Eur Respir J 1998; 12: 619–626
DOI: 10.1183/09031936.98.12030619

European Respiratory Journal
ISSN 0903 - 1936

Th2 cytokines exert a dominant influence on epithelial cell expression of the major group human rhinovirus receptor, ICAM-1

A. Bianco, S.K. Sethi, J.T. Allen, R.A. Knight, M.A. Spiteri

Th2 cytokines exert a dominant influence on epithelial cell expression of the major group human rhinovirus receptor, ICAM-1. A. Bianco, S.K. Sethi, J.T. Allen, R.A. Knight, M.A. Spiteri. ©ERS Journals Ltd 1998.

ABSTRACT: Intercellular adhesion molecule (ICAM)-1 is a cell receptor important in both human rhinovirus (HRV) attachment and immune effector cell mobilization. The level of expression of ICAM-1 by epithelial cells (EC) therefore plays a crucial role in the intricate biological phenomena underlying viral binding, host infection and consequent inflammatory events.

As T-helper (Th)2 lymphocytes predominate within the asthmatic airway, the influence was evaluated of Th2-associated mediators in the modulation of ICAM-1 expression on uninfected and HRV-infected EC. H292 EC were cultured *in vitro*, with varying concentrations of interleukin (IL)-4, IL-5, IL-10 and IL-13 for 24 h and then infected with live HRV-14. Surface ICAM-1 expression was assessed by immunocytochemistry.

Infection with HRV-14 resulted in a twofold increase in ICAM-1 expression. IL-4, IL-5, IL-10 and IL-13 produced a 2.7–5.1-fold enhancement of ICAM-1 expression of uninfected cells and caused approximately a further twofold increase in infected cells over the expression induced by HRV infection itself. Interferon-γ in combination with each Th2-associated cytokine only slightly reduced, but did not override, the Th2-induced level of ICAM-1 expression on both uninfected and virus-infected EC.

These data suggest that the effects of Th2-associated cytokines on intercellular adhesion molecule-1 expression and recovery of infectious virus are dominant over the effects of the Th1-associated cytokines such as interferon-γ. Since the airway mucosa in atopic asthma is predominantly infiltrated by Th2 lymphocytes, these results could explain both the increased susceptibility to human rhinovirus infection in asthmatic patients and the associated exacerbation of asthma symptoms.
Eur Respir J 1998; 12: 619–626.

Lung Injury and Inflammation Research Group, Dept of Respiratory Medicine, North Staffordshire Hospital Trust, Staffordshire, UK.

Correspondence: A. Bianco
Dept of Respiratory Medicine
North Staffordshire Hospital Trust
City General Site, Newcastle Road
Stoke-on-Trent ST4 6QG
UK
Fax: 44 1782 552323

Keywords: Asthma
epithelial cells
human rhinovirus
intercellular adhesion molecule-1
Th2 cytokines

Received: November 20 1997
Accepted after revision April 6 1998

Supported by the British Medical Association TV. James Fellowship (1994) to M. Spiteri and the European Respiratory Society Scientific Research Fellowship (1996) to A. Bianco.

Acute viral respiratory infections may induce clinically relevant biological and physiological changes within the lower airway in susceptible individuals [1]. Although such infections are a potent trigger of asthma symptoms, there is as yet no consensus as to whether they can initiate asthma *de novo* [2]. Eighty per cent of asthma exacerbations in school-aged children and half of all adult asthma exacerbations have been associated with viral upper respiratory infections, mostly due to human rhinovirus (HRV) [3, 4]. Studies on experimentally induced HRV infections suggest that the rhinovirus may induce a greater effect on lower airway inflammation and function in subjects with established asthma or allergy [5–8]. Separate observations indicate that atopic individuals may be more susceptible to the development of HRV infections [9]. The mechanisms of these events, however, have not been systematically investigated.

The epithelial cells (EC) are the primary target of HRV infection in human airways. EC express on their surface the intercellular adhesion molecule (ICAM)-1, which is the site of attachment for 90% of the approximately 100 HRV serotypes [10–12]. ICAM-1 interacts physiologically with leukocyte function-associated antigen (LFA)-1, expressed on leukocytes, and thus plays a vital role in the recruitment and migration of immune effector cells to sites of local inflammation. Recent reports suggest that HRV may interfere with this ICAM-1/LFA-1 binding on leukocytes and thus disrupt immune responses that are dependent on this interaction. These effects could lead to a disorder of local airway immunity with increased risk of productive viral replication [13]. Furthermore, HRV *per se* influences the expression of ICAM-1 on EC. Studies by the present authors [14] and others [15, 16] have shown that HRV significantly upregulate EC ICAM-1 expression, an effect that would facilitate viral cell attachment and entry.

HRV infection induces the local production of cytokines, known to mediate the acute-phase reactions of airway inflammation [15, 17, 18]. These cytokines [19–23] can increase the expression of ICAM-1, although the level of induced expression depends on the specific mediator and the cell type [14, 24–26]. Recently, it has been shown that tumour necrosis factor (TNF)-α, interleukin (IL)-1β and IL-8 maintain their induced increase in cell ICAM-1 expression during HRV infection; in contrast, interferon (IFN)-γ increases ICAM-1 expression in uninfected cells but produces a persistent downregulation of ICAM-1 in

HRV-infected EC [14]. This effect of IFN-γ is accompanied by parallel reductions in HRV titres. Thus, the types and time kinetics of cytokines present at the level of host cell-virus interaction may influence the pathogenesis and course of HRV infection.

Asthmatic airway inflammation is characterized by increased concentrations of the T-helper (Th)2 lymphocyte-associated cytokines IL-4 and IL-5 [27], as well as enhanced numbers of cells expressing IL-4, IL-5, IL-10 and IL-13 messenger ribonucleic acid (mRNA) transcripts [28–30], with parallel reduced expression of Th1-associated cytokine IL-12 [30]. To date, the effect of these asthma-associated mediators on host cell-HRV interaction has been less well studied.

This study explored whether Th2-associated cytokines (IL-4, IL-5, IL-10 and IL-13) are able to modulate the level of EC ICAM-1 expression and its consequence for viral binding and host infection. In addition, we have studied whether simultaneous presence of a Th2 cytokine pattern outweighs the effects of IFN-γ on the major group HRV receptor expression on EC. Finally, as IL-12 is pivotal for Th1 phenotype differentiation with consequent IFN-γ production, an evaluation was made of whether the presence of IL-12 can directly modulate EC surface ICAM-1 expression. This study used an EC line (NCI-H292) which shares many properties with human EC [31–32]. Transformed cell lines may lose certain differentiated functions [33], resulting in possible different responses to HRV and, therefore, the results will be discussed in comparison to those on nontransformed primary human nasal epithelial cells (HNEC).

Materials and methods

Epithelial cell culture

An established bronchial epithelial cell line NCI-H292 (ATCC, CRL-1848, Rockville, MD, USA), derived from human pulmonary mucoepidermoid carcinoma, was used. This cell line has been shown to share many properties in common with human bronchial EC, including the same cytokeratin expression and growth features as well as the ability to support replication of HRV [31, 32]. The NCI-H292 cells were initially grown as described previously [14]. In brief, monolayers were prepared in RPMI-1640 medium (Sigma, Poole, UK) supplemented with 10% foetal calf serum (FCS; Sigma), 10,000 units·mL^{-1} penicillin, 10 mg·mL^{-1} streptomycin and 25 mg·mL^{-1} amphotericin B (Sigma), at 37°C in humidified air containing 5% CO_2.

In all subsequent experiments freshly trypsinized EC were washed with medium to remove traces of trypsin and serum, and then plated onto 35 mm culture dishes (ICN Flow Laboratories, Thame, Oxfordshire, UK) (1×10^6 cells·well^{-1}), using a final volume of 2 mL serum-free RPMI-1640 medium supplemented with antimicrobial agents as above and cultured at 37°C in humidified air containing 5% CO_2.

Viral stocks

The main seed of rhinovirus (HRV-14) was kindly provided by J. Kent, (University of Leicester, UK). A stock solution of HRV-14 was generated by infecting confluent monolayers of HeLa Ohio cells (ECACC 84121901, Salisbury, UK). HeLa Ohio cell lines were cultured as described previously [14]. In brief, HRV-14 was propagated using confluent non-overlapping monolayers of HeLa Ohio cells. The medium was first removed and the monolayer was then inoculated with a known dilution of HRV-14 and incubated for 90 min on roller cultures at 34°C in humidified incubator air containing 5% CO_2. Residual inoculum was decanted and replaced by maintenance medium (Eagle's minimum essential medium (EMEM) supplemented with 5% FCS, 4% sodium bicarbonate, 10,000 units·mL^{-1} penicillin, 10 mg·mL^{-1} streptomycin and 25 mg·mL^{-1} amphotericin B, Sigma) and incubated using roller cultures at 34°C in humidified air containing 5% CO_2. The cultures were then checked daily for cytopathic effect (cpe); once cpe was >80%, cultures were frozen and thawed three times in order to release virus from cells. Medium containing virus was centrifuged at 600×g for 10 min after which the viral suspension was stored at -70°C until required. Supernatants from uninfected HeLa were also frozen in aliquots, to be used as a negative control.

Viral purification

Prior to use in experiments, HRV-14 was purified by an established sucrose gradient method to remove ribosomes and soluble factors of HeLa cell origin. In brief, 20 μg·mL^{-1} ribonuclease (RNase) A (Sigma) was added to the above viral suspension and incubated at 35°C for 20 min. Sodium sarkosyl (1%; Sigma) and 1 μL·mL^{-1} 2-mercaptoethanol (Sigma) were then added to the RNase A-treated HRV-14 suspension. A solution containing 20 mM Tris acetate (Sigma), 1 M NaCl and 30% (w/v) sucrose was prepared, 3 mL of which was added to a 50 mL centrifuge tube. Viral suspension (27 mL) was then overlayered onto this solution. The resulting layered solutions were centrifuged at 200,000×g at 16°C for 5 h. The supernatant was discarded and the resulting viral pellet resuspended in med-ium and stored at -70°C until required.

Rhinovirus infection of H292 epithelial cells

Medium was removed from confluent monolayers of H292 cells and inoculated with 1 mL HRV-14 at $10^{2.5}$ 50% tissue culture infective dose (TCID50)·mL^{-1}. After a 90 min incubation at 34°C, 5% CO_2/air the inoculum was removed and cells were washed. The difference between the viral titres of the inoculum used for infection and supernatants retrieved at the end of the period of infection (90 min) was used to calculate the viral uptake by the epithelial cells during the period of infection. The content of inocula after incubation in an empty well acted as a control to assess nonspecific adherence of the virus to the wells. In each case, after the 90 min incubation, the same amount of virus ($10^{2.5}$ TCID50·mL^{-1}) was recovered from the empty wells. The medium was replaced by 2 mL of serum free Eagle's medium supplemented with L-glutamine, 10,000 units·mL^{-1} penicillin, 10 mg·mL^{-1} streptomycin and 25 μg·mL^{-1} amphotericin B, and incubation continued at 34°C. The medium was not replaced further until superna-

tants were recovered at 0, 1, 4, 6, 8, 12 and 14 days after infection and stored at -70°C for viral titre analysis. Cells were recovered for immunocytochemistry and to assess cell viability. Cytospins were made using a cell suspension at $1\times10^4\cdot mL^{-1}$ spun onto alcohol-washed slides using a Shandon Cytospin 2 cytocentrifuge (Life Sciences International, Basingstoke, UK). The slides were left to air dry for 1 h, fixed in a chloroform-acetone mixture (1:1) for 10 min, and left overnight to dry.

Viral titre assay

The TCID50 method was used to calculate the concentration of virus (the viral titre) in supernatants. Serial dilutions of virus were incubated with epithelial cells using roller cultures, as described above, to assess the dilution of virus that was not able to cause cpe in cells and so quantify the amount of infectious virus. Serial 10-fold dilutions of HRV-14 suspension were prepared with med-ium (EMEM) supplemented with 5% FCS. The TCID50 was then calculated using the Karber formula:

$$TCID_{50} = L - d(S/n - 0.5)$$

where L is $\log_{10}$ of the lowest dilution, d is $\log_{10}$ of the difference between dilutions, S is the number of positive tests and n is the number of tests (duplicates) per dilution.

Cytokine pretreatment of H292 cells

Preliminary dose-response studies, using a range of concentrations including the effective dose 50 (ED50) were performed to find the optimal cytokine concentrations. Medium was removed from cultures containing confluent cells undergoing pretreatment and replaced for 24 h with serum-free RPMI supplemented with recombinant human cytokines (R&D Systems, Abingdon, UK) separately or in combination, namely: IL-4 (0.4 $ng\cdot mL^{-1}$), IL-5 (0.5 $ng\cdot mL^{-1}$), IL-13 (10 $ng\cdot mL^{-1}$), IL-10 (1 $ng\cdot mL^{-1}$), IFN-γ (1 $ng\cdot mL^{-1}$) and IL-12 (0.2 $ng\cdot mL^{-1}$). All experiments were performed in triplicate.

Immunocytochemistry

A cytokeratin immunoglobulin (Ig)G-specific monoclonal antibody (Sigma) was used to assess the purity of the H292 cell line as being epithelial in origin.

A semiquantitative, three-step indirect immunoenzymatic labelling protocol [34] was used to analyse the surface ICAM-1 expression modified from established methods [35, 36] and employed as described previously [14]. EC were incubated at room temperature for 30 min with 5 $\mu g\cdot mL^{-1}$ of a purified mouse monoclonal antihuman antibody to ICAM-1 (RR1/1.1 IgG, recognizes ICAM-1s functional domains 1 and 2; a gift from R. Rothlein; Boehringer Ingelheim, Ridgefield, CT, USA). Cells were then washed using a washing buffer and incubated with rabbit antimouse IgG at a concentration of 1 $mg\cdot mL^{-1}$ (DAKO, Cambridge, UK) for 30 min followed by pig antirabbit IgG at a concentration of 0.8 $mg\cdot mL^{-1}$ (DAKO) to amplify the staining intensity [37]. Both secondary and tertiary antibodies are conjugated to the same enzyme (peroxidase). Cells were then incubated with benzidine (Sigma) and stained with Mayer's Hemalum solution (BDH, Lutterworth, UK). Dilutions of antibody used were based on previous experiments. A specificity check was carried out using an isotype-matched mouse IgG_1 antibody as a negative control at a concentration of 10 $\mu g\cdot mL^{-1}$ ($MsIgG_1$; Coulter Clone, Luton, UK). No quenching was required for endogenous peroxidase, as background staining on control slides was negligible.

To avoid observer bias, all slides were coded prior to analysis by one observer (J.T. Allen) and read blind by two independent observers (A. Bianco and S.K. Sethi). A mean of three readings of each slide was performed. Two cytospins were assessed for each time point. Cells were studied at 400× magnification with a light microscope (Olympus CH-2 microscope, Olympus Optical Co., Tokyo, Japan). An eyepiece graticule with a graded scale was sup-erimposed on the microscopic field and cell clumps were excluded from the counts. Three hundred cells were count-ed per field. Surface ICAM-1 expression on each of these EC was scored according to a five-point rating scale based on the intensity of staining and appearance of the nucleus: 0=grey/brown, 1=light brown, 2=medium brown, 3=med-ium/ dark brown and 4=dark brown, in grades 0–2 the nucleus appears well defined, while in 3 and 4 the nucleus is partially or fully obliterated. The number of cells thus counted in each grade was then multiplied with the res-pective grade index and the resulting values were summed. The final result for surface ICAM-1 on EC was expressed as the POX score adapted from a method used by Hsi and Remick [34], defined as the difference between the sum of the specific and background staining:

$$((a\times0) + (b\times1) + (c\times2) + (d\times3) + (e\times4)) \text{ - value for the control slide = POX score}$$

where each letter represents number of cells in the respective grade. The coefficient of variability of the differences between the counts obtained from all slides by both observers was between 3 and 17%; and that between the two observers for each time point was <5%. Photographs of cells were obtained by automatic equipment (Leica DAS Mikroskop, Germany; camera Ricoh XIVKR-10 M, Japan).

Statistics

The Wilcoxon signed rank test for paired data and Mann-Whitney test for independent data was applied to the results. Probability values of <0.05 were considered significant.

Results

Effect of Th2 cytokines on ICAM-1 expression of uninfected H292 cells

All Th2-associated cytokines tested (IL-4, IL-5, IL-10 and IL-13) enhanced EC ICAM-1 expression in a time-dependent manner. ICAM-1 reached maximum plateau values from days 6–8 following pretreatment of cells with each cytokine for 24 h (table 1). Basal EC ICAM-1 ex-

Table 1. – Effect of T-helper (Th)2 cytokines on intercellular adhesion molecule (ICAM)-1 expression (POX score) of uninfected epithelial cells (H292)

	Day						
	0	1	4	6	8	12	14
IL-13 10 ng·mL^{-1}	241±22.1	301±21	478±22	783±27.9	905±26.1	870±30	899±20.9
IL-10 1 ng·mL^{-1}	149±22.3	168±25.3	281±22	354±25.1	392±20.1	451±21.5	473±22
IL-5 0.5 ng·mL^{-1}	172±26.2	192±26.1	265±21.5	496±24	502±21	491±24.5	502±23.4
IL-4 0.4 ng·mL^{-1}	199±22.5	270±22.5	351±24.2	608±21.5	631±23.1	632±27.5	632±23.8
Baseline	167±25	166±22	170±21.5	174±21.6	178±25.3	177±27.8	178±29.7

H292 were cells preincubated for 24 h with optimal concentrations of Th2-associated cytokines (interleukin (IL)-4, IL-5, IL-10 and IL-13). ICAM-1 expression was assessed at the times indicated. All experiments were performed in triplicate and data are expressed as mean score±SD (n=18).

pression remained stable throughout the 14 days of the experiment. IL-4, IL-5 and IL-10 produced a 2.7–3.6-fold increase in ICAM-1 expression of uninfected cells, whilst pretreatment with IL-13 produced a 5.1-fold increase in ICAM-1 expression over baseline. The ICAM-1 increase produced by IL-13 was significantly ($p<0.01$) greater than that produced by IL-4, -5 and -10 (table 1).

Effect of Th1 cytokines on Th2-induced ICAM-1 expression of uninfected cells

Figure 1 shows Th2 cytokine representative results obtained with IL-13 alone and in combination with IFN-γ on ICAM-1 expression of uninfected H292 cells. The Th1-associated mediator IFN-γ induced a small but significant increase in surface ICAM-1 expression in uninfected cells ($p<0.05$). The magnitude of this IFN-γ-driven upregulation of ICAM-1 was significantly lower than that observed with IL-13 ($p<0.001$) (fig. 1 and table 2). A combination of IFN-γ and IL-13 produced a slight, nonsignificant reduction in the ICAM-1 expression induced by IL-13 alone ($p>0.05$), but only at days 8, 12 and 14 (fig. 1). Similar results were obtained with combinations of IL-4 and IFN-γ, and IL-5 and IFN-γ (data not shown).

Table 2 compares the maximal differential change in cell ICAM-1 expression with all Th2 and Th1 cytokines tested. The incremental increases in uninfected cell ICAM-1 expression by single pre-exposure of IL-4, -5, -10 and 13 were all significantly ($p<0.01$) greater than those produced by either IL-12 or IFN-γ. Similarly, the effects of the Th2 cytokines on uninfected cells were significantly higher than the effects of TNF-α reported previously [14].

Effect of Th2/Th1 cytokines alone and in combination on ICAM-1 expression of HRV-infected H292 cells

The effect of IL-13 alone and in combination with IFN-γ on HRV-14 infected H292 cells is shown in figure 2. Infection with HRV-14 resulted in a 2.3-fold increase in surface ICAM-1 over baseline. Pretreatment with all Th2 cytokines tested induced a further significant increase in ICAM-1 expression over that induced by HRV-14 itself ($p<0.01$) (table 2). Maximal surface ICAM-1 overexpression was observed on IL-13 pretreated EC (2.37-fold). In contrast, IFN-γ reduced ICAM-1 expression in virally infected cells to background uninfected levels (fig. 2). IL-12 had no effect on ICAM-1 expression.

The incremental increases in ICAM-1 expression by IL-4, IL-5, IL-10 and IL-13 were all significantly ($p<0.01$)

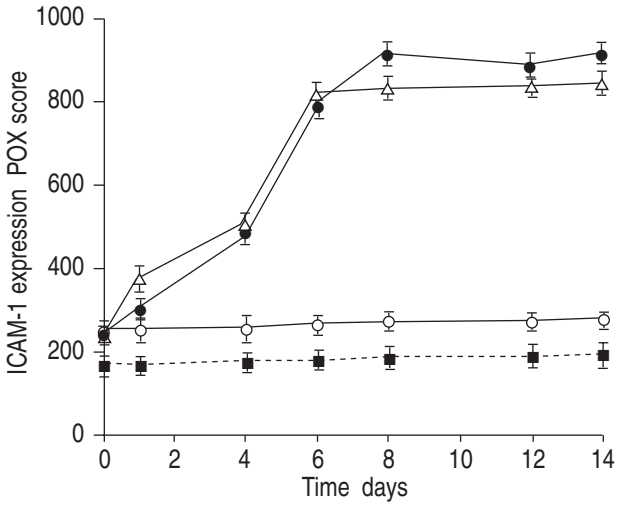

Fig. 1. – Effect of interleukin (IL)-13 (10 ng·mL^{-1}) and interferon (IFN)-γ (1 ng·mL^{-1}) alone and in combination on cell-surface intercellular adhesion molecule (ICAM)-1 (assessed by POX score) by uninfected H292 cells. ■: ICAM-1 cell-surface basal expression; ●: expression by H292 cells pretreated with IL-13 for 24 h before start of the experiment; ○: ICAM-1 expression by IFN-γ pretreated cells; ý: expression by pretreated cells with a combination of IL-13 and IFN-γ. Data are expressed as mean score±SD (n=18).

Table 2. – Maximal differential change in epithelial cell (EC) intercellular adhesion molecule (ICAM)-1 expression

		Uninfected	HRV-infected
Th2	IL-4	3.6*†#	2.13*†#
	IL-5	2.85*†#	1.75*†#
	IL-10	2.68*†#	1.99*†#
	IL-13	5.10*#	2.37*#
Th1	IL-12	1.1‡	1.0‡
	IFN-γ	1.47*‡	0.42‡
	TNF-α	2.29*	1.51*

The table shows differences between cytokine-induced and baseline ICAM-1 expression (quantified by POX score) on uninfected and human rhinovirus (HRV)-infected H292 ECs. Values represent the proportional differences in the plateau ICAM-1 expression reached during the experimental time period. Values for tumour necrosis factor (TNF)-α were obtained from a previous study [14]. All experiments were performed in triplicate. *: $p<0.05$ for effect of cytokine on EC ICAM-1 expression *versus* basal expression; †: $p<0.01$ effect of interleukin (IL)-13 *versus* IL-4, IL-5 and IL-10 on EC ICAM-1 expression; ‡: $p<0.01$ for IL-12 interferon (IFN)-γ *versus* IL-4, IL-5, IL-10 and IL-13; #: $p<0.01$ for TNF-α *versus* T helper (Th)2 cytokines.

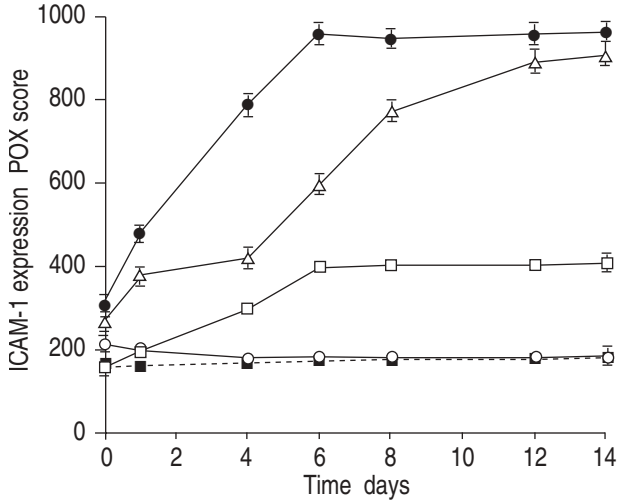

Fig. 2. – Effect of a combination of interleukin (IL)-13 (10 ng·mL^{-1}) and interferon (IFN)-γ (1 ng·mL^{-1}) on cell-surface intercellular adhesion molecule (ICAM)-1 (assessed by POX score) by human rhinovirus (HRV)-14-infected H292 cells. ■: ICAM-1 cell-surface basal expression; ❑: expression by HRV-infected cells; ○: expression by HRV-14-infected cells pretreated with IFN-γ; ●: expression by HRV-infected cells pretreated with IL-13; ý: ICAM-1 expression by HRV-infected cells pretreated with a combination of IL-13 and IFN-γ. Data are expressed as mean score±SD (n=18).

greater than those produced by either IL-12 or IFN-γ on HRV-14 infected H292 cells (table 2).

At all time points tested during HRV-14 infection, IFN-γ in combination with IL-13 delayed, but did not override, the level of ICAM-1 expression induced by IL-13 alone (fig. 2). Similar results have been observed with a combination of IL-4 and IFN-γ, and IL-5 and IFN-γ (data not shown).

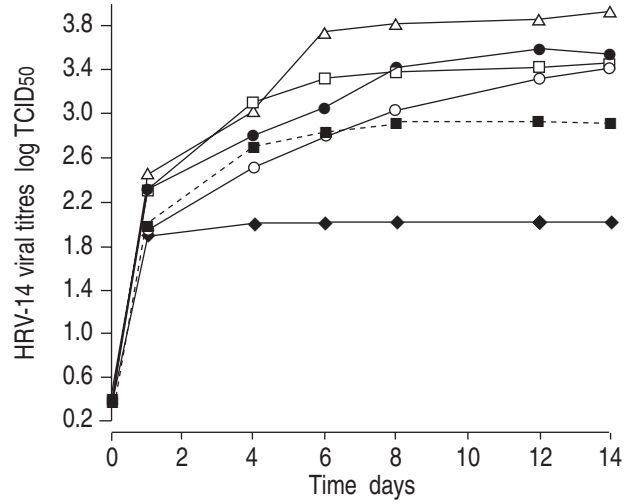

Fig. 3. – Effects of interleukin (IL)-4 (●; 0.4 ng·mL^{-1}), IL-5 (○; 0.5 ng·mL^{-1}), IL-10 (❑; 1 ng·mL^{-1}), IL-13 (ý; 10 ng·mL^{-1}) and interferon (IFN)-γ (◆; 1 ng·mL^{-1}) on viral titres in supernatants of H292 epithelial cells recovered at specific time points after infection with human rhinovirus (HRV)-14 ($10^{2.5}$ 50% tissue culture infection dose (TCID50)). ■: unstimulated infected culture supernatants. Data are expressed as mean score (n=3). At all time points the SD was ð0.22.

Table 3. – Effect of T-helper (Th)2 cytokines on human rhinovirus (HRV)-14 viral titres

Th2 cytokine	-IFN-γ	+IFN-γ
IL-13	3.92+	3.86*
IL-4	3.58	3.4*
IL-5	3.40	2.94*
IL-10	3.45	3.46*
Baseline	2.90	2.0‡

The table compares the effects of Th2-associated cytokines interleukin (IL)-4, IL-5, IL-10 and IL-13 alone and in combination with interferon (IFN)-γ, on viral titres in supernatants of H292 epithelial cells after infection with HRV-14 ($10^{2.5}$ 50% tissue culture infection dose (TCID50)). The maximal titres of HRV-14 (expressed in TCID50) recovered during the 14-day incubation are shown. All experiments were performed in triplicate and data are expressed as mean score (n=3). +: $p<0.01$ for IL-13 *versus* IL-4, IL5; *: $p>0.05$ for Th2 cytokines alone *versus* Th2 cytokines +IFN-γ; ‡: $p<0.01$ for basal epithelial cell intercellular adhesion molecule expression *versus* IFN-γ.

Effects of cytokines on viral titres

Supernatants removed after 90 min of cell incubation with virus still contained $10^{2.35}$ TCID50·mL^{-1} of HRV-14, a small reduction from the $10^{2.5}$ TCID50·mL^{-1} starting inoculum. This implies that only a very small quantity of virus was taken up by the cells. After washing, the recovered viral titre was reduced to $10^{0.4}$ TCID50·mL^{-1}. Figure 3 shows that recovered viral titres increased rapidly within 24 h from this low level. HRV infection of H292 EC resulted in further, though less marked, increases up to day 8, after which recovered viral titres remained at plateau levels.

Figure 3 also shows that pre-exposure of H292 cells for 24 h to IL-4, IL-5, IL-10 and IL-13 increased susceptibility of the cells to HRV-14 infection, resulting in significant increases in recovered viral titres compared with infected baseline conditions ($p<0.001$) (fig. 3). Pre-exposure to IL-13 produced higher recovered viral titres than pre-exposure to either IL-4 ($p<0.01$) or IL-5 ($p<0.01$) but not IL-10. In contrast, pre-exposure to IFN-γ significantly ($p<0.01$) reduced recovered viral titres (fig. 3).

The effects of Th2 alone and in combination with IFN-γ are summarized in table 3. The enhancing effects of IL-4, IL-5, IL-10 and IL-13 on plateau viral titre were not significantly affected by IFN-γ.

H292 cell viability, as assessed by trypan blue exclusion, was consistently >95% on 0–6 days in virus-infected cultures; subsequently >90% on day 8 and 75–80% viable on 12–14 days.

Discussion

In this study, it was demonstrated that Th2-associated cytokines, and particularly IL-13, produce a greater increase in uninfected and HRV-infected EC ICAM-1 expression than Th1-associated cytokines. These enhanced effects of Th2 cytokines were paralleled by greater increases in recovered viral litres. The upregulation of ICAM-1 on uninfected H292 epithelial cells induced by the Th2-associated cytokines (IL-4, IL-5, IL-10 and IL-13) was not influenced by the presence of IFN-γ in the culture

medium. Moreover, during HRV-14 infection, the effects of Th2 cytokines on the major group HRV receptor expression were only delayed but not overridden by IFN-γ, unlike previous observations with TNF-α [14]. These findings suggest that a Th2 cytokine environment can influence the host cell/HRV interaction, inducing at the level of the target cell a pronounced and relatively IFN-γ-resistant increase in the attachment site for the majority of HRV serotypes.

Parallel studies [38] on primary cultures of human nasal EC (HNEC) obtained from nonatopic healthy subjects support the above findings with the H292 epithelial cell line. In the absence of HRV-infection, Th2 cytokines such as IL-13 induce a twofold increase in ICAM-1 expression on HNEC over that with IFN-γ. When these HNEC are infected with HRV-14, virus *per se* increases cell-surface ICAM-1 significantly but does not reach the maximal levels of expression as seen with IL-13 [38]. Thus, primary human EC and the H292 cell line have similar responses to HRV-14 infection, dependent on a critical Th2/Th1 cytokine equilibrium within the target cell milieu, with consequences for viral binding and host cell infection.

Studies on bronchial biopsies and bronchoalveolar lavage (BAL) cells from asthmatic patients have provided evidence that predominance of Th2 lymphocytes within their airways and the associated cytokine secretory profile is crucial in the induction and persistence of the asthmatic inflammation. BAL cells analysed by *in situ* hybridization from atopic asthmatics show an increased proportion of cells expressing mRNA for IL-3, IL-4, IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF) compared with nonatopic control subjects [39]. These proportions of mRNA-positive BAL cells for IL-4 and IL-5 correlate well with BAL eosinophil numbers as well as with the degree of airflow obstruction and airway responsiveness in asthma [28]. Other studies have shown high mRNA expression of Th2-associated cytokines IL-4, IL-5, IL-10 but not Th1 lymphokines such as IFN-γ in BAL cells and bronchial biopsies from asthmatic individuals [28, 29]. Recently, increased numbers of IL-13 mRNA-positive cells in bronchial biopsies from asthmatic subjects have also been described, with a parallel reduced expression of IL-12 [30], a critical factor in the shift towards Th1 cell differentiation. Since the predominance of Th2-associated cytokines within the asthmatic airways would increase EC expression of ICAM-1 and thus favour HRV attachment, this may be an important mechanism to explain the enhanced susceptibility of asthmatic subjects to clinical viral respiratory infections. Indeed, studies performed with or without segmental allergen challenge, have identified significant ICAM-1 upregulation on the bronchial epithelium of asthmatic patients [40, 41]. Accordingly, HNEC from asthmatic/atopic patients exhibit a significantly greater baseline expression of ICAM-1 compared to normal healthy subjects [38]. Furthermore, when these asthmatic/atopic HNEC are infected with HRV-14, a further increase in surface ICAM-1 expression is observed over the baseline. This viral-induced level of ICAM-1 expression is enhanced further when the HNEC are pre-exposed to Th2 cytokines. The magnitude of these responses to HRV-14 infection in atopic HNEC is threefold over that observed in normal HNEC. These data support our hypothesis in this current study that dominant Th2 cytokine processes within the target cell milieu may be critical to the association between respiratory viruses and viral exacerbations of asthma.

An increase in ICAM-1 expression induced by Th2 cytokines could also facilitate recruitment of further inflammatory cells to the airway by ICAM-1/LFA-1 interactions, as well as enhancing HRV attachment and infection. Since T-cells recruited in this way will mature in an environment rich in accessory factors (such as IL-4) favouring Th2 differentiation, atopic/asthmatic inflammation within the airway will be sustained and even enhanced. Such interrelated events would serve as mechanisms for viral-induced exacerbation of atopic asthma.

This dual function of EC ICAM-1, in both enhancing susceptibility to viral infection and promoting the recruitment of further Th2 lymphocytes, suggests that therapies aimed at reducing ICAM-1 expression should reduce episodes of airway viral infection and their exacerbatory effects on asthma symptoms. There is evidence that existing effective asthma treatments act, at least in part, through these mechanisms. For example, inhaled sodium cromoglycate reduces ICAM-1 expression within the airway, with a corresponding reduction in inflammatory cell infiltration [42]. Moreover, glucocorticoids reduce ICAM-1 expression by H292 cells *in vitro*, an effect which may involve interaction between glucocorticoid receptors and the transcription factor, nuclear factor (NF)-κB [43]. These results suggest that more direct approaches using aerosolized anti-ICAM-1 antibodies or soluble ICAM-1 (sICAM-1) may be clinically useful. Indeed, intranasal administration of recombinant sICAM-1 has already been shown to reduce symptoms associated with experimentally induced HRV colds in humans [44].

Many Th1 and Th2 cytokines exert reciprocal inhibitory effects on each others production. For example, IFN-γ inhibits IL-4 production and IL-4-mediated antibody class switching to IgE [45]. Similarly, IL-12 inhibits IL-4 synthesis [46]. Conversely, both IL-4 and IL-10 inhibit IL-2 and IFN-γ production [47, 48]. In this study it has been shown, for the first time, that during HRV-14 infection, the effects of Th2 cytokines on the major group HRV receptor expression are only delayed but not overridden by the Th1 cytokine IFN-γ. In addition, it was observed that increasing the dose of IFN-γ to 2 and 5 ng·mL^{-1} had no greater effect than the standard concentration of 1 ng·mL^{-1} on ICAM-1 expression induced by Th2 cytokines (data not shown). Therefore these data suggest that the emergence of Th2-associated cytokine profile within an inflammatory milieu can override the biological action of the Th1 lymphokines.

The results of this study have important clinical implications. Firstly, the high levels of ICAM-1 expression, within a Th2 cytokine-dominated environment, would favour an enhanced HRV attachment and infection of airway EC, accounting for the increased frequency of upper respiratory HRV infections in atopic/asthmatic subjects.

Secondly, the marked upregulation of ICAM-1 expression on HRV-infected cells in the presence of IL-4, IL-5, IL-10 and IL-13 is only marginally affected by IFN-γ, concentrations of which are relatively low in the atopic/asthmatic airway [39]. This high intensity of surface ICAM-1 expression on EC would not only favour persistence of viral infection but, by recruiting further pro-inflammatory cells to the airway, produce an exacerbation of asthmatic symptoms [49].

As well as directly targeting the epithelial cell viral receptor, these observations suggest exciting therapeutic possibilities for manipulating the local cytokine environment to enhance antiviral defence.

References

1. Stark JM, Graziano FM. Lower airway responses to viruses. *In*: Busse WW, Holgate ST, eds. Asthma and Rhinitis. Boston, MA, Blackwell Scientific Publications, 1995; pp. 1229–1243.
2. Openshaw PJM, O'Donnell DR. Asthma and the common cold: can viruses imitate worms? *Thorax* 1994; 49: 101–103.
3. Johnston SL, Pattemore PK, Sanderson G, *et al.* Community study of role of viral infections in exacerbations of asthma in 9–11 year old children. *BMJ* 1995; 310: 1225–1229.
4. Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. *BMJ* 1993; 307: 982–986.
5. Fraenkel DJ, Bardin PG, Sanderson G, Lampe F, Johnston SL, Holgate ST. Lower airways inflammation during rhinovirus colds in normal and in asthmatic subjects. *Am J Respir Crit Care Med* 1995; 151: 879–886.
6. Grunberg K, Kuijpers EA, de Klerk EPA, *et al.* Effects of experimental rhinovirus 16 infection on airway hyperresponsiveness to bradykinin in asthmatic subjects *in vivo*. *Am J Respir Crit Care Med* 1997; 155: 833–838.
7. Gern JE, Calhoun W, Swenson C, Shen G, Busse WW. Rhinovirus infection preferentially increases lower airway responsiveness in allergic subjects. *Am J Respir Crit Care Med* 1997; 155: 1872–1876.
8. Grunberg K, Timmers MC, Smits HH, *et al.* Effects of experimental rhinovirus 16 colds on airway hyperresponsiveness to histamine and interleukin-8 in nasal lavage in asthmatic subjects *in vivo*. *Clin Exp Allergy* 1997; 27: 36–45.
9. Bardin PG, Fraenkel DJ, Sanderson G, *et al.* Amplified rhinovirus colds in atopic subjects. *Clin Exp Allergy* 1994; 24: 457–464.
10. Couch RB. Rhinoviruses. *In*: Fields BN, Knipe DM, eds. Virology. New York, Raven Press Ltd, 1990; pp. 608–629.
11. Greve JM, Davis G, Meyer AM, *et al.* The major human rhinovirus receptor is ICAM-1. *Cell* 1989; 56: 839–847.
12. Lineberger DW, Uncapher CR, Graham DJ, Colonno RJ. Domains 1 and 2 of ICAM-1 are sufficient to bind human rhinovirus. *Virus Res* 1992; 24: 173–186.
13. Gern JE, Joseph B, Galagan DM, Borcherding WR, Dick EC. Rhinovirus inhibits antigen-specific T cell proliferation through an intercellular adhesion molecule-1-dependent mechanism. *J Infect Dis* 1996; 174: 1143–1150.
14. Sethi SK, Bianco A, Allen JT, Knight RA, Spiteri MA. Interferon-gamma downregulates the rhinovirus induced expression of intercellular adhesion molecule-1 (ICAM-1) on human airway epithelial cells. *Clin Exp Immunol* 1997; 110: 362–369.
15. Terajima M, Yamaya M, Sekizawa K, *et al.* Rhinovirus infection of primary cultures of human tracheal epithelium: role of ICAM-1 and IL-1β. *Am J Physiol (Lung Cell Mol Physiol 17)* 1997; 273: L749–L759.
16. Papi A, Wilson S, Johnston S. Rhinoviruses increase production of adhesion molecule (CAM) and NF-κB. *Am J Respir Crit Care Med* 1996; 153: A866.
17. Subauste MC, Jacoby DB, Richards SM, Proud D. Infection of human respiratory epithelial cell line with rhinovirus. Induction of cytokine release and modulation of susceptibility to infection by cytokine exposure. *J Clin Invest* 1995; 96: 549–557.
18. Proud D, Gwaltney JM, Hendley JO, Dinarello CA, Gillis S, Schleimer RP. Increased levels of interleukin-1 are detected in nasal secretions of volunteers during experimental rhinoviral colds. *J Infect Dis* 1994; 169: 1007–1013.
19. Patel JA, Kunimoto M, Sim TC, *et al.* Interleukin-1-alpha mediates the enhanced expression of intercellular adhesion molecule-1 in pulmonary epithelial cells infected with respiratory syncytial virus. *Am J Respir Cell Mol Biol* 1995; 13: 602–609.
20. Braun M, Pietsch P, Felix SB, Baumann G. Modulation of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 on human coronary smooth muscle by cytokines. *J Mol Cell Cardiol* 1995; 27: 2571–2579.
21. Toru H, Kinashi T, Ra C, Nonoyama S, Yata J, Nakahata T. Interleukin-4 induces homotypic aggregation of human mast cells by promoting LFA-1/ICAM-1 adhesion molecules. *Blood* 1997; 89: 296–302.
22. Yao Z, Painter SL, Fanslow WC, *et al.* Human IL-17: a novel cytokine derived from T cells. *J Immunol* 1995; 155: 5483–5486.
23. Look DC, Rapp SR, Keller BT, Holtzman MT. Selective induction of intercellular adhesion molecule-1 by interferon-γ in human airway epithelial cells. *Am J Physiol* 1992; 263: L79–L87.
24. Tosi MF, Stark JM, Smith W, Hamedani A, Gruenert DC, Infecd MD. Induction of ICAM-1 expression on human airway epithelial cells by inflammatory cytokines: effects on neutrophil-epithelial cell adhesion. *Am J Respir Cell Mol Biol* 1992; 7: 214–221.
25. Bloemen PGM, van den Tweel MC, Henricks PAJ, *et al.* Expression and modulation of adhesion molecules on human bronchial epithelial cells. *Am J Respir Cell Mol Biol* 1993; 9: 586–593.
26. Mickelson JK, Kukielka G, Bravenec S, *et al.* Differential expression and release of CD54 induced by cytokines. *Hepatology* 1995; 22: 866–875.
27. Walker C, Bauer W, Braun RK, *et al.* Activated T-cells and cytokines in bronchoalveolar lavages from patients with various lung diseases associated with eosinophilia. *Am J Respir Crit Care Med* 1994; 150: 1038–1048.
28. Robinson DS, Ying S, Bentley AM, *et al.* Relationships among numbers of bronchoalveolar lavage cells expressing messenger ribonucleic acid for cytokines, asthma symptoms and airway methacholine responsiveness in atopic asthma. *J Allergy Clin Immunol* 1993; 92: 397–403.
29. Robinson DS, Tsicopoulos A, Meng Q, Durham SR, Kay AB, Hamid Q. Increased interleukin-10 messenger RNA expression in atopic allergy and asthma. *Am J Respir Cell Mol Biol* 1996; 14: 113–117.
30. Naseer T, Minshall EM, Leung DYM, *et al.* Expression of IL-12 and IL-13 mRNA in asthma and their modulation in response to steroid therapy. *Am J Respir Crit Care Med* 1997; 155: 845–851.
31. Banks-Schlegel K, Gazdar AF, Harris CC. Intermediate filament and cross-linked envelope expression in human lung tumour cell lines. *Cancer Res* 1985; 45 (3): 1187–1197.
32. Hierholzer JC, Castells E, Banks GG, Bryan JA, McEwan CT. Sensitivity of NCI-H292 human lung mucoepidermoid cells for respiratory and other human viruses. *J Clin Microbiol* 1993; 31(6): 1504–1510.

33. Gruenert DC, Finkbeiner WE, Widdicombe JH. Culture and transformation of human airway epithelial cells. *Am J Physiol (Lung Cell Mol Physiol 12)* 1995; 268: L347–L360.
34. Hsi ED, Remick DG. Monocytes are the major producers of interleukin-1β in an *ex vivo* model of local cytokine production. *J Interferon Cytokine Res* 1995; 15: 89–94.
35. Spiteri MA, Clarke SW, Poulter LW. Alveolar macrophages that suppress T-cell responses may be crucial to the pathogenetic outcome of pulmonary sarcoidosis. *Eur Respir J* 1992; 5: 394–403.
36. Mason DY, Abdulaziz Z, Falini B, Stein H. Double immunoenzymatic labelling. *In*: Polak J, van Noorden S, Wright J, eds. "Immunocytochemistry: Practical Applications in Pathology and Biology. 2nd Edn Bristol, PSG Publishing Co., 1983; pp. 113–128.
37. Boenisch T. Staining methods. *In*: Nash S, ed. Immunochemical Staining Methods, Denmark, Dako Corporation, 1989; pp. 13–18.
38. Sethi SK, Bianco A, Wilton SC, Spiteri MA. Influence of Th2 cytokines on major group human rhinovirus receptor, ICAM-1 on nasal epithelial cells from atopic subjects. *Am Rev Respir Crit Care Med* 1998; 157: A24.
39. Robinson DS, Hamid Q Ying S, *et al.* Predominant Th2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992; 326: 298–304.
40. Bloemen PGM, Henricks PAJ, Nijkamp FP. Cell adhesion and asthma (Review). *Clin Exp Allergy* 1997; 27: 128–141.
41. Chanez, P, Vignola AM, Lacoste P, Michel FB, Godard P, Bosquet J. Increased expression of adhesion molecules (ICAM-1 and LFA-1) in alveolar macrophages from asthmatic patients. *Allergy* 1993; 48: 576–580.
42. Hoshino M, Nakamura Y. The effect of inhaled sodium cromoglycate on cellular infiltration into the bronchial mucosa and the expression of adhesion molecules in asthmatics. *Eur Respir J* 1997; 10: 858–865.
43. van der Saag PT, Caldenhoven E, van de Stolpe A. Molecular mechanisms of steroid action: a novel type of cross-talk between glucocorticoids and NF-kappa B transcription factors. *Eur Respir J* 1996; 9: Suppl. 22, 146s–153s.
44. Turner RB, Wecker MT, Pohl G, *et al.* Efficacy of soluble ICAM-1 (sICAM) for prevention of rhinovirus infection and illness. Abstracts of 37th ICAAC, September 28–October 2 1997; Abstract H-85: 229.
45. Xu L, Rothman P. IFN-gamma represses epsilon germline transcription and subsequently downregulates switch recombination to epsilon. *Int Immunol* 1994; 6: 515–521.
46. Dekruyff RH, Fang Y, Wolf SF, Umetsu DT. IL-12 inhibits IL-4 synthesis in keyhole limpet hemocyanin-primed $CD4^+$ T cells through an effect on antigen-presenting cells. *J Immunol* 1995; 154: 2578–2587.
47. Tanaka T, Hu-li J, Seder RA, de St. Groth BF, Paul WE. Interleukin 4 suppresses interleukin 2 and interferon gamma production by naive T cells stimulated by accessory cell-dependent receptor engagement. *Proc Natl Acad Sci USA* 1993; 90: 5914–5918.
48. Schlaak JF, Hermann E, Gallati H, Meyer zum Buschenfelde KH, Fleischer B. Differential effects of IL-10 on proliferation and cytokine production of human gamma/delta and alpha/beta T cells. *Scand J Immunol* 1994; 39: 209–215.
49. Bianco A, Spiteri MA. A biological model to explain the association between rhinovirus respiratory infections and bronchial asthma. *Monaldi Arch Chest Dis* 1998; 53 (1): 83–87.