

Effect of glucocorticosteroids on tumour necrosis factor- α -induced intercellular adhesion molecule-1 expression in cultured primary human nasal epithelial cells

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Summary

Objective In order to confirm the direct effect of glucocorticosteroids on epithelial intercellular adhesion molecule-1 (ICAM-1) expression, we examined ICAM-1 expression on primary cultured human nasal epithelial cells (HNECs) at both protein and mRNA levels.

Material and methods HNECs were stimulated with recombinant human TNF- α (20 pg/mL–20 ng/mL) for specified time periods (0, 12, 24, and 48 h) and ICAM-1 mRNA and the soluble ICAM-1 (sICAM-1) concentrations were measured by quantitative RT-PCR and ELISA, respectively. We also evaluated surface expression of ICAM-1 by flow cytometry 48 h after stimulation and determined the effect of dexamethasone (DEX) on TNF- α -induced ICAM-1 expression.

Results Significant increases in ICAM-1 gene expression in HNECs were initially detected at 24 h, peaking at 48 h after the stimulation. The TNF-mediated-ICAM-1 mRNA and ICAM-1 surface expression at 48 h was significantly inhibited by co-incubation with human recombinant soluble TNF receptor I. Similarly, TNF- α -induced release sICAM-1 occurred in a time- and concentration-dependent manner. DEX 10^{-6} M attenuated the TNF- α -induced ICAM-1 expression at mRNA and protein levels.

Conclusions Our finding suggests a potential role for topical steroids in allergic rhinitis in suppressing inflammatory reactions in the nasal mucosa by regulating ICAM-1 expression on nasal epithelium.

Key words glucocorticosteroid, ICAM-1, nasal epithelial cell, real-time PCR, TNF

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Introduction

Inflammatory cell accumulation, including eosinophils and lymphocytes has been shown to play an important role in allergic rhinitis. It has been demonstrated that intercellular adhesion molecule-1 (ICAM-1) is an adhesion molecule implicated in the recruitment of cells expressing LFA-1 (eosinophils, neutrophils, lymphocytes), and Mac-1 (granulocytes) integrins as the complementary receptors [1]. Therefore, epithelial cells expressing ICAM-1 are likely to play a direct role in the inflammatory response by facilitating adhesion with inflammatory cells [2, 3]. Immunohistochemical studies of nasal turbinates showed that ICAM-1 was broadly distributed within the epithelium, on ciliated and basal epithelial cells and in the submucosal on endothelial and inflammatory cells [3, 4]. In allergic rhinitis, ICAM-1 expression has been observed on epithelial cells recovered from patients suffering from seasonal [2] and perennial rhinitis [3] as well as in some asymptomatic patients allergic to house dust mites after nasal challenge with allergen [5].

TNF- α has been recognized as an important regulator of a variety of cellular functions within the airway epithelium.

These TNF- α -mediated airway epithelial cell functions include enhanced IL-6, IL-8, and GM-CSF secretion [6]; enhanced inducible nitric oxide synthase expression [7]; and enhanced MUC1 mucin expression [8]. Airway epithelial ICAM-1 is also reported to be up-regulated by TNF- α [9–14]. So, it might be possible that TNF- α could regulate local leukocytes infiltration via up-regulation of epithelial ICAM-1.

Topical glucocorticosteroids have been accepted as a highly effective therapy for allergic rhinitis [15]. Despite wide use of topical glucocorticosteroids in the treatment of allergic rhinitis, mechanisms underlying the anti-inflammatory effects of these compounds have not been well clarified. Ciprandi et al. [16] have reported that topical administration of steroid to allergic rhinitis patients reduced ICAM-1 expression on nasal epithelial cells *in vivo*. However, few studies have been done to elucidate the direct effect of glucocorticosteroids on ICAM-1 expression of nasal epithelial cells. Our aim in the present study was to evaluate the effect of glucocorticosteroids on basal or TNF- α -induced nasal epithelial ICAM-1 expression *in vitro*.

Materials and methods

Tissue preparation

Human inferior turbinates were obtained after turbinectomy from 10 perennial allergic rhinitis patients with nasal

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Table 1. Demographic characteristics of perennial allergic rhinitis patients

	N = 10
Sex (male/female)	4/6
Age (years)	38 (19–65)
Specific IgE to house dust mite (d1) (kU/L)	3.4 (1.0–13)
Total IgE (kU/L)	251(10–680)
Blood eosinophils (cells/ μ L)	390(70–830)
Current nasal symptoms (number of patients)	
Nasal obstruction	10 (all patients)
Sneezing	6
Rhinoirrhoea	7

Data expressed as median values and range (in parentheses).

obstruction refractory to medical therapy. Informed consent was obtained from all patients and this study was approved by the ethics committee of Sapporo Medical University. Donors ranged in age from 19 to 65 years: four males and six females. All were non-smokers, and had perennial allergy against mites as defined by questionnaire and CAP test (Pharmacia, Uppsala, Sweden). All medications, including antibiotics, were prohibited for at least 3 weeks prior to the study. Demographic and clinical characteristics of the patients are summarized in Table 1. The nasal mucosal specimens were dissected from the cartilage, and placed into cold transfer medium (Ham's F-12 medium; Sigma, St Louis, MO, USA) for epithelial culture.

Epithelial cell culture

Human nasal epithelial cells (HNECs) were isolated from human nasal mucosa specimens according to a previously described protocol [17]. Nasal specimens were rinsed two to three times with Ham's F-12 medium (Sigma) supplemented with antibiotics and incubated in 0.1% protease type XIV (Sigma) in Ham's F-12 medium for 16 h at 4 °C. After incubation, 10% fetal bovine serum (ICN Biomedicals Inc., Aurora, OH, USA) was added to neutralize protease activity and epithelial cells were detached by gentle agitation. Cell suspensions were filtered through a 60 mesh cell dissociation sieve (Sigma) and centrifuged at 500 *g* for 10 min at room temperature. The cell pellet was then resuspended in hormonally defined Ham's F-12 culture medium (Ham's HD) with the following reagents: 100 IU/mL penicillin (Sigma), 100 μ g/mL streptomycin (Sigma), 2 μ g/mL amphotericin B (Sigma), 150 μ g/mL glutamine (Sigma), 5 μ g/mL transferrin (Sigma), 5 μ g/mL insulin (Sigma), 25 ng/mL epidermal growth factor (Sigma), 15 μ g/mL bovine pituitary extract (Upstate Biotechnology, Lake Placid, NY, USA), 200 pM triiodothyronine (Sigma), and 100 nM hydrocortisone (Sigma). Cell suspensions (10⁵ cells/well) were plated onto collagen type-I-coated six-well culture plates (Sumitomo Bakelite Co. Ltd, Osaka, Japan) in Ham's HD medium and cultured in a 5% carbon dioxide humidified atmosphere at 37 °C. The culture medium was changed at day 1 and every 2 days thereafter. Monolayer cell confluence was achieved after 6–8 days of culture. Morphologic observations using a phase contrast microscope showed the HNECs consisted primarily of epithelial cells. More than 95% of the HNECs showed positive reactions for anti-human cytokeratin antibody (Dako,

Glostrup, Denmark). HNECs grown to 80% confluency were then cultured in Ham's HD without hydrocortisone, and were used for RT-PCR analysis and flow cytometry. HNECs were exposed to recombinant human TNF- α (Sigma) over a range of concentrations (20 pg/mL–20 ng/mL) or to culture medium (control) for specified time periods (0–48 h), then the total RNA was extracted as follow.

Experiments assessing the effect of recombinant human soluble TNF receptor I (sTNF-RI) or glucocorticoid on TNF- α -mediated ICAM-1 mRNA expression were also performed. HNECs were stimulated with human recombinant TNF- α (20 ng/mL) alone or TNF- α (20 ng/mL) in combination with human recombinant sTNF-RI (2 μ g/mL: R&D Systems, Minneapolis, MN, USA) or dexamethasone (DEX) (10^{−6} M: Sigma) for 48 h. The RNA samples were aliquoted and stored at −80 °C until used.

RNA extraction and cDNA synthesis

Total RNA from HNECs was extracted by the acid-phenol guanidinium method. Reverse transcription of RNA was done in a final volume of 20 μ L containing 1 $\times$ TaqMan RT buffer (500 μ M each deoxynucleotide triphosphate, 5.5 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl, pH 8.3), 8 U of RNase inhibitor, 2.5 μ M random hexamer, 25 U of MultiScribe Reverse Transcriptase (all from PE Applied Biosystems, Foster City, CA, USA), and 1 μ g of total RNA (standard curve point samples and the target samples). The samples were incubated at 25 °C for 10 min and 48 °C for 30 min, and reverse transcriptase was inactivated by heating at 95 °C for 5 min and cooling at 5 °C for 5 min.

PCR primers and TaqMan probes for real-time PCR

The PCR primers and TaqMan probes to amplify and detect the ICAM-1 mRNA were designed using the primer express software version 1.0 (PE Applied Biosystems) as follows: forward primer 5'-CACAGTCACCTATGGCAACGA-3' (nucleotide 795–815), reverse primer 5'-CGCCGGAAGC TGTAGATG-3' (nucleotide 929–948), TaqMan Probe 5'-FAM-ACCAGAGCCAGGAGACACTGCAGACAG-TA MRA-3' (nucleotide 899–925). The PCR primers and the TaqMan probes were purchased from Amersham Pharmacia Biotech (Tokyo, Japan) and PE Biosystems Japan (Tokyo, Japan), respectively. The TaqMan-GAPDH Control Reagents (PE Biosystems) were used to amplify and detect GAPDH as recommended by the manufacturer.

Real-time PCR amplification

Real-time PCR was performed on an ABI PRISM 7700 Sequence Detector, using the ABI PRISM Sequence Detector Software 1.6 (PE Applied Biosystems) according to the manufacturer's instructions. For each PCR run, a master mix was prepared on ice with 1 $\times$ TaqMan buffer, 5 mM MgCl₂, 200 μ M dATP, dCTP, and dGTP, 400 μ M dUTP, 300 nM each primer, 150 nM TaqMan probe, and 1.25 U of AmpliTaq Gold DNA polymerase (PE Applied Biosystems). Ten microlitres of each appropriately diluted reverse transcription sample (standard curve points and target samples) were added to 40 μ L of the PCR master-mix. The thermal cycling conditions comprised an initial denaturation step at 95 °C for

10 min, 45 cycles at 95°C for 15 s, and 60°C for 1 min. Experiments were performed with duplicates for each data point. Each PCR run included the four points of the standard curve (400, 100, 50, and 12.5 ng of unstimulated HNECs total RNA). The series of diluted human unstimulated nasal epithelial cells total RNAs was aliquoted and stored at -80°C until use. For each experimental sample, the amount of ICAM-1 and GAPDH level was determined from the standard curve. Then, the target amount of ICAM-1 was divided by the GAPDH level to obtain a normalized target value. So, the level of target ICAM-1 mRNA was expressed as relative ratio against ICAM-1 mRNA in the control (unstimulated) HNECs. To analyse data for PCR products the term C_T (threshold cycle) was used to express the result: C_T , the PCR cycle at which an increase in reporter fluorescence signal above a baseline can first be detected.

Measurement of soluble ICAM-1 (sICAM-1)

One hundred microlitres aliquots of HNECs culture supernatant were analysed in duplicate for sICAM-1 using commercially available ELISA kits (Pierce Endogen, Rockford, IL, USA).

Flow cytometric analysis

For a quantitative evaluation of ICAM-1 density on the membrane of HNECs, flow cytometry was used. HNECs in the glass tubes were collected by incubation with 0.02% EDTA for 10 min at 37°C and were washed in phosphate-buffered saline (PBS) containing 0.02 mM sodium azide and 1% BSA and were incubated with an FITC-labelled monoclonal antibody for human ICAM-1 (Immunotech,

Marseille, France) diluted 1:50 in PBS or isotype-specific control antibody (Immunotech) for 30 min on ice. Cells were washed twice in PBS. A total of 10^4 cells were analysed for fluorescence by single-colour flow cytometer (FACS Calibur, Becton Dickinson, Sunnyvale, CA, USA). Surface expression of ICAM-1 was determined by subtracting the mean fluorescence intensity (MIF) measured with the control monoclonal antibody from that measured with the antibody to ICAM-1. The level of target ICAM-1 expression was expressed as relative ratio against ICAM-1 MIF in control (unstimulated) HNECs.

Statistical analysis

ICAM-1 mRNA levels, surface expression of ICAM-1 and sICAM-1 concentration were determined in duplicate from 10 separate experiments and are presented as mean $\pm$ SD. The non-parametric Mann-Whitney *U*-test was used to determine difference between TNF- α or DEX stimulated and control group. Values with $P \leq 0.05$ were considered significant.

Results

Real-time PCR

The appropriately sized 154-bp ICAM-1 cDNA RT-PCR product was visible on agarose gel electrophoresis for each calibrator. Figure 1a shows the amplification blots for detection of ICAM-1 mRNA in cultured HNECs by real-time PCR. The standard curve was constructed with total RNA extracted from unstimulated HNECs. The calibration curve of the duplicate assay values demonstrated a strong

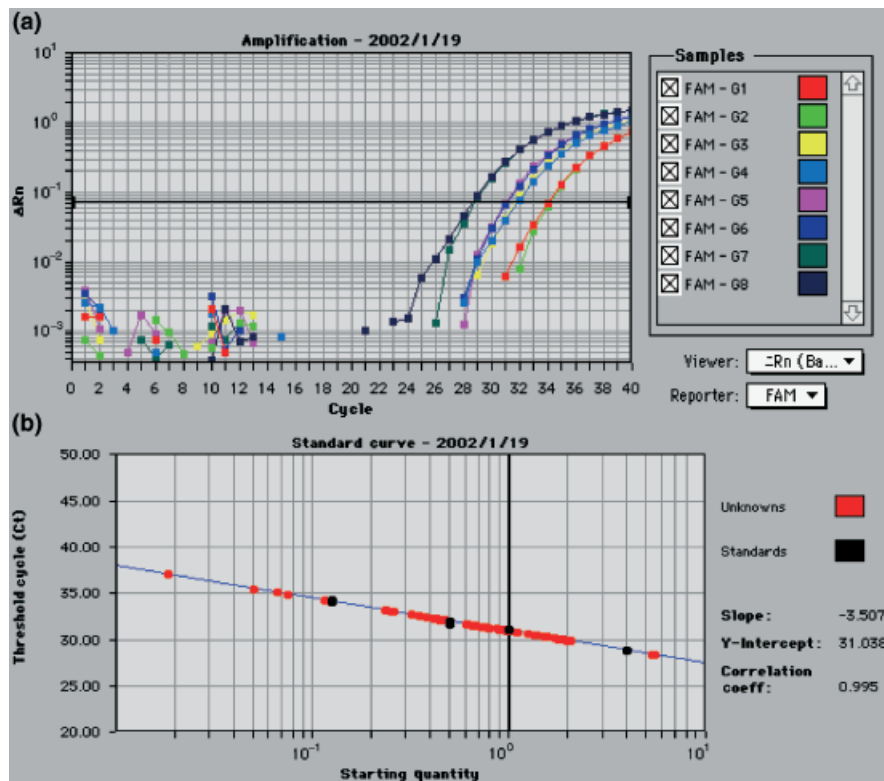


Fig. 1. (a) The amplification plots of real-time RT-PCR for detection of intercellular adhesion molecule-1 (ICAM-1) mRNA in cultured nasal epithelial cells. Cycle number is plotted vs. change in normalized reporter signal (ΔRn). The horizontal bold line indicates the fluorescence level used for the threshold cycle determination in this particular example. (b) ICAM-1 standard curve by real-time RT-PCR. ICAM-1 standard curve was composed of four points (400, 100, 50, and 12.5 ng of unstimulated human nasal epithelial cells RNA as calibrators (black circles)). Cycle threshold measurements for each of calibrator were used to determine to the quantity of ICAM-1 mRNA in the target samples (red circles).

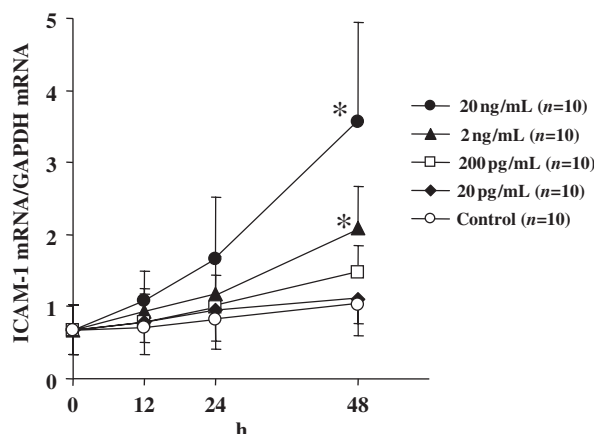


Fig. 2. The time course and dose-response induction of intercellular adhesion molecule-1 (ICAM-1) mRNA by TNF- α in cultured human nasal epithelial cells. Paired human primary nasal epithelial cell cultures were treated with either 0.02, 0.2, 2, or 20 ng/mL of recombinant human TNF- α or control medium for 12, 24, or 48 h and the level of ICAM-1 mRNA expression assessed by real-time RT-PCR. TNF- α stimulation resulted in the increase in ICAM-1 mRNA expression at 48 h. Significant difference from unstimulated cells (O) is indicated, * $P < 0.05$.

linear relationship between the threshold cycle, C_T and log starting quantity of the standard cDNA template (black circles) or target (ICAM-1) mRNA (red circles), with $r = 0.995$ (Fig. 1b).

TNF- α -induced ICAM-1 mRNA expression

In the preliminary study, IL-1 β (100 ng/mL) and INF- γ (100 ng/mL) induced minor effects on ICAM-1 mRNA expression (IL-1 β : 1.29 ± 0.63 , INF- γ : 1.18 ± 0.41 , $n = 5$). So, the effect of TNF- α on primary culture of human airway epithelial cell cultures was assessed. As shown in Fig. 2, TNF- α -mediated increases in ICAM-1 mRNA expression were concentration dependent, and ICAM-1 mRNA was maximally expressed at 48 h of TNF- α (20 ng/mL) exposure (3.56 ± 1.38 , $n = 10$). The increase in ICAM-1 mRNA was statistically significant ($P < 0.05$) for 2–20 ng/mL TNF- α .

TNF- α -induced increase in sICAM-1 concentration

The concentration of sICAM-1 in culture medium was measured periodically. As shown in Fig. 3, TNF- α -mediated increases in sICAM-1 level were time and concentration dependent, and sICAM-1 concentration was maximally increased after 48 h of TNF- α (20 ng/mL) exposure (5.53 ± 1.64 ng/mL, $n = 10$). The increase in sICAM-1 level was statistically significant ($P < 0.05$) for 2–20 ng/mL TNF- α .

Effect of sTNF-RI on TNF- α -induced ICAM-1 mRNA expression and sICAM-1 concentration

The experiments were performed to assess whether the TNF- α -mediated increase in ICAM-1 mRNA and sICAM-1 concentration could be regulated by sTNF-RI, which can neutralize the biological activity of TNF- α . As shown in Figs 4 and 5, sTNF-RI significantly inhibited the TNF- α (20 ng/mL)-mediated increases in ICAM-1 mRNA (TNF- α alone 3.56 ± 1.38 , TNF- α plus sTNF-RI 1.50 ± 0.76 , $P = 0.002$, $n = 10$) and sICAM-1 concentrations (TNF- α alone

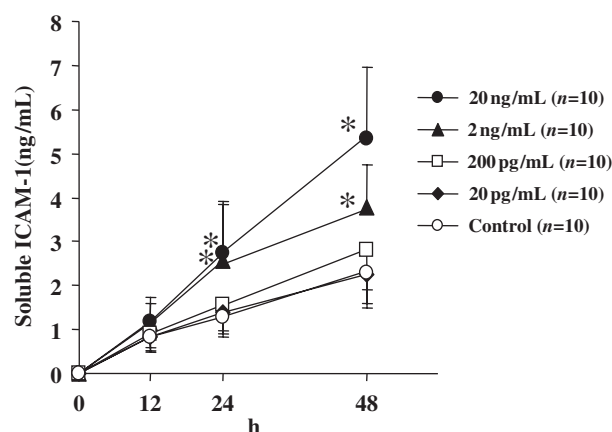


Fig. 3. The time course and dose-response release of soluble intercellular adhesion molecule-1 (ICAM-1) by TNF- α from cultured human nasal epithelial cells. Paired human primary nasal epithelial cell cultures were treated with either 0.02, 0.2, 2, or 20 ng/mL of recombinant human TNF- α or control medium for 12, 24, or 48 h and the concentration of soluble ICAM-1 was measured by a commercial ELISA kit. TNF- α stimulation resulted in the increase in soluble ICAM-1 concentration. Significant difference from unstimulated cells (O) is indicated, * $P < 0.05$.

5.52 ± 1.64 ng/mL, TNF- α plus sTNF-RI 3.30 ± 0.92 ng/mL, $P = 0.008$, $n = 10$) at 48 h. sTNF-RI alone had no significant effect on ICAM-1 mRNA (control 1.03 ± 0.42 , TNF-RI alone 0.93 ± 0.28 , $P = 0.38$, $n = 10$) nor on sICAM-1 concentration (control 2.27 ± 0.67 ng/mL, TNF-RI alone 1.92 ± 0.58 ng/mL, $P = 0.24$, $n = 10$).

Effect of DEX on TNF- α -induced ICAM-1 mRNA expression and sICAM-1 concentration

In a preliminary study, we evaluated the effect of DEX (from 10^{-8} to 10^{-6} M) on TNF- α -induced up-regulation of ICAM-1 at mRNA level. Based on the experiment, the maximum suppression of ICAM-1 mRNA by DEX was obtained at 10^{-6} M (data not shown). Results from the effect of DEX (10^{-6} M) on TNF- α -induced up-regulation of ICAM-1 expression are shown in Figs 4–6. DEX significantly attenuated the ICAM-1 mRNA expression (TNF- α alone 3.56 ± 1.38 , TNF- α plus DEX 1.53 ± 0.58 , $P = 0.001$, $n = 10$) and sICAM-1 concentration (TNF- α alone 5.53 ± 1.64 ng/mL, TNF- α plus DEX 2.98 ± 0.93 , $P = 0.005$, $n = 10$) at 48 h, whereas DEX alone had no significant effect on ICAM-1 mRNA (control 1.02 ± 0.42 , DEX alone 0.95 ± 0.32 , $P = 0.73$, $n = 10$) nor on sICAM-1 level (control 2.27 ± 0.67 ng/mL, DEX alone 2.01 ± 0.60 ng/mL, $P = 0.68$, $n = 10$) (Figs 4 and 5).

Surface expression of ICAM-1

Surface expression of ICAM-1 was also studied by flow cytometry. As shown in Fig. 6, TNF- α increased surface expression of ICAM-1 (TNF- α alone 1.69 ± 0.52 , $P = 0.002$, $n = 10$) at 48 h, which was inhibited by DEX (TNF- α plus DEX 1.21 ± 0.24 , $P = 0.01$, $n = 10$) or sTNF-RI (TNF- α plus sTNF-RI 1.23 ± 0.14 , $P = 0.008$, $n = 10$). Similar to the level of ICAM-1 mRNA and sICAM-1, DEX alone or TNF-RI alone had no significant effect on surface expression of ICAM-1 (DEX alone 0.96 ± 0.20 , $P = 0.13$, $n = 10$, TNF-RI alone 1.14 ± 0.27 , $P = 0.38$, $n = 10$).

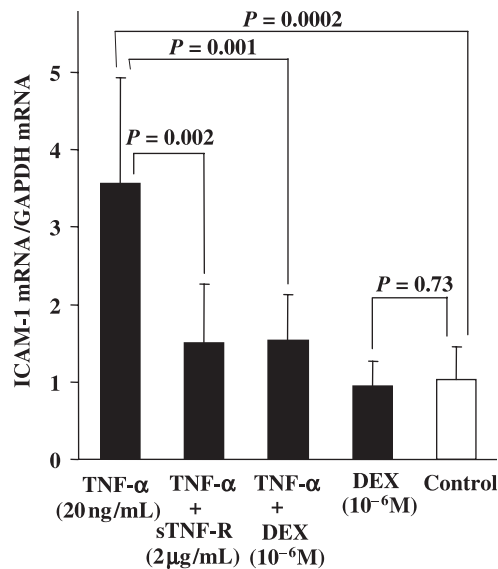


Fig. 4. Soluble TNF receptor I (sTNF-RI) or dexamethasone inhibited TNF- α -mediated intercellular adhesion molecule-1 (ICAM-1) mRNA expression in cultured human nasal epithelial cells. Primary cultures of human nasal epithelial cells were stimulated with recombinant human TNF- α (20 ng/mL) alone or recombinant human TNF- α (20 ng/mL) in combination with recombinant human sTNF-RI (2 μ g/mL) or dexamethasone (10^{-6} M) for 48 h, which represented the time of maximal TNF- α -mediated ICAM-1 mRNA expression. sTNF-RI and dexamethasone significantly inhibited the TNF- α -mediated increase in ICAM-1 mRNA. Dexamethasone alone (10^{-6} M) tended to decrease ICAM-1 mRNA expression, however, the difference was not statistically significant, $N = 10$.

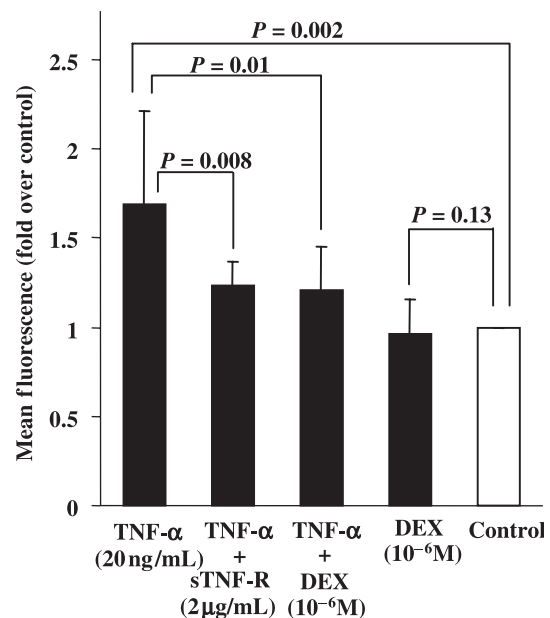


Fig. 6. Soluble TNF receptor I (sTNF-RI) or dexamethasone inhibited TNF- α -mediated surface intercellular adhesion molecule-1 (ICAM-1) (CD54) expression in cultured human nasal epithelial cells. Primary cultures of human nasal epithelial cells were stimulated with recombinant human TNF- α (20 ng/mL) alone or recombinant human TNF- α (20 ng/mL) in combination with recombinant human sTNF-RI (2 μ g/mL) or dexamethasone (10^{-6} M) for 48 h, which represented the time of maximal TNF- α -mediated ICAM-1 mRNA expression. sTNF-RI and dexamethasone significantly inhibited the TNF- α -mediated increase in surface ICAM-1 expression. Dexamethasone alone (10^{-6} M) had no significant effect on surface ICAM-1 expression, $N = 10$.

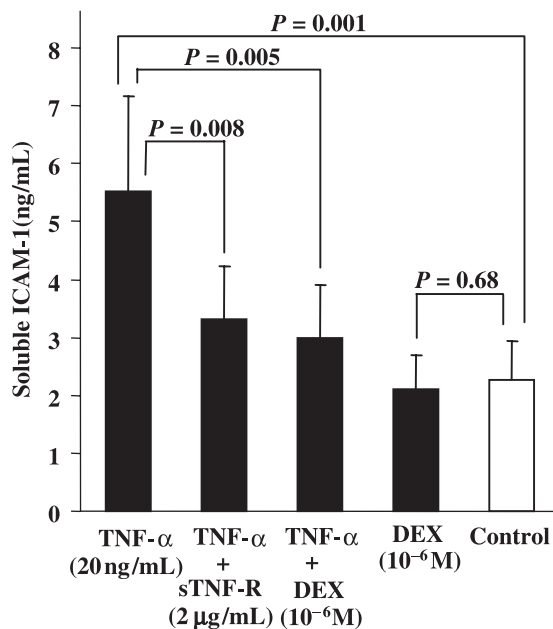


Fig. 5. Soluble TNF receptor I (sTNF-RI) or dexamethasone inhibited TNF- α -mediated soluble intercellular adhesion molecule-1 (ICAM-1) release from cultured human nasal epithelial cells. Primary cultures of human nasal epithelial cells were stimulated with recombinant human TNF- α (20 ng/mL) alone or recombinant human TNF- α (20 ng/mL) in combination with recombinant human sTNF-RI (2 μ g/mL) or dexamethasone (10^{-6} M) for 48 h, which represented the time of maximal TNF- α -mediated soluble ICAM-1 (sICAM-1) release. sTNF-RI and dexamethasone significantly inhibited the TNF- α -mediated increase in sICAM-1 concentration. Dexamethasone alone (10^{-6} M) tended to decrease sICAM-1 levels, however, the difference was not statistically significant. $N = 10$.

Discussion

Our study has shown that ICAM-1 expression of HNECs is up-regulated by TNF- α . Several mediators, including TNF- α [9–14], IL-1 β [14], and INF- γ [10, 11] have been reported to increase ICAM-1 expression *in vitro* in primary and raised human epithelial cells or cell lines. In our preliminary study, the strongest effects on ICAM-1 mRNA expression were observed by TNF- α stimulation, therefore we focused on the effect of TNF- α on ICAM-1 expression. In previous studies, the effects of TNF- α on airway epithelial ICAM-1 expression were confirmed by flow cytometry on human primary cultured tracheal [14] or bronchial [9, 11, 13] epithelial cells and on human bronchial epithelial cell lines BEAS-2B and NCI-H292 [11], by ELISA on a human alveolar epithelial cell line A549 [12], and by high-resolution electron microscopy on the human bronchial epithelial cell lines 16HBE and BEAS-2B [10]. In addition, at the mRNA level, TNF- α -induced ICAM-1 mRNA expression was confirmed in primary cultured human bronchial epithelial cells by conventional RT-PCR [9]. However, we are unable to find any other reports using real-time PCR for the analysis of ICAM-1 expression in epithelial cells. Our finding suggests that real-time PCR may be useful to analyse epithelial ICAM-1 expression, because the results from real-time PCR were almost similar to those from flow cytometry.

We showed that the soluble form of type I TNF receptor (sTNF-RI) was capable of inhibiting these TNF- α -mediated ICAM-1 mRNA expression. It is known that TNF- α exerts many of its biologic effects on cells by binding to one of two

different transmembrane receptors, TNF-RI (TNFR-B, 55 kDa) or TNF-RII (TNFR-A, 75 kDa) [18]. Previous reports showed the expression of TNF-RI on human bronchial and nasal epithelial cells [19, 20]. Binding of TNF to sTNF-RI may inactivate TNF by the formation of high affinity complexes that reduce the binding of TNF to cell membrane receptors. We suggest the hypothesis that TNF- α -mediated ICAM-1 expression in HNECs involves activation of TNF-RI.

The present study confirms a significant effect of DEX on nasal epithelial ICAM-1 expression, which is regulated predominantly at transcriptional level. It has been reported that glucocorticosteroids repression of ICAM-1 expression occurs via the nuclear factor- κ B (NF- κ B)-binding site [21]. TNF- α -induced transcriptional activation of ICAM-1 gene in human monocytic cell line U937 [22] and in human alveolar epithelial cell line A549 [23] is mainly regulated by the NF- κ B signalling pathway in the human ICAM-1 gene promoter. Therefore, it is possible that the steroid effect on ICAM-1 expression in our study involves inhibition of the NF- κ B signalling pathway. Numerous previous studies have shown that glucocorticosteroids effects vary depending on cell type, stimulus, and species, and the effects of glucocorticosteroids on airway epithelial ICAM-1 expression have been conflicting among studies using different cell types. In BEAS-2B bronchial epithelial cells, fluticasone propionate and beclomethasone dipropionate did not inhibit TNF- α -induced ICAM-1 and ICAM-1 mRNA expression [24]. In contrast, DEX was found to strongly suppress basal and TNF- α -induced ICAM-1 expression in the NCI-H292 human bronchial cell line [25]. In primary cultured human bronchial epithelial cells, fluticasone propionate, beclomethasone dipropionate, and hydrocortisone significantly attenuated TNF- α -induced release of sICAM-1 [26]. However, little information is available on the effects of glucocorticosteroids on nasal epithelial ICAM-1. *In vivo*, topical treatment with intranasal fluticasone propionate in seasonal allergic rhinitis patients decreases ICAM-1 expression on nasal epithelial cells [16]. Clinically, topical steroids have been accepted as a highly effective therapy for allergic rhinitis. Our finding suggests a potential role for topical steroids in allergic rhinitis in suppressing inflammatory reactions in the nasal mucosa by regulating ICAM-1 expression on nasal epithelium.

In the present study, we measured the level of sICAM-1 in HNECs culture medium. In a variety of inflammatory and immune disorders, it has been demonstrated that levels of sICAM-1 in body fluids correlate with the intensity of the clinical condition. A soluble form of ICAM-1 (sICAM-1) has been shown to be increased in the serum and nasal lavage fluid from patients with pollinosis under natural allergen provocation [27] and has also been detected in nasal lavage fluid during experimental rhinovirus infection [28]. In human bronchial epithelial cells, TNF- α , IL-1 α , and INF- γ increased sICAM-1 release were associated with up-regulation of ICAM-1 cell surface expression [29]. The molecular weight of sICAM-1 in human serum has been found to be similar to recombinant sICAM-1 (comprising the extracellular part of ICAM-1), and lower than that of the membrane-bound form [30], suggesting that the circulating form of ICAM-1 consists mostly of the extracellular part of ICAM-1. The origin of sICAM-1 in the nasal lavage fluid cannot be clearly identified,

however, our present results suggest that it is most likely split off from the membrane of nasal epithelial cells.

In this study, TNF- α (20 ng/mL) increased sICAM-1 concentration to 2.4 times basal level and ICAM-1 mRNA level to 3.5 times, 48 h after stimulation, suggesting TNF- α enhanced both ICAM-1 synthesis and cleavage of sICAM-1 from the cell surface. By contrast, TNF- α increased membrane-bound ICAM-1 protein only by 70% by flow cytometric analysis, suggesting that cleavage of sICAM-1 results in loss of surface ICAM-1 expression. To clarify the precise mechanisms of soluble- and membrane-bound ICAM-1 production, further studies will be needed, for example using cyclohexamide and the inhibitor of NF- κ B. On the other hand, the function of sICAM-1 has been studied using recombinant sICAM-1. Several studies showed that sICAM-1 can bind to cell surface β 2 integrins LFA-1 and Mac-1, reducing adherence of leucocytes to endothelial cells and the accompanying intracellular signalling responses [1], while binding of sICAM-1 to cells can also lead directly to their activation [31]. However, the biological significance of increased sICAM-1 levels in nasal lavage fluid is not fully understood.

In summary, we report that TNF- α increases the level of ICAM-1 expression on HNECs. In addition, the TNF- α -mediated ICAM-1 expression can be attenuated by DEX. These data suggest that topical steroids might act directly on the nasal epithelial synthesis of ICAM-1.

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