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□원 저□

The Expression of IL-8 and GROα/MGSA in HUVEC Stimulated by the TNF-α and IL-1†

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= 국문초록 =

TNF-α와 IL-1 자극에 의한 제대정맥내피세포에서의 IL-8 및 GRO/MGSA의 발현

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연구배경 :

호중구는 급성염증이 있을 때 존재하는 제일 많은 세포로서 폐의 염증이 있을 때에도 말초혈액에서부터 폐로 이동됨과 동시에 활성화된 호중구에서 분비하는 단백분해효소, 산화물 및 여러 가지 cytokine등에 의해 염증이 더욱 심해지고 호중구의 이동이 더욱 증가되는 것으로 알려져 있다. 호중구의 이동을 증가시키는 물질로 현재까지 LTB4, PAF, C5a, fMLP, TNF, IL-8 등이 밝혀져 있으며 특히 IL-8은 chemokine이라고 부르는 조그만 cytokine에 속하며 GRO/MGSA는 IL-8과 같은 C-X-C subgroup에 속하는 단백질로서 이 유전인자는 PF-4, IL-8등과 같은 chromosome 4q12-q21에 위치한다. IL-8이 호중구의 이동 및 활성화에 강력한 영향을 미치는 것은 잘 증명되어 있지만 같은 C-X-C subgroup에 속하며 최근에 발견된 GRO/MGSA의 기능 및 발현과정 등에 대해서는 잘 밝혀져 있지 않다. 내피세포는 TNFα나 IL-1β 등의 자극을 받으면 호중구의 유착을 돕는 접착분자 및 IL-8등의 생성이 증가되는등 염증반응에 능동적인 역할을 한다고 밝혀지고 있다. 저자들은 인체의 제대정맥에서 내피세포를 분리하여 TNF나 IL-1로 자극을 가하였을때 호중구의 이동을 증가시키는 IL-8이나 GRO/MGSA mRNA의 발현과 분비가 일어나는지 또한 호중구 화학주성에 미치는 각각의 기여도를 비교, 관찰하고자 하였다.

방 법 :

제대정맥에서 내피세포를 분리, 배양하고 여기에 TNF-α, IL-1β 등을 0.2, 2, 20 μg /ml 농도로 자극을 가하고

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1, 4, 8, 24시간이 경과하여 IL-8 및 GRO/MGSA mRNA의 발현을 RT-PCR로 관찰하고, 배양상층액내 IL-8 및 GRO/MGSA의 양을 ELISA로 측정하였으며 상층액의 호중구 화학주성 활성화도를 Neuro Probe 48 well chemotactic chamber를 이용하여 측정하였다.

결 과 :

제대정맥 내피세포에 TNF-α 및 IL-1β로 자극하였을 때 내피세포에서의 IL-8 mRNA는 1시간후부터, GRO/MGSA mRNA의 발현은 4시간후부터 각각 관찰되었으며 TNF-α 및 IL-1β의 자극농도에 따른 뚜렷한 차이는 차이는 없었다.

TNF-α 및 IL-1β로 자극한 내피세포의 배양상층액에서 측정한 IL-8도 1시간후부터 현저히 증가되었고 GRO/MGSA의 양은 4시간후부터 현저히 증가되어 있었다.

TNF-α 및 IL-1β로 자극한 내피세포의 배양상층액의 호중구 화학주성활성화도는 각각으로 1시간 자극후에 현저히 증가되어 있었다.

결 론 :

이상의 결과에서 제대정맥 내피세포를 TNF-α 및 IL-1β로 자극시 IL-8 및 GRO/MGSA mRNA 발현 및 단백이 분비되며, 특히 IL-8은 1시간 후에 GRO/MGSA는 4시간후에 각각 증가되고 호중구화학주성활성화는 1시간후부터 최고치로 증가됨을 관찰하여, GRO/MGSA보다는 IL-8이 호중구유주인자로서의 중요한 역할을 할것으로 생각된다. (Tuberculosis and Respiratory Diseases 1999, 46 : 338-349)

Key words : IL-8. GRO/MGSA, umbilical vein endothelial cell(HUVEC).

Introduction

The polymorphonuclear leukocyte(PMN) is the predominant inflammatory leukocytes recruited in response to acute lung injury. PMN can potentially contribute to the lung injury by releasing reactive oxygen metabolites and proteolytic enzymes[1]. In order to reach lung interstitium, neutrophils within the pulmonary vasculature must adhere to and subsequently migrate through endothelium. Migration of neutrophils occurs in response to chemotactic stimuli. Of the cytokines, the neutrophil chemotactic activity of interleukin-8(IL-8) has been best studied[2]. Recent reports of clinical studies have emphasized the role of IL-8 in the pathogenesis of ARDS[3], severe mening-ococcal infection[4], pneumocystis carinii pneumonia[5], and idiopathic pulmonary fibrosis[6]. IL-8 is a member of a novel family of small cytokines called chemo-kines[7].

Two subfamilies of chemokines are divided. The first of these, known as the CXC family, is characterized by the presence of two cysteine residues separated by a single amino acid[7]. The members of the CXC family include IL-8, GRO/MGSA, macrophage inflammatory protein-2 (MIP-2), granulocyte chemotactic peptide-2, inducible protein-10(IP-10), platelet factor-4(PF-4), platelet basic protein(PBP), neutrophil-activating protein-2 and connective-tissue activating protein III. The second, or CC family of chemokines, contains two adjacent cysteine residues in its sequence. As mentioned, IL-8 has been shown to be of major importance as a mediator of inflammation in lung diseases. Other CXC molecule, such as GRO is not fully studied until now. The GRO gene is a member of a gene superfamily encoding a set of related cytokines with inflammatory and growth regulatory properties including PF-4, neutrophil-activating pep-

tide 1/IL-8, PBP, and MIP-2. GRO, also called MGSA for melanoma growth stimulatory activity[8], was identified initially by its constitutive overexpression in spontaneously transformed Chinese hamster fibroblasts. Three related human GRO genes encoding cytokine functions were identified such as GROα, GROβ, GROγ and are located at chromosome 4q21[9]. GROα has been known to be produced by monocytes, endothelial cells, fibroblasts and synovial cells after stimulation with lipopolysac-charide, interleukin-1(IL-1), or tumor necrosis factor alpha(TNFα)[10]. GROβ and GROγ are expressed by monocytes and neutrophils, and have a high degree of homology with GROα. Inflammatory reactions require close interactions between neutrophils and vessel wall. Vascular endothelial cells play a rather active role in the increased adherence of leukocytes stimulated by chemotactic factors and IL-1 was known to induce production of IL-8 in human endothelial cells and transcriptionally activates the IL-8 gene[11]. The objective of this study was to investigate the production of IL-8 and GROα and their expression of mRNA by umbilical vein endothelial cells which were stimulated by either TNFα or IL-1β

Methods

1) Human Umbilical Vein Endothelial Cell (HUVEC) Culture.

Endothelial cells were obtained from human umbilical cord veins by the method of Jaffe[12]. The cord was severed from the placenta soon after birth, placed in a sterile container filled with cord buffer(0.14 M NaCl, 0.004 M KCl, 0.001 M phosphate buffer, pH 7.4, 0.011 M glucose), and held at 40℃ until processing. The umbilical vein was cannulated with a blunt 14 gauge needle, 2cm long. The vein was perfused with 100ml of cord buffer to wash out the blood and allowed to drain. The other end of the umbilical vein was then cannulated with a blunt, 12 gauge needle shaft over which was slipped a 4cm length polyethylene tubing. 10ml of 0.2% collagenase in cord buffer was then infused into the umbilical vein, and the polyethylene tubing was clamped shut with a hemostat. The umbilical cord, suspended by it's end was placed in a water bath containing cord buffer and incubated at 37℃ for 15 minutes.

After incubation, the collagenase solutioin containing the endothelial cells was flushed from the cord by perfusion with 30ml of cord buffer. The effluent was collected in a sterile 50ml conical centrifuge tube containing 10ml of medium 199 with 20% fetal calf serum. The yield was $0.5\sim1.5\times10^6$ cells. For subculture, cells were harvested with 0.01% EDTA-0.1% collagenase. Cells were grown in medium 199 supplemented with FCS(20%), heparin(100 μg/ml), endothelial cell growth factor(50 μg/ml), penicillin (20 U/ml), and streptomycin(20 μg/ml). Cells were passaged by trypsinization and were used before the 3rd passage. When the cells were confluent, the endothelial cells were stimulated with either TNFα or IL-1β until 24 hours, and the supernatant was collected for GROα/MGSA, IL-8 immunoassay and for the neutrophil chemotaxis.

2) Isolation of RNA and Synthesis of First Strand cDNA

Total cellular RNA was isolated by using Ultraspec-RNA(Biotecx, Houston, TX) according to the supplier's instructions. Cells were lysed with 1 ml of Ultraspec-RNA per 5×10^6 cells. The isolation and instruction were done according to the manufactur-

er's protocol. Briefly, RNA was extracted with chloroform and precipitated with isopropanol. The precipitated RNA was washed with 70% ethanol. The concentration of the extracted RNA was calculated by measuring the optical density at 260 nm. The ratio of the optical density at 260 nm to that at 280 nm was always higher than 1.8.

Aliquot's of 1 μg of RNA were reverse-transcribed using the 1st strand cDNA synthesis kit from Boehringer Manheim according to the manufacturer's instructions. Briefly, 1 μg of RNA was mixed with 2 μℓ of 10×reaction buffer(100 mM Tris, 500 mM KCl ; pH 8.3), 4 μℓ of 25 mM $MgCl_2$, 2 μℓ of 10 mM dNTP, 2 μℓ of oligo-p(dT)15 primer, 1 μℓ of RNase inhibitor, 0.8 μℓ of AMV reverse transcriptase, gelatin(0.01 mg/ml) and sterile water in a total volume of 20 μℓ. The reaction was initially incubated for 10 minutes at 25℃ and then for 60 minutes at 42℃. The reaction was terminated by incubating at 99℃ for 5 minutes and cooling to 4℃ for 5 minutes. The reaction tube was stored at -20℃ for further use in the polymerase chain reaction(PCR).

3) PCR

The reaction was performed in an thermocycler according to the manufacturer's instructions. Briefly, 5 μℓ of the first-strand cDNA was mixed with 8 μℓ of 10×reaction buffer, $MgCl_2$(1.5 mM), dNTP(0.2 mM), gelatin(0.01 mg/ml), 1 μℓ of 50 μM 5′ and 3′ primers, 0.5 μℓ (2.5 units) of Taq DNA polymerase and water in a total volume of 100 μℓ. The primer pairs for IL-8 was purchased from Maxim Biotech (San Francisco, CA). Primer pairs for GRO/MGSA and β2-microglobulin were designed from the published human cDNA sequence data[9] (Table 1).

The PCR was performed at the following setting of the thermometer : denaturing at 94oC for 1 min, annealing at 57℃ for 1 min, polymerization at 72℃ for 2 min for the 35 cycles and then extension at 72 ℃ for 5 min. Following the reaction, the amplified product was taken out and run on 2% agarose gel.

4) Neutrophil chemotaxis assay

Chemotaxis of neutrophils was conducted in triplicate using 3 μm polycarbonate membrane filter in a 48 well microchemotactic chamber(Neuro Probe,Inc., Bethesda, MD) as described by Ozaki and coworkers [13]. Neutrophils were purified from the peripheral blood of a normal volunteer by the sedimentation method described by Smith[14] and suspended at a concentration of 2×10^6 cells per ml in Hank's balanced salt solution(HBSS) containing 2% bovine serum albumin(BSA). A volume of 30 μℓ samples was introduced into the lower well of a chamber separated by a membrane filter, and the upper well was filled with 50 μℓ of a neutrophil suspension. The chamber stood

Table 1. Nucleotide sequence of the primers used in this study

GRO/MGSA		
	5′ primer	CAC TGC TGC TCC TGC TCC TGG TA
	3′ primer	CCT CCC TTC TGG TCA GTT GGA TTT G
β2-Microglobulin (control)		
	5′ primer	ACC CCC ACT GAA AAA GAT GA
	3′ primer	ATC TTC AAA CCT CCA TGA TG

for 60 min at 37℃ under 5% CO_2 in air. Then the filter was removed, and the cells on the filter were stained with Diff-Quik solution(Harleco, Gibbstown, NJ). The neutrophils that had migrated per 10 fields were counted under oil immersion by a trained technician. A positive control was assessed for each experiment using 10^{-7}M N-formyl-Met-Leu-Phe (fMLP ; Sigma) and negative control was HBSS.

5) Cytokine Measurements

GROα/MGSA and IL-8 levels in the culture supernatents were measured by the quantitative "sandwich" enzyme immunoassay technique(Quantikine, R&D, Mineapolis, USA).

Results

1. Dose-Response of TNFα and IL-1β

The secretion of IL-8 and GROα/MGSA from the HUVEC were increased when stimulated with TNFα or IL-1β from the concentration of 0.2 μg /ml to 20 μg /ml(Fig. 1 to Fig. 4). In the case of stimulation with TNFα, IL-8 concentration was markedly increased after one hour incubation, but GROα/MGSA was markedly increased after 4 hour incubation. This pattern of cytokine secretion was also same in the case of stimulation with IL-1β These results suggest that IL-8 is more rapidly secreted than GROα/MGSA by the stimulation with TNFα or IL-1β Neutrophil chemotactic activity was markedly increased from the supernatants of human umbilical vein endothelial cell(HUVEC) which was stimulated either with TNFα or IL-1β after one hour(Fig. 5 and Fig. 6). The highest chemotactic index was noted at the concentration of 0.2 μg /ml in the TNFα and 2 μg / ml in the IL-1β

2) RT-PCR Analysis of Cytokine Transcripts from HUVEC

The purified RNA from the stimulated HUVEC was reverse transcribed and the resulting cDNA was amplified using specific primers. Interestingly, IL-8 mRNA was more rapidly expressed than GROα/MGSA mRNA from the HUVEC after stimulation with either TNFα or IL-1β IL-8 mRNA is expressed from one hour and consistently expressed until 24 hours after stimulation with either TNFα or IL-1β (Fig. 7 to Fig. 10). In contrast, GROα/MGSA mRNA is not expressed after stimulation with neither TNFα nor IL-1β for one hours(Fig. 7). GROα/MGSA mRNA is expressed only from four hours and variously expressed until 24 hours depending on the concentrations of above stimulants(Fig. 8 to Fig. 10).

Overall, the chemotactic index was peak at one hour after stimulation with TNFα when the GROα/MGSA mRNA was still not expressed. Thesfindings suggest that IL-8 has much more important neutrophil chemotactic activity than GROα/MGSA in human inflammatory conditions where TNFα or IL-1β is secreted from the cells such as macrophages by various stimuli.

Discussion

Neutrophilic infiltration is a prominent characteristic of many acute and chronic inflammatory lung diseases[15]. Neutrophil migration and activation at inflammatory sites is mediated by multiple chemotactic factors. The increased expression of IL-8 gene by alveolar macrophages in patients with idiopathic pul-

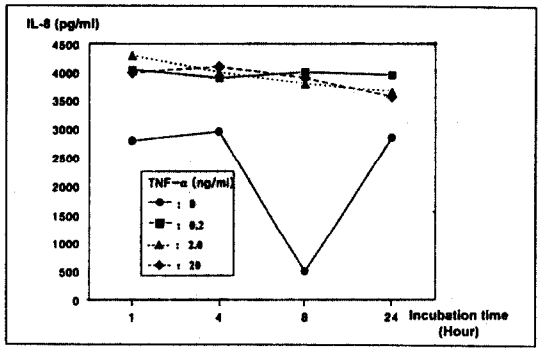

Fig. 1. The effect of TNF-α concentration and incubation time on the production of IL-8 from the HUVEC.

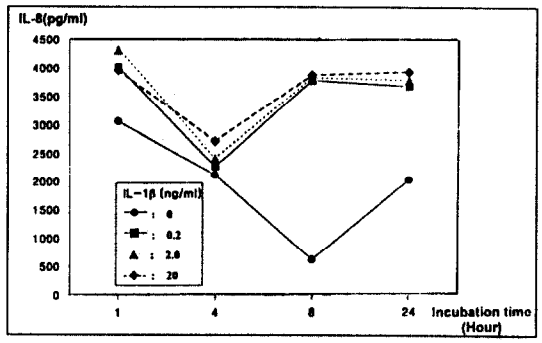

Fig. 2. The effect of IL-1β concentration and incubation time on the production of IL-8 from the HUVEC.

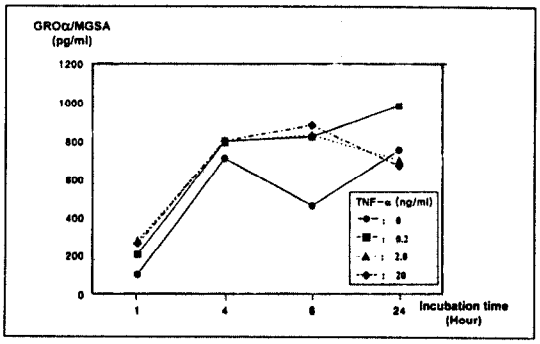

Fig. 3. The effect of TNF-α concentration and incubation time on the production of GROα/MGSA from the HUVEC.

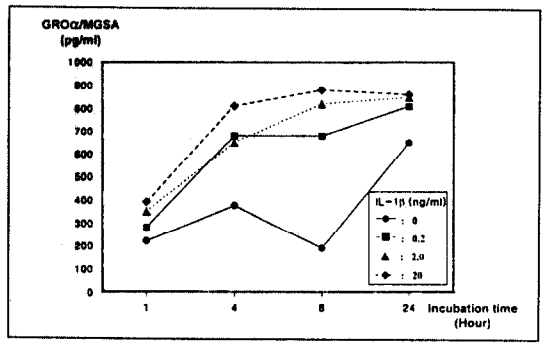

Fig. 4. The effect of IL-1β concentration and incubation time on the production of GROα/MGSA from the HUVEC.

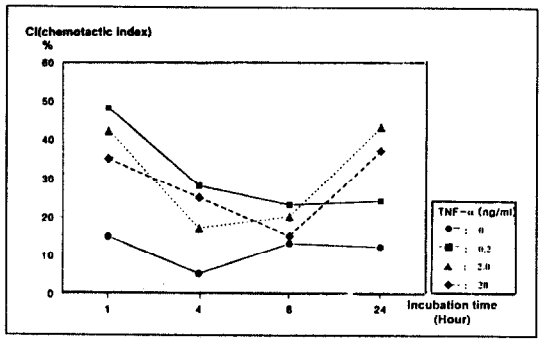

Fig. 5. Neutrophil chemotactic activity of HUVEC supernatents after stimulation with TNF-α in different concentrations and incubation time.

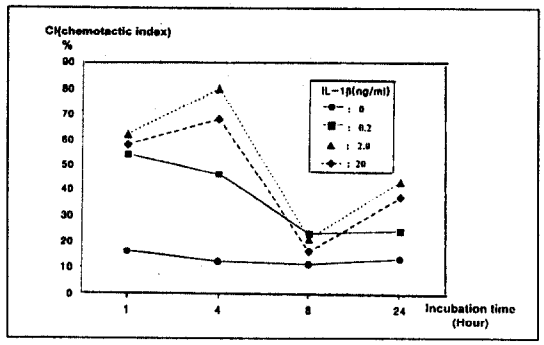

Fig. 6. Neutrophil chemotactic activity of HUVEC supernatents after stimulation with IL-1β in different concentrations and incubation time.

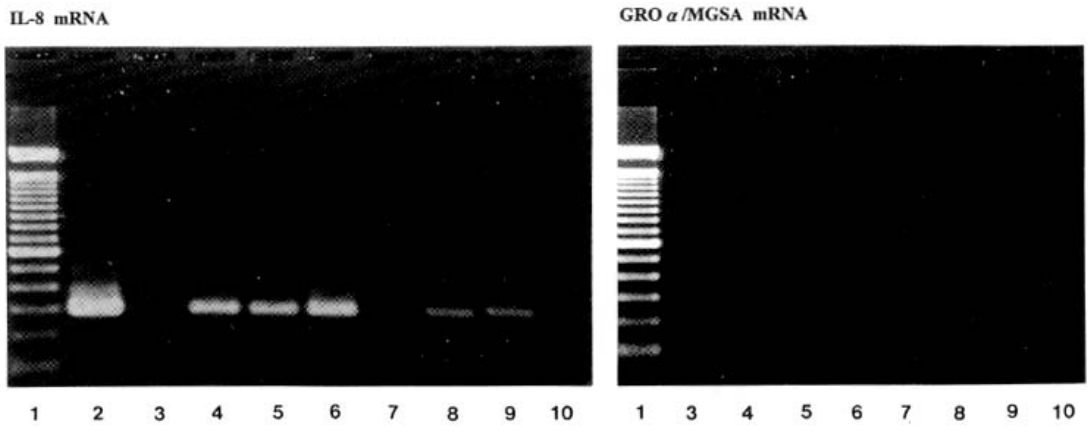

Fig. 7. Detection of IL-8 mRNA or GRO α/MGSA mRNA by RT-PCR from HUVEC after stimulation with TNF-α or IL-1β for one hour. IL-8 mRNA(upper left), Lane 1 : molecular weight marker, Lane 2 : IL-8 positive control, Lane 3 : HUVEC without stimulation, Lane 4 : TNF-α 0.2 μg /ml, Lane 5 : TNF-α 2 μg /ml, Lane 6 : TNF-α 20 μg /ml, Lane 7 : HUVEC without stimulation, Lane 8 : IL-1β 0.2 μg /ml, Lane 9 : IL-1β 2 μg /ml, Lane 10 : IL-1β 20 μg /ml. GRO α/MGSA mRNA(upper right), The stimulation order was same as in the IL-8 mRNA detection only without IL-8 positive control. GRO α/MGSA mRNA was not detected neither TNF-α nor IL-1β stimulation for one hour.

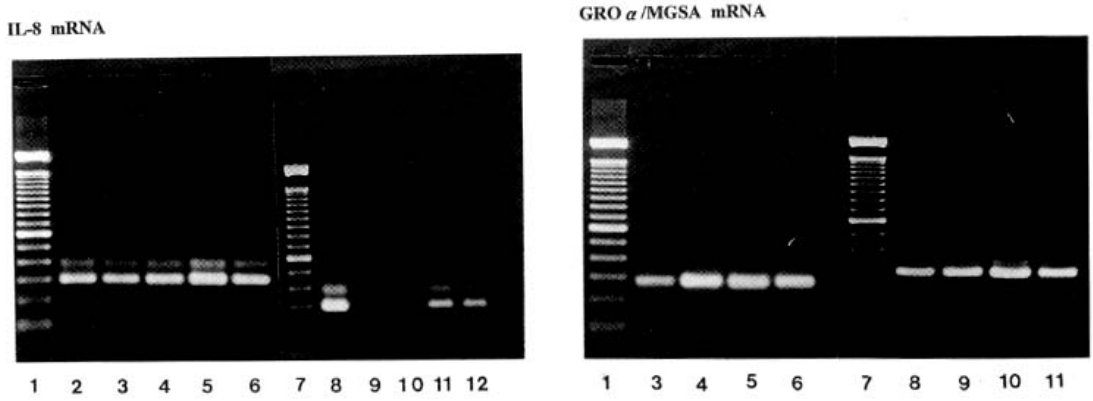

Fig. 8. Detection of IL-8 mRNA or GRO α/MGSA mRNA by RT-PCR from HUVEC after stimulation with TNF-α or IL-1β for 4 hour. IL-8 mRNA(upper left), Lane 1 : molecular weight marker, Lane 2 : IL-8 positive control, Lane 3 : HUVEC without stimulation, Lane 4 : TNF-α 0.2 μg /ml, Lane 5 : TNF-α 2 μg /ml, Lane 6 : TNF-α 20 μg /ml, Lane 7 : molecular without marker, Lane 8 : IL-8 positive control, Lane 9 : HUVEC without stimulation, Lane 10 : IL-1β 0.2 μg /ml, Lane 11 : IL-1β 2 μg /ml. Lane 12 : IL-1β 20 μg /ml. GRO α/MGSA mRNA(upper right), The stimulation order was same as in the IL-8 mRNA detection only without IL-8 positive control.

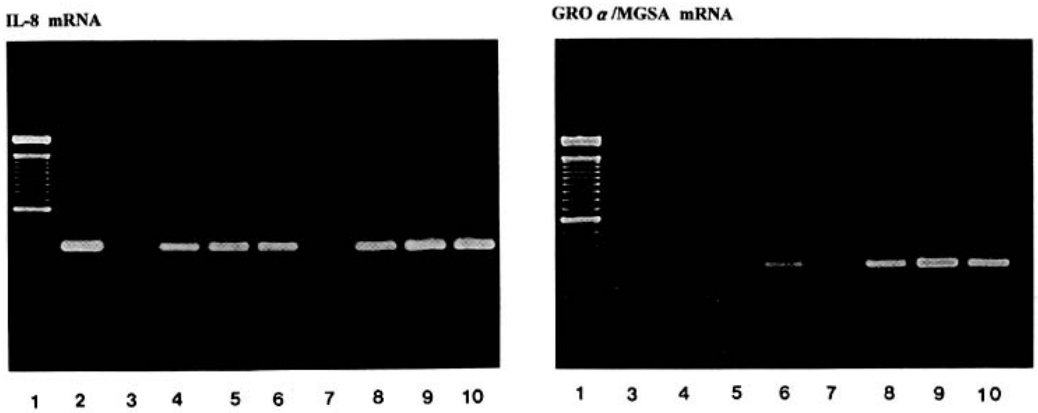

Fig. 9. Detection of IL-8 mRNA or GRO α/MGSA mRNA by RT-PCR from HUVEC after stimulation with TNF-α or IL-1β for 8 hour. IL-8 mRNA(upper left), Lane 1 : molecular weight marker, Lane 2 : IL-8 positive control, Lane 3 : HUVEC without stimulation, Lane 4 : TNF-α 0.2 μg /ml, Lane 5 : TNF-α 2 μg /ml, Lane 6 : TNF-α 20 μg /ml, Lane 7 : HUVEC without stimulation, Lane 8 : IL-1β 0.2 μg /ml, Lane 9 : IL-1β 2 μg /ml. Lane 10 : IL-1β 20 μg /ml. GRO α/MGSA mRNA(upper right), The stimulation order was same as in the IL-8 mRNA detection only without IL-8 positive control.

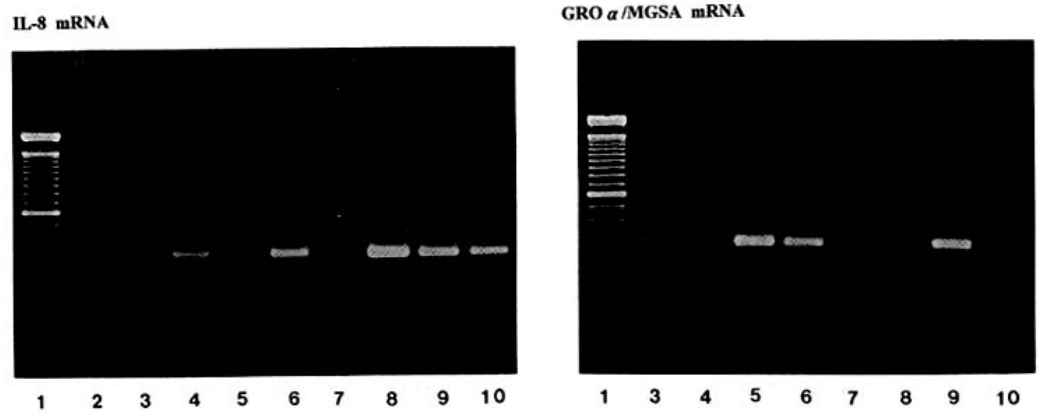

Fig. 10. Detection of IL-8 mRNA or GRO α/MGSA mRNA by RT-PCR from HUVEC after stimulation with TNF-α or IL-1β for 24 hour. IL-8 mRNA(upper left), Lane 1 : molecular weight marker, Lane 2 : IL-8 positive control, Lane 3 : HUVEC without stimulation, Lane 4 : TNF-α 0.2 μg /ml, Lane 5 : TNF-α 2 μg /ml, Lane 6 : TNF-α 20 μg /ml, Lane 7 : HUVEC without stimulation, Lane 8 : IL-1β 0.2 μg /ml, Lane 9 : IL-1β 2 μg /ml. Lane 10 : IL-1β 20 μg /ml. GRO α/MGSA mRNA(upper right), The stimulation order was same as in the IL-8 mRNA detection only without IL-8 positive control.

monary fibrosis supports a role for IL-8 in this process[9]. There is also substantial evidence that endothelial and epithelial cells are not passive in their interactions with inflammatory cells, chemoattractants, and other mediators[16]. Thus, we chose to study the expression of IL-8 and GROα/MGSA on the human umbilical vein endothelial cells(HUVEC) after stimulation with TNFα or IL-1β We also measured the

neutrophil chemotactic activity from the supernatants of the stimulated HUVEC.

In this study, dose and time response data show significantly increased IL-8 secretion was noted after one hour over a wide range of TNFα(0.2 to 20 μg/ml), but GROα/MGSA was markedly secreted only after 4 hours. This late GROα/MGSA secretion patterns were also noted in the case of IL-1β as a stimulant to the HUVEC. Interestingly, the IL-8 is more secreted after one hour than 4 hours after stimulation with IL-1β

GROα is known to be expressed from the pulmonary alveolar macrophage and bronchial epithelial cells when they are activated by TNFα. Also, GROα and IL-8 genes are colocalized on human chromosome 4q12-21. Both regions, preceding the first exon, contain similar binding motifs for several transcription factors, including nuclear factor-κB or glucocorticoid receptor, but also diverge in containing different binding domains for other nuclear factors[7]. All three GRO(GROα, GROβ, GROγ) proteins activate human neutrophils and basophils, as does IL-8, as shown by chemotaxis and the induction of an increase in the intracellular calcium concentration of these cells. By contrast, these chemokines do not induce any chemotactic response in eosinophils or monocytes.

IL-8 shows minor effects on these two types of cells[17]. In vitro studies with neutrophils and basophils suggest that IL-8 is only slightly more potent than GROα, whereas GROβ and GROγ have weaker activities than either IL-8 and GROα, despite the 86% to 90% identity between the genes for the three GROs[18]. In the bronchoalveolar lavage fluid of patients with bacterial pneumonia and adult respiratory distress syndrome, higher levels of GROα than IL-8 indicate that GROα may play an important pathogenic role in neutrophil recruitment and activation like IL-8[19]. Among the cardinal proinflammatory cytokines that have been studied, IL-1β and TNFα have been suggested to activate alveolar macrophages and to play a role in the development of IPF[20]. So, we used IL-1β and TNFα as a stimulant to the endothelial cells for the study of IL-8 and GROα expression. Although IL-8 is a potent neutrophil chemotactic factor, this cytokine also augments neutrophil protease production, oxygen radical generation[2], and 5-lipoxygenase activity[21]. In addition, IL-8 has recently been shown to be chemotactic for T lymphocytes[22].

GROα/MGSA is a 73 amino acid peptide which shares sequence characteristics of a superfamily of peptides called chemokines. Richmond and colleagues initially discovered that melanoma cells secreted autostimulatory(autocrine) growth factors[23]. They found that most of the activity was caused by a single acid stable protein of~16 KD and designated it melanoma growth stimulatory activity(MGSA). MGSA was found to be a mitogen for the melanoma cell line Hs 294T which produces this factor. cDNA for MGSA isolated from Hs 294T cells was later found to be identical to oncogene growth related peptide(GROα) gene[24]. The formal name for this protein was then designated as MGSA/GROα. MGSA/GRO α and IL-8 was induced by ultraviolet B radiation in human keratinocyte cell lines[25]. The MGSA/GROα but not IL-8 could bind to the HS 294T melanoma cell line by way of a unique receptor for MGSA/GROα[26]. However, recent studies showed that IL-8 receptor B is present in melanoma cells and plays a role in the cell growth[27]. Antileukinate (Ac-RRWWCR-NH2), which is a potent inhibitor of binding of chemokines to their receptors[28], inhibits the growth of melanoma cells by preventing MGSA/GROα from the binding to its receptors[29]. In vivo,

after 1 hour of coronary occlusion, IL-8 mRNA was markedly and consistently induced in reperfused segments of myocardium but IL-8 mRNA was not induced in control myocardial segments[30]. The adherence of alveolar macrophage itself resulted in the induction of de novo IL-8 mRNA synthesis[31].

In this investigation, we have demonstrated the gene expression of IL-8 and GROα/MGSA in HUVEC after stimulation with TNFα or IL-1β IL-8 mRNA was more rapidly synthesized than GROα/MGSA mRNA and was expressed after stimulation with cytokines for one hour. GROα/MGSA mRNA was expressed only after stimulation with cytokines for 4 hours. The neutrophil chemotactic activity of supernatants from the stimulated HUVECs showed that chemotactic index was not much different from one hour to 24 hours despite the amount of GROα/MGSA was increased after four hours. These results suggest that the neutrophil chemotactic activity of GROα/MGSA is much weaker than IL-8 in stimulated HUVEC. Further investigations into the molecular mechanism of cytokine-induced IL-8 and GROα/MGSA expression in pulmonary capillary endothelial cell should provide insight into the events of acute lung injury such as the adult respiratory distress syndrome.

Summary

Polymorphonuclear leukocytes(PMN) are the predominant inflammatory cells recruited in acute lung injury such as adult respiratory distress syndrome, pneumonia and also chronic lung disease such as idiopathic pulmonary fibrosis and pulmonary emphysema. Interleukin-8(IL-8) is an 8,000 D protein produced by many cells and has potent neutrophil chemoattractant and activating properties. The GRO, also called melanoma growth-stimulatory activity(MGSA), referring to a peptide of 73 amino acids, was reported to be mitogenic for cultured human melanoma cells. Mature GRO/MGSA has marked sequence similarity to IL-8. In view of the structural similarities to IL-8, it was of particular interest to test GRO for neutrophil activating and chemotactic properties. We found a significant release of IL-8 and GRO/MGSA from the cultured human umbilical vein endothelial cell(HUVEC) which was stimulated either with TNFα or IL-1β and also found the expression of IL-8 and GRO/MGSA mRNA. Neutrophil chemotactic activity was enhanced in accordance with the increased IL-8 and GRO/MGSA. Our study also suggest that the IL-8 is more important in the increased neutrophil chemotactic activity than GRO/MGSA when endothelial cell is stimulated with TNFα or IL-1β in vitro.

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