Human Circulating Eosinophils Secrete Macrophage Migration Inhibitory Factor (MIF)
Potential Role in Asthma

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Abstract

Macrophage migration inhibitory factor (MIF) is a potent proinflammatory mediator that has been shown to potentiate lethal endotoxemia and to play a potentially important regulatory role in human acute respiratory distress syndrome (ARDS). We have investigated whether eosinophils are an important source of MIF and whether MIF may be involved in the pathophysiology of asthma. Unstimulated human circulating eosinophils were found to contain preformed MIF. Stimulation of human eosinophils with phorbol myristate acetate in vitro yielded significant release of MIF protein. For example, eosinophils stimulated with phorbol myristate acetate (100 nM, 8 h, 37°C) released 1,539±435 pg/10⁶ cells of MIF, whereas unstimulated cells released barely detectable levels (<142 pg/10⁶ cells, mean±SEM, n = 8). This stimulated release was shown to be (a) concentration- and time-dependent, (b) partially blocked by the protein synthesis inhibitor cycloheximide, and (c) significantly inhibited by the protein kinase C inhibitor Ro-31,8220. In addition, we show that the physiological stimuli C5a and IL-5 also cause significant MIF release. Furthermore, bronchoalveolar lavage fluid obtained from asthmatic patients contains significantly elevated levels of MIF as compared to nonatopic normal volunteers (asthmatic, 797.5±92 pg/ml; controls, 274±91 pg/ml). These results highlight the potential importance of MIF in asthma and other eosinophil-dependent inflammatory disorders. (J. Clin. Invest. 1998. 101:2869–2874.) Key words: granulocyte • inflammation • lung • protein kinase C • protein synthesis

Introduction

Macrophage migration inhibitory factor (MIF) has been shown to be an important modulator of the inflammatory response (for review, see reference 1). This mediator was originally described as a T lymphocyte–derived protein that inhibited the random migration of guinea pig peritoneal macrophages (2–4). MIF has subsequently been shown to be a key modulator of both inflammatory and immune responses and has been implicated in macrophage activation and in antigen-driven T cell responses (5, 6). MIF is released from immune cells upon glucocorticoid stimulation, and once released MIF has the unique ability to override glucocorticoid-mediated inhibition of cytokine secretion by both LPS-stimulated monocytes and antigen-activated T cells (7, 8), and human alveolar cells obtained from patients with the acute respiratory distress syndrome (ARDS) (9). Animal studies have shown that MIF has the ability to overcome glucocorticoid protection against lethal endotoxemia (8). It is a mediator in septic shock and this has been highlighted by the ability of neutralizing anti-MIF antibodies to protect animals from lethal endotoxemia (10). Thus, MIF not only exerts proinflammatory effects, but also counteracts the antiinflammatory action of the glucocorticoids. MIF also has recently been shown to be secreted by the corticotrophic/thyrotrophic cells of the anterior pituitary after physiological stress or LPS stimulation (10, 11).

In this paper we report that eosinophils, after stimulation with phorbol myristate acetate (PMA), recombinant human (rh) C5a, and rhIL-5, are also an important cellular source of MIF. In addition we show that (a) MIF is stored preformed in significant quantities in eosinophils, (b) eosinophils are capable of synthesizing MIF, and (c) protein kinase C (PKC) is implicated in the regulation of MIF secretion. Given the involvement of eosinophils in allergic inflammatory disease and the role MIF plays in counteracting the antiinflammatory action of the glucocorticoids, we analyzed alveolar fluid derived from the lungs of stable asthmatic patients and found significantly elevated MIF levels compared to normal control alveolar fluid. In this paper we describe the eosinophil as a cellular source for MIF and highlight the potential importance of MIF as a modulator of the inflammatory response in asthma and other allergic inflammatory diseases.

Methods

Materials. Sodium citrate was purchased from Phoenix Pharmaceuti- cals Ltd. (Gloucester, UK). Percoll and dextran were obtained from Pharmacia Biotechnologies (St. Alans, UK). Iscove's DME, PBS, HBSS, and supplements (penicillin and streptomycin) were from Life Technologies (Paisley, UK). Sterile tissue culture plasticware was


1. Abbreviations used in this paper: ARDS, acute respiratory distress syndrome; BAL, bronchoalveolar lavage; MIF, macrophage migration inhibitory factor; PKC, protein kinase C; PMA, phorbol myristate acetate; rh, recombinant human.
purchased from CorningCostar (High Wycombe, UK) and Falcon (Baker Scientific, Runcorn, UK). PMA, rhC5a, and cycloheximide were purchased from Sigma Chemical Co. Ltd. (Poole, UK). Dynabeads (M-450) were purchased from Dynal (Mersyside, UK). Ro31,8220 was purchased from Calbiochem–Novabiochem Ltd. (Nottingham, UK) and rhIL-5 from R&D (Abingdon, UK).

Eosinophil isolation and culture. Eosinophils were isolated by a modification of a previously described method (12, 13). Briefly, eosinophils derived from the peripheral blood of normal donors were isolated by dextran sedimentation followed by centrifugation through discontinuous plasma–Percoll gradients. Granulocytes were taken from the 70%/81% layer and the eosinophils were purified from contaminating neutrophils by an immunomagnetic separation step, using sheep anti–mouse IgG Dynabeads coated with the murine antineutrophil antibody 3G8 (anti-CD16; a gift from Dr. J. Unkeless, Mount Sinai Medical School, New York). Cells were mixed with washed 3G8 Dynabeads at a bead/neutrophil ratio of 3:1 on a rotary mixer at 4°C for 20 min. Beads with bound neutrophils were removed magnetically by stationary contact (3 min) with a magnet (Magnetic Particle Concentrator MPC-1; Dynal). This procedure was repeated once to yield eosinophil populations of >98% purity and >99% viability. After purification, cells were washed sequentially in HBSS without calcium or magnesium and HBSS with calcium and magnesium before resuspending in Iscove’s DME with 2% autologous serum. Eosinophils (10⁶/ml) were cultured in Iscove’s DME with 2% autologous serum at 37°C in a 5% CO₂ atmosphere.

MIF ELISA. Immunoreactive MIF in collected cell supernatants, lysates, and patient samples were quantitated via standard ELISA as published previously (9). Plates were coated with an anti–human MIF mAb and were blocked and incubated using standard methods. Rabbit polyclonal anti-MIF sera were used as secondary antibodies, followed by goat anti–rabbit IgG conjugated to alkaline phosphatase substrate (para-NPP). MIF levels were determined by extrapolating from a quadratic standard curve using purified, human rMIF (range 0–12 ng/ml) (14). The sensitivity of this ELISA was ≈ 150 pg/ml.

Patient details. Asthmatic patients attending the Respiratory Outpatients Department at the Western General Hospital, (Edinburgh, UK) were considered eligible for study enrollment. Our normal nonsmoking control group was paramedical staff within the Western General Hospital. Informed written consent was obtained from volunteers or asthmatic patients before bronchoscopy. Asthmatic patients were eligible for enrollment if they (a) were >18 yr of age, (b) had not been prescribed oral corticosteroid medication for at least 3 mo before enrollment, (c) had a forced expiratory volume-1 (FEV1) >1 liter and (d) had oxygen saturation values on air of >90% before the procedure. This study was approved by the Lothian Health Board Ethics Committee.

Bronchoalveolar lavage (BAL) procedure. BAL sampling was performed as described previously (15). Briefly, asthmatic patients were prescribed nebulized salbutamol (2.5 mg) before bronchoscopy. Premedication consisted of phenoperidine (up to 1 mg) and midazolam (up to 3 mg) intravenously. A fiber-optic bronchoscope was introduced intranasally and passed through the vocal cords into the lungs. The distal end of the bronchoscope was wedged into either the lingula or right middle lobe. Eight 30-ml aliquots of 0.9% NaCl solution were instilled and gently aspirated immediately. All bronchoscopy procedures were performed by the same bronchoscopist (A.P. Greening). On average, 76% of instilled NaCl solution was recovered. Recovered fluid and cells were stored at 4°C until processed. All samples were processed within 1 h of collection.

For collected samples, processing initially entailed straining the lavage fluid through sterile gauze to remove mucus. The strained fluid was then centrifuged at 400 g at 4°C for 10 min to recover cells. Total cell counts were performed using a hemocytometer. Aliquots of cells were pelleted onto glass slides using a cytospin 2 (Shandon Scientific, Cheshire, UK) and then stained with Diff-Quick (Merz–Dade AG, Dudingten, Switzerland), a modified Wright–Giemsa stain. Differential counts were determined by counting 500 cells under oil im-

![Figure 1. PMA stimulates eosinophils to secrete MIF.](Image)
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The lavage fluid supernatant was resuspended at 1,000 g for 10 min at 4°C to remove cellular debris and stored at −70°C until analyzed at a later date. MIF was assayed in uncentrated, neat BAL samples and MIF results are expressed per milliliter of collected BAL fluid.

Statistical analysis. The results are expressed as mean ± SEM. Statistical analysis was performed by the Student’s t test or by one-way ANOVA followed by the Newmans–Keuls post-test. Analysis of MIF levels in BAL fluid was performed using the Spearman rank correlation. Differences were considered significant when \( P < 0.05 \). Calculations were performed using the statistical software package Statview (Abacus Concepts, Berkeley, CA).

Results

MIF secretion from PMA-stimulated eosinophils. Eosinophils were found to be efficient secretors of MIF after PMA stimulation. In 8-h cultures, human blood–derived eosinophils released a mean value of 1539 ± 435 pg/ml MIF per million cells after PMA stimulation (100 nM) (Fig. 1A) (\( P = 0.0008 \)). This secretion was concentration-dependent with 1, 10 and 100 nM PMA inducing 803 ± 230, 1,084 ± 161, and 1,718 ± 48 pg/ml MIF, respectively, in collected 8-h supernatants (Fig. 1B). The time course of MIF secretion after stimulation (Fig. 1C) revealed mean MIF release at 1, 4, and 8 h of 1,706 ± 1,259, 1,309 ± 430, and 1,886 ± 1,013 pg/ml, respectively.

MIF is stored preformed in eosinophils. The observation that MIF was found in eosinophil supernatants 1 h after PMA stimulation suggested that significant MIF resides preformed in eosinophils. Analysis of unstimulated eosinophil lysates and supernatants revealed a mean level of MIF of 111 ± 66.2 and 2,883 ± 706 pg/ml in control supernatant and lysate samples, respectively. 8 h after PMA stimulation, supernatant and lysate values were 1,793 ± 610 and 2057 ± 807 pg/ml, respectively (Fig. 2A). Pretreatment with cycloheximide (1 μM) resulted in a reduction in MIF secretion after PMA stimulation by 53% compared to untreated stimulated cells (Fig. 2B).

Influence of PKC inhibitors on MIF secretion. Preincubation of eosinophils with Ro-31,8220 (1 μM), a PKC inhibitor, resulted in an attenuation of PMA-induced MIF secretion by 72% in 8-h supernatants. PMA secretion was reduced from a mean of 2,663 ± 831 to 744 ± 254 pg/ml after preincubation with the PKC inhibitor (control, 86 ± 46 pg/ml) (Fig. 3).

IL-5 and C5a induce eosinophil MIF secretion. In relation to the pathophysiology of inflammatory lung disease and asthma, we investigated whether mediators known to be implicated in these diseases (e.g., IL-5 and C5a) could induce MIF secretion from eosinophils. Incubation of human eosinophils with rhIL-5 (100 ng/ml) resulted in a mean MIF level of 1,169 pg/ml in collected supernatants. Preincubation with rhC5a (100 nM) resulted in a mean level of 1,167 pg/ml (Table I).

MIF alveolar levels in asthma. Nonatopic normal volunteers and stable asthmatic patients were bronchoscopyed and BAL samples obtained. Significantly elevated alveolar MIF levels were found in asthmatic patients compared to controls (\( P < 0.005 \)) (asthmatic, 797.5 ± 92 pg/ml; controls, 274 ± 91 pg/ml) (Fig. 3). The mean number of total BAL cells obtained represented by \(* P < 0.05\) and differences between PMA-stimulated cells and CXH- or Ro-31,8220-treated cells are represented by \(\# P < 0.05\).

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Inflammatory cytokine production and override the anti-inflammatory effects of glucocorticoids on ex vivo ARDS alveolar cells.

This work led us to consider the potential role of MIF in an allergic inflammatory disease, namely asthma. The eosinophil has been implicated as a key cell type in the pathogenesis of allergic inflammatory diseases such as bronchial asthma, allergic rhinitis, and atopic dermatitis (16, 17). Eosinophils are recruited to inflammatory foci by the concerted effects of specific chemoattractants, adhesion molecules, and extracellular matrix proteins. Once recruited, eosinophils, if further exposed to inflammatory stimuli, become activated and can undergo a variety of functional responses including degranulation, liberation of reactive oxygen intermediates (ROI), and synthesis and release of inflammatory mediators and cytokines. Secreted products such as eosinophil cationic protein, major basic protein, eosinophil protein X, and eosinophil peroxidase, as well as reactive oxygen intermediates, assist in destruction of invading organisms and contribute to tissue damage associated with allergic diseases and asthma. For example, in asthma, elevated levels of eosinophil-derived products are found in the BAL fluid and correlate positively with the severity of the disease. Furthermore, when certain eosinophil-derived products are administered into the lungs of animals, bronchial hyperresponsiveness and lung damage can occur, further implicating the eosinophil as detrimental and contributing to the pathogenesis of asthma (for review, see references 16 and 18). Clinically, administration of glucocorticoids has been used successfully as a way of reducing inflammatory cell–induced tissue damage and amelioration of eosinophilic diseases. Although the mechanism of action of glucocorticoids in such diseases is unknown, in patients with hypereosinophilia there is a marked reduction in the number of circulating eosinophils, bronchial hyperresponsiveness and lung damage can occur, further implicating the eosinophil as detrimental and contributing to the pathogenesis of asthma (for review, see references 16 and 18). Clinically, administration of glucocorticoids has been used successfully as a way of reducing inflammatory cell–induced tissue damage and amelioration of eosinophilic diseases. Although the mechanism of action of glucocorticoids in such diseases is unknown, in patients with hypereosinophilia there is a marked reduction in the number of circulating eosinophils, bronchial hyperresponsiveness and lung damage can occur, further implicating the eosinophil as detrimental and contributing to the pathogenesis of asthma (for review, see references 16 and 18). 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rhC5a and rhIL-5 were these cells. The levels of MIF secretion after stimulation by pathogenesis (23, 24) stimulated MIF secretion from human eosinophil activators that have been implicated in asthma disease also interested in determining whether other significant eosinophil activators suggest that MIF may play a role in inhibiting maximal endogenous or exogenous steroid activity in inflammatory diseases such as asthma. This finding is potentially of therapeutic importance, as an anti-MIF strategy would not only be antiinflammatory in its own right, but would also act by removing the potential inhibitory role of MIF on glucocorticoid function. This could serve to restore maximal antiinflammatory steroid activity, and reset the balance within the pulmonary inflammatory response away from excessive inflammation, airway constriction, and tissue damage.

Acknowledgments

Seamas C. Donnelly is supported by the Scottish Hospital Endowment Research Trust (SHERT), Adriano G. Rossi by the Medical Research Council (UK), Nikhil Hirani by the Wellcome Trust, and Richard Bucala by National Institutes of Health grant AI-35931.

References