

Rhinovirus-induced alterations on peripheral blood mononuclear cell phenotype and costimulatory molecule expression in normal and atopic asthmatic subjects

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Summary

Background Rhinovirus (RV) infection is the commonest trigger of acute asthma exacerbations; however, the immune response to these viruses and any potential implications in the mechanisms leading to asthma exacerbations are not well understood.

Objective To assess the effects of *in vitro* RV infection on the phenotype and expression of costimulatory molecules on peripheral blood mononuclear cells (PBMC) from normal and atopic asthmatic subjects, as a model for RV antigen presentation.

Methods PBMC from seven normal and seven asthmatic subjects were exposed to one infectious unit/cell of RV16 for 48 h. Surface expression of CD25, CD28, CD40, CD54, CD80, CD86 and CTLA-4 was evaluated on CD3, CD4, CD8, CD14 and CD19 PBMC subpopulations by three-colour flow cytometry.

Results No changes in the percentage of CD3, CD4, CD8 or CD19 were observed. CD14 was significantly reduced by the infection and this was more pronounced in normal subjects. On Th cells CTLA-4 was increased after RV infection only in the asthmatic group. Levels of CD80 and CD86 in the control cultures were lower in the asthmatic group. RV infection induced a significant increase of CD80 on monocytes and of CD86 on B cells, which occurred in both groups but were less marked in atopic asthmatic subjects.

Conclusion Exposure of PBMC to RV is able to activate the antigen presentation machinery. Differences between normal and atopic asthmatic individuals are compatible with the hypothesis that an aberrant immune response to RV may be involved in the development of acute exacerbations in atopic asthmatic subjects.

Keywords antigen presentation, asthma, CD14, costimulation, flow cytometry, rhinovirus, surface markers

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Introduction

It is now well documented that the majority of acute asthma exacerbations, both in children and adults, follow upper respiratory tract infections [1,2]. Human rhinoviruses (RV) are the causative microorganisms for the majority of such infections [3]. However, the pathophysiology of rhinovirus-induced asthma exacerbations is not well understood. Current evidence suggests that RV infection of airway epithelium is involved in the initiation and/or augmentation of inflammatory responses related to asthma [4,5]. Nevertheless, very little is known about the immune response to these viruses and its possible implications in the development of exacerbations of asthma.

Changes in peripheral blood leucocyte numbers [6] and the lymphocyte infiltration of the bronchial mucosa after natural or experimental RV infections [7,8], indicate that both systemic

and local immune mobilization occurs. Until recently, cytokine responses to RV have been considered to be of a type-1 phenotype, with induction of IFN- γ but no IL-4 [9], even though production of IL-4 has been reported in a small number of RV-specific T cell clones [10]. However, we have recently shown that following exposure to RV, peripheral blood mononuclear cells (PBMC) can also produce type-2 cytokines, including IL-4, IL-10 and IL-13, while in atopic asthmatic individuals the response is defective, with a shift towards the type-2 phenotype, expressed as a significantly lower IFN- γ : IL-4 ratio [11]. This evidence suggests that the immune response to RV may also be involved in the induction of asthma exacerbations, through pathways with important similarities to the aberrant responses to allergens.

While some elements of the T cell response to RV can be induced by monocyte-derived soluble factors without requirement for cell contact [12], suggesting a non-specific pathway, there is no doubt that specific anti-RV T cell responses also develop [10]. In this context, activation of the antigen presentation machinery, and especially costimulation, could be important in the orchestration of this response, as well as a

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potential therapeutic target for virus-mediated airway hyper-responsiveness and asthma. Very recently we described the expression of costimulatory molecules on primary and continuous human airway epithelial cells and their modulation by RV infection [13]. In order to further test the hypothesis that RV infection would activate the antigen presentation machinery, we used RV exposure of PBMC derived from normal and atopic asthmatic subjects to model this process.

Materials and methods

Subjects

Seven healthy, non-atopic adult volunteers (three male, age range 20–54 years) and seven subjects with a history of mild to moderate atopic asthma (three male, age range 24–57 years) were included in the study. Atopy was documented by positive skin prick tests and/or specific IgE to one or more common environmental allergens, and an elevated total serum IgE (range 100–950 IU/mL). Asthma was diagnosed by history, spirometry and histamine challenge. The histamine concentration that reduced the FEV₁ by 20% (PC₂₀) was less than 8 mg/mL in all asthmatic patients and >16 mg/mL in normal subjects. Two asthmatic subjects were receiving low dose inhaled corticosteroids, while the rest of the subjects were not taking any medication at the time of the study. No concomitant diseases were present and no subject had experienced a cold or an exacerbation of asthma during the previous month. Venous blood was drawn in the morning hours from a normal and an asthmatic subject and samples were processed in parallel. Informed consent was obtained in all cases and the study was approved by the Southampton and SW Hants Joint Research Ethics Committee.

Virus preparations

RV16, a major group RV subtype with known clinical relevance [7], was initially obtained from Drs W. Busse and E. Dick (Madison, WI, USA). The identity of the virus was confirmed by neutralization with specific antiserum (ATCC, Rockville, MD, USA). RV16 was propagated in large quantities in Ohio HeLa cells, at 33 °C, in a humidified, 5% CO₂ incubator. When full cytopathic effect (CPE) developed, cells and supernatants were harvested, frozen and thawed twice, clarified, filter-sterilized, aliquoted and stored at –70 °C. For each experiment, a new vial was rapidly thawed and used immediately. The stock used in these experiments had a titre of 10⁷ tissue culture infectious doses per millilitre.

RV16 was partially purified for some experiments, in order to remove soluble factors of HeLa cell origin. An equal volume of saturated ammonium sulphate was added to RV16 suspensions, and virus was precipitated by centrifugation at 3000 g for 20 min. The precipitate was resuspended in phosphate-buffered saline (PBS), dialysed overnight at 4 °C over PBS, using a 50,000-kDa molecular weight cut off (MWCO) membrane (Sigma Chemical Co., Poole, UK), incubated for 1 h at room temperature with 50 µL/mL of *Staphylococcus* protein A-Sepharose Fast Flow (Sigma), clarified and stored at –70 °C. Titration of the virus stocks was carried out according to standard methods [4,5].

To further study the specificity of the responses, RV16 was inactivated by pre-incubation with 1 mg/mL of soluble intercellular adhesion molecule-1 (sICAM-1; kindly donated by P. Esmon, Bayer, Berkeley, CA, USA) for 1 h at room temperature, and used in some experiments in parallel with non-inactivated control preparations.

Experimental design

PBMC were obtained from whole blood by standard Ficoll centrifugation. They were washed and resuspended in RPMI-1640 medium with Glutamax (Life Technologies, Uxbridge, UK), containing 2.5% foetal calf serum (FCS) and 2.5% human AB serum, without antibiotics, at a final concentration of 2E6/mL, in 24-well plates. Virus preparations were added at a multiplicity of infection (MOI, infectious units per cell) of 1, since optimal responses have been consistently documented in this and other models with this virus concentration [5,14]. The final volume was 2 mL/well. Non-infected Ohio-HeLa cell lysates were used as negative controls. After 1 h of gentle shaking at room temperature cultures were placed in a humidified, 5% CO₂ incubator, at 33 °C. Cells were harvested 48 h after infection, washed twice with PBS containing 0.1% bovine serum albumin (BSA, Sigma), adjusted to 1E6/mL and analysed. The selection of the 48 h time-point for analysis was based on initial kinetic experiments with CD80 and CD86, as well as on its potential biological relevance in relation to *in vivo* common cold kinetics.

Flow cytometric analysis of surface markers

Surface markers were analysed by three-colour flow cytometry. Peridinin chlorophyll protein (PerCP), fluorescein-isothiocyanate (FITC) or phycoerythrin (PE)-conjugated monoclonal antibodies were added to 0.1 mL of cell suspensions (0.1E6 cells) and incubated for 30 min at 4 °C. Antibodies against CD3, CD4, CD8, CD14, CD19, CD25, CD28, CD40, CD54, CD80, CD86 and CTLA-4 (Becton Dickinson, Mountain View, CA, USA) were used. Antibodies were combined in order to quantify expression of surface markers (CD25, CD28, CD40, CD54, CD80, CD86 and CTLA-4) on different cell types (CD3, CD4, CD8, CD14 and CD19). After washing with PBS-0.1% BSA, stained cells were resuspended in 0.5 mL of the same solution and analysed on a FACScan (Becton Dickinson, San Jose, CA, USA) flow cytometer. Data from 10 000 cells were acquired and standard quadrant and histogram analyses were performed using the Lysis II software. Cells were gated by forward and side scatter characteristics and a CD3-PerCP antibody that was included in all cases in order to include or exclude T lymphocytes or B lymphocytes and monocytes, respectively. Both cell percentages and mean fluorescence intensity (MFI) were calculated.

Statistical analysis

Analysis of variance (ANOVA) was used for the comparisons of normally distributed variables, while variables that did not fit to a normal distribution were compared using Kruskal-Wallis non-parametric ANOVA. Values are expressed as mean ± standard error of mean. Calculations were performed with the Statistica 4.5 for Windows software package. Values of *P* < 0.05 were considered significant.

Results

Phenotype

In control cultures, percentages of T cells (CD3⁺), B cells (CD19⁺) and monocytes (CD14⁺) were similar between normal and asthmatic subjects. Furthermore, there were no differences between the groups for CD4⁺, CD8⁺, CD4⁺ CD8⁺ double positive or CD4-CD8-double negative T cells (Table 1). The CD4:CD8 ratio was marginally higher in the atopic asthmatic group (normal 2.54 ± 0.34 , atopic asthmatic 4.48 ± 1.38 , $P = 0.08$). Infection with RV16 did not affect the number of T cells or B cells in the culture. After the infection there was a decrease of the CD4:CD8 ratio in the asthmatic group (2.60 ± 0.67), resulting from higher CD8 and lower CD4 cell percentages in this group; however, these changes were not statistically significant. In contrast, there was a significant ($P = 0.01$) decrease in the number of CD14⁺ monocytes in infected cultures. This was more pronounced in cultures from the normal group, with a >3-fold down-regulation, than the atopic group (1.7-fold down-regulation, $P < 0.05$, Table 1).

CD25 was examined as an activation marker in the whole PBMC population, and in CD4⁺ and CD8⁺ T cells. A significant increase was observed in PBMC CD25 expression after RV infection in both groups (normal: control $8.67\% \pm 1.70$, infected $14.00\% \pm 2.57$; asthmatic: control $5.83\% \pm 1.49$, infected $12.5\% \pm 1.71$, $P = 0.005$ between uninfected and infected cultures; MFI: 11.33 ± 2.18 , 49.5 ± 11.84 , 11.17 ± 3.49 , 37.33 ± 6.44 , respectively, $P = 0.0002$, Fig. 1). When CD4⁺ and CD8⁺ T cells were analysed separately no significant differences were observed either between the groups or before and after infection, although there was a trend for an increase in CD25 expression on CD8⁺ T cells (normal: control $0.8\% \pm 0.3$, infected $2.3\% \pm 1.2$; asthmatic: control $0.8\% \pm 0.4$, infected $1.8\% \pm 0.6$, $P > 0.1$).

Costimulatory molecule expression

CD28, CTLA-4, CD54, CD40, CD80 and CD86 were examined on CD3⁺ CD4⁺ and CD3⁺ CD8⁺ T lymphocytes. There were no differences between the normal and asthmatic groups in non-infected cultures. Furthermore, RV infection did not induce any statistically significant changes on these markers in comparison with the control cultures, in normal subjects. However, in the atopic asthmatic group a significant 3.5-fold increase in CTLA-4 on CD4⁺ cells was induced by the infection (Table 2).

Considerable changes of CD80 and CD86 expression with regard to RV16 infection and atopic status were observed on

monocytes and B cells, respectively. The percentage of CD80 on CD14⁺ cells was lower in non-infected cells of atopic asthmatic patients in comparison with normal controls (asthmatic $13.5\% \pm 2.5$, normal $31.8\% \pm 6.8$, $P = 0.003$). RV16 infection induced a significant up-regulation in both groups (values post-infection: asthmatic $32.6\% \pm 8.7$, normal $70.5\% \pm 9.0$,

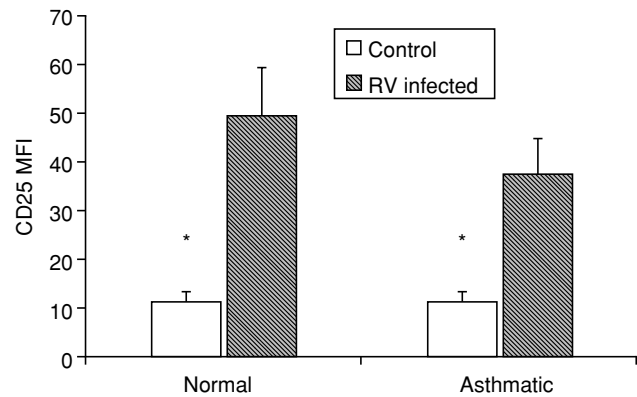


Fig. 1. Mean fluorescence intensity (MFI) of CD25 expressed on PBMC from normal or atopic asthmatic subjects 48 h after exposure to RV16 or sham-infected control. A significant virus-mediated up-regulation is observed in both groups. * $P < 0.05$.

Table 2. Expression of costimulatory molecules (% mean $\pm$ SEM) on CD4⁺ and CD8⁺ T cells from normal and atopic subjects 48 h after exposure to RV16 or HeLa cell extract alone (control)

		Normal		Asthmatic	
		Control	RV infected	Control	RV infected
CD4	CD28	94.50 $\pm$ 1.71	94.33 $\pm$ 1.33	96.83 $\pm$ 1.22	96.83 $\pm$ 1.42
	CTLA-4	1.00 $\pm$ 0.45	1.17 $\pm$ 0.60	1.17 $\pm$ 0.54	4.00 $\pm$ 1.57*
	CD54	24.20 $\pm$ 2.93	25.60 $\pm$ 4.00	24.20 $\pm$ 4.79	25.60 $\pm$ 4.63
	CD40	7.00 $\pm$ 3.78	3.50 $\pm$ 0.42	4.50 $\pm$ 1.14	3.00 $\pm$ 0.82
	CD80	1.60 $\pm$ 0.56	6.60 $\pm$ 4.50	9.60 $\pm$ 8.31	19.40 $\pm$ 16.83
	CD86	7.80 $\pm$ 3.05	6.80 $\pm$ 3.97	3.20 $\pm$ 1.51	8.60 $\pm$ 5.67
CD8	CD28	64.50 $\pm$ 7.41	58.83 $\pm$ 5.58	67.50 $\pm$ 9.94	72.17 $\pm$ 5.39
	CTLA-4	0.50 $\pm$ 0.34	1.17 $\pm$ 0.48	0.33 $\pm$ 0.21	0.50 $\pm$ 0.34
	CD54	31.60 $\pm$ 8.20	27.60 $\pm$ 8.30	18.00 $\pm$ 4.73	24.00 $\pm$ 5.35
	CD40	3.00 $\pm$ 1.73	1.50 $\pm$ 0.29	4.50 $\pm$ 2.60	4.00 $\pm$ 2.31
	CD80	4.20 $\pm$ 3.38	2.40 $\pm$ 2.19	4.60 $\pm$ 4.20	3.20 $\pm$ 2.49
	CD86	2.00 $\pm$ 0.65	1.60 $\pm$ 0.62	2.20 $\pm$ 0.84	1.00 $\pm$ 0.58

* $P < 0.05$ in comparison with non-infected control.

Table 1. Phenotype of PBMC from normal and asthmatic subjects 48 h after exposure to RV16 (infected) or HeLa cell extract (control)

	Normal		Asthmatic	
	Control	RV infected	Control	RV infected
CD3	64.50 $\pm$ 4.09	60.67 $\pm$ 4.45	62.00 $\pm$ 6.35	60.00 $\pm$ 7.32
CD4	64.33 $\pm$ 3.03	65.50 $\pm$ 3.45	69.83 $\pm$ 4.44	59.00 $\pm$ 9.60
CD8	27.33 $\pm$ 3.11	24.33 $\pm$ 3.07	21.00 $\pm$ 3.68	27.17 $\pm$ 3.54
CD4/CD8	2.54 $\pm$ 0.34	2.97 $\pm$ 0.47	4.48 $\pm$ 1.38*	2.60 $\pm$ 0.67
CD19	4.17 $\pm$ 1.49	3.00 $\pm$ 1.24	4.67 $\pm$ 1.38	3.33 $\pm$ 1.18
CD14	9.00 $\pm$ 2.22	2.83 $\pm$ 0.70†	11.00 $\pm$ 1.46	6.50 $\pm$ 1.52†

CD3, CD14 and CD19 positive cells are expressed as a percentage of total PBMC. CD4 and CD8 are percentages of T cells. * $0.05 < P < 0.1$ in comparison with normal control. † $P < 0.01$ control vs. RV infected, $P < 0.05$ normal vs. asthmatic.

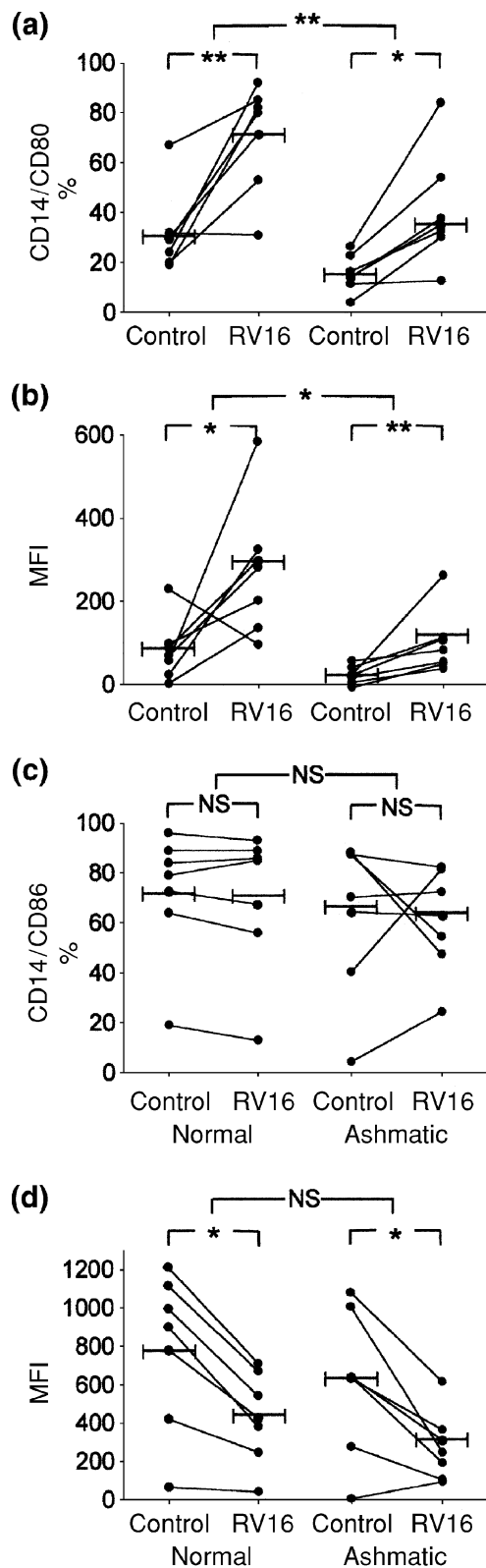


Fig. 2. CD80 (B7.1) and CD86 (B7.2) expression on CD14⁺ monocytes from normal and atopic asthmatic subjects 48 h after exposure to RV16 or sham-infected control. An RV-mediated increase of CD80% (a) and mean fluorescence intensity (MFI) (b) is observed in both groups, while reduced expression is observed in the atopic asthmatic in comparison with the normal group. The percentage of monocytes expressing CD86 (c) remained unchanged after RV16 infection, while MFI (d) was reduced by the infection in both groups. * $P < 0.05$, ** $P < 0.01$, NS = non-significant.

$P = 0.007$), which was more pronounced in the cultures from normal subjects (percentage of increase: normal $38.9\% \pm 9.1$, asthmatic $20.1\% \pm 5.3$, $P = 0.1$, Fig. 2a). Similar changes were observed when the mean fluorescence intensity (MFI) was evaluated. At baseline, the MFI of normal controls was 79.67 ± 32.91 vs. 29.33 ± 9.51 of the asthmatic group ($P = 0.001$). Infection with RV16 was able to up-regulate CD80 MFI to 270.50 ± 71.44 in normals and 119.00 ± 35.79 in asthmatics (normal vs. asthmatics, $P = 0.03$, uninfected vs. infected, $P = 0.006$, Fig. 2b). In contrast, there were no differences between the groups in the percentage of monocytes expressing CD86 (Fig. 2c), while a significant decrease of fluorescence intensity was observed after infection in both groups (normal: control 782 ± 171 , infected 431 ± 99 , asthmatic: control 620 ± 159 , infected 316 ± 77 , $P = 0.05$ between uninfected and infected cultures, Fig. 2d).

The expression of CD80 on B cells was similar in normal and asthmatic subjects and did not change significantly after RV16 infection (respective values $18.17\% \pm 3.81$, $18.00\% \pm 7.49$ before and $12.33\% \pm 4.10$, $11.50\% \pm 2.25$ after infection). In contrast, expression of CD86 on B cells was significantly induced by RV infection in both groups, while it was higher in cultures from normal than atopic asthmatic subjects for both percentage of expression (Fig. 3) and mean fluorescence intensity (respective values in normal and atopic subjects 41.0 ± 7.3 and 16.3 ± 3.6 before and 54 ± 15.2 and 55.7 ± 14.8 after infection, $P = 0.025$).

Effects of purified and inactivated virus preparations

In order to assess whether the observed changes were specifically due to RV infection and not related to virus culture by-products, purified as well as inactivated RV preparations were used. When purified RV16 was used to infect PBMC in parallel with non-purified preparations, no differences in expression of CD3, CD14, CD14/CD80 or CD19/CD86 were observed (Fig. 4). Pre-incubation of RV16 with sICAM-1 completely reversed the RV-mediated increase in CD80 on monocytes and CD86 on B cells (Fig. 4).

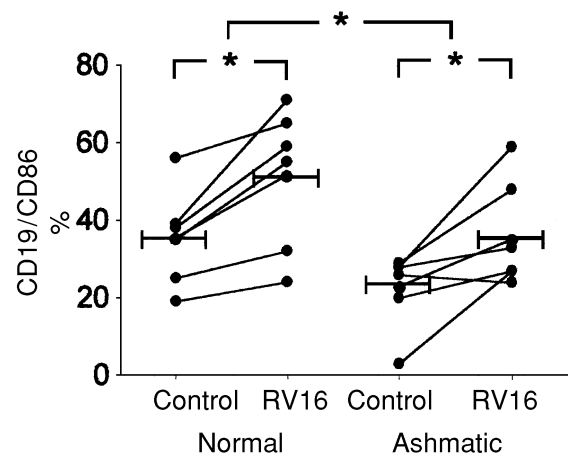


Fig. 3. Expression of CD86 (B7.2) on CD19⁺ B cells from normal and atopic asthmatic subjects exposed to RV16. Levels of CD86 were lower in the atopic asthmatic group, while an RV-mediated significant increase is observed in both groups. * $P < 0.05$.

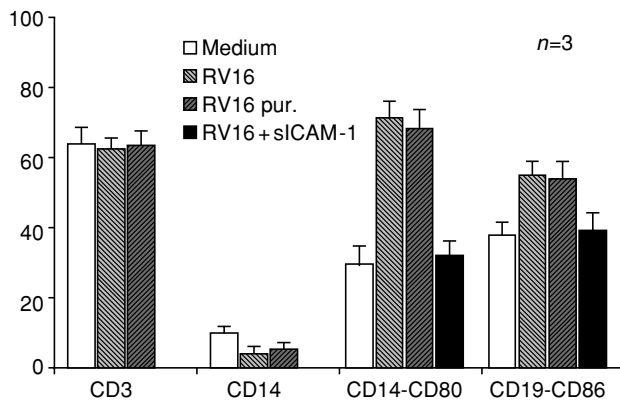


Fig. 4. Use of partially purified RV16 (RV16 pur.) produced identical results in regard to RV-mediated changes in expression of CD3, CD14, CD14/CD80 and CD19/CD86, indicating that there was no interference by unrelated HeLa cell-derived factors in the model. Pre-incubation of RV16 with soluble ICAM-1 (sICAM-1), the receptor of RV16, was able to inhibit completely virus-mediated increase of CD80 on monocytes and CD86 on B cells, suggesting that receptor-mediated viral entry is necessary for the production of these effects.

Discussion

The results of this study indicate that RVs are capable of inducing significant changes in the phenotype of immunocompetent cells, exploring for the first time the modulation of costimulatory molecules by these viruses in normal and atopic asthmatic subjects. The antigen-presenting cells in these cultures, i.e. monocytes and B cells, up-regulated their costimulatory surface antigens, in the case of the former CD80 and the latter CD86. On T cells, CTLA-4 was up-regulated by RV only on CD4⁺ cells of atopic asthmatic subjects. Inhibition experiments, as well as the use of partially purified RV, confirmed that the observed changes were virus specific and require contact of RV with ICAM-1 and probable entry of RV in the cells. The presence of serum antibodies against RV16 has not been evaluated in this study; however, it is documented that cellular immune responses to RVs develop independent to specific antibody formation and a significant cross-reactivity exists between cellular responses to different serotypes [10].

It is now well established that costimulatory signals are vital in the direction of T cell responses towards activation or anergy [15]. The CD28-CTLA-4/B7 pathway is the most well-studied costimulatory pathway and increasing evidence indicates that its modifications could be important in the induction of the aberrant immune responses observed in atopic asthma [16]. Although RVs have been recognized as the commonest trigger of asthma exacerbations in children and adults [1,2], very few data are available on the immune response against these viruses. Any potential implications of such a response in the development of acute asthma exacerbations are therefore largely unexplored. Specific T cell responses against RVs are known to develop [10], while there is indirect evidence indicating that a systemic immune response to this virus occurs *in vivo* [6].

We have recently demonstrated that RVs are capable of inducing both type-1 and type-2 responses in PBMC. Furthermore, in atopic asthmatic individuals a shift towards the type-2 phenotype was observed [11]. It is well documented that such a shift may interfere with virus clearance [17], thus

suggesting a possible mechanism through which exaggerated responses might occur in atopic asthmatic individuals. Induction of CTLA-4 on Th cells of atopic asthmatic but not normal individuals documented in this study is compatible with such a hypothesis. CTLA-4 functions as an inhibitory receptor for CD80 and CD86, probably playing a regulatory role in this costimulation pathway [18]. CTLA-4 cross-linking inhibits T cell proliferation and results in antigen-specific apoptosis [19]. It is therefore possible that increased expression of this molecule on T cells from atopic subjects during RV infection may result in an altered anti-RV response. It could also explain why antigen-induced lymphocyte proliferation is impaired, while mitogen-induced proliferation is preserved, during colds [20].

The number of monocytes expressing CD80 and B cells expressing CD86 was higher after RV infection in cultures from normal subjects; however, this was attributable both to a higher degree of up-regulation as well as higher baseline values in this group. A lower degree of costimulatory molecule up-regulation in atopic asthmatics supports the hypothesis that the antiviral response is suboptimal in these subjects. Nevertheless, decreased values after 48 h of culture in medium alone are more difficult to interpret. It should be noted that no differences between normal and asthmatic subjects were observed in freshly drawn PBMC (data not shown). Altered responses to serum components and/or baseline cytokine production, such as IL-10 [21] in cultures from allergic asthmatic subjects, may be implicated.

It is tempting to speculate that, in addition to increased CTLA-4 expression on T cells, abnormal expression of costimulatory molecules on antigen-presenting cells may affect RV presentation and therefore virus clearance in atopic individuals, explaining the amplified colds observed in these subjects [22]. However, further studies will be required to prove or reject such a hypothesis. Moreover, it should be noted that in another study, although no differences between normal and atopic subjects have been observed in CD86 on freshly drawn B cells outside the allergen season, when the same comparison was performed after the peak of the allergen season a higher level of CD86 on B cells of atopic subjects has been documented [23]. It is therefore possible that the nature of stimulus and exposure kinetics may differentially affect costimulatory molecule expression, which is nevertheless aberrant in atopic asthmatics.

Among other findings of this study, the increase of CD25 on PBMC after RV infection in both groups is not surprising, as T cell activation has been previously observed in PBMC, assessed by the increase of CD69 after incubation with RV16 [12]. In contrast, RV-mediated down-regulation of CD14 is reported for the first time in this study. CD14 is a differentiation-stage linked glycoprotein that functions as a receptor for lipopolysaccharide (LPS) [24]. Cytokines such as IFN- γ , IL-4 and GM-CSF are able to down-regulate CD14 on monocytes, while LPS induces up-regulation [25]. The modulation of this molecule is related to monocyte apoptosis since LPS-induced up-regulation inhibits apoptosis [26], while cytokine-mediated CD14 down-regulation precedes the apoptotic process [27]. In PBMC cultures, monocytes are the cells that mostly bind RV [12]. Although to date there is little evidence of RV replication or cytotoxicity in PBMC/monocytes, our findings raise the possibility that RV binding and/or infection of monocytes may

induce apoptotic signals, a hypothesis that warrants further investigation. Furthermore, down-regulation of CD14 may be related to internalization of the molecule secondary to monocyte activation. As CD14 down-regulation was less pronounced in atopic asthmatic subjects, this finding also supports the hypothesis of a reduced antiviral response in these subjects.

In conclusion, RVs are able to induce significant changes in PBMC phenotype and activate the antigen-presentation machinery, in both normal and atopic asthmatic individuals. Differences between these groups suggest that costimulation in RV infection may be impaired in atopic asthmatic subjects. A suboptimal immune response to RV, resulting in reduced viral clearance and prolonged viral presence in the airways, may be an important component of the inflammatory cascade that underlies asthma exacerbations.

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, Johnston SL. The acute exacerbation of asthma: pathogenesis. In: Holgate ST, Boushey HA, Fabbri LM, eds. *Difficult Asthma*. London: Martin Dunitz, 1999:183–204.
- Papi A, Johnston SL. Rhinovirus infection induces expression of its own receptor intercellular adhesion molecule 1 (ICAM-1) via increased NF-kappaB-mediated transcription. *J Biol Chem* 1999; 274:9707–20.
- Papadopoulos NG, Bates PJ, Bardin PG et al. Rhinoviruses infect the lower airways. *J Infect Dis* 2000; 181:1875–84.
- Levandowski R, Ou D, Jackson G. Acute-phase decrease of T lymphocyte subsets in rhinovirus infection. *J Infect Dis* 1986; 153:743–8.
- Fraenkel DJ, Bardin PG, Sanderson G et al. Lower airways inflammation during rhinovirus colds in normal and in asthmatic subjects. *Am J Respir Crit Care Med* 1995; 151:879–86.
- Trigg C, Nicholson K, Wang J et al. Bronchial inflammation and the common cold: a comparison of atopic and non-atopic individuals. *Clin Exp Allergy* 1996; 26:665–76.
- Wimalasundera SS, Katz DR, Chain BM. Characterization of the T cell response to human rhinovirus in children: implications for understanding the immunopathology of the common cold. *J Infect Dis* 1997; 176:755–9.
- Gern J, Dick E, Kelly E, Vrtis R, Klein B. Rhinovirus-specific T cells recognize both shared and serotype-restricted viral epitopes. *J Infect Dis* 1997; 175:1108–14.
- Papadopoulos NG, Stanciu L, Papi A, Holgate ST, Johnston SL. A defective type-1 response to rhinovirus in atopic asthma. *Thorax* 2002, in press.
- Gern J, Vrtis R, Kelly E, Dick E, Busse W. Rhinovirus produces nonspecific activation of lymphocytes through a monocyte-dependent mechanism. *J Immunol* 1996; 157:1605–12.
- Papi A, Stanciu LA, Papadopoulos NG, Holgate ST, Johnston SL. Rhinovirus infection induces HLA class I, but not class II, and costimulatory molecules upregulation on respiratory epithelial cells. *J Infect Dis* 2000; 181:1780–4.
- Gern JE, Dick EC, Lee WM et al. Rhinovirus enters but does not replicate inside monocytes and airway macrophages. *J Immunol* 1996; 156:621–7.
- Schwartz RH. Costimulation of T lymphocytes: the role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. *Cell* 1992; 71:1065–8.
- Robinson DS. T cell costimulation: a potential therapeutic target in asthma? *Clin Exp Allergy* 1998; 28:788–90.
- Moran TM, Isobe H, Fernandez-Sesma A, Schulman JL. Interleukin-4 causes delayed virus clearance in influenza virus-infected mice. *J Virol* 1996; 70:5230–5.
- Krummel MF, Allison JP. CTLA-4 engagement inhibits IL-2 accumulation and cell cycle progression upon activation of resting T cells. *J Exp Med* 1996; 183:2533–40.
- Gribben JG, Freeman GJ, Boussiotis VA et al. CTLA4 mediates antigen-specific apoptosis of human T cells. *Proc Nat Acad Sci USA* 1995; 92:811–5.
- Gern J, Joseph B, Galagan D, Borchering W, Dick E. Rhinovirus inhibits antigen-specific T cell proliferation through an intercellular adhesion molecule-1-dependent mechanism. *J Infect Dis* 1996; 174:1143–50.
- Adachi M, Oda N, Kokubu F, Minoguchi K. IL-10 induces a Th2 cell tolerance in allergic asthma. *Int Arch Allergy Immunol* 1999; 118:391–4.
- Bardin P, Fraenkel D, Sanderson G et al. Amplified rhinovirus colds in atopic subjects. *Clin Exp Allergy* 1994; 24:457–64.
- Hofer MF, Jirapongsananuruk O, Trumble AE, Leung DY. Upregulation of B7.2, but not B7.1, on B cells from patients with allergic asthma. *J Allergy Clin Immunol* 1998; 101:96–102.
- Heidenreich S. Monocyte CD14: a multifunctional receptor engaged in apoptosis from both sides. *J Leuk Biol* 1999; 65:737–43.
- Kruger M, Van de Winkel JG, De Wit TP, Coorevits L, Ceuppens JL. Granulocyte-macrophage colony-stimulating factor down-regulates CD14 expression on monocytes. *Immunology* 1996; 89:89–95.
- Manna SK, Aggarwal BB. Lipopolysaccharide inhibits TNF-induced apoptosis: role of nuclear factor-kappaB activation and reactive oxygen intermediates. *J Immunol* 1999; 162:1510–8.
- Heidenreich S, Schmidt M, August C, Cullen P, Rademaekers A, Pauels HG. Regulation of human monocyte apoptosis by the CD14 molecule. *J Immunol* 1997; 159:3178–88.