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An Inhibitory Effect of Dexamethasone on A549 Cell Adhesion to Neutrophils: Possible Involvement of Nuclear Factor-Kappa B

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In order to clarify the effects of dexamethasone on epithelial cells at the transcription factor level, protein synthesis level and functional level, we examined neutrophil adhesion to A549 cells (an immortalized human type II alveolar epithelial cell line), expression of intercellular adhesion molecule-1 (ICAM-1), and translocation of nuclear factor-kappa B (NF-κB) into nuclei. The intranuclear localization of fluorescently stained NF-κB and an adhesion assay between neutrophils and A549 cells was analyzed quantitatively using laser scanning cytometry. ICAM-1 expression on A549 cells was measured by flow cytometric analysis. Stimulation of A549 cells with tumor necrosis factor (TNF)-α caused a time- and dose-dependent increase in their adhesiveness for neutrophils. ICAM-1 expression on A549 cells after stimulation with TNF-α for 12 h was significantly up-regulated, compared with unstimulated cells ($P < 0.05$). Intranuclear NF-κB was induced 10 min after stimulation with TNF-α and was increased in a TNF-α-dose-dependent manner. Dexamethasone suppressed TNF-α-induced NF-κB translocation in A549 cells by approximately 60%, ICAM-1 expression on A549 cells by approximately 40%, and A549 adhesiveness for neutrophils completely. Therefore, we suggest that the inhibition of A549 adhesiveness for neutrophils by dexamethasone may be due in part to the suppression of NF-κB entry into nuclei and ICAM-1 expression in A549 cells.

Key words: alveolar epithelial cells; adhesion assay; ICAM-1; laser scanning cytometry; neutrophils; NF-κB

Injury to alveolar epithelial cells is a prominent feature in a variety of lung diseases. In adult respiratory distress syndrome, destruction of alveolar epithelial cells and increased number of neutrophils within the alveoli have been noted to be frequent and early findings (Nash et al., 1974; Bachofen and Weibel, 1977). Stark and colleagues (1996a, 1996b) reported that A549 cells infected by respiratory virus increased levels of adhesion of neutrophils. Simon and colleagues (1986) have demonstrated that neutrophils can adhere to rat pulmonary type II cells and mediate cytotoxicity. The adhesion of neutrophils to alveolar epithelial cells may be an important event of the inflammatory process.

Glucocorticoids are widely used as immunosuppressive and anti-inflammatory agents. The synthetic glucocorticoid dexamethasone suppressed nuclear factor-kappa B (NF-κB) activity of tumor necrosis factor (TNF)-α-activated A549 cells (Adcock et al., 1996). NF-κB is a ubiquitous transcription factor involved in the regulation of a variety of genes encoding pro-inflammatory mediators, especially intercellular adhesion molecule-1 (ICAM-1). It was reported that dexamethasone suppressed the

Abbreviations: AM, acetoxymethylester; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle medium; FITC, fluorescein isothiocyanate; GM-CSF, granulocyte-macrophage colony-stimulating factor; HBSS, Hanks' balanced salt solution; ICAM-1, intercellular adhesion molecule-1; LSC, laser scanning cytomety; NF-κB, nuclear factor-kappa B; PBS, phosphate buffered saline; PI, propidium iodide; PLP, periodate lysine paraformaldehyde; TNF, tumor necrosis factor

expression of ICAM-1 on TNF-α-activated A549 cells (Rothlein et al., 1988) and the neutrophil-to-bronchial epithelial cell line adhesion (Kawasaki et al., 1998). However, in respiratory epithelial cells the effect of dexamethasone on transcription factor level, protein level, and function is still unclear.

Traditionally, adhesion assays to detect cell-to-cell interaction have been measured by the counting of ^{51}[Cr] labeled cells. Recently, the fluorogenic dye calcein acetomethylester (AM) has provided a fast and highly sensitive method for analyzing labeled cells which adhere to cell monolayers (Clerck et al., 1994; van Kessel et al., 1994; Martens et al., 1995). Calcein AM is a non-fluorescent compound, and once loaded into cells, is cleaved by endogenous esterases to produce the highly fluorescent and well-retained dye calcein. In this method, the number of labeled cells has been assessed by a fluorometer after cell lysis. It is difficult to assess cell-to-cell adhesion fairly when unlabeled cells from monolayers such as A549 cells are undergoing proliferative and rapid growth. To count the number of monolayer cells may be an important method for assessing adhesion interaction objectively. So we quantitatively analyzed and visualized the adhesion between calcein-stained neutrophils and DNA-stained epithelial cells using laser scanning cytometry (LSC).

The aim of the present study was to assay for adhesion between neutrophils and A549 cells by LSC. Furthermore, we examined the effects of dexamethasone on neutrophil-to-A549 cell adhesion, ICAM-1 expression, and NF-κB translocation in A549 cells.

Materials and Methods

Human respiratory epithelial cell cultures

A549 (American Type Culture Collection, Rockville, MD), the epithelial type II cell line derived from a patient with alveolar cell carcinoma (Lieber et al., 1976) was selected as a source of respiratory epithelial cell for these studies. A549 cells were cultured in culture dishes (Falcon 3002; Becton Dickinson, San Jose, CA) in Dulbecco's modified Eagle medium/nutrient mixture F-12 (DMEM/F12; Gibco BRL, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (Cansera, Rexdale, ON, Canada), 100 units/mL penicillin, and 100 mg/mL streptomycin (Bio Whittaker, Walkersville, MD) in an incubator containing 5% CO_2 at 37°C. At confluency, the cells were detached with trypsin-EDTA (Kurabo Co., Ltd., Osaka, Japan) and subcultured on 22×24 mm^2 coverglasses (Matsunami Glass Ind., Ltd., Osaka) in 35 mm diameter dishes (Falcon 1008; Becton Dickinson) and then incubated to allow the formation of semi-confluent monolayers. The culture medium was replaced with serum free medium (DMEM/F12) and incubated over night, which resulted in confluent monolayers.

Neutrophil isolation

Neutrophils were obtained from heparinized venous blood of healthy volunteers. Thirty minutes after sedimentation of erythrocytes in 6 % dextran (Nycomed As, Oslo, Norway)-saline, the supernatant layer containing leukocytes was centrifuged in Ficoll-Paque (Pharmacia, Uppsala, Sweden) for 30 min at $400 \times g$ at room temperature. The supernatant containing lymphocytes was discarded, and contaminating erythrocytes within the pellet were lysed by hypoosmosis with hypotonic saline (0.2%) for 15 s. Hypertonic saline (1.6%) was added immediately to maintain isotonicity. This procedure was repeated twice. After washing in phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+} (Handai Bacterial Research, Osaka) and centrifugation, the pellet was resuspended in 1 mL Hanks' balanced salt solution (HBSS; Gibco BRL) without Ca^{2+} and Mg^{2+}. Viability was > 95% by trypan blue exclusion and > 95% of the cells were neutrophils by May-Giemsa staining.

Calcein AM labeling of neutrophils

Stock solutions were prepared by adding 100 mL of fresh dimethyl sulfoxide (Wako Life Science Co., Ltd., Osaka) to 1 mg of calcein AM (Molecular Probes, Eugene, OR). Aliquots

of the stock solution were stored at –20°C. Before the assay, the calcein AM was dissolved and added to the neutrophil suspension at a final concentration of 1 μM for 30 min at 37°C and 5% CO_2. Incubation time was set taking into account the possible increase in the release of calcein after this optimal period (van Kessel et al., 1994). Labeled cells were washed twice in HBSS without Ca^{2+} and Mg^{2+} and resuspended in HBSS with Ca^{2+} and Mg^{2+} at 2.5×10^6 cells per mL before the cell-to-cell adhesion assay.

Fixation and preparations

A549 cells were stimulated with or without TNF-α (Genzyme Corporation, Cambridge, MA) for 12 h for adhesion assay and detection of ICAM-1, or 30 min for detection of NF-κB. Cells in the dexamethasone group were pretreated with dexamethasone (Sigma Chemical Co., St. Louis, MO) for 30 min at various concentrations before stimulation with TNF-α. After washing twice in PBS, the cells were fixed at room temperature in freshly prepared periodate lysine paraformaldehyde (PLP) fixative that consisted of 37 mmol/L phosphate buffer, pH 7.4, containing 10 mmol/L sodium m-per-iodate (Wako), 75 mmol/L lysine (Wako) and 2% paraformaldehyde (Wako). This procedure has been shown to preserve the antigenicity of many protein and carbohydrate determinants (McLean and Nakane, 1974). After removing the supernatant, the monolayers were washed twice with PBS. Then they were washed with 100 mM glycine, and 0.1% bovine serum albumin (BSA; Sigma) in HBSS without Ca^{2+} and Mg^{2+} (blocking solution). The dishes were stored at 4°C with blocking solution. Before use in the adhesion assays they were washed with PBS, and then 1 mg/mL ribonuclease A (Sigma) and 100 mg/mL propidium iodide (PI) (Sigma) were added to each dish for 30 min at 4°C. After removing the supernatant, the monolayers were washed twice with PBS.

Adhesion assay of neutrophils to A549 monolayers

For the adhesion assay, 10 ng/mL granulocyte-macrophage colony-stimulating factor (GM-CSF) was added to 2.5×10^6 of calcein-labeled neutrophils supplemented with 1 mL of HBSS with Ca^{2+} and Mg^{2+}. After incubation for 30 min at 37°C in 5% CO_2, the plates were washed six times with 1 mL of HBSS without Ca^{2+} and Mg^{2+} to remove nonadherent neutrophils. We confirmed the adhesion of neutrophils to A549 cells under microscopy. A coverglass in each dish was mounted cell-side down on each slide and sealed with nail polish. The slide was placed on the microscope stage.

NF-κB staining of A549 cells

A549 cells in the dexamethasone group were pretreated with dexamethasone at various concentrations 30 min before TNF-α stimulation. After washing twice in PBS, the cells were prefixed in 2% formalin (Wako) in PBS, pH 7.4, for 10 min at room temperature. After thorough washing in PBS, the cells were fixed with methanol-acetone at –20°C for 10 min. Nonspecific antibody binding sites were then blocked with 10% normal goat serum (Chemicon, Temecula, CA) in PBS with 1% BSA. Coverglasses were incubated with anti-Rel A rabbit polyclonal antibody (SC-109; Santa Cruz Biotechnology, Santa Cruz, CA,) at a final dilution of 1:100 for 1 h at room temperature. After washing thoroughly 3 times with PBS, cells were incubated for 30 min at room temperature with 5 mg/mL goat anti-rabbit IgG fluorescein isothiocyanate (FITC)-conjugated antibody (Santa Cruz). After each incubation with antibodies, cells were extensively washed with PBS. Nuclei were stained for 10 min with 50 mg/mL PI and 200 mg/mL ribonuclease A and washed again. As a negative control, rabbit IgG (Santa Cruz) was used in place of the first antibody at the same dilution.

Detection of adhesion and of intranuclear NF-κB by LSC

For adhesion assay and measuring intranuclear NF-κB staining, LSC (LSC101, Olympus Co., Tokyo, Japan) was used as previously described (Newton and Millette, 1992; Clatch et al., 1996).

Dual-channel imaging was performed with 2 photomultipliers, using a band-pass filter specifically designed to detect FITC fluorescence and a long-pass (590 nm) filter for detecting PI fluorescence. Images were generated using integral computational and display software. To assess the mean fluorescence intensity of NF-κB translocated into nuclei, each nucleus was identified by the areas counter stained with PI. For the adhesion assays, staining with FITC for neutrophils and PI for A549 cells was used for cell counting. Firstly, we confirmed the accuracy of the instrument. When cells of the same sample and the same area were counted repeatedly by LSC, the liability to error, calculating the mean value of the squares of these errors, was 0.4% (n = 10). Secondly, we chose 1 mm^2 as the scan area in this experiment. This is because the cell number counted by LSC closely correlated to the scan area (r = 0.998, P < 0.0001) (Fig. 1A) and the scan time would be too long if the area were over this size. Thirdly, we checked the correlation between the A549 cell number and the neutrophil number in adhesion to each other since A549 cells were immortalized and were rapid-growth cells. The result showed that the number of A549 cells was positively correlated with the adherent neutrophils (Fig. 1B). Then the adhesion assay was performed. After counting the number of A549 cells, the number of adherent neutrophils was examined in the same scan area. Each sample was counted in triplicate. The rate of the adhesion of neutrophils to A549 cells was calculated as follows:

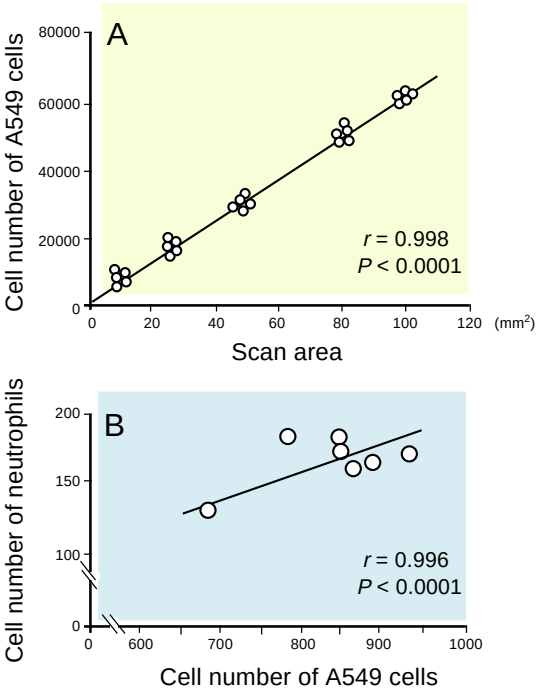

Fig. 1. Relationship between the cell number of A549 cells and scan area (**A**), or between the cell number of A549 cells and that of neutrophils (**B**). For the relationship between A549 cells number and scan area, the number of PI-stained cells was measured at different sites on the same sample, which was confluent on the glass, by LSC. For the relationship between A549 cell number and neutrophil number, we analyzed a 1 mm^2 scan area after adhesion of GM-CSF-stimulated and calcein-stained neutrophils to 50 ng/mL TNF-α-stimulated and PI-stained A549 cells.

$$\text{Adherent rate (\%)} = \frac{\text{the total number of adherent neutrophils}}{\text{the total number of A549 cells}} \times 100$$

Detection of ICAM-1 on A549 cells by flow cytometry

A549 cells were pretreated with dexamethasone 30 min before 50 ng/mL TNF-α was added. At the end of the 12 h incubation period, the cells were washed twice with PBS. After trypsinization, the cells were centrifuged for 5 min at 400 × g, and at 4°C. The supernatant was discarded and the pellet was suspended with PBS. This procedure was repeated twice. Then the cells were fixed in PLP fixative at room temperature for 10 min. After thorough washing in PBS, the cells were resuspended in 300 mL PBS containing 0.1% sodium azide (Wako) and 1% BSA (staining buffer). Then, they were incubated with FITC-conjugated mouse anti-human-ICAM-1 monoclonal antibody (B-C14, IgG_1; Serotec, Oxford, United Kingdom) at a final dilution of 1:100 for 1 h in the dark at 4°C. Next they were washed twice in staining buffer and resuspended in staining buffer. Flow cytometry was performed with 1.0 × 10^6 cells per sample by FACSCalibur (Becton Dickinson,

Mountain View, CA). Fluorescence measured in the fluorescence-1 channel was quantified as mean fluorescence intensity units.

Statistical analysis

Statistical analysis was performed with the Stat View 4.0 statistics package (Abacus Concepts, Berkeley, CA). Data are expressed as mean ± SD. The nonparametric Mann-Whitney *U* test was used for statistical analysis of the unpaired data. In addition, the relationship between scan area and the number of A549 cells, and that between the A549 cell number and the neutrophil number were analyzed by Spearman's rank sum test.

Results

The adhesiveness of A549 cells for neutrophils

Fluorescent microscopy

Figure 2 is a representative micrograph of neutrophil adhesion to a confluent monolayer of A549 cells, which was pretreated without (Fig. 2A) or with TNF-α (Fig. 2D). Neutrophil adhesion increased significantly on exposure to TNF-α. In addition, LSC was available to visualize and separate A549 cells (Figs. 2B and E) and neutrophils (Figs. 2C and F) by calcein- and PI-staining, respectively.

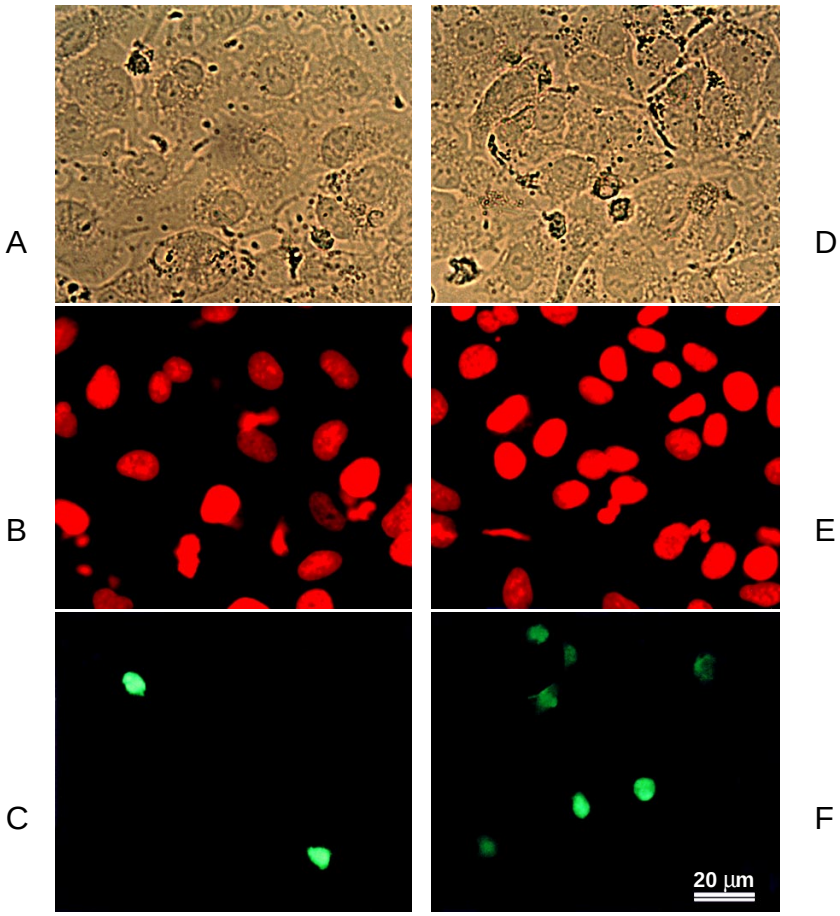

Fig. 2. Neutrophil adhesion to an underlying confluent monolayer of A549 cells after no treatment (**A**, **B** and **C**) and after pretreatment with TNF-α (50 ng/mL) for 12 h (**D**, **E** and **F**). These were shown by microscopic image (**A** and **D**), red fluorescence image (**B** and **E**) for PI stained A549 cells, and fluorescence image (**C** and **F**) for calcein-stained neutrophils from the same sample.

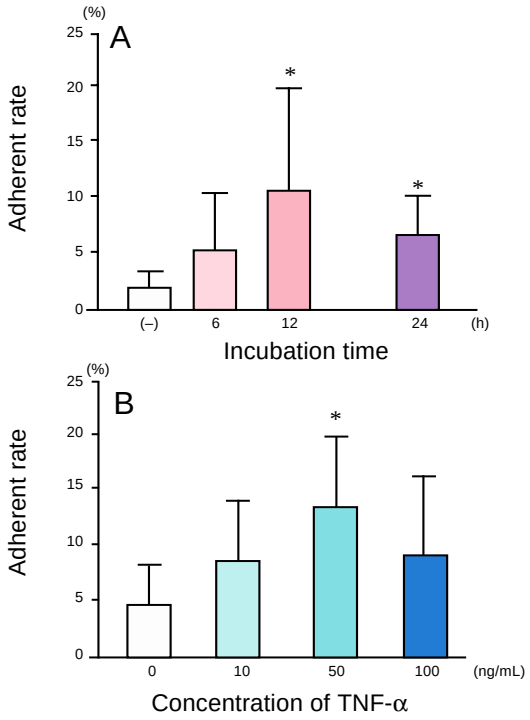

Fig. 3. A549 adhesiveness for neutrophils after stimulation with TNF-α (50 ng/mL) for different periods (**A**) and that with different concentrations of TNF-α (**B**) for 12 h. Results are expressed as mean ± SD; $n = 6$. *$P < 0.05$, compared with no stimulation.

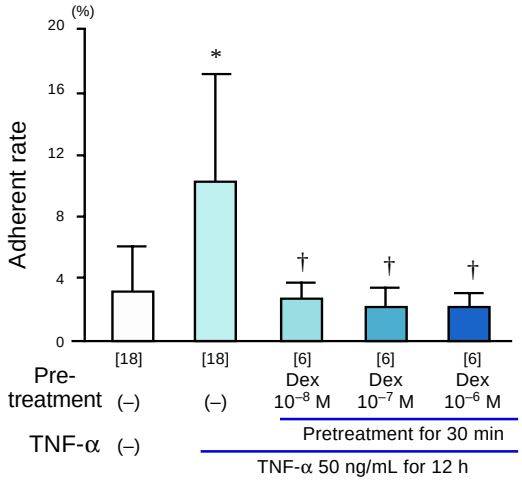

Fig. 4. Suppression of A549 adhesiveness for neutrophils by dexamethasone (Dex). A549 cells in each group except for the controls were treated with TNF-α (50 ng/mL) for 12 h. Cells in the Dex group were incubated with various concentrations of Dex 30 min before treatment with TNF-α. Results are expressed as mean ± SD. [ ] = number of samples. *$P < 0.05$, compared with no stimulation. †$p < 0.05$, compared with TNF-α stimulation.

Effect of TNF-α on the adhesiveness of A549 cells for neutrophils

Stimulation of A549 cells with TNF-α caused a time-dependent increase in their adhesiveness for GM-CSF stimulated neutrophils (Fig. 3A). The adherent rate began to increase after stimulating A549 cells with TNF-α for 6 h compared with the control group (2.1 ± 1.6% versus 5.4 ± 5.2%) and reached its peak at 12 h (10.6 ± 9.2%). When stimulation lasted for 24 h, no further increase was observed (6.8 ± 3.5%). The effect of different doses of TNF-α on A549 adhesiveness for neutrophils is shown in Fig. 3B. TNF-α pretreatment increased the adherent rate in a dose-dependent manner within 50 ng/mL. Although there was a trend for the adherent rate to decline with high doses of TNF-α (100 ng/mL) compared with 50 ng/mL, this difference did not reach statistical significance.

Effect of dexamethasone on the adhesiveness of A549 cells for neutrophils

Pretreatment with dexamethasone at a low dose (10^{-8} M) could inhibit the TNF-α-induced neutrophil adhesion to A549 cells ($P < 0.05$). As shown in Fig. 4, 10^{-7} M dexamethasone completely inhibited neutrophil adhesion to A549 cells.

ICAM-1 expression on A549 cells

Concentration-related dexamethasone suppression of TNF-α-induced ICAM-1 expression on A549 cells is shown in Fig. 5. TNF-α stimulation caused a significant increase in ICAM-1 expression compared with the unstimulated group (mean fluorescence intensity; 13.3 ± 5.6 versus 103.4 ± 15.8, $P < 0.05$). At a dose of 10^{-7} M, dexamethasone suppressed TNF-α-induced ICAM-1 up-regulation by approximately 40%. Using 10^{-6} M of dexamethasone did not increase the suppression, indicating that dexamethasone only partially blocks the TNF-α-induced ICAM-1 up-regulation.

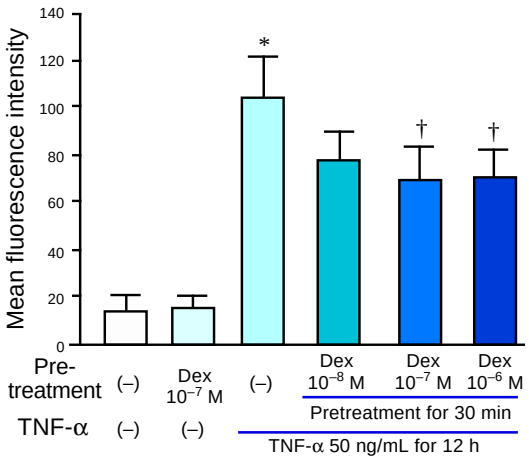

Fig. 5. Suppression of TNF-α-induced ICAM-1 expression on A549 cells by dexamethasone (Dex). Cells were incubated with various concentrations of Dex 30 min prior to addition of 50 ng/mL TNF-α. ICAM-1 levels were measured by flow cytometry analysis. Results are expressed as mean ± SD; $n = 5$. *$P < 0.05$, compared with no stimulation. †$P < 0.05$, compared with TNF-α stimulation.

NF-κB translocation into nuclei of A549 cells

Intranuclear NF-κB of A549 cells

NF-κB was located in the cytosol of unstimulated A549 cells and exposure to TNF-α caused NF-κB to translocate into the nuclei in a time- (Table 1) and dose- (Table 2) dependent manner. Intranuclear p65 staining was induced 10 min after stimulation with TNF-α, without a decline in staining intensity over the subsequent 1 h.

Table 1. Time response of translocation of p65 into nuclei

Stimulation time (min)	Fluorescence intensity
0	17.6 ± 4.8
10	51.6 ± 7.2*
30	51.9 ±10.3*
60	44.1 ± 3.2*

A549 cells were stimulated with 50 ng/mL TNF-α for various times.
Data are expressed as mean ± SD of 8 individual experiments.
* $P < 0.01$, compared with no stimulation.

Effect of dexamethasone on NF-κB translocation into nuclei

Pretreatment with dexamethasone suppressed translocation of NF-κB into nuclei in the presence of TNF-α stimulation. Quantitative image analysis using LSC revealed that the green fluorescence level in A549 cell nuclei from the unstimulated cells and the cells treated by dexamethasone alone was significantly less than the cells treated by TNF-α ($P < 0.01$). Dexamethasone suppressed the TNF-α-induced translocation of NF-κB into nuclei by approximately 60% (Table 3).

Table 2. Dose response of translocation of p65 into nuclei by TNF-α

TNF-α (ng/mL)	Fluorescence intensity
0	19.6 ± 6.1
0.1	26.2 ± 1.9†
1.0	32.2 ± 3.8*
10	34.4 ± 3.8*
50	53.0 ± 8.9*

A549 cells were stimulated with various concentrations of TNF-α for 30 min.
Data are expressed as mean ± SD of 10 individual experiments.
* $P < 0.01$, compared with no stimulation.
† $P < 0.05$, compared with no stimulation.

Table 3. Inhibition of translocation of p65 into nuclei by dexamethasone

Dexamethasone (M)	TNF-α (ng/mL)	Fluorescence intensity
(–)	(–)	17.8 ± 3.3
10^{-7}	(–)	17.4 ± 3.2
(–)	50	41.8 ± 4.1†
10^{-8}	50	34.0 ± 2.2*
10^{-7}	50	28.0 ± 2.5*
10^{-6}	50	28.9 ± 1.2*

A549 cells were pretreated with various concentrations of dexamethasone for 30 min, and then were stimulated with 50 ng/mL TNF-α for 30 min.
Data are expressed as mean ± SD of 8 individual experiments.
* $P < 0.01$, compared with TNF-α stimulation.
† $P < 0.01$, compared with no stimulation.

Discussion

We assessed the adhesion of neutrophils to A549 cells using the quantitative and visual method, LSC. The results have demonstrated that pretreatment of A549 cells with dexamethasone suppressed simultaneously their adhesiveness for neutrophils, ICAM-1 expression and nuclear translocation of NF-κB in the presence of TNF-α.

NF-κB controls genes encoding proinflammatory cytokines, chemokines and adhesion molecules, and is therefore an important regulatory factor in immune and inflammatory responses. NF-κB is capable of being transported into nuclei and interacting with specific NF-κB-like binding sites in the promoters of responsive genes (Siebenlist et al., 1994). Previous work and our present results have demonstrated that the proinflammatory cytokines, TNF-α and interleukin-1β cause NF-κB activation in A549 epithelial cells (Newton et al., 1996). In this study, we only assessed the translocation of NF-κB into nuclei and did not directly analyze DNA-NF-κB binding with electrophoretic mobility shift assay. We found that nuclear translocation of NF-κB by TNF-α occurred within the first 30 min of stimulation, and decayed rapidly and then declined more slowly. These results are in agreement with a previous study using electrophoretic mobility shift assay (Newton et al., 1996). Dexamethasone has been reported to reduce ICAM-1 messenger RNA levels and its expression in bronchial epithelial cells (van de Stolpe et al., 1993). We demonstrated that dexamethasone suppressed the TNF-α-induced translocation of NF-κB and ICAM-1 expression to a similar extent in A549 cells. These results suggest one action of dexamethasone at the transcriptional level. There are multiple binding sites for transcription factors, such as NF-κB, activating protein 1 and CCAAT/enhancer-binding protein β in the upstream regions of ICAM-1 genes, which also encode important targets for glucocorticoid repression (Adcock and Newton, 1998). So, we suggest that dexamethasone partially suppresses ICAM-1 expression on A549 cells by partial suppression of NF-κB translocation and the action of transcription factors other than NF-κB.

The present study showed that TNF-α increases the adhesiveness of alveolar epithelial cells for neutrophils. The cell-to-cell adhesive interactions are mediated through adhesion molecules. Tosi and coworkers (1992) described that enhanced neutrophil adhesion to TNF-α-treated airway epithelial cells was dependent on neutrophil CD18 integrins and epithelial cell expression of ICAM-1. Our results are coincident with Burke-Gaffney and Hellewell (1996) who demonstrated that TNF-α caused ICAM-1 up-regulation in A549 epithelial cells. As for adhesion assays, we found that A549 cells adhesiveness for neutrophils after stimulation with TNF-α increased significantly. This is different from a previous report (Thornhill et al., 1993) which showed that stimulation of A549 cells with TNF-α for 4 h could not increase neutrophil adhesion. As to this discrepancy, two reasons could be considered. First, stimulation for 4 h may be too short to up-regulate adhesion molecules. Atsuta and colleagues (1997) reported that ICAM-1 messenger RNA expression was induced in the bronchial epithelial cell line after TNF-α treatment for 2 h. Secondly, the neutrophils used in our study were stimulated with GM-CSF. Previous reports indicated that GM-CSF-treated neutrophils increased the expression of adhesion molecules such as CD11b/CD18 (Yong and Linch, 1992; Novella et al., 1997). In addition, our results showed that the suppressive role of dexamethasone exhibited at the functional level and protein level is different. This indicates the possibility that ICAM-1-mediated adhesion of neutrophils to epithelial cells requires these adhesion molecules to be up-regulated to some extent.

The increase in adhesiveness of airway epithelial cells for neutrophils following stimulation by cytokines is considered to be an important mechanism whereby neutrophils injury and exfoliate epithelial cells at sites of inflammation (Tosi et al., 1992). Simon and associates (1986) have demonstrated that neutrophils can adhere to alveolar epithelial cells and medi-

ate cytotoxicty. Glucocorticoids are widely used as immunosuppressive and anti-inflammatory agents. Repression of NF-κB-dependent gene expression is one of the key characteristics by which glucocorticoids exert their effects. It was previously reported that in mild asthmatic subjects treatment with corticosteroids suppressed the activation of NF-κB in the bronchial mucosa obtained by bronchial biopsy (Demoly et al., 1995). The mechanisms of anti-inflammatory effects of dexamethasone are not well-known. We agree with the reports of Shimoyama and colleagues (1997) who demonstrated that the suppressive effect of dexamethasone on NF-κB translocation into nuclei in corneal endothelium was dissociated from that of the endothelium adhesiveness for neutrophils. In our results, 10^{-7} M dexamethasone was able to completely inhibit adhesiveness for neutrophils whereas the suppression of NF-κB was approximately 60%. We suggested that this dissociation of suppressive effects might be attributed to the dissociation between protein level and functional level. Further study is necessary to solve the suppression mechanisms of the dexamethasone from transcription factor level to functional level.

For quantitative detection of the adherent rate, we used non-radiolabeled fluorochromes to stain cells and visualize them by LSC. It was possible for LSC to discriminate each different kind of cells by staining the cells one after another, as calcein-stained neutrophils and PI-stained A549 cells. LSC is a useful tool for performing immunophenotypic analysis of cells on a slide rather than in a fluid stream (Clatch et al., 1996). We confirmed that LSC could quantitatively count the number of cells and might be an optimal instrument for adhesion assays when monolayers are proliferative cells, such as cancer cell lines, because the number of adherent neutrophils was dependent on the number of cells in the A549 monolayer.

In conclusion, we measured adherent rates of calcein-stained neutrophils to PI-stained A549 cells using the LSC quantitative method. It might also be a useful method for proliferative monolayer cells. We also demonstrated that the inhibition of A549 cells adhesiveness for neutrophils by dexamethasone might be due in part to the suppression of NF-κB entry into nuclei and the subsequent suppression of the ICAM-1 expression.

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