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Adhesion molecule expression on epithelial cells infected with respiratory syncytial virus

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Adhesion molecule expression on epithelial cells infected with respiratory syncytial virus. S-Z. Wang, P.G. Hallsworth, K.D. Dowling, J.H. Alpers, J.J. Bowden, K.D. Forsyth. © ERS Journals Ltd 2000.

ABSTRACT: Respiratory epithelium is both a target and an effector of airway inflammation. Adhesion molecules on epithelium play an important role in a variety of airway diseases. Respiratory syncytial virus (RSV) is the most important pathogen for airway diseases in infants. The expression of adhesion molecules on epithelium in RSV infection, however, is unclear.

The expression of selected adhesion molecules and major histocompatibility complex (MHC) class I and II antigens on a human alveolar type II epithelial cell line (A549) infected with RSV was investigated by means of flow cytometry and immunocytochemistry.

The results showed that intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) were expressed on A549 cells at a low level. E-cadherin and MHC class I antigen were constitutively expressed on the cells. RSV infection of A549 cells significantly upregulated the expression of ICAM-1, VCAM-1 and MHC class I and II antigens on these cells. RSV infection also altered the expression of E-cadherin on A549 cells. Immunostaining showed that E-cadherin was mainly upregulated around or in RSV-induced giant cells.

These data suggest that respiratory syncytial virus infection of respiratory epithelial cells enhances the expression of adhesion molecules and major histocompatiblity complex antigens. These changes may play an important role in the pathophysiology of respiratory syncytial virus disease.

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The respiratory epithelium is not only a physical barrier between environmental noxious agents and the internal body milieu but also a metabolically active physiochemical structure [1]. Respiratory epithelial cells are both "target" and "effector" cells in airway inflammation [2]. As target cells, epithelial cells can be infected and damaged by various pathogens, such as respiratory syncytial virus (RSV) [3]. As effector cells, epithelial cells can express adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) [4–6], and can produce a wide array of cytokines [2, 7], and thereby be involved in inflammation and immune responses.

RSV is the most frequent cause of bronchiolitis and pneumonia in infants requiring hospitalization [8]. Moreover, ≥50% of infants who have acute viral bronchiolitis due to RSV have subsequent episodes of wheezing consistent with asthma [9] and it seems that there is a strong link between RSV bronchiolitis and asthma in epidemiology and immunology, reviewed by WANG and FORSYTH [10]. It has previously been reported that RSV infection can damage epithelial cells to a certain extent and neutrophils can augment the epithelial damage and detachment induced by RSV [3]. However, the pathophysiology of RSV disease, especially the role of respiratory epithelial cells in RSV infection, is unclear. Although the cytokine production profile of respiratory epithelial cells in RSV infection has been well studied [7, 11–14], the expression of adhesion molecules on epithelial cells in RSV infection is poorly understood [3, 5].

It has been demonstrated that both primary cultured bronchial epithelial cells and the cultured epithelial cell line BEAS-2B, constitutively express ICAM-1 and leukocyte function-associated molecule-3, but only ICAM-1, expressed on both cell types can be upregulated after stimulation [15]. Besides ICAM-1, human bronchial epithelial cells constitutively express major histocompatibility complex (MHC) class I antigen and other molecules [16]. Culture of BEAS-2B cells with tumour necrosis factor-β (TNF-α) and interleukin (IL)-1β was found to enhance ICAM-1 and induce *de novo* vascular cell adhesion molecule-1 (VCAM-1, CD106) expression [16].

Although it has been reported that RSV infection upregulates ICAM-1 expression on epithelial cells and ICAM-1 is the only known ligand on epithelial cells for neutrophil binding to A549 cells [5], it is unclear whether RSV infection could induce the upregulation of ICAM-1 on A549 cells in a dose- and time-dependent pattern. Moreover, VCAM-1 is a recently reported adhesion molecule expressed on airway epithelial cells [16], and the effect of RSV infection on VCAM-1 expression is unknown.

E-cadherin is heavily distributed on the basolateral domain of alveolar type II cells [17] and mediates epithelial cell/cell adhesion. However, there is no report of the expression of E-cadherin on respiratory epithelial cells in RSV infection. The authors speculate that RSV infection may upregulate the expression of E-cadherin on A549 cells. Such upregulation may be involved in the formation of giant cells in RSV infection.

As epithelial cells are likely to be "nonprofessional" antigen-presenting cells (APCs) [5, 18], the expression of MHC class I and II antigens on A549 cells in RSV infection is also of interest.

It was hypothesized that RSV infection may upregulate the expression of adhesion molecules on epithelial cells, which may contribute to the pathophysiology of RSV disease. Therefore, the aim of this study was to investigate the expression of adhesion molecules on respiratory epithelial (A549) cells infected with RSV. In addition to ICAM-1, VCAM-1, and E-cadherin, the expression of MHC class I and II antigens on A549 cells in RSV infection were also investigated.

Materials and methods

Epithelial cell culture

A549, an immortalized human alveolar type II epithelial cell line (American Type Culture Collection (ATCC), Rockville, MD, USA), was selected as the source of respiratory epithelial cells for these studies [5, 19], because of its standardized use as a respiratory epithelial cell line, and its low baseline expression of ICAM-1. A549 cells were cultured in Dulbecco′s modified Eagle′s medium (DMEM) plus 5% foetal calf serum (FCS). Each well (9.62 cm^2) of a six-well plate (Becton Dickinson Labware, Franklin Lakes, NJ, USA) was seeded with 0.75×10^6 cells and each chamber (1 cm^2) of an eight-chamber plate (Nunc Inc., Naperville, IL, USA) was seeded with 0.75×10^5 cells. After 24–36 h of culture in an atmosphere of 5% in a humidified incubator CO_2/air 37°C, the cells in the wells or chambers formed confluent monolayers, 1.5×10^6 cells·$well^{-1}$ and 1.5×10^5 cells·$chamber^{-1}$, respectively.

Study design

Two methodologies were utilized at the same time: flow cytometric analysis to quantify the intensity of expression of adhesion molecules; and immunocytostaining to visualize the expression of adhesion molecules on A549 cells [20]. Different doses of RSV (at multiplicities of infection (MOIs) of 0.01, 0.1 and 1.0) were used in the infection of epithelial cells in six-well plates for flow cytometric analysis. RSV at a MOI of 1.0 was used to infect the cells in eight-chamber slides for immunocytostaining. The effect of RSV was investigated at 6, 24 and 48 h of infection. Sterile culture medium was used as a negative control. Inactivated RSV (RSVi) was not employed as a control, as a previous study had shown that active RSV had a significantly stronger effect than RSVi on leukocyte/epithelial adhesion [3].

Antibodies

The following monoclonal antibodies (mAbs) were used in this study: anti-ICAM-1 (Clone 84H10 (IgG1); Immunotech, Marseille, France), anti-VCAM-1 (Clone 1G11 (IgG1); Immunotech), anti-E-cadherin (Clone 67A4 (IgG1); Immunotech), anti-MHC class I antigen (Clone FMC16, (IgG2a/k); Flinders Medical Center, Adelaide, Australia), anti-MHC class II antigen (Clone FMC52 (IgG1/k); Flinders Medical Center), and negative control (Clone X63 (IgG1); ATCC). The dilutions of mAbs used for immunocytostaining were: anti-ICAM-1, 1:50; anti-VCAM-1, 1:50; anti-E-cadherin 1:50; anti-MHC class I antigen, 1:10 of supernatant; and anti-MHC class II antigen, 1:10 of supernatant.

Virus preparation

The characterized long strain of RSV (88:RS4) was originally obtained from P. Young of the Sir Albert Sakzewski Virus Research Center, Royal Children′s Hospital, Brisbane, Australia. The virus was propagated from a frozen stock by inoculating fresh HEp-2, an immortalized human laryngeal epithelial tumour cell line (ATCC, Rockville, MD), cells with the virus and incubating them for 3 days; the virus-infected cells were then harvested, sonicated and stored in aliquots in liquid nitrogen. The titre was determined by means of the median tissue culture infective dose (TCID50) [21] and fluorescent focus assays. The two assays have been shown to correlate well in determining RSV titre. However, the fluorescent focus assay is much quicker to perform than the TCID50 assay and is more commonly used. Briefly, cultures of Hep-2 cells were inoculated with dilutions of the virus, incubated in 5% CO_2 for 18 h at 37°C, and then stained with fluorescein isothiocyanate (FITC)-labelled anti-RSV (Bartels, Issaquah, WA, USA). The fluorescing cells were counted and the virus titre was calculated assuming each fluorescent focus represented 1 infectious unit of virus. Typically virus preparations contained 1×10^7–6×10^7 fluorescent focus-forming units of RSV·mL^{-1}. Crude RSV extracts were used in these experiments in keeping with previously published data.

Viral infection of epithelial cells

Different doses of RSV (MOIs of 0.01, 0.1 and 1.0) were used in the infection of epithelial cells [3]. RSV diluted in DMEM plus 2% FCS were added to the confluent A549 monolayers in each well or chamber and incubated in humidified 5% CO_2/air for 2 h at 37°C (to allow virus adsorption). The supernatant was removed and all wells or chambers were washed once with DMEM. DMEM plus 2% FCS, 5 or 0.5 mL, was added to each well or chamber respectively and cultured to specific time points (6, 24, and 48 h) in the incubator.

Flow cytometric analysis

A549 cells in wells were scraped with sterile scrapers and 5×10^5 cells in 50 μL DMEM were incubated with an excess concentration of various mAbs (anti-CD54 (anti-ICAM-1), anti-CD106 (anti-VCAM-1), anti-E-cadherin, and anti-MHC class I and class II antigens; with X63 used as a negative control) for 30 min on ice. The cells were washed twice with phosphate-buffered saline (PBS) containing sodium azide and labelled with Silenus antimouse $F(ab')_2$ fragment conjugated to FITC (AMRAD, Boronia,

Australia) diluted 1:100. The cells were incubated for another 30 min on ice. After two washes with PBS/sodium azide, the cells were analysed by means of FACScan flow cytometry (CellQuest, Los Angeles, CA; Becton Dickinson, LA, CA) using standard settings. Ten thousand cells from each sample were analysed. The mean fluorescence intensity (MFI) on cells in the gated epithelial cell area was measured by means of flow cytometry as a marker of cell surface expression of the above molecules. All data were collected from three separate experiments.

Immunocytostaining

The reagent kit was purchased from Signet Laboratories Inc. (Dedham, MA, USA). Briefly: 1) all primary antibodies were prepared in blocking reagent (normal serum in buffer, multispecies); 2) the specimens (A549 cells in eight-chamber slides) were fixed in 95% ethanol for 15 min and washed in tris (hydroxymethyl) amino methane (Tris)-buffered saline (TBS) for 5 min; 3) the specimens were incubated with the correct titres of primary antibody diluted in blocking reagent in a humid incubation tray overnight at 4°C, and were washed in three changes of TBS, each for 5 min; 4) the endogenous peroxidase activity was blocked in 1% H_2O_2 diluted in absolute ethanol for 10 min and the specimens were washed in TBS for 5 min; 5) the specimens were incubated in linking reagent (biotinylated antimouse plus antirabbit immunoglobulins in buffer, multispecies) for 30 min at room temperature and were washed three times in TBS; 6) the specimens were incubated in labelling reagent (ultra streptavidin/peroxidase labelled in buffer) for 30 min at room temperature and were washed three times in TBS; 7) the specimens were incubated in 3′3′-diaminobenzidine tetrahydrochloride (DAB) solution for 5 min at room temperature and were washed well in TBS followed by tap water; 8) the slides were stained in Lillie Mayer′s haematoxylin for 2 min, differentiated briefly in 0.5% acid alcohol, blued in aqueous lithium carbonate, dehydrated in ethanol, cleared in xylol and mounted in PIX; and 9) photomicrographs were taken.

Statistical analysis

Data are presented as mean±SEM. Differences in results between groups were examined using one-way analysis of variance. The p-values between groups were corrected using Bonferroni′s test. Differences were considered to be significant at a p-value of <0.05.

Results

The expression of intercellular adhesion molecule-1 on A549 cells in respiratory syncytial virus infection

ICAM-1 was weakly expressed on normal A549 cells compared with negative control X63 (MFI 36.8±1.3 *versus* 30±1.6) (fig. 1). At 48 h of infection, RSV upregulated ICAM-1 expression in a dose-dependent pattern and reached a maximum level at a MOI of 1.0 (MFI 93±6.1) (fig. 1). However, RSV infection at a MOI of 1.0 upregulated ICAM-1 to the maximum level even at 6 h of infection (MFI 102±11.9), although there was no significant ICAM-1 upregulation after RSV infection at a MOI of 0.01 and 0.1 for 6 and 24 h. Immunostaining studies showed that ICAM-1 was strongly expressed around or in RSV-induced syncytial cells or giant cells (fig. 2).

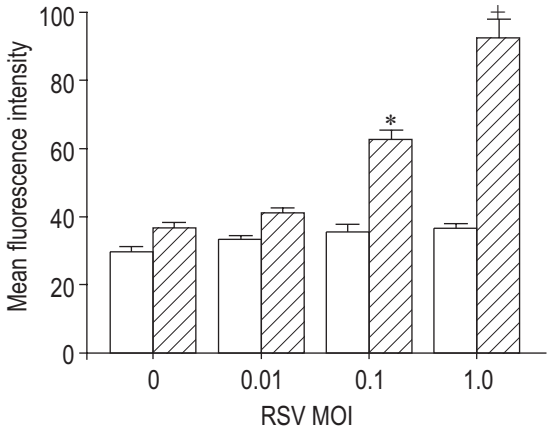

Fig. 1. – Intensity of intercellular adhesion molecule-1 expression on A549 cells infected with respiratory syncytial virus (RSV) at different multiplicities of infection (MOIs) for 48 h (▨). X63 (□) was used as negative control. Data are presented as mean±SEM (n=3). *: p<0.05 *versus* MOI of 0 group; +: p<0.05 *versus* MOI of 0, 0.01 and 0.1 groups.

The expression of vascular cell adhesion molecule-1 on A549 cells infected with respiratory syncytial virus

VCAM-1 is also weakly expressed on normal A549 cells compared with X63 (MFI 45±1.3 *versus* 30±1.6, p>0.05) (fig. 3). At 48 h of RSV infection, VCAM-1 expression was upregulated in a dose-dependent manner and was at its highest with RSV at a MOI of 1.0 (MFI 73±4) (fig. 3). However, RSV infection at a MOI of 1.0 could upregulate VCAM-1 to the maximum level even at 6 h of infection (MFI 83±2.9), although there was no significant VCAM-1 upregulation after RSV infection at MOIs of 0.01 and 0.1 for either 6 or 24 h. Similarly to ICAM-1, VCAM-1 was also strongly upregulated around or in RSV-induced giant cells (fig. 2).

The expression of major histocompatibility complex class I and II antigens on A549 cells in respiratory syncytial virus infection

Class I antigen is constitutively expressed on normal A549 cells compared with X63 (MFI 405±40 *versus* 30±1.6), and RSV infection for 48 h could upregulate class I expression on these cells in a dose-dependent pattern and reached a maximum level with RSV at a MOI of 1.0 (MFI 918±43) (fig. 4). There is effectively a doubling of class I antigen expression after RSV infection. However, there was no significant class I antigen upregulation at 6 h of RSV infection at a MOI of 1.0. At 24 h of RSV infection with a MOI of 1.0, class I antigen expression reached its peak (916±39) and there was no more upregulation at 48 h of infection.

Class II antigen was weakly expressed on normal A549 cells compared with X63 (MFI 36±1.3 *versus* 30±1.6), and RSV infection for 48 h at a MOI of 1.0 could upregulate class II antigen expression slightly compared with control cells and RSV infection at a MOI of 0.01 (MFI 45.4±1.5 *versus* 36±1.3, 39±0.7, p<0.05, respectively) (fig. 5). Both

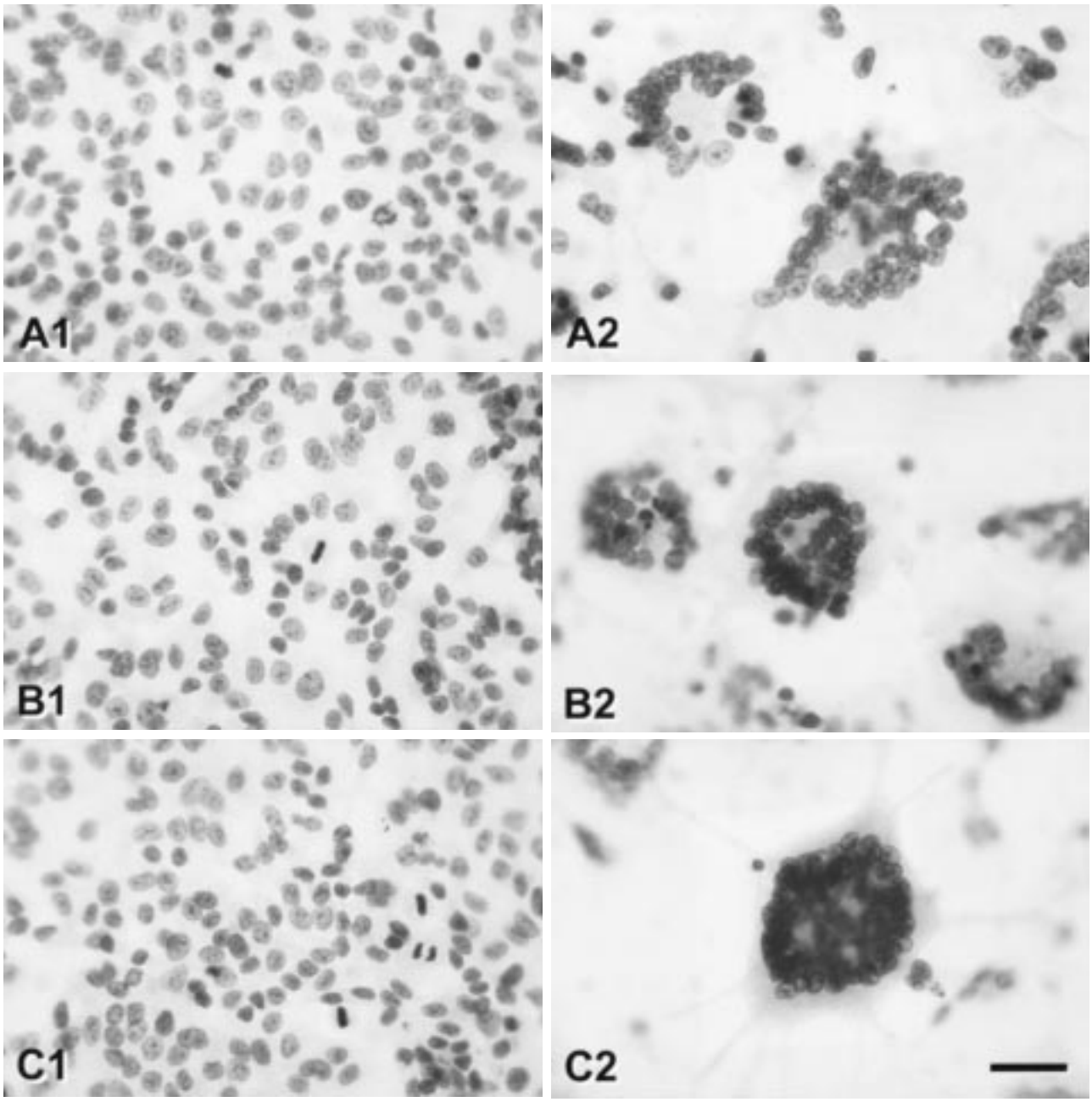

Fig. 2. – Respiratory syncytial virus (RSV)-induced syncytial (giant) cells and upregulation of adhesion molecules (intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-cadherin) on A549 cells. A549 cell monolayers were infected with RSV at a multiplicity of infection of 1.0 for 48 h (A2, B2 and C2), with noninfected A549 cell monolayers (A1, B1 and C1) as controls: A1, A2 negative controls; B1, B2 ICAM-1 expression; and C1, C2 VCAM-1 expression. (Internal scale bar=20 μm.)

MHC class I and II antigens were mainly upregulated around or in the giant cells (fig. 6).

The expression of E-cadherin on A549 cells infected with respiratory syncytial virus

E-cadherin expressed on A549 cell cultures was downregulated with the extension of culture time in both control and infection groups. However, at 48 h of infection, RSV could upregulate E-cadherin expression even at a MOI of 0.01 (MFI 144±8.6 *versus* 100±9.0, $p<0.05$). Immunostaining supports the view that E-cadherin is mainly upregulated on the RSV-induced giant cells (fig. 2).

Discussion

The present data show that RSV infection upregulates the expression of ICAM-1, VCAM-1 and MHC class I and II antigens on cultured A549 cells, and could also affect the expression of E-cadherin on these cells. These findings may not necessarily be highly specific for RSV, as RSVi as an additional control was not used [3].

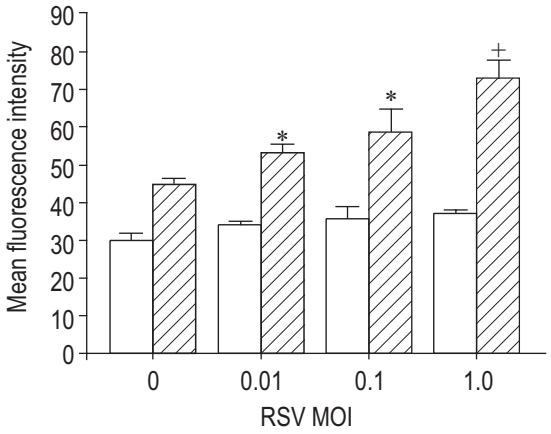

Fig. 3. – Intensity of vascular cell adhesion molecule-1 expression on A549 cells infected with respiratory syncytial virus (RSV) at different multiplicities of infection (MOIs) for 48 h (▨). X63 (□) was used as negative control. Data are presented as mean±SEM (n=3). *: $p<0.05$ *versus* MOI of 0 group. +: $p<0.05$ *versus* MOI of 0.01 and 0.1 groups.

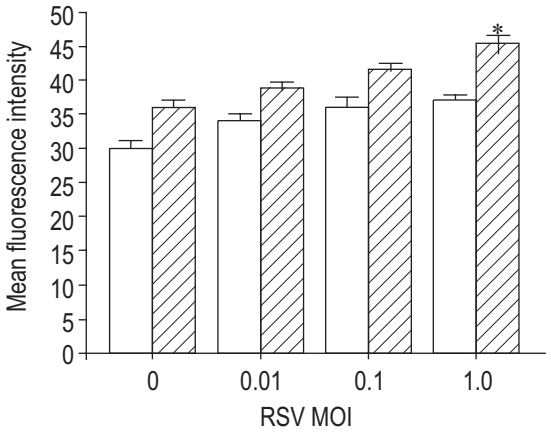

Fig. 5. – Intensity of class II antigen expression on A549 cells infected with respiratory syncytial virus (RSV) at different multiplicities of infection (MOIs) for 48 h (▨). X63 (□) was used as negative control. Data were presented as mean±SEM (n=3). *: $p<0.05$ *versus* MOI of 0 group and X63 with a MOI of 1.0 groups.

ICAM-1 is expressed on a variety of cells including endothelial cells, epithelial cells, fibroblasts and leukocytes [15]. Endothelial/epithelial cell ICAM-1 participates in the migration of leukocytes out of the blood in response to airway inflammation [22]. Epithelial ICAM-1 is also partially responsible for the enhanced neutrophil adhesion to cultured A549 cells in RSV infection and ICAM-1 is the only known ligand expressed on A549 cells for neutrophil CD18 [5]. The upregulation of ICAM-1 on cultured respiratory epithelial cells in RSV infection could help explain the high percentage of neutrophils in both upper and lower airway washings in infants with RSV bronchiolitis [23], and the increased neutrophil adhesion to A549 cells in RSV infection *in vitro* [3, 5]. The present data show that RSV-induced ICAM-1 expression is time- and dose-dependent, and that high-dose RSV could induce ICAM-1 expression to the maximum level at an early stage. These findings support the results of a previous neutrophil/epithelial adhesion and neutrophil damage study, demonstrating that RSV infection increases neutrophil adhesion to epithelial cells in a time- and dose-dependent pattern, RSV infection alone can damage and detach epithelial cells to a limited extent, and neutrophils can significantly augment the RSV-induced epithelial damage and detachment [3]. The upregulation of CD18 and CD11b on neutrophils in RSV infection may also contribute to the increased neutrophil/epithelial adhesion and neutrophil-induced epithelial damage [24].

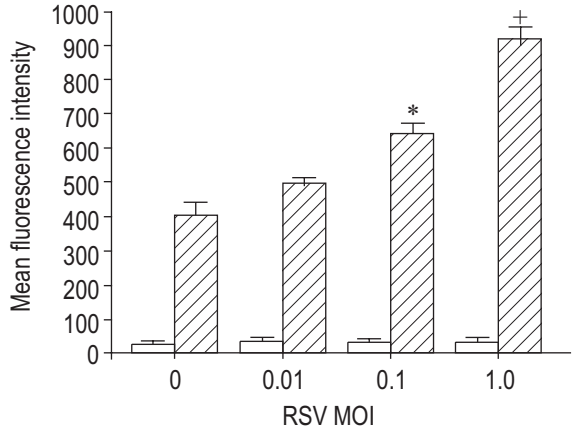

Fig. 4. – Intensity of class I antigen expression on A549 cells infected with respiratory syncytial virus (RSV) at different multiplicities of infection (MOIs) for 48 h (▨). X63 (□) was used as negative control. Data are presented as mean±SEM (n=3). *:$p<0.05$ *versus* MOI of 0 group. +: $p<0.05$ *versus* MOI of 0, 0.01 and 0.1 groups.

There are several possible mechanisms of ICAM-1 upregulation on respiratory epithelial cells in RSV infection. It has been reported that RSV induces the synthesis of IL-1α, IL-1β, and TNF-α, and that the enhanced ICAM-1 expression in RSV-infected epithelial cells is mediated primarily by IL-1α [25]. Another study showed that the upregulation of ICAM-1 on lung epithelial cells, after airway instillation of lipopolysaccharide, was mediated through TNF-α and IL-1 [26]. A more recent study indicated a critical role of the activation of transcription factors NF-κB and C/EBP in RSV-induced ICAM-1 expression by A549 cells [27].

The present finding that RSV-infected A549 cells express VCAM-1 is in contrast to a previous study [5], which showed no upregulation of VCAM-1 on A549 cells after RSV infection. More recently, another report showed that cytokine (TNF-α, IL-1β and IL-4) activation can induce the expression of both ICAM-1 and VCAM-1 on BEAS-2B epithelial cells [16]. The inability of STARK *et al.* [5] to detect the induction of VCAM-1 on A549 cells by RSV may be due to their use of an enzyme immunoassay. The present study employed DAB as a substrate and investigated the reaction by means of microscopy. Furthermore, the intensity VCAM-1 expression was detected and quantified by flow cytometric analysis, which is more sensitive than immunohistochemical techniques [16]. In the study reported here, video image analysis for quantifying epithelial antigen expression in the cell monolayers was unreliable due to significant epithelial cell detachment from the monolayers. Finally, STARK *et al.* [5] used A549 cells restricted to passage numbers 82–95, whereas cells were used before passage 45 in the present study; it has been shown that cells older than passage 50 failed to respond to stimulation [16].

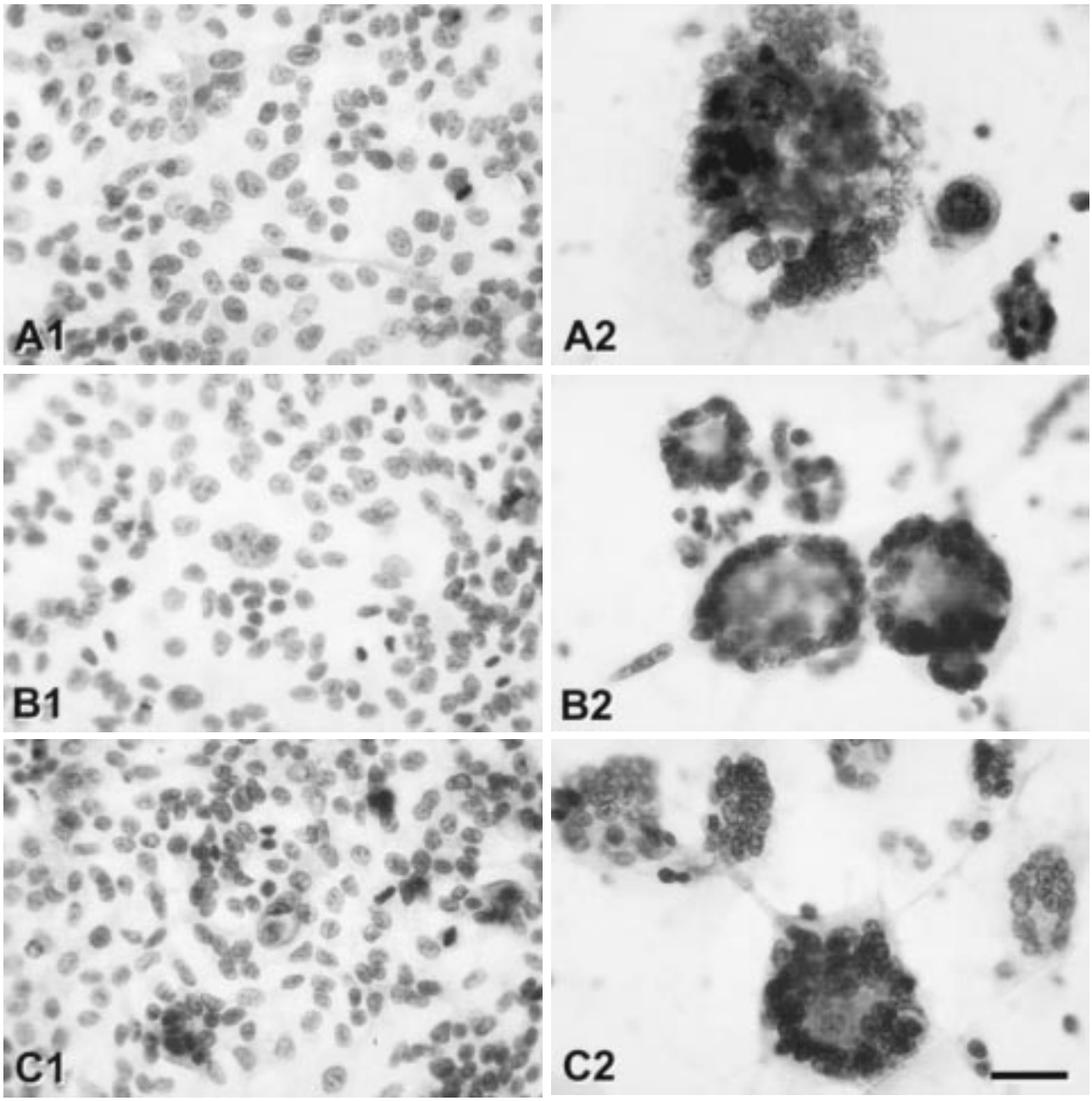

Fig. 6. – Respiratory syncytial virus (RSV)-induced syncytial (giant) cells and upregulation of major histocompatibility complex (MHC) class I and II antigens and E-cadherin on A549 cells. A549 cell monolayers were infected with RSV at multiplicities of infection of 1.0 for 48 h (A2, B2 and C2), with noninfected A549 cell monolayers (A1, B1 and C1) as controls: A1, A2 MHC class I antigen expression; B1, B2 MHC class II antigen expression; and C1, C2 E-cadherin expression. (Internal scale bar = 20 μm.)

The molecular mechanism of RSV-induced VCAM-1 expression on A549 cells is unclear. It has been reported that A549 cells infected with RSV could produce IL-1α, IL-1β, and TNF-α [25], and both TNF-α and IL-1β are strong inducers of VCAM-1 expression [16]. The expression of VCAM-1 on respiratory epithelial cells may have important clinical significance. T-lymphocytes and eosinophils express very late activation antigen-4 (VLA-4, CD49d/CD29, α4/b1) and can therefore bind to VCAM-1 [28–30]. The role of VLA-4 in the emigration of eosinophils was studied in an *in vivo* model [31]. Interestingly, VLA-4 was important for eosinophil rolling after intraperitoneal injection with IL-1β. It has been suggested that L-selectin and VLA-4 mediate eosinophil rolling in a sequential manner [31]. More recently, it was also shown that VLA-4/VCAM-1 was involved in the transendothelial migration of lymphocytes in bancroftian filariasis [32]. Most recently, it is reported that both selectin ligands and α_4 integrins participate in T-lymphocyte recruitment into the airspace during the pulmonary immune response [33]. Taken together, these studies suggest that the upregulation of VCAM-1 on epithelial cells in RSV infection may be

involved in T-lymphocyte and eosinophil adhesion to epithelial cells and the recruitment of these cells into the airways.

The upregulation of MHC class I antigen expressed on A549 cells in RSV infection confirmed previous observations [5, 34]. The association of viral antigen with MHC class I antigen in the presence of ICAM-1 supports an interaction with CD8+ cytotoxic T-cells [5, 34]. Both MHC class I antigen-restricted cytotoxic T-lymphocytes and lymphoproliferative responses were observed after RSV infection [36]. In studies of BALB/c mice, it has been shown that CD8 and CD4 T-cells are important for clearing virus infection [37, 38]. However, cellular immunity also appears to contribute to illness with reinfection by RSV and RSV vaccine-augmented disease [36, 39]. Since CD8 cytotoxic T-cells recognize antigen-laden MHC class I molecules on the target cells, the upregulation of MHC class I antigen on epithelial cells in RSV infection would facilitate the recognition and lysis of the infected epithelial cells by RSV-specific CD8 T-cells. Under these conditions, CD8 T-cells may clear RSV within the epithelial cells and damage or kill the epithelial cells concurrently.

The slight enhancement of MHC class II antigen expression on A549 cells at 48 h of RSV infection *in vitro* was an unexpected finding. Another recent study showed that, starting on day 3 of *in vivo* Sendai virus infection, rat tracheal epithelial cells expressed increasing levels of MHC class II antigen and that this expression was maximal at day 5 and declined rapidly thereafter [40]. As CD4 T-cells recognize antigen-laden MHC class II molecules on the target cells, the expression of MHC class II antigen on A549 cells in RSV infection may enable these cells to have the potential ability to present antigen to CD4 T-cells [41]. This may enable the respiratory epithelium to function as a "nonprofessional" APC, inducing immune inflammatory responses in RSV infection.

The disadvantage of the present *in vitro* cell model is that it is not possible to keep the cells infected with RSV at a MOI of 1.0 for >3 days, because most infected cells will be damaged and detached on the third day of RSV infection and there are limited numbers of cells for flow cytometric and image analysis. Therefore, further study on the expression and role of MHC class II antigen in RSV infection would depend on the establishment of a stable animal model.

The expression of MHC class I and II antigens, ICAM-1 and, VCAM-1, together with the expression of viral antigens on the cell surface [5], may allow the respiratory epithelium to function as a "nonprofessional" APC [18].

E-cadherin is a member of the family of calcium-dependent cell adhesion molecules. It is well established that E-cadherin functions as an epithelial adhesion component and is uniformly present at the lateral surfaces of all epithelia independently of their germ layer origin [42, 43]. More recently, it has been reported that E-cadherin is heavily distributed on the basolateral domain of alveolar type II cells [17].

E-cadherin, together with other adhesion molecules, mediate cell/cell or cell/matrix interactions in the lung and play a crucial role in the maintenance of lung tissue architecture [44]. E-cadherin mediates mainly homotypic (to a cell of the same type) epithelial cell-cell adhesion. As E-cadherin is also expressed on the basal side of epithelial cells [17, 45], E-cadherin may also be responsible for epithelial adhesion to the basal membrane.

The role of E-cadherin in cancer has been studied widely and it has been confirmed that impaired or reduced E-cadherin expression on tumour cells is associated with dedifferentiation, local invasiveness, lymph node metastasis and unfavourable prognosis [46–49]. However, the expression and clinical significance of E-cadherin on respiratory epithelial cells in RSV infection is unclear. The present immunocytostaining showed that E-cadherin is downregulated on some A549 cells, which may tend to detach later. However the expression of E-cadherin in/around RSV-induced giant cells is greatly enhanced. Therefore, the downregulation of E-cadherin on A549 cells with increasing culture time may help explain the increasingly enhanced detachment of A549 cell monolayers with an extended culture course [3]. Syncytia induced by RSV infection are usually formed on the second or third day of RSV infection *in vitro*. The upregulation of E-cadherin on A549 cells at 48 h of RSV infection may be responsible for the formation of syncytial giant cells. Clearly, the roles of E-cadherin in epithelial detachment and in the formation of giant cells in RSV infection requires further study.

The important RSV infection in infants is bronchiolitis. There is now ample evidence that the lower airway inflammation in infants with bronchiolitis is substantially due to host immune factors [10]. The studies described here may help to explain some of the contribution of the respiratory epithelial cell to this inflammation. Clearly *in vivo* correlates will be necessary to confirm these observations.

In summary, this study suggests that respiratory syncytial virus infection upregulates the expression of intercellular adhesion molecule-1, vascular cell adhesion molecule-1, vascular cell adhesion molecule-1 and major histocompatibility class I and class II antigens on A549 cells. Respiratory syncytial virus infection can also enhance the expression of E-cadherin on A549 cells, especially in or around the giant cells. These changes may play an important role in the pathophysiology of respiratory syncytial virus disease.

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