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MIF attenuates the suppressive effect of dexamethasone on IL-6 production by nasal polyp

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Abstract. – BACKGROUND: Nasal polyposis (NP) is a chronic inflammatory disease of the upper airways, that characterized by inflammatory cells infiltration, extracellular matrix accumulation and oedema. Interleukin-6 (IL-6) is a multifunctional cytokine, implicated in various inflammatory conditions, including NP pathogenesis. Macrophage migration inhibitory factor (MIF) is a pro-inflammatory mediator able to antagonize the inhibitory effects of glucocorticoids on the expression of various cytokines and growth factors.

AIM: To investigate the presence of MIF in nasal polyp tissues and the influence of a MIF activity inhibitor on dexamethasone effects on IL-6 production.

PATIENTS AND METHODS: Nasal polyps were resected by functional endoscopic sinus surgery for treatment of chronic sinusitis with polyposis and healthy nasal mucosa was taken during nasal septoplasty-chochoplasty. MIF and IL-6 levels were determined by ELISA. The expression of MIF and IL-6 at the mRNA level was ascertained by RT-PCR.

RESULTS: MIF was detected in all polyp tissue extracts and tissue cultures conditioned media. MIF and IL-6 expression were significantly higher in polyp tissues as compared to normal nasal mucosa tissues. Dexamethasone at concentration 1-100 microM caused a statistically significant dose-dependent suppression of IL-6 production by polyp tissue cultures. Inhibition of MIF by (*S,R*)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester (ISO-1), an inhibitor of MIF tautomerase activity, significantly enhanced the dexamethasone suppressive effect on IL-6 production.

CONCLUSIONS: MIF, presence in polyp tissue, attenuates the suppressive effect of dexamethasone on the production of IL-6 by this tissue, since the simultaneous use of its inhibitor ISO-1 leads to an enhancement of dexamethasone activity. Therefore, it is reasonable to propose that the utilization of MIF inhibitors together with glucocorticoids in clinical practice may be beneficial in the treatment of NP.

Key Words:
Nasal polyps, Macrophage migration inhibitory factor (MIF), ISO-1, Dexamethasone, IL-6.

Introduction

Nasal polyposis (NP) is a chronic inflammatory disease of the upper airways, featuring inflammatory cell infiltration, modifications of epithelial differentiation, and tissue remodeling including basement membrane thickening, gland modifications, extracellular matrix (ECM) accumulation and oedema. It has been reported that polyp formation involves ECM swelling through an initial localized epithelial defect[1-3]. Interactions between epithelial, stromal and inflammatory cells could then perpetuate further polyp growth. Mucosal inflammation, especially containing an infiltration of eosinophils, is probably the most important factor in the development of NP. The intensity of the eosinophilic inflammation differs considerably among patients with NP. The degree of the tissue eosinophilia in NP is reported to correlate with the severity of the polyposis, involvement of the lower respiratory tract by asthma or other respiratory mucosal diseases and the probability of polyp recurrence[4-6]. Although eosinophils are thought to play a significant role in nasal polyposis, the cascade of events leading up to polyp growth and the relationship between the various cell types are still controversial[1-3]. The advent of topically administered corticosteroids has improved the treatment of NP and rhinitis. Their clinical efficacy is achieved by a combination of anti-inflammatory effects along with their ability to reduce airway eosinophilic infiltration by preventing their increased viability and activation[3]. The polyp stroma contains vari-

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ous mediators, including cytokines, chemokines, growth factors, adhesion molecules, proteolytic enzymes, and other proteins, and some of these mediators participate in a series of events that culminate in chronic inflammation and the subsequent development of a polyp. Among them the IL-6 is involved.

IL-6 is a multifunctional cytokine that is implicated in a variety of inflammatory conditions. One of its central roles is the modulation of host immunity such as inducing the final differentiation of B cells into antibody-producing plasma cells and stimulating T-cells proliferation with the subsequent leukocytosis[7]. IL-6 is also a Th2-type cytokine that stimulates fibroblast proliferation, increases collagen deposition, and/or decreases collagen breakdown. According to several studies, IL-6 may participate in the pathology of chronic sinusitis and NP[8-16]. Elevated levels of IL-6 have been demonstrated in nasal polyps compared with the normal controls sampled from inferior turbinate[10]. Macrophages, eosinophils, lining epithelium and fibroblasts were the main cellular sources of IL-6[10]. According to Liu et al[13] pathogenesis of nasal polyp involves IL-6 secretion by cells which modulates the activation of immune response, inducing the plasma cell formation, synthesis of stroma components, promoting collagen deposition, and tissue remodeling.

Macrophage migration inhibitory factor (MIF) is a pro-inflammatory mediator with the ability to induce various immunomodulatory responses and override glucocorticoid-driven immunosuppression[17-21]. Some of these functions have been linked to the unusual enzymatic properties of the protein, namely tautomerase and oxidoreductase activities[22,23]. Among them, the *in vitro* glucocorticoid counter-regulatory activity, the chemotactic activity on monocytes, the induction of IL-8 and TNFα production by macrophages, the superoxide generation by neutrophils and the upregulation of matrix metalloproteinase 1 (MMP-1) and MMP-3 expression by synovial fibroblasts, are included[24-27].

Various molecules able to inhibit the MIF tautomerase activity have been designed, including (*S,R*)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester (ISO-1)[24,28]. ISO-1 suppresses several MIF biological activities, including MIF counter-regulation of suppression of TNFα, prostaglandin E_2 (PGE_2) and cyclooxygenase-2 (COX-2) production by glucocorticoid in human monocytes, MIF-stimulated cytoplasmic phospholipase A2 activation in fibroblasts and arachidonic acid release, and TNFα secretion from lipopoblysaccharides (LPS)-treated macrophages[24,28].

The literature regarding the biological role of MIF in the nasal polyps is limited. In previous studies, using immunohistochemical analysis, it was found that MIF is expressed in eosinophils and epithelial cells in allergic rhinitis and NP[29,30]. Taken to account the glucocorticoid counter-regulatory activity of MIF, the significant role of IL-6 in NP pathogenesis[8-16] and the ability of glucocorticoids, which are used in NP treatment, to suppress the IL-6 production[8,31,32], the aim of our study, therefore, was to evaluate the presence of MIF in polyp tissue, and to investigate its influence on the suppressive effect of dexamethasone on IL-6 production in this tissue.

Patients and Methods

Biologic Materials

Nasal polyps were resected by functional endoscopic sinus surgery from patients suffered from chronic sinusitis with polyposis, who were referred to Department of Otorhinolaryngology, University Hospital of Patras, Patras, Greece. The diagnosis of nasal polyposis was established on the basis of medical history and symptoms, endoscopic examination of the nose, and a computed tomographic scan of nasal fossa and paranasal sinuses. The patients had no history of nasal allergy and asthma, confirming from negative skin prick tests and negative radioallergosorbent test (RAST), and diagnosis of a pneumonologist, respectively. None of the subjects had history of aspirin sensitivity and they had no regularly taken topical or oral medication within three weeks. Patients with ciliary dyskinesia and cystic fibrosis were also excluded. The control group consisted of healthy subjects from whom healthy nasal mucosa was taken during nasal septoplasty-inferior turbinoplasty. The study was approved by the local Ethical Committee on human experimentation of the University Hospital of Patras and an informed consent was obtained from each patient and control subject before the surgical procedure.

Histomorphological Studies

Biopsy specimens were dyed with haemotoxilin/eosin and examined under the light microscope. Eosinophils, polymorphonuclear (PMN) leucocytes, lymphocytes, and mast cells were

identified. A percentage 1-5% of a cell type population was considered as small portion of the total cells population in the specimens.

Tissue Extraction and Culture

Immediately after harvesting, a portion of each specimen was subjected to extraction as previously described[33], and the remainder was treated and cultured according to Syggelos et al[34]. Tissue cultures were performed in Dulbecco's modified Eagle's minimum essential medium (DMEM), supplemented with 1% ITS (contains insulin from bovine pancreas, human transferin and sodium selenite) liquid media supplement (Sigma Aldrich Chemical Co., St Louis, MO, USA). The tissue specimens were initially cultured for 24h, the conditioned medium was removed and replaced by fresh medium and the culture was continued for an additional 72h, without or with dexamethasone at various concentrations in the absence or presence of ISO-1 (kindly provided by Dr Y. Al-Abed, The Picower Institute for Medical Research, Manhasset, NY, USA). At the end of the incubation period, tissue suspensions were centrifuged (10,000 g at 4°C for 20 min) and the conditioned media were collected and stored at –20°C until further use.

Determination of IL-6 Levels by Sandwich ELISA

The levels of IL-6 in conditioned media were determined by sandwich ELISA, using a matched pair of monoclonal antibodies, according to the manufacturer's instructions (ENDOGEN, Inc. Woburn, MA, USA), as previously described[34].

Determination of MIF Levels by Sandwich ELISA

The levels of MIF in conditioned media of tissue culture and tissue extracts were determined by sandwich ELISA, using a matched pair of monoclonal antibodies, according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA).

Polystyrene plates (GREINER, Frickenhausen, Germany) were coated with capture antibody (2 μg/ml) in 10 mM phosphate buffer, pH 7.4/0.14M NaCl (PBS) (100 μl/well), overnight at 37°C. Empty sites in plates were blocked with 1% bovine serum albumin (BSA) in PBS, containing 5% sucrose (200 μl/well), for 1h at 37°C. After washing 3 times with PBS-0.05% Tween 20 (PBS-T), the standards or samples, directly or after dilution in PBS, were added (100 μl/well), and the plates incubated for 1.5h at 37°C. After washing 3 times with PBS-T, the biotin-labeled detecting antibody (200 ng/ml) in 50 mM Tris-HCl buffer, pH 7.4/0.15M NaCl (TBS), containing 1% BSA (100 μl/well) was added and the plates were further incubated for 1.5h at 37°C. The plates were washed again with PBS-T, streptavidin-peroxidase conjugated (Chemicon, Temecula, CA, USA) at dilution 1:4000 in TBS/1% BSA was added (100 μl/well) and they were incubated at 37°C for 40 min. After washing twice with PBS-T and once with 0.1 M citrate buffer pH 5.0, the peroxidase substrate (0.03% H_2O_2, 2 mg/ml o-phenyl-diamine in 0.1 M citrate buffer pH 5.0) was added (100 μl/well) and the plates were kept in the dark at room temperature for 30 min. The reaction was stopped by the addition of 2 M H_2SO_4 (100 μl/well) and the absorbance at 492 nm was measured.

The concentration of MIF was estimated using a standard curve, constructed with standard solutions of human recombinant MIF (R&D Systems, Minneapolis, MN, USA). The sensitivity of the method was estimated to be 17 pg/ml and the relationship between absorbance at 492 nm and MIF concentration was linear from 31.25 to 2000 pg/ml.

All samples were tested in duplicate.

Western Blot Analysis

Samples of polyp tissue extracts and polyp tissue cultures conditioned media were initially enriched in MIF by solid phase extraction. After adjustment of their pH to 2.8 by addition of trifluoroacetic acid (TFA), the samples were subjected to solid phase extraction on Bond Elut C18 cartridges (Varian, Harbor City, CA, USA), equilibrated in 0.02 %TFA (pH 2.8). The cartridges were washed with 0.02% TFA and then eluted with 80% acetonitrile (CH_3CN) in 0.02% TFA. The 80% CH_3CN fractions were lyophilized, dissolved in Laemmli sample buffer[35], treated with 2-mercaproethanol and then subjected to sodium dodecyl sulphate-polyacrylamide gels (SDS-PAGE) on 15% polyacrylamide gels followed by western blotting, as previously described[33]. Incubation with anti human MIF monoclonal antibody (R&D systems, Minneapolis, MN, USA) (1 μg/ml) was performed overnight at 4°C, followed by incubation with peroxidase-conjugated anti-mouse IgG (Sigma Aldrich Chemical Co., St Louis, MO, USA) (1/4000) for 2h at room temperature. The immunoreacted proteins were de-

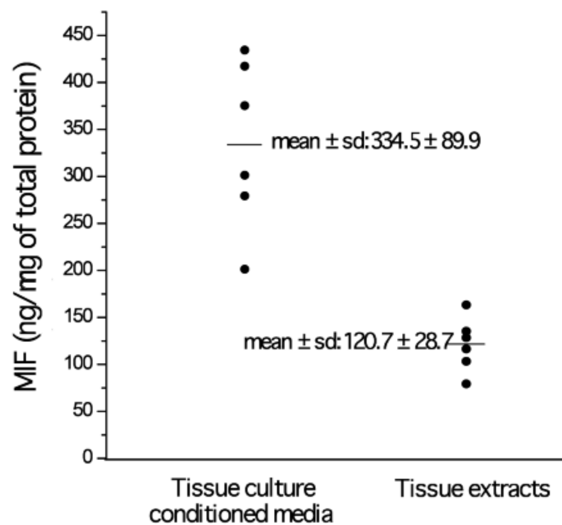

Figure 1. Levels of MIF in the polyps tissue extracts and 3-day polyps explant cultures conditioned media. Each sample was analyzed in duplicate. The mean values ± SD of MIF levels in extracts and conditioned media are given.

tected by the enhanced chemiluminescence (ECL) method, according to the manufacturer's instructions (Pierce, North Meridian Road, Rockford, IL, USA).

RNA Isolation and RT-PCR Analysis

Total RNA was extracted from polyp and normal nasal mucosa tissues using the RNAeasy spin mini-column kit purchased from Qiagen Inc. (Stanford, Valencia, CA, USA), according to the manufacturer's instructions (RNeasy Mini Handbook, Qiagen, 2001). Up to 100 mg of tissue were used. Reverse transcription polymerase chain reaction (RT-PCR) was carried out using the PrimeScript One Step RT-PCR kit Ver. 2 (Takara, Japan). Total RNA, 20 ng for MIF and IL-6, and 4 ng for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was added to each reverse transcription (RT) reaction containing the kit mix and the appropriate PCR primers (15 pmole) in 50 μl total volume. RT was carried out for 30 min at 50°C followed by a 15 min step at 95°C. Amplifications were performed with 30 cycles. Each cycle included denaturation at 95°C for 1min, annealing at 58°C for 1 min and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min. The PCR primers used for MIF, [(sense) 5'-CTC-TCC-GAG-CTC-ACC-CAG-CAG-3' and (antisense) 5'-CGC-GTT-CAT-GTC-GTA-ATA-GTT-3'], for IL-6, [(sense) 5'-GCC-CAG-CTA-TGA-ACT-CCT-TCT-C-3' and (antisense) 5'-GAG-TTG-TCA-TGT-CCT-GCA-GCC-3'], and GAPDH, [(sense) 5'-ACA-TCA-TCC-CTG-CCT-CTA-CTG-G-3' and (antisense) 5'-AGT-GGG-TGT-CGC-TGT-TGA-AGT-C-3'], were synthesized by MWG-Biotech AG (Ebersberg, Germany). The PCR amplification products 255 bp, 560 bp and 263 bp for MIF, IL-6 and GAPDH, respectively, were analyzed and visualized by electrophoresis on 2% agarose gel in the presence of 0.01% GelRed Nucleic Acid Gel Stain (Biotium Inc. Hayward, CA, USA). The intensity of PCR products was measured using the Scion Image PC software and expressed in arbitrary units (pixels).

Protein Determination

The protein determination was performed by the Bradford method[36], using BSA as the standard.

Statistical Analysis

Statistical analysis of the results was performed by Student's non-paired *t*-test. $p < 0.05$ was considered statistically significant.

Results

Expression of MIF in Nasal Polyps

Polyp tissue extracts and polyp tissue cultures conditioned media from six patients were tested

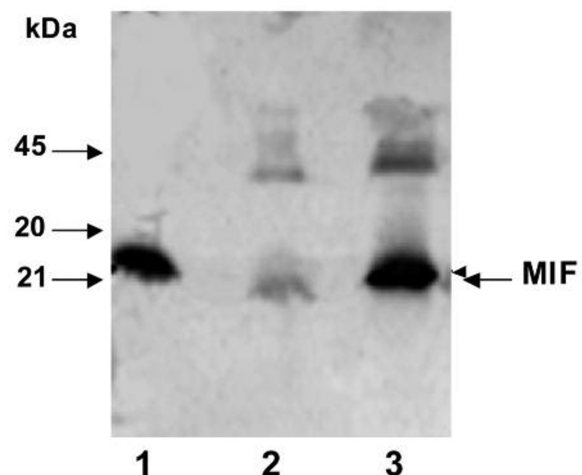

Figure 2. Representative Western Blotting of a polyps tissue extract (lane 2), and a 3-day polyps explant culture conditioned medium (lane 3), using a monoclonal antibody against human MIF. Recombinant human MIF, subjected to same treatment, was electrophorised and immunoblotted in parallel (lane 1). Markers of known molecular mass are shown to the left.

for the presence of MIF by sandwich ELISA. As shown in Figure 1, MIF was detected in all tissue extracts and cultures conditioned media. MIF levels in tissue conditioned media were higher as compared to tissue extracts (334.5 ± 89.9 and 120.7 ± 28.7 ng/mg of total protein, respectively). Histomorphological study showed that the five of polyp tissues contained mainly eosinophils and a small portion of lymphocytes, whereas one polyp tissue contained mainly lymphocytes and eosinophils comprised the minority. Very small was the portion of neutrophils in all tissues. Notably, the lower levels of MIF were detected only in the culture conditioned medium of polyp tissue containing mainly lymphocytes and a small portion of eosinophils.

The above data were further confirmed by western blotting. The monoclonal antibody employed in the ELISA as capture antibodies, was used. As shown in Figure 2 only one major immunoreactive band of approximately 12.5 kDa, migrating as the human recombinant MIF, was detected in polyp tissue extracts and culture conditioned media. The minor immunoreactive band of molecular mass ~37 kDa may represent MIF homotrimers, as previously reported[37].

The detection of MIF in the tissue cultures conditioned media, suggests that MIF is produced and secreted by polyps tissue cells. Taking into account that the higher levels of MIF were present in the cultures conditioned media of polyp tissues containing mainly eosinophils and a small portion of lymphocytes, it seems that eosinophils are the major producers of MIF in nasal polyp. This is in accordance with previous findings that in nasal mucosa from patients with NP, MIF is expressed in the surface and glandular epithelium, with eosinophils to form the largest population of MIF-expressing cells[29,30].

In order to support the above data the expression of MIF at the mRNA levels in polyp tissues was examined. For comparison, expression of MIF was also examined in nasal mucosa from normal individuals. For this purpose total RNAs, isolated from five samples of polyps tissue obtained from different patients (all tissues contained mainly eosinophils and a small portion of lymphocytes), and five samples of normal mucosa tissue obtained from different individuals, were analyzed by RT-PCR. Human MIF specific primers were used and the amplifying products were subjected to agarose gel electrophoresis (Figure 3). In all samples a 255 bp band was visualized, corresponding to human MIF, indicating that MIF is expressed by cells of polyp tissues. Importantly, the expression of MIF in polyp tissues was significantly higher ($p < 0.05$) as compared to normal nasal mucosa tissues. This result suggests that MIF may be implicated in the pathogenesis of NP.

Expression of IL-6 in Nasal Polyps and Effect of Dexamethasone on IL-6 Production

The growth of nasal polyps, although not fully understood, is a result of compound intercellular reactions mediated by overproduction of various cytokines including proinflammatory interleukins such as IL-6 and IL-8. Elevated levels of IL-6 have been demonstrated in nasal polyps compared with the normal controls[10]. Therefore, the expression of IL-6 at the mRNA levels in the same as above polyp and normal tissues was also examined by RT-PCR, using specific primers for human IL-6, and the amplifying products were subjected to agarose gel electrophoresis (Figure 4). As shown in Figure 4, IL-6 is significantly higher expressed (about twofold) in polyp tissues as compared to normal nasal mucosa tissues ($p < 0.0002$). These results suggest that apart from MIF, IL-6 may also be an important player implicated in NP pathogenesis.

It has been reported that glucocorticoids, such as dexamethasone, which are widely used to treat some types of nasal polyps, inhibit the expression of IL-6 in various cell types[8,31,32]. As was ascertained by immunohistochemical analysis, upon oral administration of corticosteroids in patients with chronic rhinosinusitis, the levels of IL-6 were significantly decreased when compared with pretreatment levels[38]. More recently, it was reported that dexamethasone at concentration 10 μM suppressed the production of IL-6 in nasal polyps organ culture[39]. In another study, using immunohistochemical analysis, no significant differences in the levels of IL-6 among the treated and untreated subjects were found after topical application of corticosteroids in patients with NP[40].

We, therefore, evaluated the effect of dexamethasone on the expression of IL-6 in nasal polyps. For this purpose a polyp tissue immediately after harvesting was cultured in the absence or presence of different concentrations of dexamethasone (1, 10 and 100 μM), for 72h and the IL-6 levels in the culture conditioned media were determined by ELISA (Figure 5). As shown in Figure 5, dexamethasone exhibits a substantial dose-dependent suppression of IL-6

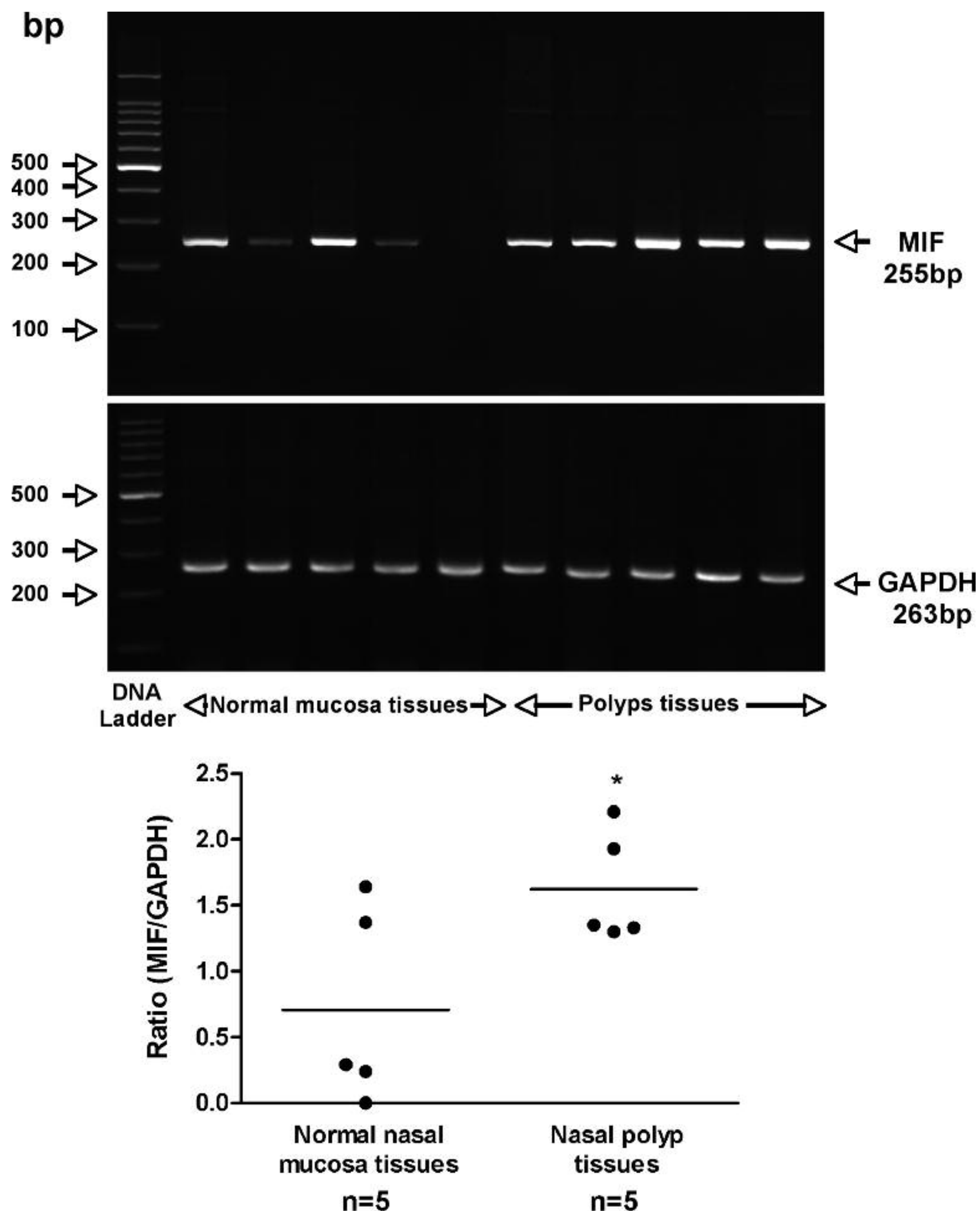

Figure 3. Agarose gel electrophoresis of RT-PCR products using total RNA from polyps and normal mucosa tissues. Total RNA was extracted from the tissues, as described in Patients and Methods, and used for RT-PCR analysis of MIF and GAPDH mRNA steady-state levels. The ratio of MIF mRNA level to that of the house-keeping gene GAPDH was established from the densitometric values of transcript scanning, using results within the linear part of amplification. *Indicates a statistically significant difference between polyp tissues and normal mucosa tissues ($p < 0.05$).

production. Particularly, significant suppression ($p < 0.0008$) of approximately 14%, 20% and 23% at dexamethasone concentrations 1, 10 and 100 μM, respectively, was noted. Since the higher suppression of IL-6 production was achieved by the concentration 100 μM of dexamethasone, this concentration was used in the followed experiments.

MIF Inhibition Enhances the Dexamethasone-Induced IL-6 Suppression

It is established that MIF attenuates the glycocorticoid-induced inhibition of inflammatory factors[32,41-44]. Therefore, we found of improve to evaluate its effect in the dexamethasone-induced IL-6 production. For this purpose, the MIF activity inhibitor ISO-1, which it was known that in-

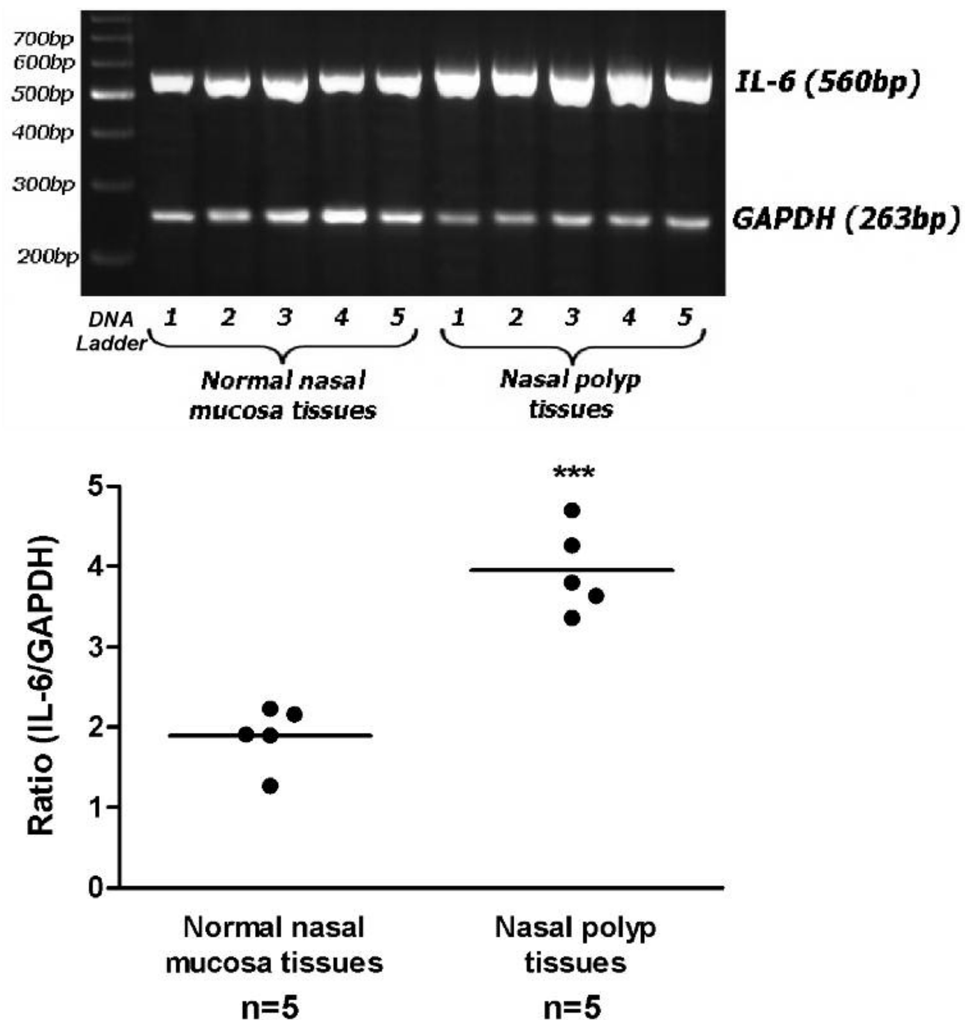

Figure 4. Agarose gel electrophoresis of RT-PCR products using total RNA from polyps and normal mucosa tissues. Total RNA was extracted from the tissues, as described in Patients and Methods and used for RT-PCR analysis of IL-6 and GAPDH mRNA steady-state levels. The ratio of IL-6 mRNA level to that of the house-keeping gene GAPDH was established from the densitometric values of transcript scanning, using results within the linear part of amplification. ***Indicates a statistically significant difference between polyp tissues and normal mucosa tissues ($p < 0.0002$).

hibits several MIF biological activities, including MIF counter-regulation of suppression of various factors production by glucocorticoid[24,28], was used. Particularly, six polyp tissues (five containing mainly eosinophils and a small portion of lymphocytes and one containing mainly lymphocytes and a small portion of eosinophils) were cultured with or without dexamethasone (100 μM) in the absence or presence of ISO-1 in two concentrations (100 and 200 μM) for 72h and IL-6 levels in the conditioned media were determined by ELISA. Dexamethasone caused statistically significant ($p < 0.0005$ – $p < 0.05$) suppression, ranging from 22.7 to 27.7 % (25.1% ± 2.1%, mean ± sd), in the IL-6 levels of all different polyp tissue cultures (Figure 6A-F). Notably, no significant differences were observed in the effect of dexamethasone between the six cultures, indicated that the variation in the type of cells in the tissues does not play a role in this effect. In the presence of ISO-1 (200 μM) an increased suppression of IL-6 levels by dexamethasone was observed, ranging from 37.8 to 46.5% (42.6% ± 2.7%, mean ± SD), in the six different polyp tissue cultures. ISO-1, at concentration of 100 μM, did not significantly affect the dexamethasone suppressive effect (Figure 6A-F). Given that, except of the fourth culture (Figure 6D), ISO-1 alone did not cause statistically significant decrease of IL-6 levels, an enhancement of dex-

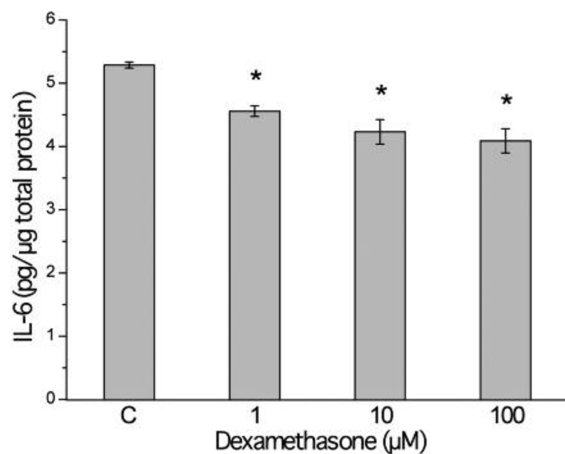

Figure 5. Levels of IL-6 in conditioned media obtained from a 3 day polyps explant culture without or with dexamethasone at different concentrations (1-100 μM). Values represent the mean ± SD of three independent experiments. C: Control. *Indicates a statistically significant decrease ($p < 0.0008$) as compared to control (without dexamethasone).

amethasone inhibitory effect in the presence of ISO-1 was achieved, ranging from 15.0 to 20.5% (17.5% ± 2.2%, mean ± SD).

Discussion

In the present study the expression of MIF in polyp tissue, as well as the effect of dexamethasone on the production of IL-6, in the absence or presence of MIF activity inhibitor ISO-1, were investigated.

The expression of MIF in NP and allergic rhinitis has been previously ascertained by immunohistochemical analysis[29,30]. Immunostaining of surface and glandular epithelium as well as of eosinophils was observed. In the present study, MIF expression in polyp tissues was confirmed by various biochemical tools such as RT-PCR analysis, western blot analysis and ELISA. Apart from eosinophils and epithelial cells, many other cells, such as fibroblasts, inflammatory cells and endothelial cells can produce MIF[17]. MIF expression in polyp cells does not necessarily mean that these cells also secrete it. It is known that significant amounts of preformed MIF protein exist in intracellular pools, mainly in macrophages, that can be rapidly released upon cell stimulation[45]. The presence of MIF in the polyp tissue cultures conditioned media in our study indicates that it was produced by the polyp cells and secreted in the extracellular matrix, where it may stimulate other cells to produce various inflammatory factors. It has been reported that MIF is able to induce the production by various cell types of cytokines, growth factors and prostaglandins[46-50] and to up-regulate the expression of IL-1 and TNF receptors[51] as well as of matrix metalloproteinases[52-54]. Therefore, MIF may contribute to the pathogenesis of NP by interfering with the function of such factors, which play an important role in polyp formation[1-3].

Regarding the expression of IL-6 in polyp tissues the results of present study are in accordance with the results of previous studies[10,13], further supporting the aspect that this cytokine may play a significant role in NP pathogenesis. The effects of dexamethasone on the production of IL-6, from tissue polyp cultures were, therefore, studied. The concentrations of dexamethasone used (1-100 μM), it was previously used in polyp organ culture[39]. Despite several reports demonstrating that glucocorticoid treatment downregulates proinflammatory and proangiogenic cytokines, including IL-6, in nasal polyps[39,55,56], no quantitative data were presented. Here, we show that dexamethasone at concentration 100 μM caused suppression of IL-6 levels in polyps tissue cultures conditioned media 25.1% ± 2.1% (mean ± sd). Even though high concentrations of dexamethasone were used, the achieved suppression of IL-6 production was lower than that previously achieved with lower dexamethasone concentrations in various cell cultures: i) 83% by 0.1 μM of dexamethasone in corneal fibroblasts stimulated by LPS[57], ii) about 50% by 10 μM in epithelial cells from nasal polyps or healthy nasal mucosa[8], and iii) 84% by 2.5 μM in pituitary folliculo-stellate-like cells stimulated by LPS[32]. This observation indicates that various factors, including MIF, which are present in nasal polyp may antagonize dexamethasone effect.

When nasal polyp tissue cultures took place in the presence of both the dexamethasone (100 μM) and the MIF inhibitor, ISO-1 (200 μM), an enhancement in the dexamethasone suppressive effect was observed. A further decrease 17.5% ± 2.2% (mean ± sd) of the levels of IL-6 in conditioned media was achieved. These observations suggest that the MIF produced by nasal polyps attenuates the inhibitory effect of dexamethasone on IL-6 production, and ISO-1, which inhibits the activity of MIF, restores the suppressive ability of dexamethasone. Although it has been previously reported that MIF induces the production of IL-6 by synovial fibroblasts from patients with rheuma-

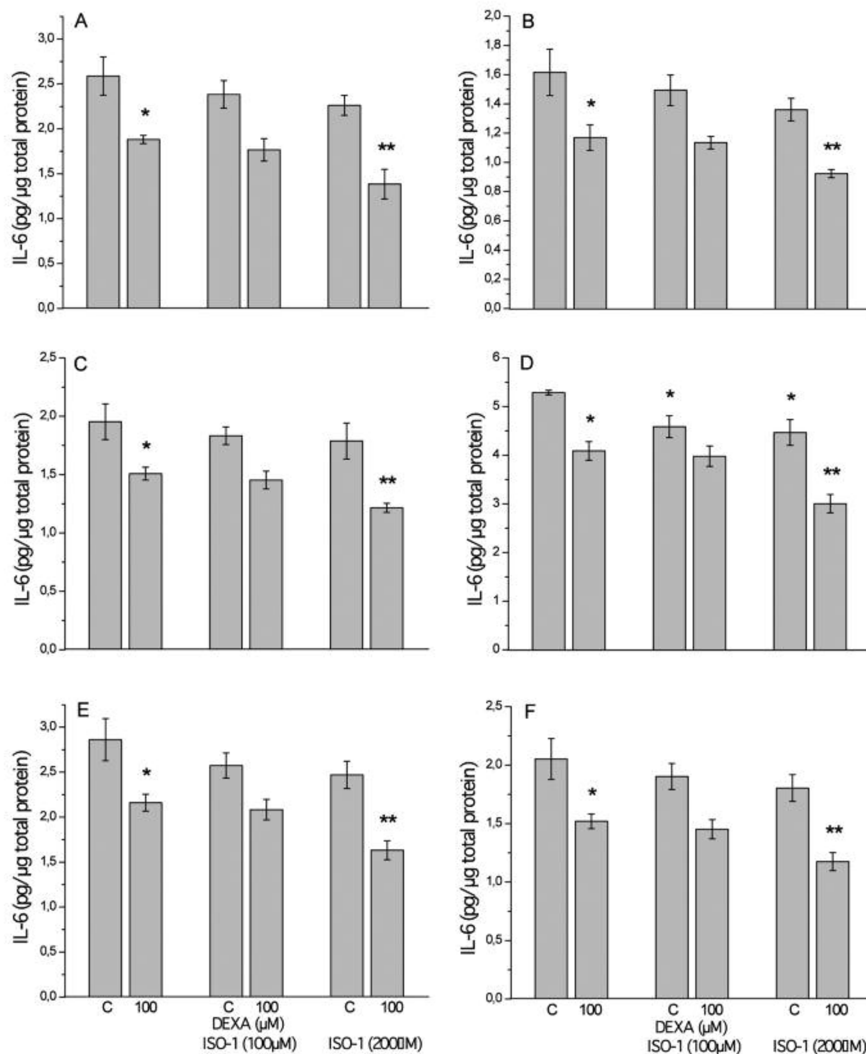

Figure 6. Levels of IL-6 in conditioned media obtained from 3-day polyps explant cultures of six different patients ***(A, B, C, D, E*** and ***F)*** without or with dexamethasone (DEXA) at concentration 100 μM, in the absence or presence of MIF-tautomerase activity inhibitor ISO-1 (100 and 200 μM). Values represent the mean ± SD of three independent experiments. C: Controls. *Indicates a statistically significant decrease ($p < 0.0005 – p < 0.05$) as compared to control (without dexamethasone). **Indicates a statistically significant decrease ($p < 0.003 – p < 0.03$) as compared to dexamethasone alone treatment.

toid arthritis[50,52], in nasal polyps seems that it has very low contribution on IL-6 production. ISO-1 alone did not exhibit statistically significant suppressive effect on the production of IL-6. Known that dexamethasone at low concentrations (~ 1-100 nM) stimulates the production of MIF in a variety of cells, including fibroblasts and macrophages, whereas at concentrations higher than 0.1 μM it suppresses MIF production[32,44], it was expected that the levels of MIF in nasal polyp culture conditioned media to be low, since the concentration of dexamethasone used was high (100 μM). However, the use of high concentrations of ISO-1 in order to restore the dexamethasone inhibitory ability suggests that MIF levels in culture conditioned media remain high, despite the presence of high concentrations of dexamethasone. It is, therefore, possible that some cell types in nasal polyps, such as eosinophils, lymphocytes and epithelial cells, that highly express MIF, may be not sensitive to high concentrations of dexamethasone. This possibility is under consideration.

It is known that glucocorticoids exert their inhibitory actions on gene transcription, through a variety of mechanisms, including: (1) the interaction of their receptor (GR) monomer with transcription factors such as nuclear factor-κB (NF-κB) and activator protein-1 (AP-1), prevent-

ing both receptors and transcription factors from binding to their respective DNA response elements (transrepression); (2) the inhibition of mitogen activated protein (MAP) kinases and other signaling pathways, which it seems to be mediated by an increased expression, as well as a decreased degradation by proteasome of the MAP kinase phosphatase-1 (MKP-1)[55].

MIF is considered a pro-inflammatory cytokine, but it is also able to antagonize the inhibitory effects of glucocorticoids, on the expression of various cytokines and growth factors, including IL-6[44]. The molecular pathways involved in the regulation of glucocorticoids-sensitivity by MIF have now been elucidated. MIF is able to suppress the glucocorticoids-induced expression of MKP-1, thereby indirectly inhibiting the dephosphorylation of MAPKs and restoring their activation. Through its capacity, MIF may antagonize the transrepressive effect of glucocorticoids on AP-1 activation. In addition MIF impairs glucocorticoids-induced transactivation of IκB-α, an inhibitor of NF-κB activation[44]. These MIF effects implicate that pharmacological glucocorticoids may not exert optimal effects when MIF is present. The antagonistic effect of MIF can be neutralized using inhibitors of MIF activity, such as ISO-1, which was used in this study. Indeed, the observed significant enhancement of dexamethasone inhibitory effect on the production of IL-6 in the presence of ISO-1, suggests that MIF present in nasal polyps attenuates the inhibitory effect of dexamethasone. This MIF ability may be explained by virtue of the presence one response element for each AP-1 and NF-κB in IL-6 promoter[58].

Conclusions

MIF is expressed in nasal polyp cells and secreted in extracellular matrix. Its expression in polyp tissue is higher than in normal nasal mucosa, indicated that it may play an important role in NP pathogenesis. Dexamethasone is able to suppress the IL-6 production by nasal polyps, and this ability is enhanced in the presence of MIF inhibitor ISO-1, indicated that the endogenous MIF attenuates the suppressor effect of dexamethasone. Known that IL-6, which is highly expressed in nasal polyps, is implicated in NP pathogenesis, the combination of dexamethasone and ISO-1 should be beneficial in the treatment of this disorder. Therefore, it is reasonable to propose that a formulation of glucocorticoids drugs, which includes a MIF inhibitor, would be more effective in the treatment of NP. Such MIF inhibitors, more specific and effective from ISO-1, have already been synthesized[59,60] and may be useful in clinical practice for the treatment of the disease.

Conflict of Interest

None to declare.

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