Allergology International (1996) 45: 97–103

Original Article

Expression of ICAM-1 on human bronchial epithelial cells after influenza virus infection

Satoshi Matsukura, Fumio Kokubu, Hiromichi Noda, Hiroyuki Watanabe, Kunihiko Fukuchi, Kunio Gomi and Mitsuru Adachi

First Department of Internal Medicine and Clinical Pathology, Showa University School of Medicine, Shinagawa, Tokyo, Japan

ABSTRACT

Damage of bronchial epithelium is a feature of airway viral infection and airway inflammatory disease, such as bronchial asthma. Adhesion molecules, which are expressed on bronchial epithelium, play an important role in the pathogenesis of epithelial damage and airway inflammation. We analysed ICAM-1 and VCAM-1 expression on human bronchial epithelial cell line, NCI-H292, after influenza virus A infection. ICAM-1 was expressed on control cells constitutively. Influenza virus A infection caused a three-fold increase in ICAM-1 expression on NCI-H292 cells. Supernatant of virus-infected cells was analysed for the concentration of IL-1β and TNF-α but these cytokines were not detected. VCAM-1 was not expressed on control cells and did not change after cytokine stimulation or virus infection. These findings suggest that influenza virus infection may induce ICAM-1 expression on bronchial epithelium without intervention of leukocytes, and ICAM-1 expressed on epithelium plays a major part in the pathophysiology of airway inflammatory disease caused by viral infection.

Key words: adhesion molecule, airway inflammation, bronchial asthma, bronchial epithelial cells, influenza virus, ICAM-1

INTRODUCTION

Recent reports indicate that adhesion molecules play an important role in the pathogenesis of airway inflammatory diseases, such as asthma. The interaction between leukocytes and adhesion molecules expressed on endothelial cells has been clearly defined.[1,2] Adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin, were expressed on bronchial microvessels and expression of these adhesion molecules was increased in asthmatics.[3–6] Wegener *et al.* demonstrated ICAM-1 expression on epithelium of a primate model of airway inflammation caused by chronic antigen challengen.[7] Recently, ICAM-1 expression on bronchial epithelial cells has been reported.[8] It is thought that ICAM-1 expressed on bronchial epithelial cells may facilitate adhesion between inflammatory cells and bronchial epithelial cells. Inflammatory cells recruit and consequently damage the epithelium in the process of airway inflammation. Therefore adhesion molecules expressed on bronchial epithelium are important in the pathogenesis of airway inflammation. Investigators have reported that cytokines, such as interleukin-1 beta (IL-1β), tumour necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) increased ICAM-1 expression on bronchial epithelial cells and augmented leukocyte adhesion to epithelial cells.[9–11] In the airway, the major source of these cytokines are thought to be macrophages.

Influenza virus is a major pathogen of airway viral infection and causes inflammation and epithelial damage.[12–15] Adhesion molecules expressed on epithelium may contribute to pathogenesis of airway inflammatory disease caused by viral infection. In viral infections of the airway, cytokines, which are derived from macrophages, possibly stimulate ICAM-1 expression on epithelium. But ICAM-1 may be an important initiator of inflammatory cell accumulation, therefore we hypothesized that increased ICAM-1 expression on bronchial epithelium is needed for the process of airway inflammation before recruitment of inflammatory cells. If this is correct then increased ICAM-1 expression needs other stimulator, which is different from leukocyte-derived cytokines, in the initial stage of viral airway inflammation. We thought that influenza virus may initiate up-regulation of ICAM-1 expression on epithelium cells, and examined ICAM-1 expression on human bronchial epithelial cells infected with influenza A virus.

Correspondence: Dr Satoshi Matsukura, First Department of Internal Medicine, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan.

Received 20 September 1995. Accepted for publication 28 March 1996.

MATERIALS AND METHODS

Cell cultures

The bronchial epithelial cell line NCI-H292 is derived from human lung mucoepidermoid carcinoma (American Type Culture Collection, ATCC). NCI-H292 was cultured in RPMI 1640 (Gibco, Grand Island, NY, USA) containing 10% heat-inactivated foetal bovine serum (FBS; Gibco), 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco). Cultures were incubated at 37°C in 5% CO_2.

MDCK cells were cultured for influenza virus propagation. This cell line is derived from a kidney of a canine (ATCC). MDCK cells were grown and maintained in Eagles Minimal Essential Media (MEM; Gibco) containing 2% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin. Stock cultures of NCI-H292 and MDCK cells were frozen at −80°C.

Virus stock

Influenza A virus/Sisen/2/92 (a kind gift from Dr M. Toda, Showa University, Tokyo, Japan) was grown in MDCK cells in MEM containing 2% FBS at 33.5°C in 5% CO_2. Infected culture fluid was centrifuged at 20 × *g* for 5 min to give a clear supernatant (containing virus particles) which was stored in aliquots at −80°C.

Vitus titration

MDCK cells were grown in six-well culture plates (Falcon, Becton Dickinson, Lincoln Park, NJ, USA) to confluence. Serial 10-fold dilutions of virus stock were prepared in MEM supplemented with 100 U/mL penicillin, 100 μg/mL of streptomycin and 2 μg/mL trypsin (Gibco). The supernatant of each culture plate was removed and 100 μL of each dilution was added to the plates which were rocked every 10 min to facilitate adsorption of virus to the cells. After 1 h, supernatant was removed and replaced with 2 mL of MEM containing 1% agarose, 1% DEAE dextran and 4 μg/mL trypsin. Cultures were incubated for 3–4 days at 33.5°C in 5% CO_2. Cells were fixed with 10% formalin and stained with methylene blue, and plaques on each plate were counted and the number of plaque forming units (PFU) determined.

Infection of cell culture with influenza A virus

NCI-H292 cells were grown to confluence in sterile tissue culture plates (3.5 diameter). Influenza A virus (H3N2) was added to the plates (100 μL of 1×10^6/mL PFU) and allowed to adsorb to cells for 1 h with gentle rocking of the plates every 10 min. After 1 h, virus was removed, 2 mL of fresh medium RPMI 1640 (Gibco) with 2% of FBS, 100 U/mL penicillin and 100 μg/mL streptomycin, was added and infected cells were grown at 37°C in 5% CO_2. Culture medium containing cells was collected 6, 24, 48 and 72 h after infection.

Stimulation of the epithelial cells by TNF-α

NCI-H292 cells were grown to confluence in 3.5 cm diameter culture plates and incubated in RPMI 1640 medium with 2% of FBS, 100 U/mL penicillin and 100 μg/mL streptomycin. As a positive control of ICAM-1 expression, 100 U/mL of TNF-α (R&D systems, Minneapolis, MN, USA) was added to the medium. After 6, 24, 48 and 72 h, the culture of supernatant and cells were collected.

Flow cytometry analysis of expression of adhesion molecules

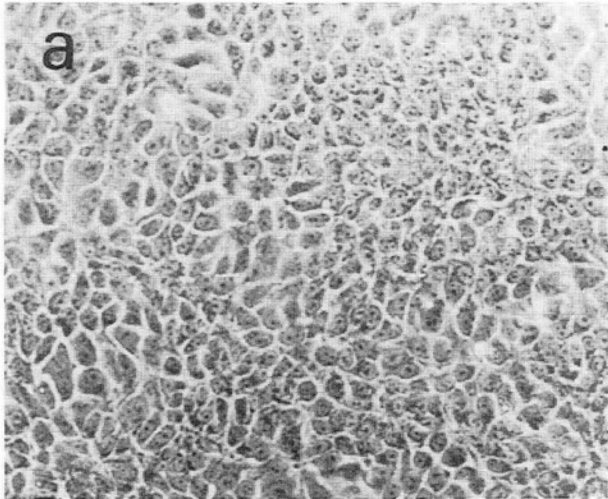
NCI-H292 cells were harvested following treatment with trypsin-EDTA. They were suspended in PBS containing 2% BSA and 0.02% sodium azide, and 10^6 cells were incubated with mAb to ICAM-1 or VCAM-1 (Seikagaku Corporation, Tokyo, Japan) on ice for 30 min. After three washes in PBS, cells were incubated with fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Gam-FITC, Seikagaku) on ice for 30 min. After another three washes in PBS, cells were resuspended in PBS and 10^4 cells were analysed for fluorescence intensity by flow cytometry (FACScan, Becton Dickinson). Mean ICAM-1-specific fluorescence was determined by subtracting the mean background fluorescence (negative control, Gam-FITC) from that measured with the mAb to ICAM-1. In preliminary experiments, trypsin did not alter the fluorescence of surface ICAM-1.

Assay of cytokine concentration in culture medium

IL-1β and TNF-α concentration in medium collected at 6, 24, 48 and 72 h after infection was analysed using a commercially available enzyme-linked immunosorbent assay (ELISA) system (Amersham International, UK). Briefly, samples and horseradish-peroxidase-conjugated anti-cytokine antibody were added to the 96-wells of a microtitre plate coated with anti-cytokine antibody. After washing with PBS, substrate was added and absorbance was measured at 450 nm. A standard curve was generated by plotting the logarithm of optical density versus logarithm of the concentration standards, and from this the concentration of each sample was determined.

Expression of ICAM-1mRNA

Control cells, TNF-α-cells stimulated cells and cells infected with influenza A virus were harvested using a scraper 24 h after cytokine stimulation or virus infection. Total RNA was extracted from samples of 2×10^6 cells using guanidium thiocyanate. First strand cDNA was synthesized by incubating 2 μg of RNA in the presence of Moloney-Murine Leukemia Virus reverse transcriptase (Pharmacia, USA), random primers and reaction buffer at 37°C for 1 h. After cDNA denaturation at 94°C for 5 min, reverse transcription polymerase chain reaction (RT-PCR) was used to amplify part of the ICAM-1 gene using 5′-and 3′-primers.[16] The sequence selected for amplification was the region between 288–1059bp.



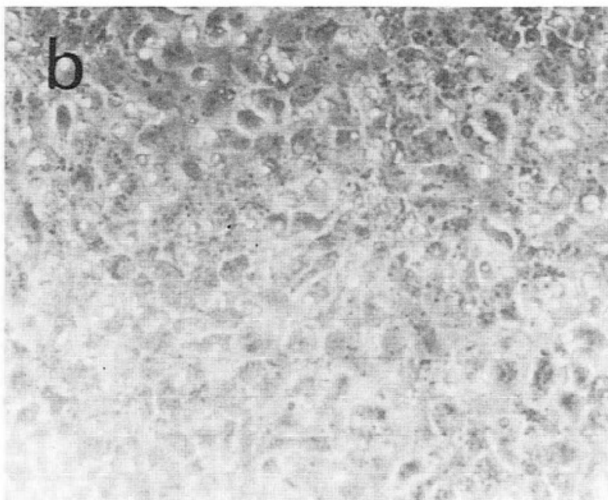


Fig. 1 Cytopathology of influenza virus in bronchial epithelial cells. Human bronchial epithelial cells, NCI-H292, were infected with influenza virus A(H3N2). Phase-contrast photomicrographs of cultured cells were obtained. (a) Non-infected control cells. (b) At 48–72 h after infection.

RT-PCR was performed using 5 pmol of 5'- ICAM-1 primer and 5 pmol of 3'-ICAM-1 primer, 5 μL cDNA, 0.6 U *Taq* polymerase (Boehringer-Mannheim, Germany), 2.5 μL PCR reaction buffer (Tris-HC1 100 mmol/L $MgCl_2$ 15 mmol/L KCl 500 mmol/L; Boehringer-Mannheim), and distilled water to a final volume of 25 μL. Amplification was performed for 30 cycles with denaturation at 94°C for 45 s, annealing at 61°C for 45 s, and extension at 72°C for 1 min (Perkin-Elmer Cetus, Norwalk, CT, USA). The β actin gene was amplified as mentioned above using 5'-β actin primer (5'-GTGGGGCGCCCCAGGCACCA-3') and 3'-β actin primer (5'-CTCCTTAATGTCACGCACGATTTC-3') for normalization. After incubation at 72°C for 10 min, amplified products were analysed by electrophoresis on 2% agarose and ethidium bromide staining, followed by visualization with an ultraviolet transilluminator.

Statistics

The paired *t*-test was used to evaluate statistical significance of the data in the analysis of mean fluorescence intensity and cytokine production.

Results

Infection of epithelial cells by influenza A virus

At 24 h after influenza virus inoculation, remarkable cytopathic change was not noted when compared with control cells. At 48–72 h after inoculation, cytopathic changes like rounding of cells and cellular fusion were seen in infected NCI-H292 cells (Fig. 1).

Expression of adhesion molecules on bronchial epithelial cells

Expression of adhesion molecules on cultured human bronchial epithelial cells, NCI-H292 cells, was analysed with flow cytometry. Histograms for 10 000 cells from a representative experiment are shown in Figure 2. ICAM-1 was constitutively expressed on NCI-H292 cells (mean fluorescence ±s.e.m., = 36.5 ± 8.2, n=4). VCAM-1 was not expressed on NCI-H292 cells (data not shown). ICAM-1 expression on NCI-H292 cells was increased by stimulation of TNF-α time-dependently and there was a two-fold increase in mean fluorescence at 48–72 h after stimulation (Fig. 3a), as has been shown previously.[17] There was a time-dependent increase in ICAM-1 expression after influenza A virus (H3N2) infection, and three-fold increase in mean fluorescence 72 h after infection (Fig. 3b). There was no change in VCAM-1 expression on NCI-H292 following TNF-α stimulation nor by influenza virus infection (data not shown).

Cytokines concentration in culture medium

The concentration of cytokines in the medium of cultured NCI-H292 cells was analysed using ELISA. IL-1 β and TNF-α were not detected in the culture medium of control cells or cells which were infected with influenza A virus (H3N2) at any time (data not shown).

Expression of ICAM-1 mRNA

ICAM-1 mRNA was detected by RT-PCR. The sample obtained from control epithelial cells revealed a faint cDNA band, whereas samples from cells harvested 24 h after TNF-α stimulation or influenza A virus infection, had a strong cDNA band compatible with the expected size of the product (772 bp). Every product of β actin 548 bp) from control cells, TNF-α-stimulated cells and infected cells was detected (Fig. 4).

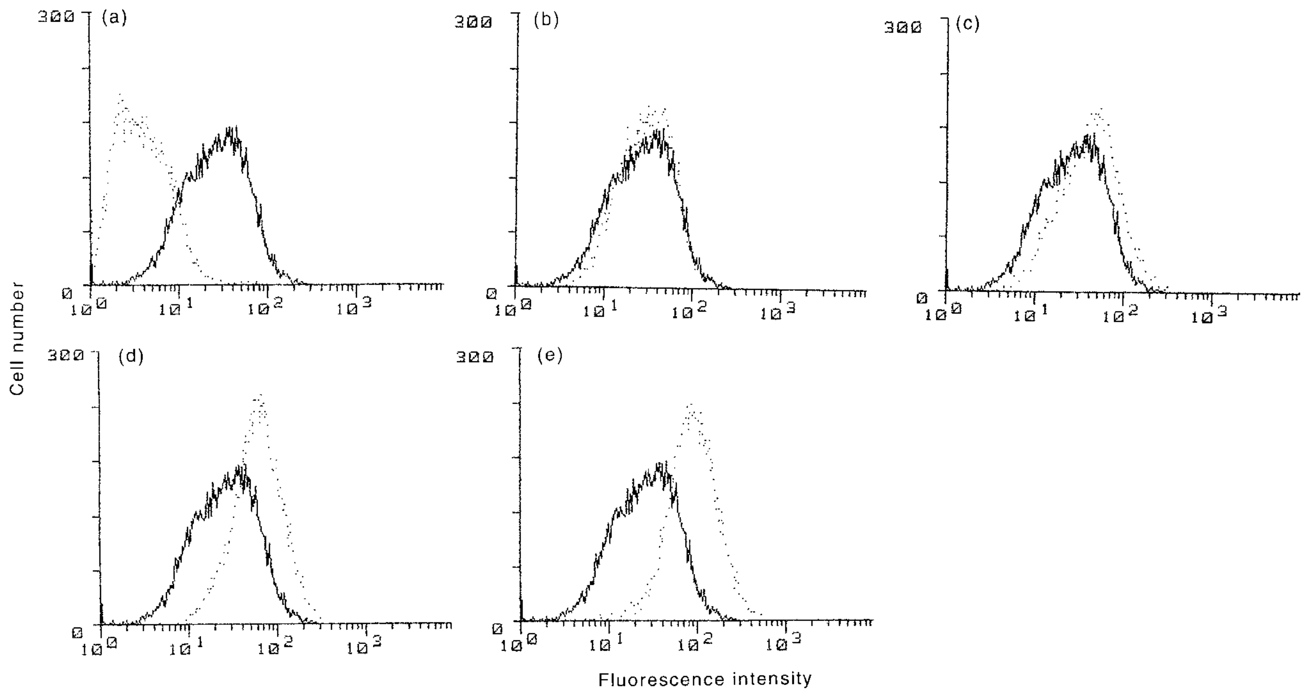


Fig. 2 Flow cytometry analysis of ICAM-1 expression on NCI-H292 cells. The thick line of the representative flow cytometry histogram for 10 000 cells indicates basal ICAM-1 expression on control cells. The thin line indicates (a) non-specific fluorescence intensity of control cells, (b) ICAM-1 expression on infected cells at 6 h, (c) at 24 h, (d) at 48 h, (e) at 72 h after influenza A virus infection.

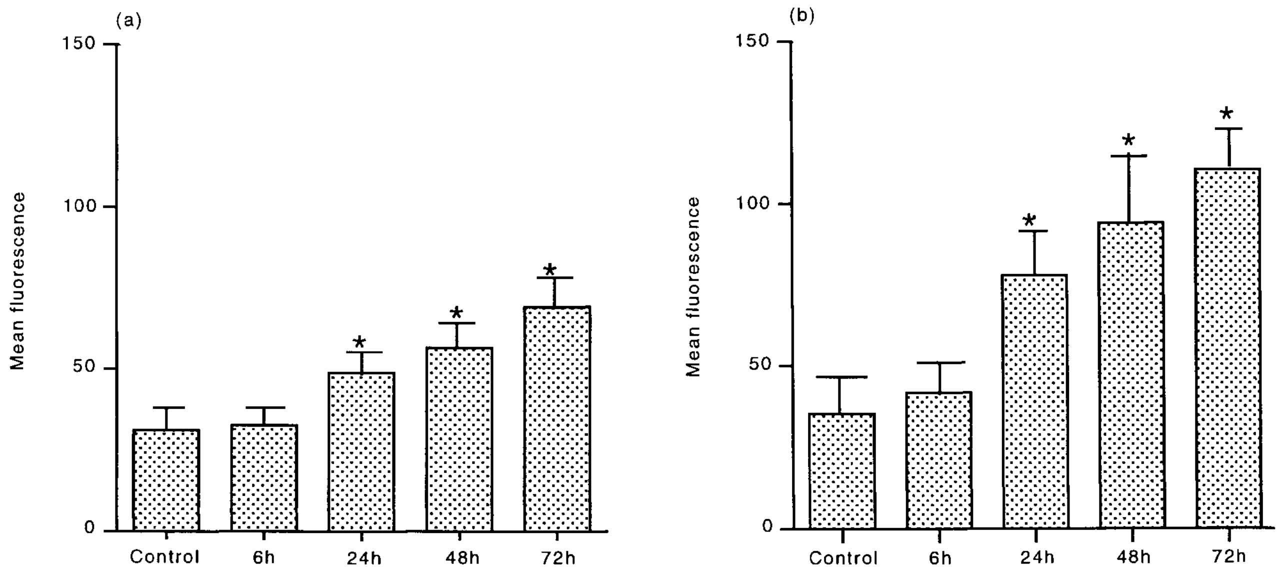


Fig. 3 Mean fluorescence of flow cytometry analysis. Time course of ICAM-1 expression on NCI-H292 cells which were (a) stimulated with 100 U/mL of TNF-α, and (b) infected with influenza virus A. n=4. *$P<0.05$.

Discussion

NCI-H292 is an established line of mucoepidermoid carcinoma cells derived from the human lung.[18,19] It is reported that NCI-H292 cells are available for cultivation of paramyxoviruses and may be sensitive to other viruses including influenza virus.[20] On the other hand, NCI-H292 shares some characteristics of adhesion molecule expression and cytokine production with normal bronchial epithelial cells. We have examined the production of IL-6 and IL-8 by NCI-H292 cells and found that it was increased by IL-1β or TNF-α (data not shown). In this report, we demonstrated that TNF-α stimulated ICAM-1 expression on NCI-H292

cells, and the data are similar to that obtained for normal bronchial epithelial cells.[9,24,25] Therefore, we used NCI-H292 as a model of bronchial epithelium in airway inflammation.

At 48–72 h after virus inoculation, cytopathic effects, such as rounding of cells and cellular fusion, were observed in the present study. These pathologic changes indicate the damage to bronchial epithelial cells following virus infection.[12]

It has recently been suggested that cell adhesion molecules potentially mediate leukocyte migration, leukocyte activation, and leukocyte–airway constitutive cell interactions in inflammatory airway diseases such as asthma. Wegener *et al.* demonstrated enhanced ICAM-1 expression on bronchial microvessels and epithelium in a primate model of chronic airway inflammation, and showed an amelioration of inflammatory response to antigen exposure after administration of blocking antibodies against ICAM-1.[7] Recent evidence has also demonstrated that soluble ICAM-1 and soluble E-selectin were increased in the bronchoalveolar lavage fluid after antigen challenge.[5] Increased serum levels of soluble ICAM-1 and soluble E-selectin were detected in asthmatic patients.[6] ICAM-1, E-selectin and VCAM-1 were expressed in bronchial tissue from asthmatics and an increase in inflammatory cells, e.g. eosinophils, mast cells and lymphocytes, was associated with increased expression of ICAM-1 and E-selectin.[4] It has been documented that IL-1 β, TNF-α and IFN-γ induced up-regulation of ICAM-1 expression on human bronchial epithelial cells and increased leukocyte adhesion to epithelial cells.[9–11] These mediators may be important in the pathogenesis of inflammatory airway disease such as bronchial asthma or virus infection.

We hypothesized that virus infection of the airways may cause an increase in ICAM-1 expression on bronchial epithelium cells and this increase may possibly contribute to epithelial damage and airway inflammation. Viral infection causes bronchial inflammation and exacerbates bronchial asthma because of increased hyper-reactivity of the bronchial wall.[14,15,21] Of these viral infections, influenza virus type A (H3N2) is common. It is a major cause of infection-induced asthma in adults. Investigators have documented several reports about the mechanism of virus-induced asthma, but the process by which inflammatory cells infiltrate the bronchial mucosa remains undefined. It has been reported that viruses such as respiratory syncytial virus, parainfluenza virus and adenovirus induce leukocyte adhesion and up-regulation of ICAM-1 expression on epithelial cells.[22,23,26,27] The report documents that leukocyte adherence to MDCK cells was increased by influenza virus injection.[28] But within our knowledge, it has not been clarified that influenza virus infection increases ICAM-1 expression on human bronchial epithelial cells. In our study, we demonstrated, using flow cytometry, an increase in ICAM-1 expression on bronchial epithelial cells, when they were infected with influenza A virus. Mean fluorescence of ICAM-1 expression increased time-dependently and this data was consistent with an increase in ICAM-1 PCR product from the cells 24 h after infection compared with the product from control cells. Because of the importance of influenza virus in airway infection, ICAM-1 up-regulation induced by the virus is useful to understand the mechanism of airway inflammatory disease induced by influenza.

Adhesion molecules which are expressed on bronchial epithelium play an important role in the process of airway inflammation caused by viral infection. Because the bronchial epithelial cell is a primary site, an increase in ICAM-1 expression by influenza virus may be significant in the initial inflammatory response. ICAM-1 expression on bronchial epithelium may induce leukocyte–epithelial cell interaction and epithelial damage. We have already documented that IL-1β, TNF-α and IFN-γ stimulated ICAM-1 expression on human bronchial epithelial cells and NCI-H292 cells.[17] These results are similar to past reports documenting human primary bronchial epithelial cells.

We demonstrated that influenza virus infection induced ICAM-1mRNA up-regulation on bronchial epithelial cells without leukocyte intervention. In our result, IL-1β and TNF-α were not detected in the supernatant of infected cells, therefore it is possible that influenza virus infection directly induced ICAM-1 expression on epithelial cells without intervention of cytokines. Recent reports have indicated that wild-type adenovirus infection augmented ICAM-1 expression on human bronchial epithelial cells,

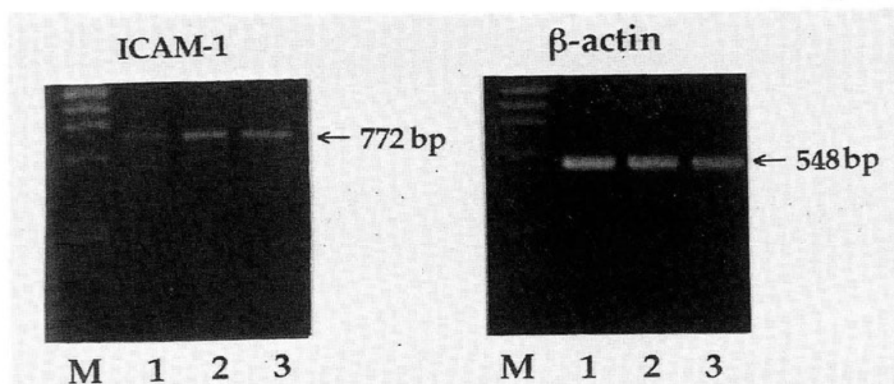


Fig. 4 Amplified DNA products from NCI-H292 cells. Cells were stimulated by 100 U/mL of TNF-α or infected with influenza virus A. At 24 h after stimulation or infection, epithelial cells were harvested and mRNA of ICAM-1 and β-actin was assayed by RT-PCR. Amplified products appeared as a single band of appropriate size. M, size marker . 1, control; 2, stimulated by TNF-α; 3, infected with influenza virus A. Representative of three experiments.

but recombinant adenovirus did not change ICAM-1 expression. Considering this report, increased ICAM-1 expression on epithelial cells are relative to cell response to virus replication or cell cytotoxicity Phal *et al.* have documented that influenza virus haemagglutinin activated NF-κB,[29] a transcription factor of the ICAM-1 gene [30] Therefore, it is possible that influenza virus infection increased ICAM-1 expression on NCI-H292 cells by activating NF-κB. But there is also the possibility that other cytokines or mediators contributed to ICAM-1 expression, and the mechanism of ICAM-1 regulation is complicated, therefore further investigation is required

In conclusion, we have demonstrated that influenza virus infection increased ICAM-1 expression on bronchial epithelial cells and its mechanism may be a direct effect of the influenza virus It has been documented that ICAM-1 expression contributed to the pathogenesis of atopic asthma But airway ICAM-1 expression induced by viral infection *in vivo* has not been fully investigated We hypothesized that ICAM-1 expression on epithelium may similarly contribute to airway inflammatory disease such as nonatopic asthma. But further studies are needed to understand the regulation and function of adhesion molecules in the airway

Acknowledgments

We thank K Kobayashi, M Iwata, H Tsujiyama and M Toda for helpful discussions and A. Yasuda, M. Okamoto, and H. Takeuchi for skilled technical assistance. This study was supported by the Pollution-related Health Damage Compensation and Prevention Association

References

1 Pilewski JK, Albelda SM Adhesion molecules in the lung *Am Rev Respir Dis* 1993, **148** (suppl) S31–7
2 Gundel RH, Wegner CD, Torcellini CA *et al* Endothelial leukocyte adhesion molecule-1 mediates antigen-induced acute airway inflammation and late-phase airway obstruction in monkeys *J Clin Invest* 1991, **88** 1407–11
3 Montefort S, Gratziou C, Goulding D *et al* Bronchial biopsy evidence for leukocyte infiltration and upregulation of leukocyte-endothelial cell adhesion molecules 6 hours after local antigen challenge of sensitized asthmatic airways *J Clin Invest* 1994, **93** 1411-21
4 Ohkawara Y, Yamauchi K, Maruyama N *et al* *In situ* expression of the cell adhesion molecules in bronchial tissues from asthmatics with air flow limitation *In vivo* evidence of VCAM-1/VLA-4 interaction in selective eosinophil infiltration *Am J Respir Cell Mol Biol* 1995, **12** 4–12
5 Takahashi N, Liu MC, Proud D, Yu XY, Hasegawa S, Spannhake EW Soluble intercellular adhesion molecule-1 in bronchoalveolar lavage fluid of allergic subjects following segmental antigen challenge *Am J Respir Crit Care Med* 1994, **150** 704–9
6 Montefort S, Lai CKW, Kapahi P *et al* Circulating adhesion molecules in asthma *Am J Respir Crit Care Med* 1994, **149** 1149–52
7 Wegener CD, Gundel RH, Reilly P, Haynes N, Letts LG, Rothlein R Expression and modulation of adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma *Science* 1990, **247** 456–9
8 Mette SA, Pilewski J, Buck CA, Albelda SM Distribution of integrin cell adhesion receptors on normal bronchial epithelial cells and lung cancer cells in vitro and in vivo *Am J Respir Cell Mol Biol* 1993, **8** 562–72
9 Bloemen PGM, Tweel MC, Henricks PAJ *et al* Expression and modulation of adhesion molecules on human bronchial cells *Am J Respir Cell Mol Biol* 1993, **9** 586–93
10 Look DC, Rapp SR, Keller BT, Holtzman MJ Selective induction of intercellular adhesion molecule-1 by interferon-γ in human airway epithelial cells *Am J Physiol* 1992, **263** L79–L87
11 Tosi MF, Stark JM, Smith CW, Hamedani A, Gruenert DC, Infeld MD Induction of ICAM-1 expression on human airway epithelial cells by inflammatory cytokines Effect of neutrophil-epithelial cell adhesion *Am J Respir Cell Mol Biol* 1992, **7** 214–21
12 Walsh JJ, Dietlein LF, Low FN, Burch GE, Mogabgab WJ Bronchotracheal response in human influenza *Arch Int Med* 1960, **108** 376–88
13 Minor TE, Dick EC, Baker JW, Ouellette JJ, Cohen M, Reed CE Rhinovirus and influenza type A infections as precipitants of asthma *Am Rev Respir Dis* 1976, **120** 149–53
14 Weiss ST, Tager IB, Munoz A, Speizer FE The relationship of respiratory infections in early childhood to the occurrence of increased levels of bronchial responsiveness and atopy *Am Rev Respir Dis* 1985, **131** 573–8
15 Welliver RC Upper respiratory infections in asthma *J Allergy Clin Immonol* 1983, **72** 341–6
16 Stauton DE, Marlin SD, Starotowa C, Dustin ML, Springer TA Primary structure of ICAM-1 demonstrates interaction between members of the immunoglobulin and integrin supergene families *Cell* 1988, **52** 925–33
17 Yasuda M, Kokubu F, Izumi H *et al* Effect of dexamethasone on intercellular adhesion mollecule-1 expression on cultured bronchial epithelial cells stimulated by inflammatory cytokines (in Japanese) *Jap J Allergol* 1995, **44** 100–3
18 Banks-Schlegel SP, Gazdar AF, Harris CC Intermediate filament and cross-linked envelope expression in human lung *Cancer Res* 1985, **45** 1187–97
19 Carney DN, Gazdar AF, Bepler G, Guccion JG, Marangos PJ, Moody TW Establishment and identification of small cell lung cancer cell lines having classic variant features *Cancer Res* 1985, **45** 2913–23
20 Castells E, Velma GG, Hierholzer JC NCI-H292 as an alternative cell line for the isolation and propagation of the human paramyxoviruses *Arch Virol* 1990, **115** 277–88
21 Empey DW, Laitinen LA, Jacobs L, Gold WM, Nadel JA Mechanisms of bronchial hyperreactivity in normal subjects following upper respiratory tract infection *Am Rev Respir Dis* 1976, **113** 131–9
22 Tosi MF, Stark JM, Hamedani A, Smith CW, Gruenert DC, Huang YT Intercellular adhesion molecule-1 (ICAM-1)-dependent and ICAM-1 independent adhesive interactions between polymorphonuclear leukocytes and human airway epithelial cells infected with parainfluenza virus type 2 *J Immunology* 1992, **149** 3345–9
23 Faden H, Hong JJ, Ogra PL Interaction of polymorphonuclear leukocytes and viruses in humans Adherence of polymorphonuclear leukocytes to respiratory syncytial virus-infected cells *J Virol* 1984, **52** 16–23
24 Takizawa H, Ohtoshi T, Ohta K *et al* Interleukin 6/B Cell Stimulatory factor-II is expressed and released by normal and transformed human bronchial epithelial cells *Biochem Biophys Res Commun* 1992, **187** 596–602

25 Nakamura H, Yoshimura K, Jaffe HA, Crystal RG. Interleukin-8 gene expression in human bronchial epithelial cells. *J. Biol. Chem.* 1991; **266**: 19 611–17.

26 Okamoto Y, Kudo K, Ishikawa K. Presence of respiratory syncytial virus genomic sequences in middle ear fluid and its relationship to expression of cytokines and cell adhesion molecules. *J. Infect. Dis.* 1993; **168**: 1277–81.

27 Pilewski JM, Sott DJ, Wilson JM, Albelda SM. ICAM-1 expression on bronchial epithelium after recombinant adenovirus infection. *Am. J. Respir. Cell. Biol.* 1995; **12**: 142–8.

28 Ratcliffe DR, Nolin SL, Cramer EB. Neutrophil interaction with influenza-infected epithelial cells. *Blood* 1988; **72**: 142–9.

29 Phal HL, Baeuerle PA. Expression of influenza virus hemagglutinin activates transcription factor NF-kappa *B. J. Virol.* 1995; **69**: 1480–4.

30 Baeuerle PA, Henkel T. Function and activation of NF-κB in the immune system. *Annu. Rev. Immunol.* 1994; **12**: 141–79.