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Full Paper

Glycyrrhizin Inhibits Interleukin-8 Production and Nuclear Factor–κB Activity in Lung Epithelial Cells, but Not Through Glucocorticoid Receptors

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Abstract. This study was designed to examine the glucocorticoid-like inhibitory effect of glycyrrhizin (GL) on interleukin (IL)-8 production in A549 lung epithelial cells. GL, as well as dexamethasone (DEX) inhibited both tumor necrosis factor (TNF)-α– and IL-1β–induced IL-8 production, mRNA expression, and promoter activity in A549 cells. Both GL and DEX inhibited transactivation of nuclear factor (NF)-κB, without inhibiting translocation of the NF-κB p65 subunit to the nucleus. However, the effect of GL was insensitive to RU486, a GR antagonist, and to GR knockdown by siRNA. Furthermore, only GL inhibited DNA binding of p65 to the IL-8 promoter region. These findings indicated that GL had a glucocorticoid-like inhibitory effect on IL-8 production via a mechanism that differs from that of glucocorticoids.

Keywords: glycyrrhizin, glucocorticoid, nuclear factor (NF)-κB, interleukin (IL)-8, A549 cell

Introduction

Glucocorticoids are the most effective therapy for the treatment of inflammatory diseases such as asthma, a chronic inflammatory disease of the airways (1 – 3). Functionally, they act partly by inducing some anti-inflammatory genes, such as lipocortin-1 (4), secretory leukocyte proteinase inhibitor (5), and interleukin (IL)-1–receptor antagonist (6), but mainly by repression of inflammatory genes, such as IL-8 and eotaxin (7, 8). They act by binding to a cytosolic glucocorticoid receptor (GR) and rapidly induce the translocation of GR to the nucleus (9). Within the nucleus, GR either induces gene transcription by binding to specific DNA elements in the promoter/enhancer regions of responsive genes or reduces gene transcription by functional interaction with pro-inflammatory transcription factors such as nuclear factor (NF)-κB (p65/p50 heterodimers) (2, 9 – 11). Despite their effectiveness, the use of glucocorticoids in lung diseases is limited because of their severe side effects.

Glycyrrhizin (GL), a triterpene glycoside from licorice root (*Glycyrrhiza glabra*), is composed of a molecule of glycyrrhetic acid (GA) and two molecules of glucronic acid. In Japan, GL has been used clinically for more than 20 years in patients with chronic hepatitis (12, 13). So far, GL has been reported to have a variety of pharmacological activities, including anti-inflammatory, anti-allergic, and anti-viral activities. For instance (14 – 17), it has been reported that orally administrated GL inhibits asthmatic responses in obalbumin (OVA)-sensitized mice and reduces levels of inflammatory cytokines IL-4 and IL-5 in the bronchoalveolar lavage fluid (15), which suggests the usefulness of GL, as well as glucocorticoids, for lung inflammation. GL has been shown to be transformed to the metabolite GA by glucuronidase in intestinal bacteria (18). The mechanism of action of GL in vivo has been believed for some time to be due to this GA, which can inhibit 11β-hydroxysteroid dehydrogenase (11β-HSD) (19).

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However, intravenously administrated GL has also been shown to inhibit the lipopolysaccharide (LPS)-induced severe acute respiratory syndrome in mice (16), which suggests that biological transfer to GA by intestinal bacteria is not necessary for its glucocorticoid-like effects. In addition to this, GL and its related compounds have been shown to inhibit IL-8 and eotaxin production induced by pro-inflammatory cytokines in cultured fibroblast cells (20), although the mechanism has not been elucidated. Taking these findings together, we presumed that GL itself may have glucocorticoid-like action.

Therefore in the present study, we evaluated the glucocorticoid-like effect of GL by examining the effects of GL on TNF-α– and IL-1β–induced IL-8 production, mRNA expression, and its promoter activity and compared them with the effects of dexamethasone (DEX). We also examined the effects of GL and DEX on NF-κB transcription factor activation.

Materials and Methods

Reagents

GL was supplied by Minophagen Pharmaceutical Co. (Tokyo) as a powder of >99% purified glycyrrhizin-NH_4. DEX, TNF-α, IL-1β, and RU-486 were purchased from Sigma (St. Louis, MO, USA).

Cell culture

A549 human lung carcinoma cells (Riken Bioresourse Center, Tsukuba) were grown in Dulbecco's Modified Eagle Medium (DMEM; Nisssui, Tokyo) supplemented with 5% fetal bovine serum (FBS; HyClone Laboratories, Logan, UT, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. For assays, the cells were cultured with DMEM containing charcoal-stripped 5% FBS and antibiotics.

ELISA for IL-8

Culture-conditioned media were centrifuged at 3000 rpm for 5 min and stored at −80°C until use. IL-8 in culture-conditioned media was assayed by commercially available ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the instructions of the manufacturer.

RNA isolation and quantitative real-time RT-PCR for IL-8 mRNA

Total RNA was isolated with Trizol (Invitrogen, Carlsbad, CA, USA). Random-primed cDNA was prepared from 0.5 μg total RNA using the Prime Script Reverse Transcriptase (Takara Bio, Otsu). PCR amplification was carried out with SYBR® Premix Ex Taq™ (Takara Bio) and analyzed in 96-well optical reaction plates, using the threshold cycle (Ct) method (21). An IL-8 cDNA fragment was amplified with the 5'-catgact tccaagctggccg-3' and 5'-tttatgaattctcagccctc-3' primer pair. A GAPDH cDNA fragment was amplified with the 5'-accatcttccaggagcgaga-3' and 5'-cagtcttctgggtggca gtg-3' primer pair. The PCR conditions were set at 95°C for 3 min to activate the TaqDNA polymerase, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. The fluorescence was monitored at the end of each cycle to obtain a measure of the amount of PCR product formed. The thermocycler PCR data were analyzed with the software installed in the iCycler (Bio-Rad Laboratories, Hercules, CA, USA), which determines the cycle number at which each sample reaches this threshold. The cycle number corresponding to the fluorescence threshold is inversely related to logarithm of the initial template concentration. Given that amplification occurs in an exponential manner, a difference of 1 in the cycle threshold corresponds approximately to a twofold difference in relative transcript abundance. The amount of IL-8 mRNA was normalized by that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA and is presented in arbitrary units, with 1 unit corresponding to the value in control cells treated with vehicle.

Reporter plasmid, transient transfection and luciferase assay

The IL-8–luc and mouse mammary tumor virus (MMTV)-luc reporter gene contains the –422/+54-bp region of the IL-8 gene and the –224/+100-bp region of the MMTV gene driving firefly luciferase expression vector pGL2 basic, respectively (22). The NF-κB–luc construct was purchased from Stratagene (La Jolla, CA, USA). Transient transfection with the DNA plasmid was performed using the HilyMax reagent (Dojindo, Kumamoto) in accordance with the manufacturer's recommendations. Briefly, cells cultured in 24-well plates were transfected with the DNA-transfection reagent mixture at 70% – 80% confluency. At 24 h after transfection, cells were treated with drugs and harvested for detection of luciferase activity. Luciferase activity was measured with a luminometer (Lumat LB9507; EG&G Berthold, Oak Ridge, TN, USA) using Dual Luciferase Assay kits (Promega, San Luis Obispo, CA, USA). Co-transfection with phRL-TK (Promega), which expresses *Renilla* luciferase, was performed to enable normalization of data for transfection efficiency. Transfection with GR siRNA (Dharmacon, Lafayette, CO, USA) was performed with Lipofectamine (Invitrogen), in accordance with the manufacturer's recommendations.

Immunofluorescence of NF-κB p65

Cells cultured on glass-bottomed dishes were treated with drug, after which cells were fixed with 4% paraformaldehyde and permeabilized with 0.5% Triton-X. Cells were blocked with 1% BSA in PBS for 20 min and incubated with mouse anti-p65 NF-κB antibody for 1 h (Santa Cruz Biotechnology, Santa Cruz, CA, USA). After the primary antibody reaction, cells were washed with PBS and incubated with fluorescein isothiocyanate-conjugated anti-mouse IgG for 30 min. Cells were visualized and analyzed with a Fluoview FV300 confocal laser scanning microscope (Olympus, Tokyo). A semi-quantitative analysis of cytoplasmic p65 was performed in a blinded fashion by scoring 100 – 120 cells on one coverslide.

Chromatin immunoprecipitation (ChIP)

Cells were fixed with 1% formaldehyde, scraped, and collected by centrifugation. Collected cells were resuspended in SDS lysis buffer [50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 1% SDS, and 1% (v/v) protease inhibitor cocktail] and incubated on ice for 20 min. The lysates were then sonicated on a Bioruptor UCD-250 (Cosmo Bio, Tokyo) with a power output setting of 250 W. The samples were centrifuged and the supernatants were diluted 10-fold in ChIP dilution buffer [50 mM Tris/HCl (pH 8.0), 167 mM NaCl, 1.1% Triton X-100, 0.11% deoxycholate, 1% (v/v) protease inhibitor cocktail], and pre-cleared with salmon sperm DNA/protein G agarose slurry for 4 h at 4°C. Cross-linked chromatin was incubated with 4 μg anti-p65 antibody overnight at 4°C. Immuno-complexes were collected by immunoprecipitation with salmon sperm DNA/protein G agarose slurry for 3 h at 4°C. The beads were washed sequentially in the following buffers: low salt wash buffer [50 mM Tris/HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% SDS, 0.1% deoxycholate]; high salt wash buffer [50 mM Tris/HCl (pH 8.0), 500 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% SDS, 0.1% deoxycholate]; LiCl wash buffer [10 mM Tris/HCl (pH 8.0), 0.25 M LiCl, 1 mM EDTA, 0.5% NP-40, 0.5% deoxycholate]; and Tris/EDTA buffer. Immuno-complexes were extracted from the beads with ChIP elution buffer [10 mM Tris-HCl (pH 8.0), 300 mM NaCl, 5 mM EDTA, 0.5% SDS]. Cross-linking was reversed by incubation at 65°C for 6 – 12 h. The eluants were digested with proteinase K at 55°C for 1 h, and then they were further subjected to phenol/chloroform extraction. The DNA was purified by ethanol precipitation. Real-time PCR was performed to determine the percentage of p65 in the genomic region of IL-8 precipitated with antibodies against input DNA. The oligonucleotide primers used to amplify the genomic region of IL-8 (−121/+61) were as follows: 5'-gggccatcagttgcaaatc-3' (sense) and 5'-ttccttccggtggtttc ttc-3' (antisense).

Statistical analyses

Differences in means ± S.E.M. values among groups were assessed by Dunnett's test. All statistical tests were two-tailed, and $P<0.05$ was considered significant.

Results

Effect of GL on IL-8 production

To examine the effect of GL on IL-8 production, IL-8 protein levels in spent culture media of A549 cells treated with either TNF-α (2.5 ng/ml) or IL-1β (1 ng/ml) were measured by ELISA. As shown in Fig. 1, IL-8 levels in these cytokine-treated media were significantly greater compared with those in control media, and pretreatment of DEX (100 nM) markedly blocked these increases in IL-8 release. As well as the effect of DEX, pretreatment of GL (0.1, 0.3, and 1 mM)

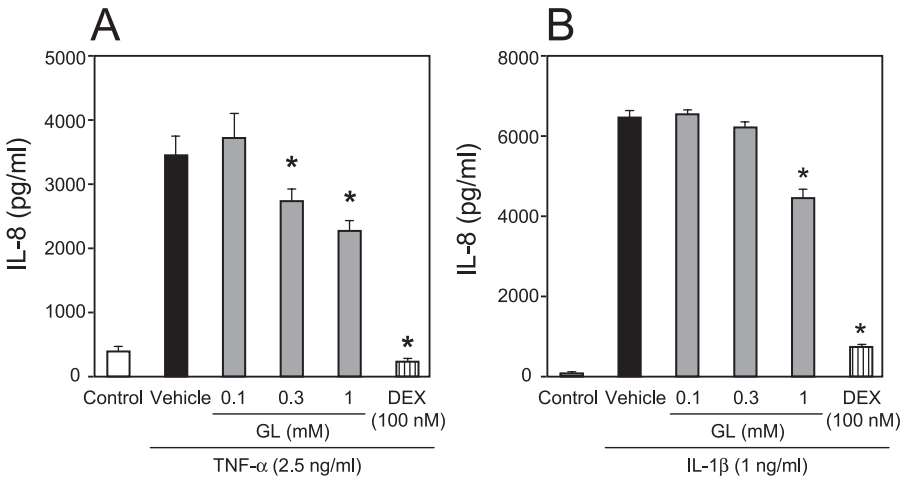

Fig. 1. Effects of GL and DEX on IL-8 production. Confluent A549 cells were serum-starved for 6 h, and pretreated with GL (0.1, 0.3, and 1 mM) or DEX (100 nM) for 1 h; then the cells were treated with either TNF-α (2.5 ng/ml, A) or IL-1β (1 ng/ml, B) for 6 h. The level of IL-8 in culture media was measured by ELISA. Results represent the means ± S.E.M. from three independent experiments. *$P<0.05$, compared with TNF-α or IL-1β alone.

concentration-dependently blocked both TNF-α– and IL-1β–induced IL-8–release, with the most pronounced inhibition being noted for 1 mM GL (35% and 32% of TNF-α and IL-1β alone, respectively) (Fig. 1). IL-8 mRNA level was determined by real-time PCR. TNF-α, as well as IL-1β, caused a 10- and 16-fold increase in IL-8 mRNA level compared with that in the controls (Fig. 2). Pretreatment of DEX blocked these increases in

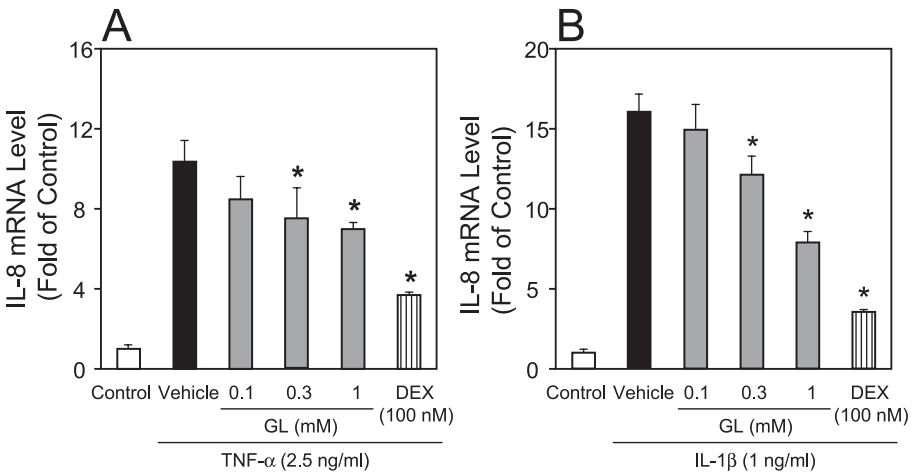

Fig. 2. Effects of GL and DEX on IL-8 mRNA expression. Confluent A549 cells were serum-starved for 6 h; pretreated with GL (0.1, 0.3, and 1 mM) or DEX (100 nM) for 1 h; and then treated with either TNF-α (2.5 ng/ml, A) or IL-1β (1 ng/ml, B) for 4 h. Cells were then harvested and total RNA was prepared. The expression levels of IL-8 mRNA were measured by real-time PCR and normalized to GAPDH. Results represent the means ± S.E.M. from four independent experiments. *P<0.05, compared with TNF-α or IL-1β alone.

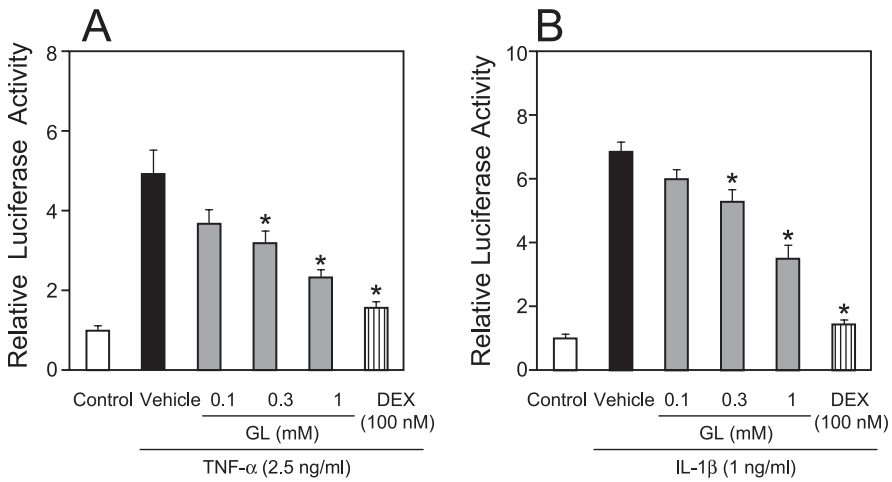

Fig. 3. Effects of GL and DEX on transcriptional activity of IL-8 promoter gene. A549 cells (80% confluent) were transiently transfected with IL-8 promoter and incubated for 24 h. Cells were then serum-starved for 6 h; pretreated with either GL (0.1, 0.3, and 1 mM) or DEX (100 nM) for 1 h; and then treated with TNF-α (2.5 ng/ml, A) or IL-1β (1 ng/ml, B) for 6 h. Cells were lysed and luciferase activity in the cell lysates was measured. Results represent the means ± S.E.M. from three independent experiments. *P<0.05, compared with TNF-α or IL-1β alone.

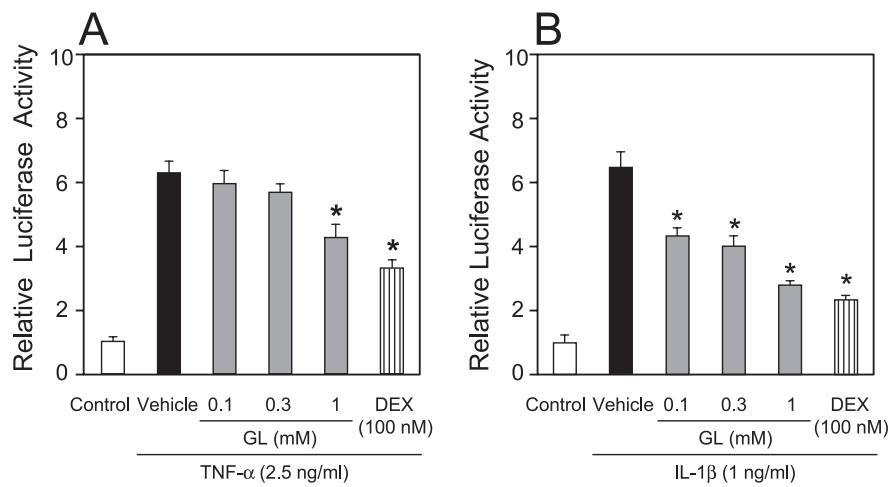

Fig. 4. Effects of GL and DEX on transactivation of NF-κB. A549 cells (80% confluent) were transiently transfected with NF-κB–responsive luciferase plasmid and incubated for 24 h. Cells were then serum-starved for 6 h; pretreated with either GL (0.1, 0.3, and 1 mM) or DEX (100 nM) for 1 h; and then treated with TNF-α (2.5 ng/ml, A) or IL-1β (1 ng/ml, B) for 6 h. Cells were lysed and luciferase activity in the cell lysates was measured. Results represent the means ± S.E.M. from four independent experiments. *P<0.05, compared with TNF-α or IL-1β alone.

IL-8 mRNA, although the inhibition was not complete. GL (0.1, 0.3, and 1 mM) also concentration-dependently blocked both TNF-α– and IL-1β–induced increases in IL-8 mRNA. GL (1 mM) significantly inhibited by 30% and 50% compared with TNF-α and IL-1β alone, respectively (Fig. 2). These data suggested that GL inhibited IL-8 production through inhibition of IL-8 mRNA expression.

Effect of GL on IL-8 promoter activity

To examine whether GL inhibited IL-8 transcription, A549 cells were transfected with luciferase reporter plasmid containing human IL-8 promoter, and the effect of GL was examined by luciferase assay. In good agreement with the changes in the levels of IL-8 protein and mRNA, TNF-α and IL-1β activated the IL-8 promoter gene. The TNF-α responses and the IL-1β ones induced five- and seven-fold increases in luciferase activity, respectively (Fig. 3). In addition, DEX significantly blocked the activation of the IL-8 promoter gene by these stimulants. GL also blocked these promoter activations in a concentration-dependent manner. GL (1 mM) significantly decreased that by 50% compared with TNF-α and IL-1β alone (Fig. 3). It has been shown that both TNF-α and IL-1β activate IL-8 promoter gene through the activation of NF-κB transcription factor (23, 24). Therefore, luciferase assay was performed with artificial promoter plasmid containing three NF-κB

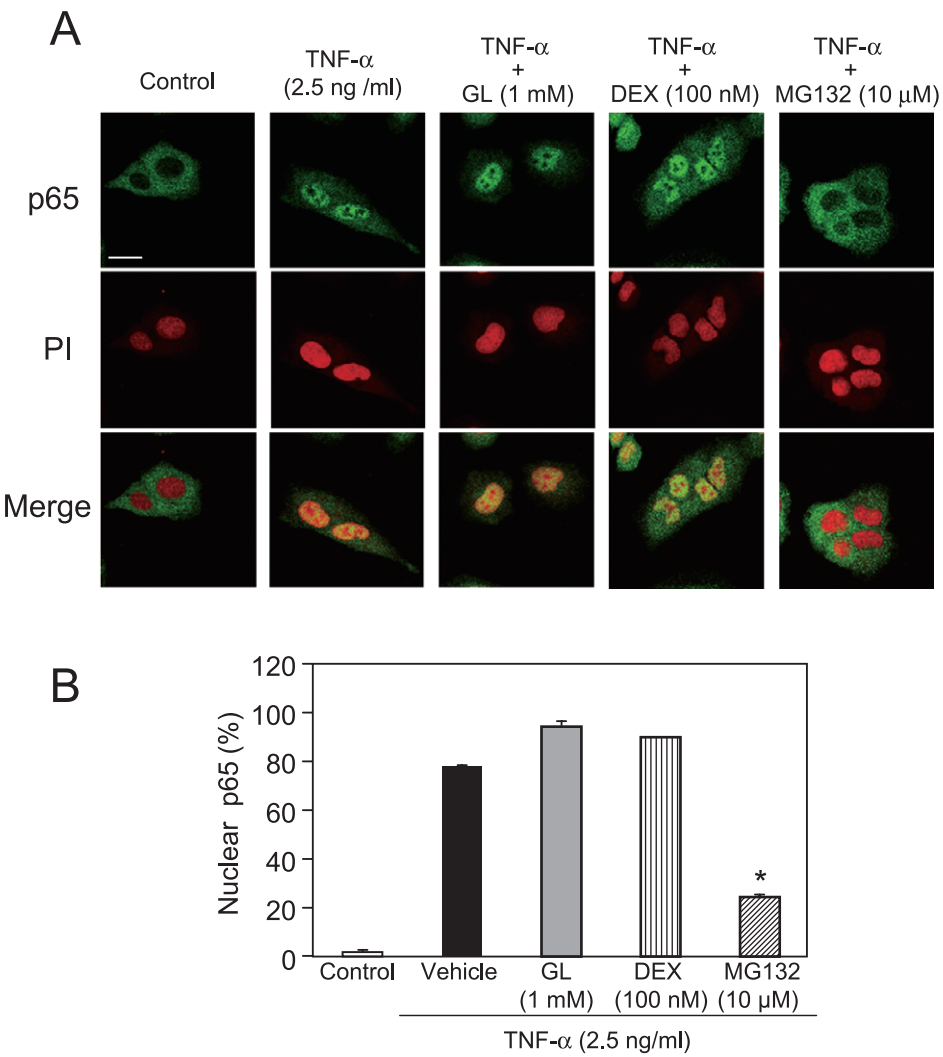

Fig. 5. Effects of GL and DEX on nuclear translocation of p65. A549 cells (80% confluent) were serum-starved for 6 h; pretreated with either GL (1 mM), DEX (100 nM) or MG132 (10 μM) for 1 h; and then treated with TNF-α (2.5 ng/ml) for 1 h. Cells were then fixed, permeabilized, and stained with anti-p65 antibody and propidium iodine (PI). A: Fluorescent images of p65 (green) and PI (red). B: Quantification of the effect of GL on nuclear translocation of p65. The percentage of cells with nuclear p65 is plotted relative to the total cell number. Results represent the means ± S.E.M. from three independent experiments. *P<0.05 *vs* TNF-α alone.

sites, instead of the IL-8 promoter gene. Both GL and DEX significantly inhibited the activation of the NF-κB promoter gene induced by TNF-α and IL-1β (Fig. 4), which suggests that GL, as well as DEX, inhibits NF-κB-dependent gene transcription.

Effect of GL on nuclear translocation of NF-κB component p65

TNF-α and IL-1β activate IκB kinases (IKKs) and subsequently IκB-α is degraded (25). Then, the NF-κB component p65 is translocated from the cytoplasm to the nucleus, and activates transcription of target genes (25). To examine whether GL inhibits nuclear translocation of p65, the p65 in A549 cells was visualized by immunofluoresence. In control cells, most p65 signals were associated with the cytoplasm (Fig. 5A). In contrast, after 1-h treatment with TNF-α, the p65 signals in the cytoplasm were markedly decreased and increased nuclear signals were observed (Fig. 5A). The number of cells that had nuclear p65 signals was counted, and the ratio to the total number of cells is shown in Fig. 5B. In the TNF-α–treated cells, translocalization to the nuclei was observed in 78% of cells. GL and DEX did not inhibit p65 translocation, whereas MG132, an inhibitor of IκBα degradation, considerably decreased the number of cells that had nuclear p65. These data suggested that both GL and DEX inhibited NF-κB–dependent gene expression after translocation to the nucleus.

Possibility that the effect of GL is mediated by glucocorticoid receptors (GRs)

As a result of the structural similarity between GL and DEX, it may be thought that the DEX-like inhibitory effects of GL are mediated through GRs. To assess this possibility, the influence of GR antagonists and GR knock down on the effects of GL and DEX were examined in an IL-8 promoter reporter assay. RU486 (100 nM), a GR antagonist, completely abolished the inhibitory effect of DEX on TNF-α–induced IL-8 promoter activation (Fig. 6A). In contrast, RU486 did not antagonize the effect of GL (Fig. 6A). In addition, GL inhibited TNF-α–induced IL-8 promoter gene activation in both control and GR siRNA–transfected knock down cells, whereas the inhibition by DEX was completely abolished (Fig. 6B). Furthermore, GL did not activate MMTV-promoter, a glucocorticoid-dependent gene, in the luciferase reporter assay (Fig. 6C). These data clearly suggested that GL inhibited

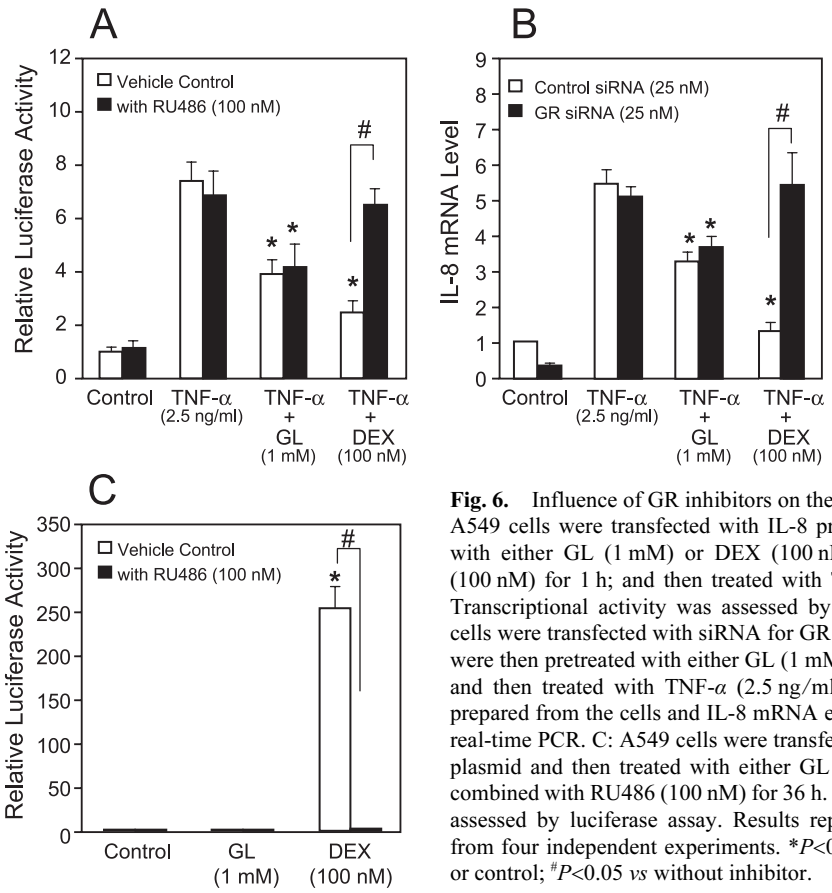

Fig. 6. Influence of GR inhibitors on the effects of GL and DEX. A: A549 cells were transfected with IL-8 promoter plasmid; pretreated with either GL (1 mM) or DEX (100 nM) combined with RU486 (100 nM) for 1 h; and then treated with TNF-α (2.5 ng/ml) for 6 h. Transcriptional activity was assessed by luciferase assay. B: A549 cells were transfected with siRNA for GR and cultured for 72 h. Cells were then pretreated with either GL (1 mM) or DEX (100 nM) for 1 h and then treated with TNF-α (2.5 ng/ml) for 4 h. Total RNA was prepared from the cells and IL-8 mRNA expression was measured by real-time PCR. C: A549 cells were transfected with MMTV promoter plasmid and then treated with either GL (1 mM) or DEX (100 nM) combined with RU486 (100 nM) for 36 h. Transcriptional activity was assessed by luciferase assay. Results represent the means ± S.E.M. from four independent experiments. *P<0.05, compared with TNF-α or control; $^{\#}P$<0.05 *vs* without inhibitor.

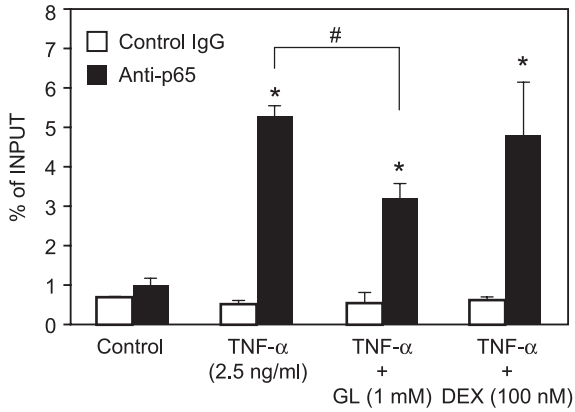

Fig. 7. Effects of GL and DEX on DNA-binding of p65. A549 cells were pretreated with either GL (1 mM) or DEX (100 nM) for 1 h and treated with TNF-α (2.5 ng/ml) for 2 h. The soluble chromatin extracts from the cells were immunoprecipitated with anti-p65 or control IgG and then subjected to real-time PCR with primer pairs for the IL-8 promoter gene. The amount of DNA binding was normalized by "Input" that was the crude chromatin extracts prior to immunoprecipitation. Results represent the means ± S.E.M. from three independent experiments. *$P<0.05$ and $^{\#}P<0.05$ *vs* control and TNF-α alone, respectively.

NF-κB–dependent gene transcription, but not through GR activation.

Effect of GL on DNA binding to p65

Finally, the effects of GL and DEX on DNA binding to p65 were examined. In the ChIP assay, DNA binding to p65 in TNF-α–treated cells was significantly increased compared with that in control cells (Fig. 7). GL, but not DEX, inhibited DNA binding to p65 (Fig. 7), which suggests that GL-induced inhibition of IL-8 transcription may be due to p65 binding.

Discussion

Airway epithelial cells are not only a passive barrier, but also play a crucial role in the inflammatory response by producing various mediators (26). IL-8 is one of the most important mediators from airway epithelial cells, which activates neutrophil influx into lung tissue (26). The inhibition of IL-8 production by glucocorticoids, based on the inhibition of NF-κB, is related to their anti-inflammatory effects. In the present study, we examined the effect of GL on IL-8 production, mRNA expression, and promoter activation induced by TNF-α and IL-1β, to evaluate its glucocorticoid-like effect. The data clearly indicated that GL, as well as DEX, inhibits IL-8 production. GL inhibited both TNF-α– and IL-1β–induced IL-8 production (Fig. 1). Therefore, we can exclude the possibility that GL inhibits the effects of these pro-inflammatory cytokines at their receptors. Both GL and DEX decreased the level of IL-8 mRNA and IL-8 promoter activity (Figs. 2 and 3), in good agreement with the decrease in the amount of IL-8 protein. In addition, both GL and DEX inhibited the activation of artificial-promoter-containing NF-κB sites (Fig. 4). We assumed therefore, that GL, as well as DEX, inhibited IL-8 gene expression through the inhibition of NF-κB transcription factor. We also assume that GL may inhibit expression of not only IL-8, but also other NF-κB–dependent inflammatory genes such as inducible nitric oxide synthase (iNOS), cyclooxigansae-2, TNF-α, and IL-1β. To support this idea, GL also inhibited mRNA expression of iNOS in our preliminary study (data not shown), although we did not examine other genes.

The inhibitory effect of glucocorticoids on NF-κB is thought to be due to an intranuclear event (27, 28). Glucocorticoids act by binding to cytosolic GRs, which upon binding, become activated and translocate to the nucleus (9). It is known that GR rapidly reduces p65-associated histone acetyltransferase activity by the recruitment of histone deacetylase-2 to the nucleus, which results in inhibition of NF-κB–dependent transcription (29 – 31). In the present study, DEX did not inhibit the translocation of p65 to the nucleus, which suggests that inhibition of IL-8 promoter activation by DEX is due to these intranuclear events. Similarly, GL did not affect p65 translocation to the nucleus (Fig. 5). Therefore, we assume that GL, as well as DEX, inhibits NF-κB–dependent transcription in the nucleus.

Despite these similarities between the effects of GL and those of DEX, the data in the present study exclude the possibility that GL acts at GRs. Both the combined treatment of RU-486 with GL and GR knockdown by siRNA failed to abolish the effect of GL (Fig. 6: A and B). In addition, GL did not activate glucocorticoid-dependent gene transcription by itself, as assessed by the MMTV-promoter reporter gene assay (Fig. 6C). Earlier studies have shown that a degradation product of GL, GA, enhances the effect of glucocorticoids by inhibiting 11β-HSD (19). Therefore, we also examined the effect of GA in an IL-8 reporter gene assay. GA (10 μM) did not inhibit IL-8 promoter activation by TNF-α and IL-1β at all, and it was toxic to the cells at higher concentrations. Therefore, we can also exclude the possibility that the effect of GL shown here was due to contaminated GA.

Recently, it has been reported that GL directly binds to high-motility group box-1 protein (HMGB1) and inhibits its chemoattractant activity (32). HMGB1 is a nuclear protein that acts as an architectural chromatin-binding factor (33, 34). It has been shown that HMGB1

directly interacts with the p50 subunit of NF-κB and facilitates the binding to DNA of the p65/p50 complex (35). In their report, TNF-α–stimulated VCAM-1 expression in embryonic fibroblasts derived from HMGB1-deficient mice was considerably decreased, compared with that in control cells. In addition to these intranuclear functions, HMGB1 acts as an important extracellular inflammatory mediator, which is released from necrotic cells and stimulated monocytes (36). Extracellular HMGB1 interacts with plasma membrane receptors, such as receptor for advanced glycogen end-products, and activates signaling pathways, including MyD88, TRAF6, TAK1, and NF-κB activation (37). Considering the importance of HMGB1 in the inflammatory response, the inhibition of NF-κB–dependent gene expression by GL shown here might have been due to inhibition of HMGB1. In the present study, GL decreased DNA binding to p65 in the ChIP assay, without changing the nuclear translocation of p65. Furthermore, Mollica et al. (32) have shown that the addition of GL to extracellular medium can inhibit intranuclear DNA binding of HMGB1 in fibroblasts. Therefore, we assume that the inhibition of intranuclear HMGB1 may be a possible mechanism for NF-κB inhibition by GL. However, further studies are clearly needed to prove this hypothesis.

In summary, the present study clearly indicates that GL has a glucocorticoid-like inhibitory effect on pro-inflammatory-cytokine–induced gene expression in lung epithelial cells. It is likely that this effect is, at least partly, due to the inhibition of DNA binding of NF-κB in the nucleus, which is different from the mechanism of glucocorticoids. The concentration of GL we used in this study is much higher than the blood concentration in clinical therapeutics. Therefore, it is uncertain whether the inhibition of NF-κB that we showed here can explain the clinical effect of GL or not. However, further characterization of the effects of GL may provide a new insight into treating chronic inflammatory lung diseases.

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