaining activity of 10 pM GM-CSF (9). At 100 μ g/ml, anti-GM-CSF did not cross-react with IL-3 and IL-5. Replicate samples of normodense eosinophils were cultured for 3 d in enriched medium alone, or in enriched medium supplemented with 10 pM GM-CSF, 50% endothelial cell-conditioned medium, or 50% IL-1 α -stimulated endothelial cell-conditioned medium. Eosinophil viability was 10±4, 74±4, 44±4, and 68±5% (mean±SEM, $n = 3$), respectively. Preincubation of these same media with 100 μ g/ml of anti-GM-CSF decreased eosinophil survival to 7±2, 10±8, 12±8, and 12±9%, respectively ($P < 0.05$ compared with cultures without antibody) (Fig. 1). In two experiments in which the preincubation of 10 pM IL-3 with anti-IL-3 (1:250 final dilution) decreased the 3-d survival of replicate eosinophils from 82 and 56% with IL-3 alone to 12 and 10%, respectively, the survival of eosinophils maintained in 25% endothelial cell-conditioned medium was unaffected. In similar fashion, preabsorption by anti-IL-5 (20 μ g/ml) of 1 pM IL-5 decreased eosinophil viability from 63 and 63%, to 3 and 5%, respectively. However, the viability of eosinophils maintained with 25% endothelial cell-conditioned medium was unaffected by preabsorption.

Influence of dexamethasone on GM-CSF elaboration by endothelial cells. As quantitated by ELISA, the constitutive generation of GM-CSF by endothelial cells over 48 h was 3.2±0.8

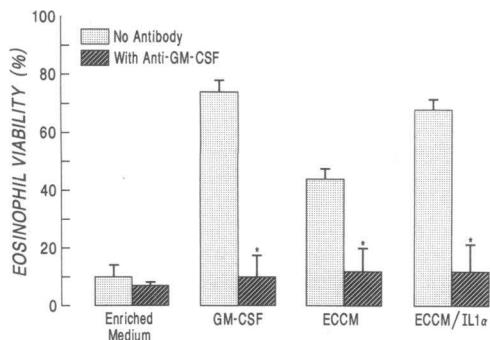


Figure 1. Neutralization by anti-GM-CSF of the eosinophil viability-sustaining activity obtained from enriched medium alone, 10 pM recombinant GM-CSF, 50% constitutive endothelial cell-conditioned medium (ECCM), and 50% IL-1 α -stimulated endothelial cell-conditioned medium (ECCM/IL1 α). The percentage of viable eosinophils after 3 d of culture is expressed as mean \pm SEM for three experiments. *Statistically significant decrease in eosinophil viability for cells cultured with neutralizing antibody compared with replicate cells cultured without antibody ($P < 0.05$).

pM (mean \pm SEM, $n = 4$). After stimulation of the endothelial cells with 5 U/ml of IL-1 α , GM-CSF elaboration increased to 8.2 ± 0.9 pM ($n = 4$). Treatment of replicate unstimulated endothelial cell cultures with 100 nM dexamethasone for 48 h reduced constitutive GM-CSF elaboration by $49 \pm 5\%$ ($n = 4$) to 1.7 ± 0.6 pM ($P < 0.001$). In contrast, dexamethasone (100 nM) treatment for 48 h of replicate IL-1 α -stimulated endothelial cells did not result in a reduction in GM-CSF elaboration, which was maintained at 7.8 ± 0.8 pM. Increasing the concentration of dexamethasone to 10 μ M did not result in a decrease in the augmented quantity of GM-CSF elaborated by the IL-1 α -stimulated endothelial cells.

Influence of dexamethasone on eosinophil viability. The viability of eosinophils cultured for 3 d in enriched medium alone, in endothelial cell-conditioned medium, and in IL-1 α -stimulated endothelial cell-conditioned medium was 22 ± 5 , 58 ± 1 , and $75 \pm 1\%$, respectively ($n = 4$) (Fig. 2). The pretreatment of replicate endothelial cell monolayers for 24 h with 100 nM

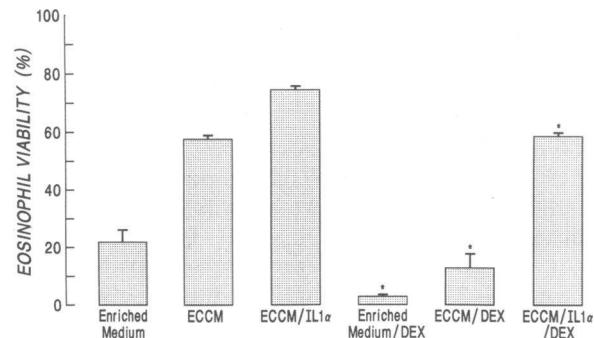


Figure 2. Influence of dexamethasone (DEX) on the 3-d eosinophil viability-sustaining activity observed for enriched medium alone or for 50% endothelial cell-conditioned medium (ECCM) collected 48 h after various treatments. The results expressed are the mean \pm SEM of four experiments. *Statistically significant decrease in eosinophil viability for replicate cells cultured in 48-h conditioned medium from 24 h dexamethasone-pretreated endothelial cultures, compared with untreated cultures ($P < 0.01$).

dexamethasone decreased the viability of replicate eosinophils to 3 ± 1 , 14 ± 4 , and $59 \pm 1\%$, respectively ($P < 0.05$ for differences in eosinophil viability for replicate cell cultures with or without dexamethasone).

The negative effect on eosinophil viability of IL-1 α -stimulated endothelial cell-conditioned medium prepared in the presence of dexamethasone was inconsistent with the failure of this glucocorticoid to suppress the stimulated release of GM-CSF. Thus, it seemed likely that dexamethasone had an additional direct effect on eosinophils. Replicate eosinophils were cultured for up to 3 d with or without 100 nM dexamethasone in enriched medium alone or medium supplemented with 1 and 10 pM GM-CSF. By 24 h of culture, and extending to 3 d for cells cultured with 1 pM GM-CSF, statistically significant differences in eosinophil viability were observed for cells that were cultured with dexamethasone in enriched medium alone or with 1 pM GM-CSF as compared to replicate cells cultured without dexamethasone (Fig. 3). The 24-h viability of replicate eosinophils cultured in enriched medium alone was reduced from $43 \pm 6\%$ ($n = 3$) to $21 \pm 7\%$ with dexamethasone ($P < 0.05$), and for eosinophils cultured in 1 pM GM-CSF was reduced from $75 \pm 2\%$ ($n = 3$) to $54 \pm 6\%$ with dexamethasone ($P < 0.05$). In contrast, the viability of eosinophils cultured in 10 pM GM-CSF was unaffected by the inclusion of dexamethasone (Fig. 3).

The glucocorticoid-induced antagonism of eosinophil viability augmented by GM-CSF was evaluated further by culturing replicate normodense eosinophils with incremental concentrations of IL-3 (0.1–100 pM) and IL-5 (0.01–10 pM) in the presence and absence of 100 nM dexamethasone. Similar to GM-CSF, dexamethasone-induced viability antagonism at 3 d was abolished by culturing the eosinophils with higher concentrations of cytokine (Fig. 4).

To determine the steroid specificity of dexamethasone-induced antagonism of the eosinophil hematopoietins, replicate eosinophils were cultured for 3 d with 1 pM GM-CSF in the presence and absence of 100 nM dexamethasone, prednisolone, hydrocortisone, and testosterone. In comparison to dexamethasone, which decreased eosinophil viability at 3 d from $42 \pm 3\%$ to $8 \pm 2\%$ (assigned a value of 1.0), prednisolone, hydrocortisone, and testosterone decreased the eosinophil viability-sustaining activity of 1 pM GM-CSF by the relative values of 0.7 ± 0.2 , 0.2 ± 0.2 , and 0 ± 0 , respectively ($n = 3$).

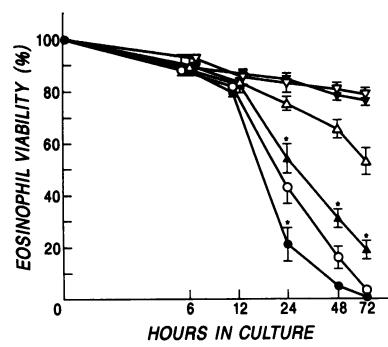


Figure 3. Effect of dexamethasone on the viability of replicate eosinophils cultured in enriched medium alone (●), or in medium supplemented with 1 pM (▲) or 10 pM (▼) GM-CSF. Replicate eosinophils, which were cultured under the same conditions in the absence of dexamethasone, are represented by the corresponding open symbol. The results are expressed as the mean \pm SEM of three experiments. *Statistically significant decrease in the viability of eosinophils cultured in the presence of dexamethasone, as compared to replicate cells cultured under the same conditions without dexamethasone ($P < 0.05$).

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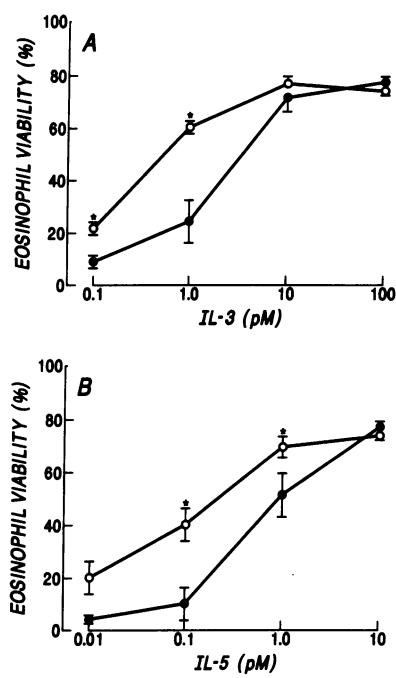


Figure 4. Influence of dexamethasone on IL-3 (A) and IL-5 (B) augmented eosinophil survival after 3 d of culture. Solid symbols correspond to eosinophils cultured in the presence of 100 nM dexamethasone; open symbols correspond to replicate eosinophils cultured in the absence of dexamethasone. *Statistically significant decrease in the viability of eosinophils cultured in the presence of dexamethasone, as compared to replicate cells cultured under the same conditions without dexamethasone ($P < 0.05$).

Influence of GM-CSF, IL-3, and IL-5 on DNA fragmentation of eosinophils. As visualized by agarose gel electrophoresis, DNA obtained from freshly isolated eosinophils was intact. However, by 20 h of culture in enriched medium alone, the eosinophil DNA was degraded with a characteristic apoptotic "ladder" pattern of fragmentation in which prominent fragments of < 1,000 bp were observed with decrements of ~ 200 bp (Fig. 5A). DNA extracts obtained from replicate eosinophils cultured in 10 pM GM-CSF revealed a marked reduction in DNA fragmentation ($n = 2$). Furthermore, 10 pM GM-CSF attenuated DNA fragmentation in eosinophils cultured for 24 h even in the presence of 100 nM dexamethasone ($n = 4$) (Fig. 5B). After 24 h of culture in the absence of GM-CSF, DNA fragmentation was of such magnitude that it was difficult to define additional fragmentation due to the presence of dexamethasone. The capacity of eosinophil-directed hematopoietic cytokines to prevent DNA fragmentation in normodense eosinophils was examined by culturing replicate eosinophils for 24 h with enriched medium alone, 10 pM IL-3, 10 pM IL-5, and 1,000 U/ml TNF α . Prominent DNA fragmentation characteristic of apoptosis was observed in eosinophils cultured in medium alone or with TNF α , but was absent in the eosinophils cultured with IL-3 and IL-5 (data not shown).

Discussion

The in vitro viability and the physical and functional phenotype of human peripheral blood eosinophils are regulated by the action of GM-CSF, IL-3, and IL-5 (1–3, 5, 6). In the idiopathic hypereosinophilic syndrome and the tryptophan-associated eosinophilia/myalgia syndrome, the presence of hypodense eosinophils in the blood is associated with excessive serum levels of IL-5 (9, 10, 12). Because T lymphocytes produce each of these hematopoietins (13), the T cell has been the focus for IL-5 elaboration in these hypereosinophilic states (14–16). When stimulated with the appropriate cytokines, both human umbilical vein endothelial cells (8, 17, 18) and human fibroblasts (19) elaborate GM-CSF. Because unstimulated human endothelial cells elaborate a soluble activity capable of regulating the viability and phenotype of human eosinophils (7), we sought to characterize this activity, and to utilize this model cell system to determine the endothelial cell-mediated interactions of IL-1 α and dexamethasone on eosinophil viability.

Both the constitutive and the IL-1 α -stimulated endothelial cell-derived eosinophil viability-sustaining activity acted in a dose-dependent manner. Based upon complete antibody neutralization with monospecific antibody, this activity was demonstrated to be GM-CSF (Fig. 1). Antibodies against IL-3 and IL-5 had no effect. As quantitated by ELISA, the mean 48-h constitutive generation of GM-CSF was 3.2 pM, and with IL-1 α stimulation, this level was increased 2.5-fold. Monocyte-derived IL-1 was shown to augment the generation of endothelial cell-derived eosinophil viability-sustaining activity (20), and GM-CSF mRNA and protein increase when endothelial cells are stimulated with IL-1 (8, 17, 18).

Because glucocorticoids are commonly used in the therapy of disorders associated with tissue and peripheral blood eosinophils (12), we examined the capacity of dexamethasone to attenuate the generation of GM-CSF by the endothelium and to influence eosinophil viability. At a concentration of dexamethasone (100 nM) that decreased the constitutive generation of

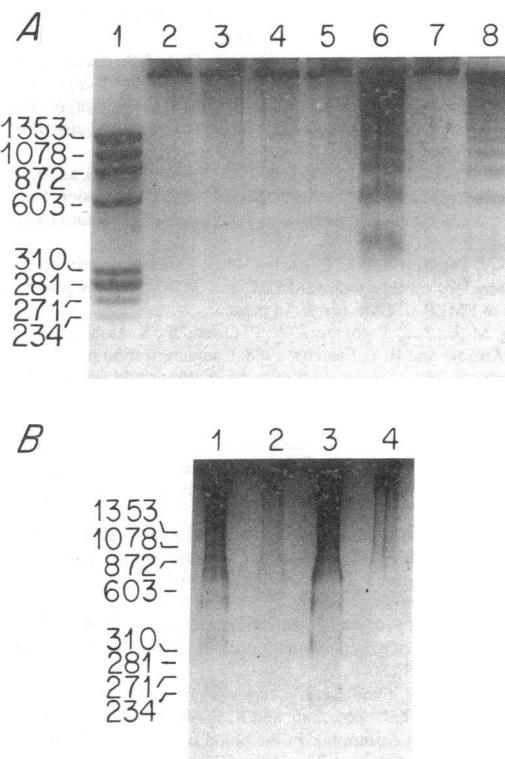


Figure 5. Influence of GM-CSF on eosinophil DNA fragmentation. (A) DNA was extracted from 1.5×10^6 freshly isolated eosinophils (lane 2) and replicate cells that were cultured for 10, 20, and 38 h in enriched medium alone (lanes 4, 6, and 8, respectively) or for the same intervals with 10 pM GM-CSF (lanes 3, 5, and 7, respectively). Lane 1 was loaded with DNA of standard sizes (PhiX174 RF DNA-Hae III digest). (B) DNA was extracted from 1.5×10^6 replicate eosinophils that were cultured for 24 h in enriched medium alone (lane 1), or with 10 pM GM-CSF (lane 2), 100 nM dexamethasone alone (lane 3), or GM-CSF and dexamethasone (lane 4).

GM-CSF by endothelial cells by ~ 50%, the effect on IL-1 α -stimulated augmented elaboration of GM-CSF was negligible. Increasing the concentration of dexamethasone to 10 μ M did not diminish IL-1 α -elicited GM-CSF levels. However, even when the GM-CSF concentration was not reduced by dexamethasone, the viability of eosinophils maintained with dexamethasone was significantly diminished (Fig. 2). Thus, as has been previously suggested, dexamethasone appeared to have an antagonistic effect on the cytokine-mediated maintenance of eosinophil viability (20). Additionally, dexamethasone had an indirect effect to diminish eosinophil viability by a decrease in the constitutive production of GM-CSF by endothelial cells.

Dexamethasone accelerated the loss of viability of eosinophils maintained in enriched medium alone or in enriched medium supplemented with 1 pM GM-CSF, a suboptimal concentration as defined by its capacity to extend eosinophil viability ex vivo and to induce the acquisition of the hypodense eosinophil phenotype (1, 5). If the eosinophils were cultured with a concentration of GM-CSF that was optimal for eliciting these effects (10 pM) (1, 5, 21), their viability in vitro was unaffected by the inclusion of dexamethasone (Fig. 3). In a similar manner, optimal concentrations of IL-3 and IL-5 (2, 3, 5) were observed to block the effect of dexamethasone (Fig. 4). The effect of dexamethasone was demonstrated to be specific for the glucocorticoids based upon the absence of an effect by the androgenic steroid testosterone, and a rank order of potency of dexamethasone, prednisolone, and hydrocortisone in this assay that was similar to their antiinflammatory potency.

Glucocorticoid-induced lymphocytolysis is preceded by a pattern of DNA cleavage characterized by DNA fragmentation into ~ 200-bp multiples (22, 23), and this "ladder" pattern of fragmentation is associated with endonuclease-specific programmed cell death (apoptosis) (11, 24, 25). The sizes of the DNA fragments from cytokine-deprived eosinophils exhibited the same ~ 200-bp "ladder" pattern of DNA degradation (Fig. 5 A). However, eosinophil DNA was protected from this process by the inclusion of GM-CSF during the culture, even in the presence of dexamethasone (Fig. 5 B). Both IL-3 and IL-5 were also capable of preventing programmed cell death in normodense eosinophils. In contrast, TNF α , which is capable of priming select eosinophil functions (21) but is without eosinophil viability-sustaining activity (2), was unable to prevent endonuclease-specific DNA degradation. Thus, the three hematopoietins with the capacity to maintain the ex vivo viability of normodense eosinophils, and to convert them to the functionally primed hypodense phenotype, are selectively able to prevent apoptosis. The occurrence of programmed cell death after the withdrawal of IL-3 from hematopoietic precursor cell lines has been described (26). However, the protective role of these hematopoietins against programmed cell death in eosinophils has not been previously recognized.

The present study of a model cell system identifies GM-CSF as the constitutive and IL-1 α -stimulated endothelial cell-derived eosinophil viability-sustaining factor. Glucocorticoids suppress the constitutive production of GM-CSF by the endothelium and antagonize the viability sustaining effect of suboptimal concentrations of GM-CSF. However, glucocorticoids do not suppress IL-1 α -elicited GM-CSF, and these higher concentrations of GM-CSF, and IL-3 and IL-5, prevent glucocorticoid-mediated antagonism of eosinophil viability. These findings demonstrate that, in addition to mediating a phenotypic

change of mature normodense eosinophils to the primed hypodense phenotype which occurs over several days in culture (1–3, 5), GM-CSF, IL-3, and IL-5 have an acute cytoprotective effect for normodense eosinophils which is associated with the prevention of programmed cell death (apoptosis). Thus, stimulus-elicited cytokine production by another cell type might provide concentrations of these eosinophil-directed hematopoietins which are sufficiently great to produce steroid-resistant eosinophils. The resulting eosinophils would be capable of a conversion to the highly pathobiologic hypodense phenotype, and this sequence of events may explain the differential responsiveness of select eosinophilic disorders to intervention with corticosteroids.²

Acknowledgments

We are grateful to Dr. R. J. Soberman for his helpful discussions.

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2. While the manuscript for this article was in review, two additional groups of investigators reported that GM-CSF is the constitutive eosinophil viability-sustaining activity derived from human endothelial cells and human bronchial epithelium, and that glucocorticoids antagonize the viability effects of this cytokine in the presence of suboptimal concentrations (27, 28). Programmed cell death was not examined in either paper.

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