

ORIGINAL PAPER

Modulation of the epithelial inflammatory response to rhinovirus in an atopic environment

M. Xatzipsalti*, F. Psarros†, G. Konstantinou*, M. Gaga‡, D. Gourgiotis*, P. Saxoni-Papageorgiou* and N. G. Papadopoulos*

*Allergy Department, 2nd Pediatric Clinic, University of Athens, Athens, Greece, †Allergy Department, Naval Hospital, Athens, Greece and ‡Respiratory Department of Medicine, 'Sotiria' General Hospital, Athens, Greece

Clinical and Experimental Allergy

Summary

Background Immune responses to rhinovirus (RV) as well as direct effects of RV on respiratory epithelium may contribute to the induction of asthma exacerbations.

Objective To evaluate the effect of the environment resulting from an atopic immune response on RV-induced epithelial inflammation, replication and cytotoxicity.

Methods Peripheral blood mononuclear cells (PBMC) from atopic asthmatic subjects and matched controls (12 pairs) were isolated and stimulated by RVs. Human bronchial epithelial (BEAS-2B) cells were infected with RV in the presence of conditioned media from RV-stimulated PBMC cultures. IL-6, IL-8, RANTES and TGF- β 1 levels were measured by ELISA, RV-induced cytotoxicity by a colorimetric method and RV titres on Ohio-HeLa cells.

Results RV-induced epithelial production of IL-6, IL-8 and RANTES was significantly lower, while TGF- β 1 was higher when cells were exposed to conditioned media from atopic asthmatic subjects compared with those from normal controls. Exposure to the 'atopic' environment also resulted in elevated RV titres and increased RV-induced cytotoxicity.

Conclusions Under the influence of an atopic environment, the epithelial inflammatory response to RV is down-regulated, associated with increased viral proliferation and augmented cell damage, while TGF is up-regulated. These changes may help explain the propensity of atopic asthmatic individuals to develop lower airway symptoms after respiratory infections and indicate a mechanism through which viral infections may promote airway remodelling.

Keywords atopy, immune response, rhinovirus

Submitted 5 February 2007; revised 19 September 2007; accepted 2 November 2007

Correspondence:

Maria Xatzipsalti, 23–27 Makrigianni Str., 11742 Makrigianni, Athens, Greece.
E-mail: marax5873@hotmail.co.uk

Introduction

Epidemiological studies during the last decade have convincingly demonstrated that the majority of acute asthma exacerbations, especially in children, follow upper respiratory viral infections, of which around 60% are caused by human rhinoviruses (RVs) [1, 2]. However, the mechanisms by which RV may exacerbate asthma are still incompletely understood. Direct effects of the virus on the respiratory epithelium, abnormal immune responses and neurogenic reflexes have been implicated as parts of a complicated mechanism leading from upper airway infection to reactive lower respiratory disease [3, 4].

Innate and adaptive immune responses serve to limit the extent of infection and clear virus-infected epithelial cells. Recent evidence has shown that the nature of immune responses to RV differs between asthmatic and normal

subjects, expressed as deficient production of IFN- γ and IL-12 as well as suboptimal costimulation in atopic asthmatic patients compared with normal subjects [5, 6]. Clinical symptoms and viral clearance after experimental infection with RV correlate well with the balance of airway T-helper type 1 (Th1) and Th2 cytokines in sputum [7]. Furthermore, strong *in vitro* IFN- γ responses to RV could predict virus shedding after experimental infections [8].

Nevertheless, the bronchial epithelium is a major locus of inflammation induction and control. We have recently shown that RV is able to reach and replicate in the bronchial epithelium [9], in which, under some circumstances, it may also induce cytotoxicity. Epithelial infection leads to inflammation, demonstrated by the induction of pro-inflammatory cytokines and chemokines, including IL-1, IL-6, IL-8 and RANTES [10, 11]. Furthermore, several events related to viral infection, including cytotoxicity, or

the induction of angiogenic factors such as vascular endothelial growth factor after RV infection [12, 13] may be involved not only in exacerbation but also in perpetuation of asthma symptoms. In this respect, the presence of Th2 or combinations of Th1 and Th2 cytokines during RV infection of epithelial cells has been shown to alter epithelial cytokine production, intercellular adhesion molecule (ICAM)-1 expression and RV titre [14–17]. A Th2-skewed environment may also affect the life–death cycle of the respiratory epithelium, crucial in the induction of airway remodelling [18].

Based on the above, we hypothesized that the abnormal immune responses of atopic asthmatic individuals to RV may influence the epithelial response to the virus. The aim of this study was to evaluate the effect of an atopic environment, modelled by stimulation of immune cells from normal or atopic asthmatic individuals with RV, on the induction of epithelial inflammation, RV replication in epithelial cells and RV-induced cytotoxicity.

Methods

Subjects

Venous blood was drawn from 24 subjects. Twelve were patients with asthma, diagnosed and classified according to the 2004 GINA guidelines, and the other 12, examined in parallel, were healthy non-atopic, non-asthmatic subjects. There were eight men and four women in each group, 19–40 years of age (mean: 30 years). All subjects were non-smokers. No subject had experienced symptoms of a common cold during the last 4 weeks before the study and no subjects were receiving inhaled corticosteroids or other pharmaceutical treatment for asthma prevention for the same period of time. Subjects suffering from any other acute or chronic diseases were excluded. Written informed consent was obtained in all cases, and the study was approved by the institutional Ethics Committee.

Preparation of peripheral blood mononuclear cells (PBMC)

PBMC were obtained from whole blood by Ficoll centrifugation [5]. Major or minor RV subtypes (RV16, RV1b) were added at a final concentration of 5 infectious units per cell [multiplicity of infection (MOI)]. Supernatants were harvested after 48 h and stored at -70°C until further use. These conditioned media from normal or atopic asthmatic individuals were labelled as 'normal environment' or 'atopic environment', respectively.

Exposure of epithelial cells to RV under the influence of PBMC supernatants

The above supernatants (0.8 mL) were subsequently added to cultures of human bronchial epithelial cells (BEAS-2B),

at a confluence of 80%, grown in minimum essential medium (MEM) with Glutamax and 3% FSC. The cultures were placed in a humidified 5% CO_2 incubator at 37°C for 18 h. Then, a part of the supernatant (0.4 mL) was kept on ice, cells were exposed to the same RV serotype at a concentration of 1 MOI for 1 h with gentle shaking at room temperature, cultures were washed and cells were re-suspended in MEM containing 4% FSC, 4% tryptose phosphate, 1% MgCl_2 and the kept supernatant. Cell cultures were placed in a humidified 5% CO_2 incubator at 33°C . Supernatants were harvested and cells were obtained at 6 and 48 h, and stored at -70°C until further use.

In some experiments, cultures were supplemented with $10\text{ }\mu\text{g/mL}$ of neutralizing anti-IFN- γ mAb (R&D Systems Europe, Abingdon, UK) [19].

Cytotoxicity assay

RV-induced cytotoxicity was measured in 48-well plates of BEAS-2B cells exposed to PBMC supernatants and infected with RV at MOI = 5, as previously described [20]. After 48 h, BEAS-2B cells were stained with filtered crystal violet and de-stained with methanol, and the optical density (OD) was measured spectrophotometrically at 620 nm. RV-induced cytotoxicity was expressed as a proportion in comparison with a parallel well with non-infected cells, using the algorithm $(1 - \text{OD of infected cells} / \text{OD of non-infected cells}) \times 100$, as previously described [20].

Cytokine assays

Levels of IFN- γ , IL-10, IL-6, IL-8, RANTES and TGF- β 1 in PBMC and BEAS-2B supernatants were measured using commercially available ELISAs (DuoSet ELISA development system, R&D Systems) according to the manufacturer's recommended concentrations and ELISA protocols.

RV titration

BEAS-2B cells were lysed at 6 and 48 h and RV was titrated with serial 10-fold dilutions on Ohio-HeLa cells.

Statistical analysis

Data are presented as median (interquartile range). All data were log transformed because of their skewed distributions ($|\text{skewness}| > 0.7$). All variables' log transformations resulted in normal distributions. Comparisons were obtained using ANOVA followed by Student's *t*-test in the log-transformed variables. *P*-values $< 5\%$ were considered significant.

Results

Characterization of RV-stimulated PBMC supernatants from atopic asthmatic and normal subjects

Production of IFN- γ from RV-stimulated PBMC was significantly higher in normal than in atopic asthmatic subjects at 48 h, while levels of IL-10 were higher in cultures from asthmatic subjects compared with normal subjects, confirming our previous observations [5] (Table 1).

RV infection also resulted in a significant increase of IL-8 and a non-significant increase of IL-6 production from PBMC, and a parallel non-significant TGF- β 1 reduction in both atopic asthmatic and normal subjects (Table 1).

The RV-stimulated PBMC supernatants were tested with RT-PCR for the presence of vRNA before being added to BEAS-2B and were found to be negative (data not shown). These RV-stimulated PBMC supernatants were used as conditioned media in subsequent experiments, modelling either 'normal' or 'atopic asthmatic' immune environments.

RV-induced epithelial production of pro-inflammatory mediators under the influence of conditioned media from normal or atopic PBMC

BEAS-2B cells were infected or not with RV, with or without the presence of conditioned media from RV-stimulated PBMC. RV infection significantly up-regulated the baseline epithelial production of IL-6, IL-8 and RANTES at 48 h, as previously described [21]. Epithelial RV infection in the presence of conditioned media resulted in a statistically significant further up-regulation of IL-6, IL-8 and RANTES production (Fig. 1). This up-regulation was observed after correcting the effect of RV in PBMC by removing the RV-produced cytokines

in PBMC supernatants. However, significantly lower mediator levels were observed under the influence of the atopic medium compared with the normal conditioned medium [IL-6: 3.34 (12.86) ng/mL vs. 7.03 (27.41) ng/mL; IL-8: 10.2 (16.72) ng/mL vs. 10.64 (49.4) ng/mL; RANTES: 5.79 (10.17) ng/mL vs. 8.27 (3.58) ng/mL] (Figs 1a–c).

Furthermore, RV marginally up-regulated epithelial production of TGF- β 1 [baseline: 1.4 (3.4) pg/mL; 48 h after exposure: 4.3 (10.2) pg/mL]. Contrary to the above mediators, epithelial production of TGF- β 1 was significantly higher under the influence of the atopic environment in comparison with the normal environment [7.36 (7.4) pg/mL vs. 1.6 (3.1) pg/mL] (Fig. 1d).

Effect of IFN- γ neutralization on epithelial production of IL-6, IL-8, RANTES and TGF- β 1

To address the possibility that epithelial cytokine production is IFN- γ -dependent, cultures from three pairs of asthmatic and normal subjects were supplemented with neutralizing anti-IFN- γ mAb. Anti-IFN- γ mAb failed to reverse the RV-induced, PBMC supernatant-conditioned, epithelial production of IL-6, IL-8, RANTES or TGF- β (Table 2).

Effect of an atopic environment on RV replication in epithelial cells

RV titres increased at 6 h and peaked at 48 h post-infection of BEAS-2B cells. Higher viral titres were observed under the influence of the atopic rather than the normal environment at 6 h (2.7 ± 0.2 vs. 2.3 ± 0.1 , $P = 0.03$) as well as at 48 h (2.8 ± 0.4 vs. 1.9 ± 0.3 , $P = 0.01$) (Fig. 2).

Effect of an atopic environment on RV-induced epithelial cytotoxicity

By 48 h, epithelial cells seeded at 50% confluence and infected with 5 MOI of RV1b had a 43% (20%) reduction in viability, as previously described [20]. Exposure of epithelial cells to conditioned media significantly reduced RV-mediated cytotoxicity to 26% (17.1%) ($P = 0.001$). This PBMC-derived protective activity was significantly lower under the influence of an atopic environment compared with a normal [cytotoxicity of 38% (20.2%) vs. 14% (14%), respectively, $P \leq 0.001$] environment (Fig. 3).

Discussion

This study suggests that, under the influence of an atopic immune response, the epithelial inflammatory response to RV becomes suboptimal, associated with reduced viral clearance and higher RV-associated cytotoxicity. The preponderant view on the mechanism of RV-induced asthma exacerbations has been associated with the induction of epithelial inflammation [7, 22–25]. Despite the fact

Table 1. Pro-inflammatory mediators in peripheral blood mononuclear cell (PBMC) supernatants from atopic asthmatic and normal individuals before and after exposure to rhinovirus (RV)

Cytokines	Normal		Atopic	
	Not infected	Infected	Not infected	Infected
RANTES (ng/mL)	3.31 (2.44)	3.2 (3.19)	3.31 (2.23)	3.75 (3.04)
IL-6 (ng/mL)	0.62 (5.69)	2.06 (23.7)	0.10 (9.66)	1.25 (10.49)
IL-8 (ng/mL)	21.00 (51.61)	50.79 (43.85)*	33.19 (62.36) [†]	44.03 (46.95) [‡]
IL-10 (pg/mL)	96 (327)	70 (370)	87 (251)	248 (234) [‡]
IFN- γ (ng/mL)	0 (0)	0.77 (1.21)*	0 (0)	0.27 (0.69) ^{‡,§}
TGF- β 1 (ng/mL)	2.92 (2.85)	2.33 (1.04)	2.61 (3.83)	1.81 (2.08)

Data are log transformed and presented as median (inter-quartile range). All values are presented as median (inter-quartile range).

* $P < 0.05$, non-infected vs. infected in normal individuals.

[†] $P < 0.05$, atopic vs. normal individuals, non-infected PBMC.

[‡] $P < 0.05$, non-infected vs. infected in atopic individuals.

[§] $P < 0.05$, atopic vs. normal individuals, infected PBMC.

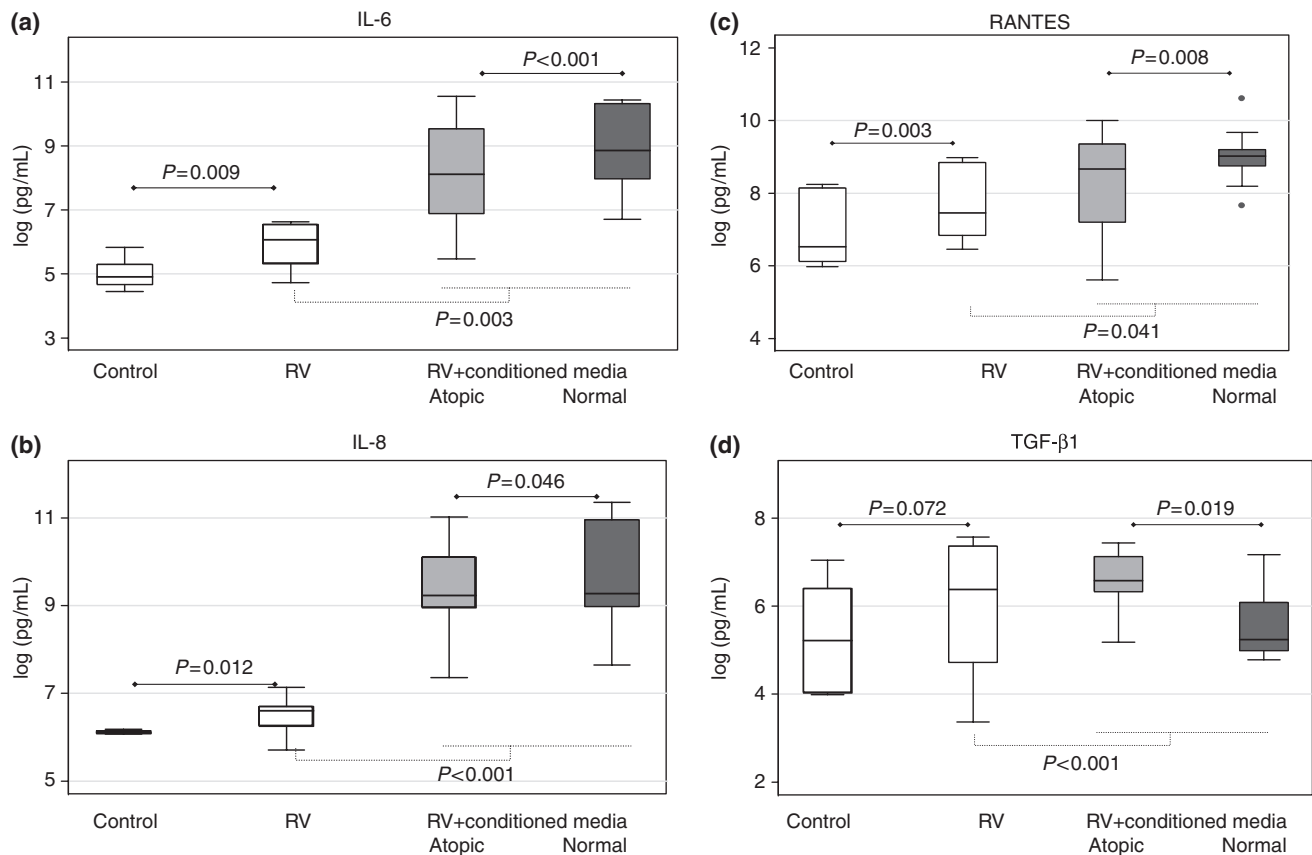


Fig. 1. Epithelial production of IL-6 (a), IL-8 (b), RANTES (c) and TGF-β1 (d) 48 h after rhinovirus (RV) infection and under the influence of conditioned media from RV-stimulated peripheral blood mononuclear cells from atopic asthmatic or normal subjects. Data are log transformed and presented as median. RV induced the baseline epithelial production of all mediators, which was further increased in the presence of conditioned media. Lower induction of IL-6, IL-8 and RANTES and higher induction of TGF-β1 were observed under the influence of the atopic environment in comparison with the normal one.

Table 2. Effect of IFN-γ neutralization on epithelial production of IL-6, IL-8, RANTES and TGF-β1 after stimulation of infected BEAS-2B with conditioned media from normal or atopic peripheral blood mononuclear cells (PBMC)

Cytokines	Atopic	Atopic + anti-IFN-γ	Normal	Normal + anti-IFN-γ
RANTES (ng/mL)	7.53 (6.82)	6.94 (8.72)	14.15 (6.37)	14.24 (9.42)
IL-6 (ng/mL)	4.43 (10.06)	3.98 (11.33)	15 (2.6)	14.02 (10.77)
IL-8 (ng/mL)	11.22 (30.42)	9.66 (29.73)	31.57 (35.06)	35.19 (47.09)
TGF-β1 (ng/mL)	1.49 (1.18)	2.26 (1.77)	1.42 (1.15)	1.48 (0.82)

There are no statistically significant differences between the groups. Data are log transformed and presented as median (inter-quartile range).

that bronchial epithelial cells are presumed to be the first site of RV infection, viraemia may occur during RV respiratory infections in normal children and is rather common in the early course of acute asthma exacerbations [4]. Bidirectional interactions between airway mucosal dendritic cells (DCs) and T cells in response to live virus *in situ* have been demonstrated [26]. Immediate recruitments of large numbers of DCs appear at the site of inflammation [27]. Furthermore, a systemic immune response develops rapidly. Therefore, the immune response to RV, both local and systemic, develops rapidly and in parallel to the bronchial epithelial response,

potentially conditioning the latter. Our system is intended to model this condition.

Our findings suggest that atopic asthmatic individuals may actually respond with less acute inflammation to RV, with respect to production of cytokines and chemokines such as IL-6, IL-8 and RANTES. This suboptimal acute response is associated with inadequate viral clearance and increased virus-induced damage, which may lead to the induction of airway hyperresponsiveness.

We have recently reported that a defective type-1 immune response against RV occurs in atopic asthmatic individuals [5]. Furthermore, RV-mediated induction of

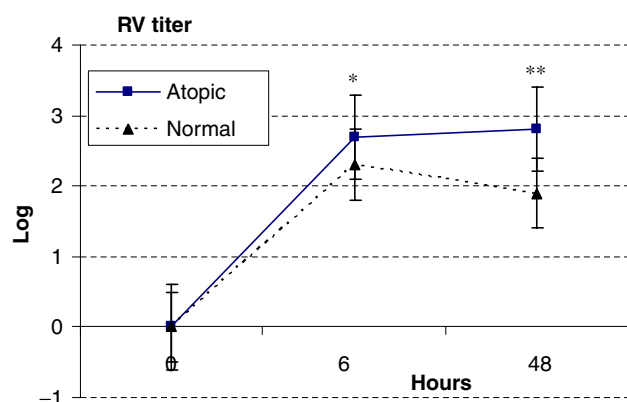


Fig. 2. Replication of rhinovirus (RV) in epithelial cells under the influence of peripheral blood mononuclear cell-conditioned media from atopic asthmatic or normal individuals. Significantly higher RV titres occur at 6 and 48 h post-infection in cells exposed to the atopic environment (* $P=0.03$, ** $P=0.01$).

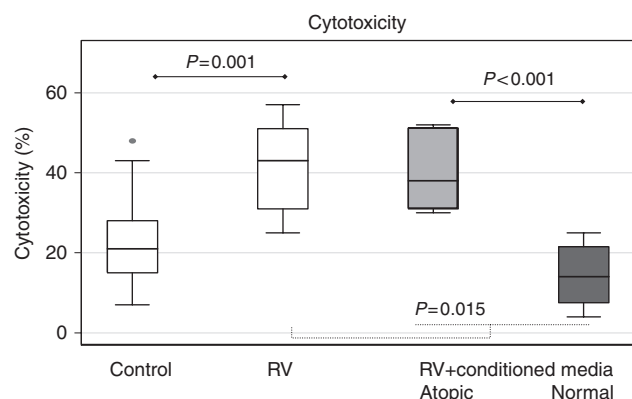


Fig. 3. Rhinovirus (RV) infection is able to induce epithelial cytotoxicity. Peripheral blood mononuclear cell supernatants from both atopic asthmatic and normal subjects protect epithelial cells from such cytotoxicity. However, this protection is significantly lower under the influence of the atopic rather than the normal environment.

bronchial inflammation has been documented in several instances. Assuming the validity of the above observations, an interaction between the immune and epithelial responses may be crucial to the clinical outcome of RV infection. Pre-treatment of epithelial cells with different Th1 or Th2 cytokines alone or in combination, before infection with RV, has been reported to induce considerable changes in both RV replication and inflammatory response [14, 28, 29]. However, the essential difference of the model used in this study is the use of conditioned media, which contain a complex mediator mixture, possibly closer to the *in vivo* situation, and also at more relevant relative concentrations. While the effect of each individual cytokine may be important to a different extent in the fate of RV epithelial infection, it is their combined – synergistic or contrasting – effects that would determine the outcome of infection.

Using this model it was shown that RV-mediated epithelial IL-6, IL-8 and RANTES production is decreased under the influence of PBMC-conditioned media from atopic asthmatic patients. In line with these results, previous studies have shown that treatment of epithelial cells with Th1 cytokines tends to induce increased epithelial release of IL-6, IL-8 and RANTES [14, 30, 31], while Th2 cytokines have the opposite result [31, 32]. However, these findings were not always reproducible [33], a discrepancy that could be explained by differences in the amounts of added cytokines or their combinations. In addition, experimental RV infection in healthy individuals has led to higher levels of sputum IL-6 and IL-8 during the acute cold, in comparison with patients with atopic asthma, although not significantly, possibly due to the small number of subjects in that study [34]. This early intense inflammatory response observed in subjects with a 'normal' immune response may lead to viral clearance, followed by fast clinical resolution, in line with our observation of reduced viral titres in epithelial cells exposed to the 'normal' conditioned media. Almost identical with the findings of the current study, Wark et al. [35] very recently demonstrated that primary human epithelial cells from asthmatic patients have an abnormal innate response to RV infection, resulting in increased viral replication, as compared with epithelial cells from healthy controls. In that study, cytokine responses did not differ between cells from normal or asthmatic subjects; however, ICAM-1, another marker of the epithelial inflammatory response, was also more highly inducible in normals [35]. Until recently, RV was considered unable to induce epithelial cytotoxicity. However, in a recent study we have shown that this may occur even extensively, in sparsely cultured epithelial cells, modelling the disrupted asthmatic epithelium [20, 36]. Here, we demonstrate that RV-induced cytotoxicity is higher when epithelial cells are exposed to an atopic environment, once again in agreement with the findings of Wark et al. [35] who went on to demonstrate that resistance of cells from asthmatics to early apoptosis, associated with reduced IFN- β production, may account for these differences. The fact that conditioning of a continuous cell line by a normal or atopic asthmatic immune environment resulted in similar findings to a model of primary cells from atopic asthmatic and normal subjects, particularly with respect to RV replication and epithelial cell cytotoxicity, suggests that the primary cell defect in asthmatic subjects may be the result of a similar conditioning occurring *in vivo*. It should be noted that the asthmatic patients included in the study of Wark et al. [35] were all atopic and that cells were used for the experiments while on passage 2.

In conclusion, we propose that when an atopic asthmatic host is infected with RV, a Th2-skewed immune response predisposes epithelial cells to a reduced inflammatory

response associated with increased viral proliferation and augmented virus-mediated cell damage, while a pro-fibrotic, remodelling-associated response is promoted. These evidences provide a novel insight into the mechanisms of virus-induced asthma exacerbations and support the possibility that viral infections may be involved in the induction of airway remodelling.

Acknowledgements

Funding: This study was supported by the 2002–3 Research Scholarship in Pediatric Pulmonology by GlaxoSmithKline, Hellas (ELKE 70/3/7106).

References

- 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**:1225–8.
- Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. *BMJ* 1993; **307**:982–6.
- Papadopoulos NG, Papi A, Psarras S, Johnston SL. Mechanisms of rhinovirus-induced asthma. *Paediatr Respir Rev* 2004; **5**: 255–60.
- Xatzipsalti M, Kyrana S, Tsolia M *et al*. Rhinovirus viremia in children with respiratory infections. *Am J Respir Crit Care Med* 2005; **172**:1037–40.
- Papadopoulos NG, Stanciu LA, Papi A, Holgate ST, Johnston SL. A defective type 1 response to rhinovirus in atopic asthma. *Thorax* 2002; **57**:328–32.
- Bossios A, Xatzipsalti M, Manoussakis E, Psarras F, Saxoni-Papageorgiou P, Papadopoulos NG. Expression of costimulatory molecules in peripheral blood mononuclear cells of atopic asthmatic children during virus-induced asthma exacerbations. *Int Arch Allergy Immunol* 2004; **134**:223–6.
- Gern JE, Vrtis R, Grindle KA, Swenson C, Busse WW. Relationship of upper and lower airway cytokines to outcome of experimental rhinovirus infection. *Am J Respir Crit Care Med* 2000; **162**:2226–31.
- Parry DE, Busse WW, Sukow KA, Dick CR, Swenson C, Gern JE. Rhinovirus-induced PBMC responses and outcome of experimental infection in allergic subjects. *J Allergy Clin Immunol* 2000; **105**:692–8.
- Papadopoulos NG, Bates PJ, Bardin PG *et al*. Rhinoviruses infect the lower airways. *J Infect Dis* 2000; **181**:1875–84.
- Johnston SL, Papi A, Bates PJ, Mastrorade JG, Monick MM, Hunninghake GW. Low grade rhinovirus infection induces a prolonged release of IL-8 in pulmonary epithelium. *J Immunol* 1998; **160**:6172–81.
- Papadopoulos NG, Papi A, Meyer J *et al*. Rhinovirus infection up-regulates eotaxin and eotaxin-2 expression in bronchial epithelial cells. *Clin Exp Allergy* 2001; **31**:1060–6.
- Psarras SVE, Xatzipsalti M, Bossios A *et al*. Vascular endothelial growth factor-mediated induction of angiogenesis by human rhinoviruses. *J Allergy Clin Immunol* 2006; **117**:291–7.
- Ghildyal R, Dagher H, Donninger H *et al*. Rhinovirus infects primary human airway fibroblasts and induces a neutrophil chemokine and a permeability factor. *J Med Virol* 2005; **75**:608–15.
- Konno S, Grindle KA, Lee WM *et al*. Interferon-gamma enhances rhinovirus-induced RANTES secretion by airway epithelial cells. *Am J Respir Cell Mol Biol* 2002; **26**:594–601.
- Sethi SK, Bianco A, Allen JT, Knight RA, Spiteri MA. Interferon-gamma (IFN-gamma) down-regulates the rhinovirus-induced expression of intercellular adhesion molecule-1 (ICAM-1) on human airway epithelial cells. *Clin Exp Immunol* 1997; **110**:362–9.
- Fischer JE, Johnson JE, Kuli-Zade RK *et al*. Overexpression of interleukin-4 delays virus clearance in mice infected with respiratory syncytial virus. *J Virol* 1997; **71**:8672–7.
- Kostense S, Sun WH, Cotter R *et al*. Interleukin 12 administration enhances Th1 activity but delays recovery from influenza A virus infection in mice. *Antiviral Res* 1998; **38**:117–30.
- Davies DE. The bronchial epithelium: translating gene and environment interactions in asthma. *Curr Opin Allergy Clin Immunol* 2001; **1**:67–71.
- Varga SM, Beckman NA, Chu M, Braciale TJ. Sensitive detection and quantitation of mouse eosinophils in tissues using an enzymatic eosinophil peroxidase assay: its use to rapidly measure pulmonary eosinophilia during experimental respiratory syncytial virus infection of mice. *J Immunol Methods* 2002; **262**:111–20.
- Bossios A, Psarras S, Gourgoutis D, Constantopoulos GA, Saxoni-Papageorgiou P, Papadopoulos NG. Rhinovirus infection induces cytotoxicity and delays wound healing in bronchial epithelial cells. *Respir Res* 2005; **6**:114.
- Zhang L, Redington AE, Holgate ST. RANTES: a novel mediator of allergic inflammation? *Clin Exp Allergy* 1994; **24**:899–904.
- 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** (Part 1):879–86.
- Grunberg K, Sterk PJ. Rhinovirus infections: induction and modulation of airways inflammation in asthma. *Clin Exp Allergy* 1999; **29** (Suppl. 2):65–73.
- Trigg CJ, Nicholson KG, Wang JH *et al*. Bronchial inflammation and the common cold: a comparison of atopic and non-atopic individuals. *Clin Exp Allergy* 1996; **26**:665–76.
- Holgate ST, Davies DE, Lackie PM, Wilson SJ, Puddicombe SM, Lordan JL. Epithelial-mesenchymal interactions in the pathogenesis of asthma. *J Allergy Clin Immunol* 2000; **105** (Part 1): 193–204.
- Huh JC, Strickland DH, Jahnsen FL *et al*. Bidirectional interactions between antigen-bearing respiratory tract dendritic cells (DCs) and T cells precede the late phase reaction in experimental asthma: DC activation occurs in the airway mucosa but not in the lung parenchyma. *J Exp Med* 2003; **198**:19–30.
- Stumbles PA, Strickland DH, Pimm CL *et al*. Regulation of dendritic cell recruitment into resting and inflamed airway epithelium: use of alternative chemokine receptors as a function of inducing stimulus. *J Immunol* 2001; **167**:228–34.
- Subauste MC, Jacoby DB, Richards SM, Proud D. Infection of a 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**:549–57.
- Sedgwick JB, Menon I, Gern JE, Busse WW. Effects of inflammatory cytokines on the permeability of human lung microvascular

- endothelial cell monolayers and differential eosinophil transmigration. *J Allergy Clin Immunol* 2002; **110**:752–6.
- 30 Striz I, Mio T, Adachi Y, Carnevali S, Romberger DJ, Rennard SI. Effects of interferons alpha and gamma on cytokine production and phenotypic pattern of human bronchial epithelial cells. *Int J Immunopharmacol* 2000; **22**:573–85.
- 31 Striz I, Mio T, Adachi Y, Romberger DJ, Rennard SI. Th2-type cytokines modulate IL-6 release by human bronchial epithelial cells. *Immunol Lett* 1999; **70**:83–8.
- 32 Fujisawa T, Kato Y, Atsuta J *et al.* Chemokine production by the BEAS-2B human bronchial epithelial cells: differential regulation of eotaxin, IL-8, and RANTES by TH2- and TH1-derived cytokines. *J Allergy Clin Immunol* 2000; **105** (Part 1):126–33.
- 33 Striz I, Mio T, Adachi Y, Robbins RA, Romberger DJ, Rennard SI. IL-4 and IL-13 stimulate human bronchial epithelial cells to release IL-8. *Inflammation* 1999; **23**:545–55.
- 34 Fleming HE, Little FF, Schnurr D *et al.* Rhinovirus-16 colds in healthy and in asthmatic subjects: similar changes in upper and lower airways. *Am J Res Crit Care Med* 1999; **160**:100–8.
- 35 Wark PAJS, Bucchieri F, Powell R *et al.* Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med* 2005; **201**:937–47.
- 36 Papadopoulos NG, Bossios A, Xatzipsalti M, Karpathios T, Saxoni-Papageorgiou P, Gourgiotis D. Rhinovirus-induced cytotoxicity on bronchial epithelial cells is serotype and cell-density dependent. *Allergy* 2001; **56**:36.