Expression of Three Forms of Melanoma Growth Stimulating Activity (MGSA)/gro in Human Retinal Pigment Epithelial Cells

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Purpose. To characterize mRNA expression and protein production of the cytokine MGSA/gro in human retinal pigment epithelial (RPE) cells and to determine whether expression of MGSA/gro is modulated by serum and the cytokines interleukin 1β (IL-1β), tumor necrosis factorα (TNFα), or transforming growth factor β (TGFβ) mediators implicated in proliferative vitreoretinopathy (PVR).

Methods. Reverse-transcription polymerase chain reaction was used to determine the steady-state mRNA expression of three forms of MGSA/gro, α, β, and γ, by cultured human RPE cells in the presence or absence of recombinant IL-1β, TNFα, or TGFβ, or when serum-starved cells were re-fed with medium containing serum. Immunocytochemistry was used to characterize RPE cell-associated MGSA/gro protein, and immunoprecipitation of MGSA/gro from cell-conditioned medium was used to demonstrate MGSA/gro secretion.

Results. MGSA/gro mRNA was expressed minimally under basal conditions. Expression for all three forms of MGSA/gro mRNA was induced in a dose- and time-dependent manner after exposure to IL-1β, to a lesser extent after exposure to TNFα, but not after exposure to TGFβ. Serum induced MGSA/gro α and γ transcripts, but not β transcripts. Cell-associated MGSA/gro was identified on RPE cells grown in the absence of cytokines, but MGSA/gro was not secreted under these conditions. Exposure to IL-1β did not consistently cause increased cell-associated MGSA/gro; however, IL-1β induced secretion of MGSA/gro in a time-dependent manner.

Conclusion. MGSA/gro is produced by human RPE in response to mediators implicated in PVR. Because MGSA/gro is a pleiotropic modulator of cell proliferation and inflammation, it may contribute to the intraocular wound healing response that characterizes PVR. Invest Ophthalmol Vis Sci 1993;34:2776–2785.

Melanoma growth stimulating activity/gro (MGSA/gro), is a recently described cytokine that was originally identified as an autocrine growth factor for melanoma cells.[1,2] Subsequently, a gene termed gro (for growth responsive) was identified in transformed hamster cells, human fibroblasts, and human tumor cells,[3] and a role for gro as an early response gene in cell growth and as a mediator of the IL-1β-induced inflammatory response was hypothesized.[4] Sequence analysis revealed that the MGSA gene and the gro gene were one and the same.[3,5] In addition to the gro gene originally described (gro α), two additional gro genes—gro β and gro γ—have been described.[6,7] These genes are identical to the recently described human homologues of the murine MIP-2 gene, MIP-2α

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and MIP-2β, respectively.[8] MGSA/gro β and γ share 93% and 87% identity with MGSA/gro α at the nucleotide level. The differences in nucleotide sequence among these genes result in 11 amino acid substitutions in gro β and 15 amino acid substitutions in gro γ. The functional significance of these alterations remains to be determined. MGSA/gro is a member of a family of proteins denoted CXC, which describes the arrangement of the first two cysteines. Other members of the CXC family include IL-8,[9] platelet factor 4, platelet basic protein and its cleavage products β-thromboglobulin and connective tissue activating protein III,[10] chicken-v-src-inducible proteins (9E3),[10,11] rat CINC,[12] γ-interferon inducible protein 10,[13] mouse macrophage inflammatory protein 2,[14] and the KC gene product.

Proliferative vitreoretinopathy (PVR) is a disease characterized by breakdown of the blood-retinal barrier associated with an intraocular wound healing response that leads to the formation of fibrocellular scar tissue on the surface and undersurface of the retina. Cell-mediated contraction of this tissue causes traction or rhegmatogenous retinal detachment. Key cell types, including retinal pigment epithelial (RPE) cells, macrophages, and retinal glial cells have been associated with this wound healing response.[15,16] Cytokines have been increasingly implicated in the pathogenesis of PVR. For example, elevated levels of the cytokines tumor necrosis factor (TNFα),[17] transforming growth factor β (TGF-β),[18] and interleukin 1[19] have been found in the vitreous of patients with PVR. In rabbit eyes, PVR is induced when serum components or TGFβ in combination with fibronectin[18] are injected into the vitreous cavity.

To date, MGSA/gro has not been identified in ocular tissue. In nonocular tissues, MGSA/gro is a pleiotropic cytokine that can influence cellular activities relevant to PVR such as cell proliferation and migration. We hypothesized that the RPE cell, an important cell in PVR, would express MGSA/gro under the influence of mediators that are found in increased quantities in the vitreous of patients with PVR. Here, we characterize mRNA expression and protein production of MGSA/gro in human RPE cells and determine whether expression of MGSA/gro is modulated by the presence of serum or the cytokines IL-1β, TNFα, or TGF-β.

MATERIALS AND METHODS

Cell Culture

Human cadaveric donor eyes were obtained from the North Carolina Organ Donor and Eye Bank within 24 hours of death. Cells were harvested from these eyes as previously described.[20] Cells were grown in Eagle's minimal essential medium (MEM) with 10% fetal bovine serum (FBS) at 37°C in a humidified environment containing 5% CO_2. For experiments, second to fifth passage cells were seeded at a density of 30,000 to 40,000 cells/ml in 24-well culture plates or in 35-mm or 60-mm culture dishes and were grown to visual confluence. Purity of RPE cell cultures was confirmed by cytokeratin staining as previously described.[21] Cells used in these experiments formed a contact-inhibited monolayer, exhibited polygonal morphology characteristic of RPE cells, and were stained uniformly for cytokeratins.

RNA Extraction

Cells were grown to visual confluence in 60-mm tissue culture dishes in MEM with 10% FBS. For experiments, cells were exposed to MEM + 10% FBS, alone or in combination with varying dosages of recombinant human IL-1β, TGFβ (Collaborative Research, Bedford, MA), TNFα (Amgen Biologicals, Thousand Oaks, CA), or cycloheximide (1 μg/ml, Sigma, St. Louis, MO) at 37°C, 5% CO_2 for varying time intervals. At the end of the incubation period, cells were rinsed twice with MEM, and total RNA was extracted as previously described.[22] RNA purity was determined by measuring OD_{260}/OD_{280}, and RNA quantity was estimated from OD_{260}.

cDNA Synthesis

Total RNA was converted to cDNA using a modification of a previously described technique.[23] One microgram of total RNA in 10 μl of DEPC-treated water was added to 10 μl of reverse transcription mixture consisting of 2 μl of 10X PCR buffer (500 mM KCl, 100 mM Tris HCL pH 9, 15 mM MgCl, 0.01% [wt/vol] gelatin), 4 μl of 5 mM dNTP (Pharmacia LKB Biotech. Inc, Piscataway, NJ), 1 μl of Maloney Murine Leukemia virus reverse transcriptase (200 U/μl; BRL, Gaithersburg, MD), 1 μl of random hexamers (100 pmol/μl; Pharmacia LKB Biotech, Inc.), 0.5 μl of RNasin (40 000 units/ml; Promega, Madison, WI), and 1.5 μl of DEPC-treated water. This mixture was incubated for 45 minutes at 37°C and then for 5 minutes at 90°C. cDNA was stored frozen at −70°C.

PCR Amplification

One microliter of cDNA mixture was added to a PCR reaction mixture consisting of 1X PCR buffer, 2.5 pmol dNTP, 5 pmol of MGSA/gro α, β, or γ or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primer pairs, 1.25 unit TAQ polymerase (Promega), and distilled water in a total volume of 50 μl. The sequences of the MGSA/gro and GAPDH primer pairs have been previously described.[6,24] GAPDH primer pairs were synthesized at the Nucleic Acid Synthesis and Analysis Facility at Lineberger Cancer Center (Chapel Hill, NC). The reaction mixture was overlaid

with mineral oil and amplified in a PCR thermal cycler (Perkin-Elmer/Cetus, Norwalk, CT) as follows: denaturation at 94°C for 1 minute, primer annealing at 55°C for 2 minutes, and extension at 72°C for 3 minutes. Only the inner wells of the PCR thermal cycler were used to minimize variations in temperature across the heating block. To verify that equivalent quantities of mRNA in unknown samples were reverse transcribed to cDNA, samples were also incubated with GAPDH primer pairs as previously described.[24] cDNA from adherent human macrophages was used as positive control PCR template for MGSA/gro α, β, and γ. PCR reaction mixtures without the addition of cDNA template were used as negative controls. In some experiments, positive control cDNA template obtained from RPE cells stimulated with IL-1β was serially diluted and amplified with corresponding specific primer pairs in parallel with unknown samples. To be certain that the amount of cDNA in the standard dilution curve bracketed the amount in the unknown samples, the standard dilution curve was prepared beginning with 3 μl of cDNA in the PCR mixture (1 μl of unknown sample cDNA was used in the PCR mixture). All cDNA samples were amplified in parallel with dilution series in the same PCR run to eliminate run-to-run variation. Ten μl aliquots were removed from the reaction mixture at different cycles to insure that PCR products were evaluated during the exponential phase of the amplification process. PCR products from the unknown samples and dilution series were run on a 2% agarose gel (Ultrapure, BRL, Gaithersburg, MD) with TBE (TBE: Tris 21 g/l, boric acid 11 g/l, EDTA .002 M, pH = 8) running buffer. Gels were stained with ethidium bromide and photographed with Polaroid film (type 55) (Polaroid, Cambridge, MA).

The relative intensity of the bands was measured using densitometric measurements obtained from the photographic negatives with a high-resolution black-and-white camera (Dage/MTI, Michigan City, IN) coupled to an image processor (Imaging Technology, Woburn, MA), driven by JAVA densitometric software (Jandel Scientific, San Rafael, CA) as previously described.[25,26] Relative differences in mRNA expression among samples were determined by comparison with the standard dilution curve as previously described.[27-30] Measurements obtained from the dilution series were fit to a regression line using Cricket Graph software on a Macintosh computer (Cupertino, CA). The relative concentration of mRNA was determined by comparison with the calculated regression line.

Restriction Enzyme Analysis

Restriction enzyme analysis was performed to confirm the identity of PCR products as previously described.[27,31] MGSA/gro α, β, and γ PCR products have unique restriction sites for HinD III, Msp I, and Hpa I, respectively. Samples of MGSA/gro α, β, and γ PCR products were digested with HinD III, Msp I, and Hpa I (Promega), respectively. Restriction digests were run on agarose gels, and bands were visualized with ethidium bromide.

Immunocytochemistry

To examine the distribution of MGSA/gro in cultured RPE, cells were seeded at 20 000 cells/ml in 8-well plastic chamber slides (Lab Tek, Napier IL). Cells were grown in MEM + 10% FBS. After 72 hours, cells were washed twice with MEM, and medium was replaced with fresh MEM + 0.2% lactalbumin hydrolysates (LH; Sigma, St. Louis, MO), MEM + 1% FBS, or MEM + 10% FBS, in the presence or absence of IL-1β (5 U/ml). After varying time periods, cells were fixed with 10.7% formalin/0.2% glutaraldehyde/0.6% Tris buffered saline (pH = 7.5). Cells were stained for MGSA using monoclonal antibody FB2AH7 (1:2 dilution of supernatent fluid) and the Vectastain ABC kit (Vector-Lab, Burlingame, CA) as previously described.[5] For controls, cells in parallel wells were processed identically except that a mouse monoclonal antibody (IgM_κ) to granulocyte associated antigen (1:10 dilution; DAKO-M1; Dakopatts a/s, Glostrup, Denmark) was substituted for FB2AH7 (IgM_κ). This antibody was used at a 1:10 dilution to deliver an IgM concentration equivalent to that of FB2AH7 at the 1:2 dilution.

Metabolic Labeling and Immunoprecipitation of MGSA/gro Protein

Retinal pigment epithelial cells were seeded in 35-mm culture dishes and grown to visual confluency in MEM + 10% FBS. Cells were rinsed twice with phosphate buffered saline, once with cysteine and methionine-free MEM (Lineberger Cancer Institute Tissue Culture Facility, Chapel Hill, NC) and incubated for 24 hours with 750 μl of cysteine and methionine-free MEM and ^{35}S-labeled cysteine and methionine (150 μCi/ml; ICN) in the presence or absence of IL-1β. After incubation, PMSF was added to a final concentration of 1 mM, and MGSA/protein was immunoprecipitated using a modification of a previously described technique,[32] as follows: Samples were pretreated with 10 μl of normal goat serum and a suspension of 4 mg protein A-Sepharose in 40 μl of 20 mM HEPES pH = 7.4 at 4°C for 1 hour. The protein A-Sepharose beads were removed by centrifugation, and the pretreated samples were incubated overnight at 4°C with MGSA/gro α antiserum raised in rabbits to the KLH-coupled peptide C-PIVKKIIEKMLNSD-KSN at a 1:200 dilution. The next day, samples were incubated for 1 hour with a suspension of 4 mg protein A-Sepharose beads in 40 μl of 20 mM HEPES (pH = 7.4). The protein A-Sepharose beads were pelleted and then washed four times in Tris-buffered RIPA (10

mM Tris, pH = 7.4, 1% Triton X-100, 1 mM PMSF, 30 mM pyrophosphate, 50 mM NaCl, 50 mM NaF, 0.1 M Na_3VO_4, 10 μg/ml aprotinin). The washed beads were heated with 100 μl of SDS gel electrophoresis loading buffer at 100°C for 5 minutes. The beads were removed by centrifugation, and the supernatent was loaded on a 15% SDS-polyacrylamide gel.[33]

RESULTS

MGSA/gro mRNA Expression

Semiquantitative PCR was used to determine the relative amount of MGSA/gro mRNA expressed in the presence or absence of IL-1β (5 U/ml for 4 hours), TNFα (10 ng/ml for 4 hours), or TGFβ (10 ng/ml for 4 hours) in 10, 6, and 5 different human RPE cell lines, respectively. Results from this type of analysis are similar to those determined from Northern blot analyses (Jaffe GJ, unpublished data, 1990). For each cell line tested, transcripts for MGSA/gro either were not detected or were expressed at low levels in the absence of cytokines, but all three forms (MGSA/gro

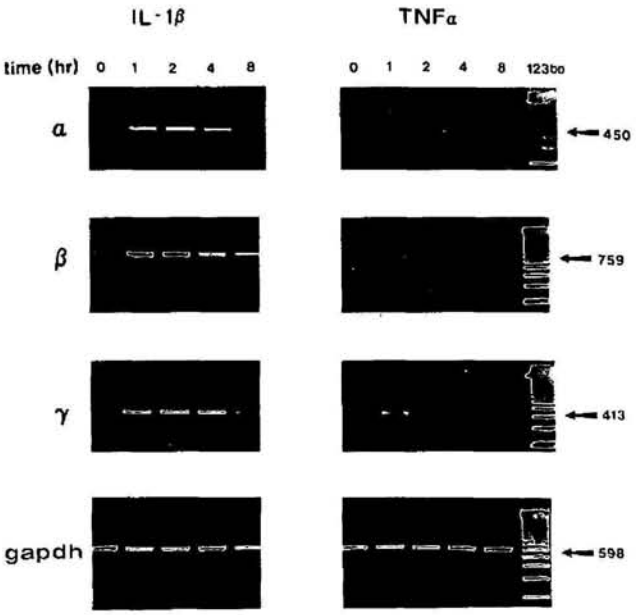

FIGURE 2. PCR-cDNA gel showing the effect of varying exposure periods to IL-1β (5 U/ml) or TNFα (10 ng/ml) in a representative cell line (cell line T-31). PCR amplification for 35 cycles.

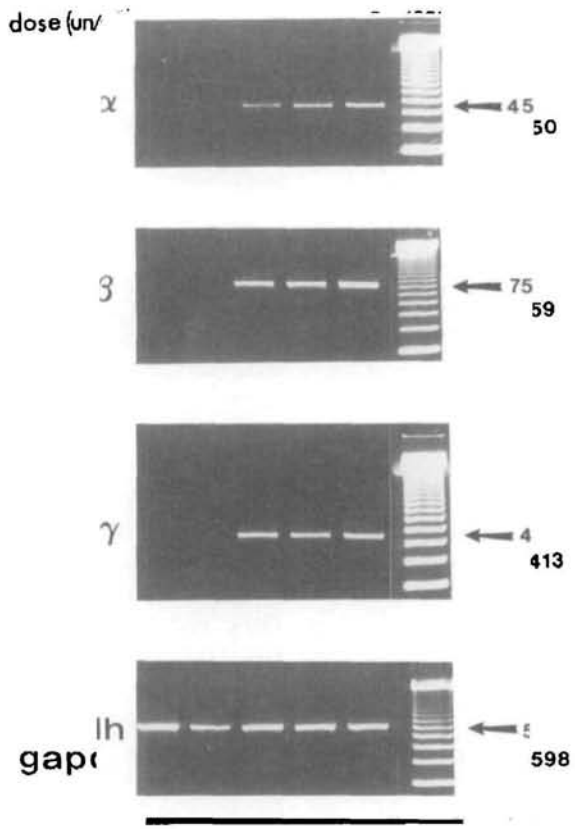

FIGURE 1. PCR-cDNA gel showing the effect of varying concentrations of IL-1β on the expression of MGSA/gro mRNA in a representative cell line (cell line T-35). Cells were exposed to IL-1β for 4 hours. PCR amplification for 35 cycles.

α, β, and γ) were induced after exposure to IL-1β and TNFα. In contrast, TGF-β did not consistently induce MGSA/gro expression. Induction of MGSA/gro transcripts was dose- and time-dependent. Maximal induction occurred after exposure to 5 U/ml IL-1β (Fig. 1) and 10 ng/ml TNFα (not shown). Induction occurred rapidly; steady state transcripts were detected by 1 hour and were maximal 2 to 4 hours after cells were exposed to recombinant cytokines. By 8 hours, steady state mRNA expression had begun to decrease (Fig. 2). IL-1β consistently induced higher levels of MGSA/gro than TNFα (Table 1). To determine whether new protein synthesis was important for cytokine-mediated induction of MGSA/gro mRNA, cells were exposed to IL-1β (5 U/ml) in the presence or absence of the protein translation inhibitor cycloheximide. Exposure of cells to IL-1β in the presence of CHX resulted in superinduction of MGSA/gro mRNA, compared to the levels produced by exposure to IL-1β alone (Fig. 3). Exposure to CHX alone (1 μg/ml) produced moderate induction of MGSA/gro.

When cells were grown continuously in serum containing medium, only minimal levels of MGSA/gro were expressed. However, when cells were serum-starved and re-fed with medium containing 10% FBS, there was a time-dependent induction of MGSA/gro α and γ similar to that observed after exposure to IL-1β and TNF α; MGSA/gro α and γ expression was first detected at 1 hour, was maximal at 4 hours, and declined thereafter (Fig. 4). In contrast, serum did not induce MGSA/gro β expression.

TABLE 1. Amount of MGSA/gro mRNA Induced by IL-1β Relative to TNFα*

Target mRNA	*Relative Induction*
MGSA/gro α	4.4
MGSA/gro β	10
MGSA/gro γ	3.8

* Values represent the ratio of the maximal amount of PCR product detected in time-course experiments for IL-1 induction to the maximal amount of PCR product measured in time-course experiments for TNFα. The amount of PCR product was determined by comparison with a standard dilution curve, as described in Materials and Methods.

Immunocytochemistry

In three RPE cell lines, the immunocytochemical staining pattern of cell-associated MGSA/gro was evaluated in the presence or absence of IL-1β and in conjunction with measurement of MGSA/gro mRNA expression. MGSA/gro was detected immunocytochemically in all three cell lines (Table 2). The response to IL-1β varied among the cell lines. In two lines, there was no significant difference in the intensity of staining when cells were exposed to IL-1β (5 U/ml; Figs. 5A and B). In a third cell line, there was a time-dependent increase in the intensity of staining up to a maximum at 8 hours after cells were exposed to IL-1β (5 U/ml; Figs. 5C and D). The proportion of positively stained cells also varied among cell lines. In two of three lines, a higher percentage of cells was stained after exposure to IL-1β, whereas in a third line, there was no difference in the percentage of cells stained after exposure to IL-1 (Table 2). In all cell lines, staining was distributed fairly evenly throughout the cytoplasm (Fig. 5).

Staining was observed among cells exposed to MEM + 0.2% LH, MEM + 1% FBS, or MEM + 10% FBS. The staining pattern (percentage of positively stained cells and distribution of stain within individual cells) was similar for cells grown in each of these media; however, the overall intensity of the staining was

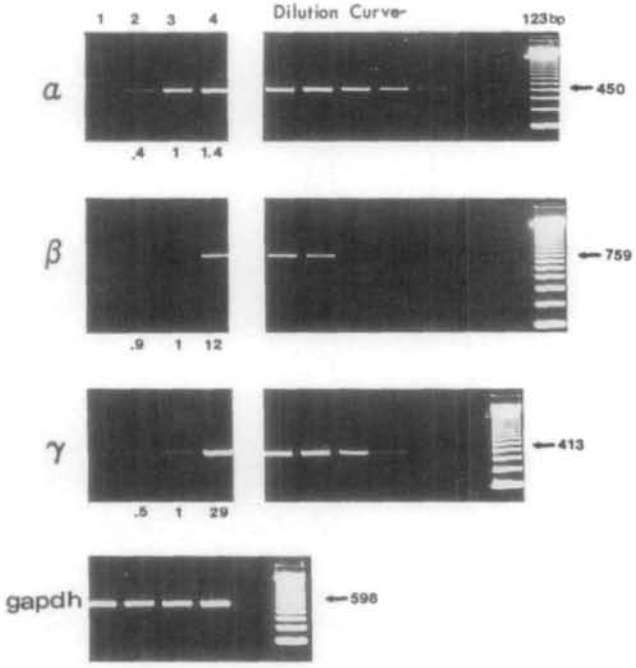

FIGURE 3. PCR-cDNA gel showing the effect of cycloheximide (CHX) on MGSA/gro α, β, and γ mRNA expression in a representative cell line (cell line T-61). Cells were exposed for 4 hours to medium alone (lane 1), or medium containing CHX (1 μg/ml, lane 2), IL-1β (5 U/ml, lane 3), or IL-1β (5 U/ml) + CHX (1 μg/ml); (lane 4). Concentration of mRNA in samples treated with CHX alone or IL-1β + CHX relative to samples treated with IL-1β alone indicated below corresponding lanes. Lanes from dilution curve correspond to serial threefold dilutions of positive control template. Positive control template consisted of cDNA (1 μg/ml) that was reverse transcribed from RNA from human RPE stimulated for 4 hours with IL-1α (5 U/ml) + CHX (1 μg/ml). The most concentrated sample was obtained by using 3 μl of positive control template in the PCR reaction.

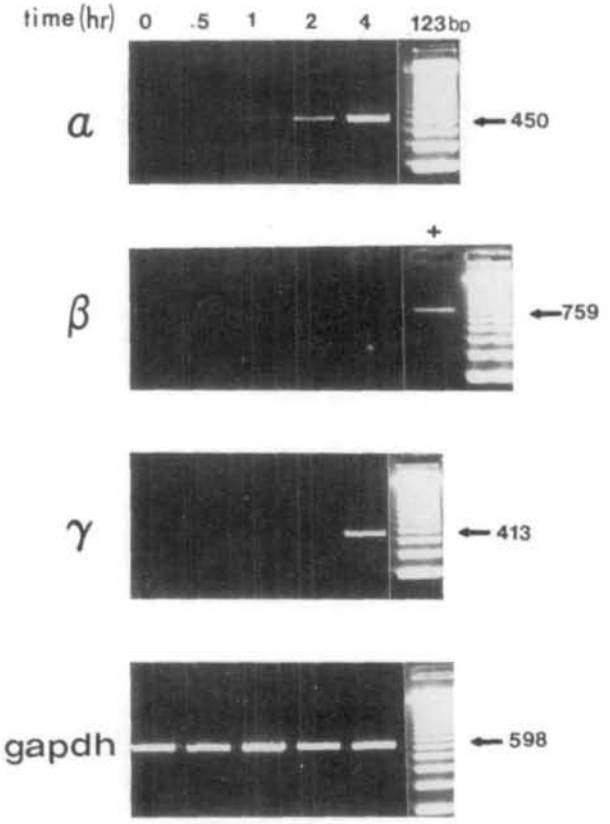

FIGURE 4. PCR-cDNA gel showing the effect of serum on MGSA/gro mRNA expression (cell line T-93). Cells exposed to serum-free medium (MEM + 0.2% LH) for 24 hours, then re-fed for indicated time periods with serum-containing medium (MEM + 10% FBS). + = positive control. cDNA template for positive control was obtained from reverse transcription of total RNA extracted from human RPE stimulated for four hours with IL-1β (5 U/ml).

TABLE 2. Relative Staining Intensity and Percentage of Positively Stained Cells for Three Different RPE Cell Lines Stained Using Monoclonal Antibody to MGSA/gro α

	*Staining Intensity**		*Percentage of Stained Cells†*	
Cell Line	*Unstimulated*	*IL-1β-Induced*	*Unstimulated*	*IL-1β-Induced*
T-77	0–+	++	84	94
T-85	++–+++	++–+++	98	98
T-76	++	++	55	98

*Graded on a 0 to +++ scale. 0 = no staining; + = slight staining; ++ = moderate staining; +++ = intense staining.
† Number expressed as percentage of positively stained cells out of total of 300 cells counted.

greatest for cells in MEM + 10% FBS and least for cells in MEM + 0.2% LH.

In contrast to the pattern observed when cells were stained using a primary antibody specific for MGSA/gro, controls with an antibody to granulocyte-associated antigen showed minimal staining. With control antibody, staining was nondetectable in two of three cell lines under all conditions tested (Figs. 5E and F). In the third cell line, there was light diffuse staining of cells exposed to IL-1β for 8 hours that was much less intense than that observed in cells stained with MGSA/gro-specific antibody. In this cell line, staining was undetectable for all other time points with control antibody.

MGSA/gro Secretion

To determine whether RPE cells secrete MGSA/gro protein, MGSA/gro α protein was immunoprecipitated from RPE-conditioned medium obtained from three separate cell lines. MGSA/gro α was not detected in conditioned medium from any of the cell lines when cells were grown in medium alone. In contrast, when cells were exposed to medium containing IL-1β, all three cell lines secreted MGSA/gro α that was readily detected by immunoprecipitation (Fig. 6). The time course of MGSA/gro secretion was examined in one cell line. MGSA/gro was first detected in RPE-conditioned medium by 4 hours and increased thereafter during the collection period (Fig. 7).

DISCUSSION

We have shown that in RPE cells, MGSA/gro mRNA is minimally expressed under basal conditions. In contrast, mRNA expression is induced for all three forms —MGSA/gro α, β, and γ—after cells are exposed to the inflammatory cytokines IL-1β and TNFα, and mRNA for gro α and γ is induced after serum-starved cells are re-fed serum. The pattern of mRNA expression for each of the specific forms of MGSA/gro is cell-type specific. Like RPE cells, human umbilical vein endothelial cells, human foreskin fibroblasts, and mammary epithelium do not express MGSA/gro under basal conditions, but all three forms are induced after exposure to IL-1 or TNFα.[4,6,34,35] In contrast, adherent macrophages express MGSA/gro α, β, and γ constitutively, whereas polymorphonuclear leukocytes and lymphocytes only express MGSA/gro α constitutively. The significance of these varying expression patterns is unclear. Differential regulation of MGSA/gro mRNA among different cell-types suggests possible functional differences in the translated proteins. Although differences in protein structure and function have been predicted on the basis of differences in the amino acid sequences coded by MGSA/gro α, β, and γ mRNA,[6] little is actually known regarding the full range of biologic activities of each of these peptides.

Cycloheximide alone induced MGSA/gro mRNA and caused superinduction of IL-1 mediated expression. Superinduction of MGSA/gro also has been observed for other cell types.[4,5,19] Similarly, we have observed CHX-mediated superinduction of macrophage colony stimulating factor[27] and IL-1[28] in human RPE. These results indicate that new protein synthesis is not required for MGSA/gro expression. Furthermore, as previously suggested, expression may be regulated post-transcriptionally by a repressor protein that recognizes AU-rich sequences that mediate rapid cytokine mRNA degradation.[3,5]

The mechanism by which cytokines and serum activate MGSA/gro mRNA in RPE cells remains to be determined. Steady state mRNA levels can be increased by transcriptional activation or by stabilization of MGSA/gro mRNA. Our study design did not allow us to distinguish between these two mechanisms. However, in fibroblasts, IL-1 and TNFα transcriptionally activate all three forms of MGSA/gro via the cytokine response element NF-kB. This cytokine-mediated response differs from the growth-mediated activation of transcription induced by serum.[36] In fibroblasts, MGSA/gro α, β, and γ mRNA also are stabilized by IL-1, thereby prolonging their respective half-lives.[34] We propose that in RPE, expression of MGSA/gro may be similarly regulated transcriptionally and post-

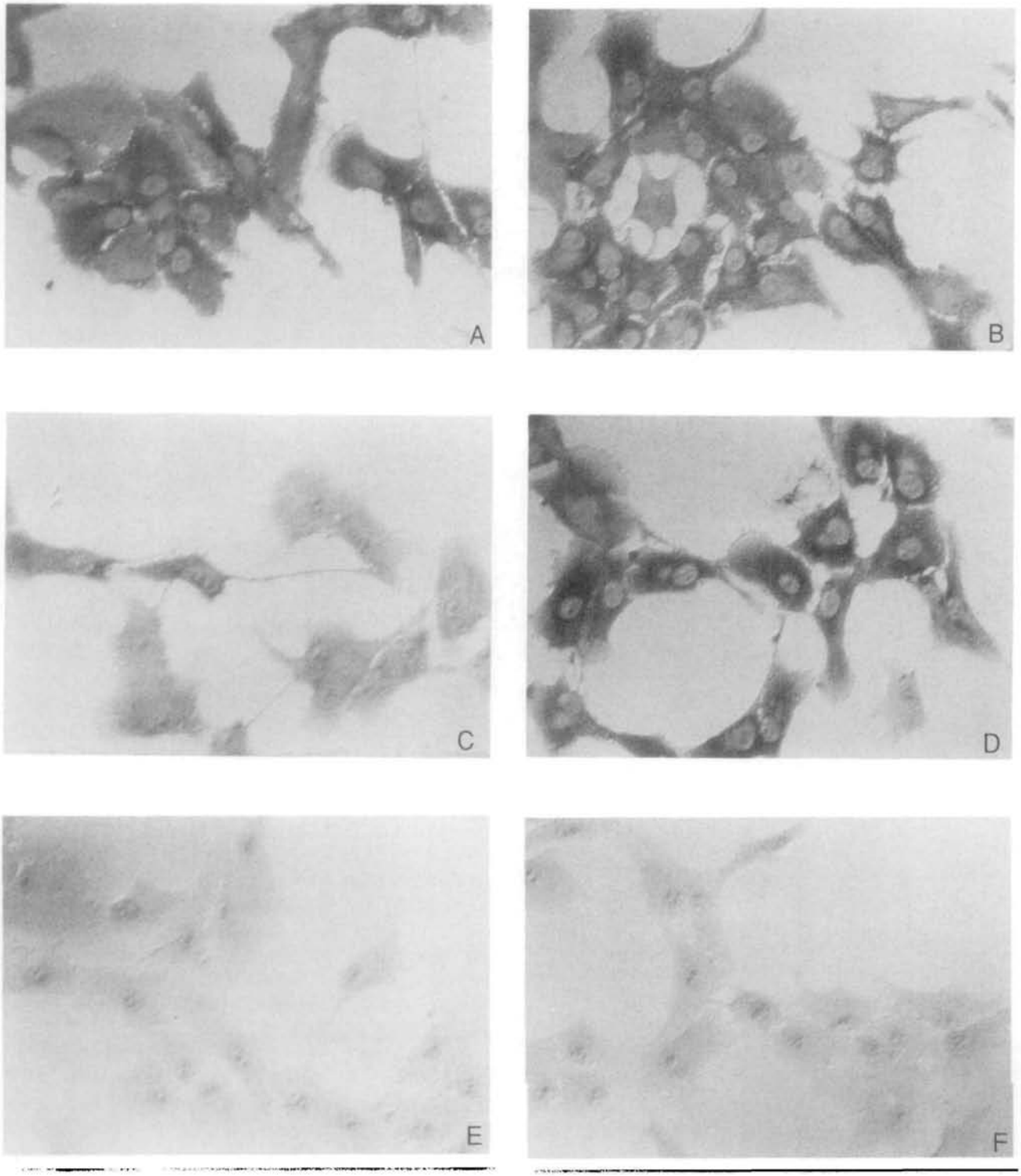

FIGURE 5. Immunocytochemical staining of two representative RPE cell lines for MGSA/gro. Cells were exposed to medium alone (MEM + 10% FBS; **A, C, E**), or to medium containing IAB-1β (U/ml) for 8 hours (**B, D, F**), then stained with the MGSA/gro primary antibody FB2AH7 (**A, B, C, D**) or with a nonspecific antibody (**B, E**). (**A, B**) From cell line T-76. (**C, D, E, F**) From cell line T-77.

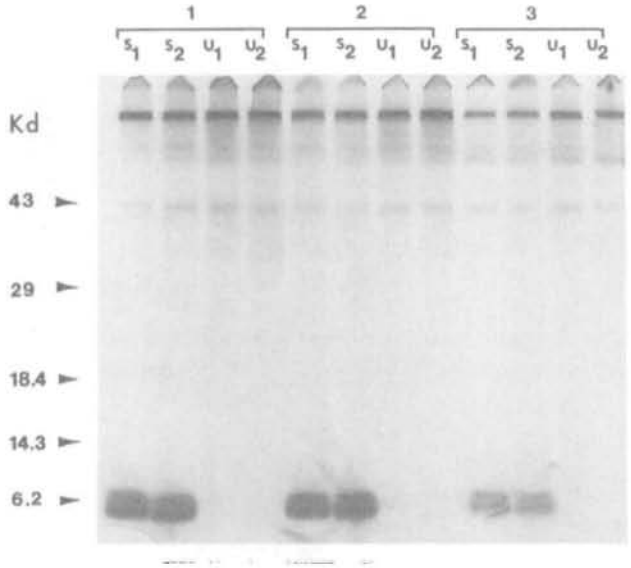

FIGURE 6. Autoradiogram of MGSA/gro α protein immunoprecipitated from RPE-conditioned medium. Samples from cells in duplicate culture dishes incubated for 24 hours in the presence or absence of IL-1β (5 U/ml) were obtained from three different cell lines, T-91, T-85, and T-35, as indicated by numbers above lanes—1 to 3, respectively. S = samples from cells stimulated with IL-1β (5 U/ml). U = samples from unstimulated cells.

transcriptionally by serum, IL-1, and TNFα. Experiments are currently under way to test this hypothesis.

MGSA/gro protein secretion paralleled mRNA expression; MGSA/gro was secreted only after cells were exposed to IL-1β. In contrast, cell-associated MGSA/gro was detected by immunocytochemistry, in the absence of cytokines, in all cell lines examined, but the response to IL-1 was variable; only one of three cell lines consistently showed a time-dependent increase in cell-associated MGSA after exposure to IL-1β. In the remaining two cell lines, it is possible that our immunocytochemical technique was not sensitive enough to detect small IL-1β-induced increases in cell-associated MGSA/gro. Alternatively, it is possible that in these cell lines, IL-1β stimulated MGSA/secretion at a rate that was approximately equivalent to new protein synthesis, resulting in no net accumulation of cell-associated MGSA/gro. The proportion of cells staining for MGSA/gro in uninduced and IL-1β-induced cultures also differed among cell lines, suggesting possible cell cycle variations. In any case, it is likely that the differences observed among cell lines reflect inherent biologic variability among donors. Previously, we have observed similar donor variability in RPE protein synthesis for the cytokines IL-1α and macrophage colony stimulating factor.[27,28]

The RPE cell, thought to be a key cell type involved in PVR, is a potential source for a wide variety of cytokines. In vitro, RPE cells constitutively produce basic fibroblast growth factor, transforming growth factor-β,[18] insulin-like growth factor, macrophage colony stimulating factor,[27] monocyte chemoattractant and activating factor (MCAF),[38] and a platelet-derived growth factor-like activity.[37] Other cytokines, including interleukin 1α and 1β,[28] interleukin 8,[38] and, as shown in the current report, MGSA/gro, are not expressed basally but are produced rapidly in response to exogenous inflammatory peptides such as IL-1 and TNFα. Because TNFα and IL-1 are present in elevated quantities in the vitreous from patients with PVR,[17,19] and IL-1 activity is present in subretinal fluid from patients with retinal detachment,[40] these cytokines may serve as potential stimuli for induction of IL-1, IL-8, and MGSA/gro during the evolution of PVR. Although increased quantities of TGF-β have been found in the vitreous of patients with PVR, we did not consistently observe induction of MGSA/gro mRNA in response to TGFβ. In PVR, it is likely that TGF-β influences aspects of the wound healing response that may be unrelated to modulation of MGSA/gro expression. PVR is associated with breakdown of the blood-retinal barrier.[15] We found that serum stimulated MGSA/gro mRNA expression in RPE cells. Thus, in PVR, serum components that leak into the vitreous could act alone or in concert with cytokines like IL-1β and TNFα to induce expression of MGSA/gro in RPE.

Interleukin 8, MGSA/gro, and MCAF are members of a rapidly emerging group of small peptides that have been recently termed the "chemokine" family.[41] IL-8 and MGSA/gro are particularly closely related; genes encoding these proteins are located on chromosome 4.[42] Both proteins competitively bind to the neutrophil IL-8 receptor[41] and mediate several functions of polymorphonucleocytes.[43,44] Additionally, both cytokines influence the activities of monocytes,[44] an important cell type implicated in PVR and certain types of uveitis. In the current report, we have

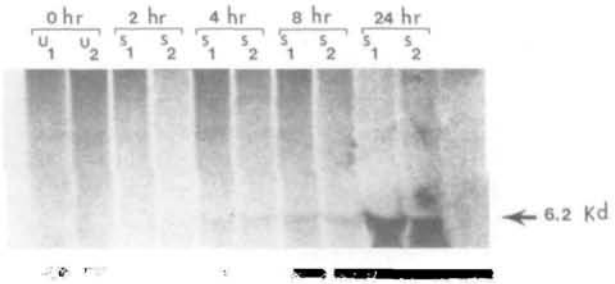

FIGURE 7. Autoradiogram of MGSA/gro α protein immunoprecipitated from RPE-conditioned medium. Samples from cells in duplicate culture dishes (cell line T-85) were incubated for varying time periods in the presence or absence of IL-1β. S = samples from cells stimulated with IL-1β (5 U/ml) for varying time periods (hours) as indicated above lanes. U = samples collected from cells grown in IL-1β-free medium for 24 hours.

demonstrated that MGSA/gro is produced by RPE cells in response to IL-1β and TNFα with a time course similar to that observed for IL-8. It is not immediately evident why two closely related cytokines would be produced by RPE cells under similar conditions and would then antagonize one another's activity at the receptor level. However, the complete range of factors that mediate transcription, translation, and secretion of these peptides has yet to be determined; depending on the situation, one may be produced preferentially. Furthermore, the MGSA receptor appears to have different physicochemical properties than the IL-8 receptor,[45] and activation of the receptor may initiate different second messengers than those transduced by the IL-8 receptor. Although some of the biologic activities elicited by MGSA/gro and IL-8 overlap, their full spectra of bioactivities are far from understood and may differ significantly. Recently, purified recombinant human MGSA/gro α and γ have been obtained from mammalian and insect expression systems, respectively,[32,46] and recombinant IL-8 is now commercially available. With the availability of relatively large quantities of purified protein, it will be possible to begin to clarify the role of these agents in ocular disease.

Key words

MGSA/gro, retinal pigment epithelial cells, proliferative vitreoretinopathy, polymerase chain reaction, cytokines

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