Eur Respir J 1998; 11: 630–635
DOI: 10.1183/09031936.98.11030630

European Respiratory Journal
ISSN 0903 - 1936

Local cytokine messenger ribonucleic acid expression and *in vitro* allergic late phase responses in Brown-Norway rats

E.M. Minshall, R.J. Dandurand, Q. Hamid, D.H. Eidelman

Local cytokine messenger ribonucleic acid expression and in vitro *allergic late phase responses in Brown-Norway rats. E.M. Minshall, R.J. Dandurand, Q. Hamid, D.H. Eidelman. ©ERS Journals Ltd 1998.*

ABSTRACT: The events subsequent to antigen challenge in allergic asthmatics involve the synthesis of pro-inflammatory cytokines. However, little is known how cytokine gene activation prior to allergen challenge may influence this series of events, nor how cytokine gene expression is related to antigen-induced alterations in lung function. Using a novel *in vitro* explant technique, we hypothesized that the local expression of cytokines influenced the development of antigen-induced late-onset airway responses, and that alterations in cytokine messenger ribonucleic acid (mRNA) expression were associated with antigen-induced changes in airway luminal area.

Explants were prepared from excised lungs of ovalbumin-sensitized Brown-Norway rats. Airways were challenged by direct application of ovalbumin or an irrelevant control antigen. Cryostat sections of explants were used for *in situ* hybridization and mRNA for interleukin (IL)-2, IL-4 and interferon (IFN)-γ were detected using radiolabelled probes.

We found that the presence of high numbers of cells expressing IFN-γ and IL-2 mRNA within the airways attenuated the development of antigen-induced late airway responses in sensitized rat lung explants. Furthermore, we observed that cytokine mRNA for IL-4 was significantly increased following allergen exposure in sensitized lung explants exhibiting late airway responses.

This study implicates the local expression of interferon-γ and interleukin-2 messenger ribonucleic acid in the failure of sensitized rat lung explants to exhibit late airway responses, and provides evidence linking local interleukin-4 messenger ribonucleic acid expression to the sequelae of events occurring as a result of antigen exposure within the airways.

Eur Respir J 1998; 11: 630–635.

Meakins-Christie Laboratories, Montreal Chest Institute Research Center, Royal Victoria and Montreal General Hospitals, McGill University, Montreal, Quebec, Canada.

Correspondence: D.H. Eidelman
Meakins-Christie Laboratories
McGill University
3626 St. Urbain Street
Montreal, Quebec
Canada H2X 2P2
Fax: 1 514 3987483

Keywords: Cytokines
late airway response
lung explants
lymphocytes
mast cells

Received: September 26 1997
Accepted after revision October 29 1997

EMM is supported by a CLA/MRC fellowship. DHE and QH are supported by Chercheur Bousier awards from the Fonds de la Recherche en Santé du Québec. This work was supported by the Medical Research Council of Canada, Inspiraplex and the J.T. Costello Memorial Fund.

Allergic disorders such as atopic asthma are associated with the infiltration and activation of inflammatory cells, such as eosinophils and lymphocytes [1, 2]. These lymphocytes are predominantly CD4-positive T-cells expressing "Th2-type" cytokines such as interleukin (IL)-4 and IL-5 [3, 4]. It has been suggested that the local release of these cytokines contributes towards the development and maintenance of this allergic inflammation. Indeed, there is considerable support for the involvement of these mediators in the pathway of events leading to eosinophil accumulation, late-onset airway responses and hyperresponsiveness. Nevertheless, their role in contributing to the heterogeneity of airway responses to allergen, particularly with reference to the late response remains unclear.

Cytokines such as IL-4 and IL-5 have been implicated in the pathophysiology of asthma by virtue of their ability to elicit the differentiation and maturation of eosinophils [5, 6], and their capacity to promote the isotype switching of B-cells towards IgE [7]. In order to elucidate the actions of these cytokines in orchestrating the events subsequent to allergen exposure, recent studies have focused on the presence of these cytokines following acute allergen challenge. Both animal studies and bronchial biopsies from asthmatic individuals have indicated that the presence of IL-5 is associated with the acute development of airways eosinophilia, late-onset airway responses and hyperresponsiveness following allergen challenge [8, 9]. Moreover, the presence of IL-4 has been linked to allergic inflammation, and particularly the recruitment of eosinophils into the airways *via* an upregulation of vascular cell adhesion molecule-1 (VCAM-1) expression [10].

We recently developed a system in which late asthmatic responses (LARs) can be elicited in antigen-challenged explanted airways from sensitized Brown-Norway (BN) rats, which preferentially produce antigen-specific immunoglobulin (Ig)E [11]. In this system, LARs are produced in the absence of *de novo* inflammatory cell recruitment, underscoring the importance of resident inflammatory cells. One advantage of this *in vitro* preparation is the ability to assess local cytokine expression during allergen-induced changes in airway calibre [9]. Since only approximately two thirds of all sensitized BN rats will exhibit LARs, this technique also permits us to examine the baseline cytokine expression with respect to the subsequent changes in airway luminal area following allergen challenge.

In order to better understand the relationship between local inflammation and the *in vitro* LARs, we investigated the production of cytokines which play a role in IgE production (IL-4 and interferon-γ (IFN-γ)) and T-lymphocyte activation (IL-2). Our hypothesis was that at baseline the local expression of IL-2 and interferon-γ (IFN-γ) messenger ribonucleic acid (mRNA) would influence the subsequent response to allergen, and that the IL-4 mRNA production would be upregulated in lung explants following allergen challenge. To achieve these objectives, we studied the expression of IL-4, IL-2 and IFN-γ mRNA in allergen-sensitized rat lung explants prior to and 6 h after allergen challenge. By determining changes in airway luminal area, we also examined whether the cytokine mRNA expression could be associated with the magnitude of the LAR.

Material and methods

Animal protocol

Eight week old Brown-Norway rats were obtained from a commercial supplier (Charles River, St Constant, Quebec, Canada) and housed in a conventional animal facility at our laboratory. Fifteen animals were sensitized and six served as unsensitized, sham controls. The rats were sensitized to ovalbumin (OVA), as described previously [11], using a protocol designed to elucidate allergen-specific IgE synthesis and late-onset airway responses. Sham-sensitized animals were sensitized using an identical protocol, but the OVA was omitted.

Study design

Animals were studied at 10 weeks of age, 14 days after allergen or sham-sensitization. Lung explants were prepared from allergen and sham-sensitized rats to examine for allergen-induced changes in airway luminal area. Cytokine mRNA expression was investigated in lung explants prior to and 6 h after OVA exposure using the technique of *in situ* hybridization. To determine the relationship between the presence of allergen-induced late-onset airway responses and the cytokine expression of the lung, we examined mRNA for IL-2, IL-4 and IFN-γ in lung explants, and associated these with the observed changes in airway luminal area. The cytokine mRNA expression in allergen-sensitized animals following challenge with an irrelevant antigen, bovine serum albumin (BSA), and in sham-sensitized animals following OVA exposure were used to examined for the effects of specific antigen exposure.

Methods

A sterile bicarbonate buffered culture medium (BCM; pH 7.3) suitable for the preparation and short-term culture of rat lung explants was prepared as described previously [11]. Agarose type VII solutions (2 and 4 % weight/volume (w/v); Sigma, Oakville, ON, Canada) were prepared in BCM and stored at 4°C. Sterile 100 μg·mL^{-1} solutions of OVA and BSA were freshly prepared in BCM and the pH adjusted to 7.4. All solutions were used within 1 week of preparation.

Lung explants were prepared aseptically as described previously [11]. Briefly, animals were administered a lethal dose of pentobarbital sodium (0.8 mg·kg^{-1} *i.p.*), intubated by tracheotomy with a 9 cm length of sterile polyethylene tubing and exsanguinated by inferior vena caval section. To remove excess blood, the pulmonary circulation was perfused *via* the right ventricle with 20 mL of sterile Ca^{2+} and Mg^{2+}-free Hank's solution. The excised lungs were maintained at a volume equivalent to the total lung capacity by inflation with 48 mL·kg^{-1} of a 1% agarose-BCM solution at 37°C. Once cooled, the lungs were isolated and placed upright in a sterile 35 mL syringe from which the needle end had been cut off and embedded in 4% agarose-BCM solution. The lung-agarose block was then sectioned into 0.5–1.0 mm transverse slices, using a hand-held microtome blade (model 818, Cambridge Instruments, Buffalo, NY). The resulting explants were inspected using an inverted microscope (IMT-2; Olympus, Tokyo, Japan). Explants were selected for study if: 1) they contained a lobar or segmental airway; 2) the entire epithelial-luminal junction of the airways could be focused; 3) the airways were free of agarose; and 4) they demonstrated beating cilia. The lung explants were incubated overnight in 2 mL BCM at 37°C in 5% CO_2/95% air.

Suitable airways in explants from 15 OVA-sensitized and six sham-sensitized rats were identified. The culture dish inserts containing the lung explants were transferred to six-well plates (Costar) containing 2 mL of BCM, placed on the stage of an inverted microscope (IMT-2; Olympus) and maintained at 37°C with a stage warmer (Physitemp, Clifton, NJ, USA). Individual airways were imaged with a video camera (CDS; Sony, Nagano, Japan) and recorded with a video disk recorder (TQ2026F; Panasonic, Osaka, Japan). After recording baseline images of the airways, a 20 μL aliquot of a sterile solution containing either 100 μg·mL^{-1} of OVA or BSA in BCM was applied directly to the airways. The explants were then incubated at 37°C in 5% CO_2, and were re-imaged 6 h after antigen application at which time near-maximal constriction was observed [11].

The stored images were digitized and subjected to measurements with the Galileo Image Processing Software (Inspiraplex, Montreal, Canada). The lumen area was taken as the area enclosed by the epithelial luminal border. Calibration was performed with a 0.01 mm graticule, imaged with the same equipment at the same magnification. A late response was defined as a constriction exceeding 11% of the baseline area as defined previously [11].

Tissue explants were taken for *in situ* hybridization either prior to or 6 h after allergen challenge [9]. Briefly, explant slices were fixed in 4% paraformaldehyde, washed, blocked and sectioned at 10 μm thickness onto poly-L-lysine coated slides. The sections were hybridized with ^{35}S-uridine triphosphate (UTP) labelled antisense and sense probes coding for rat IL-2, IL-4 and IFN-γ mRNA. Prior to the application of the probe, the sections were permeabilized with proteinase K and then prehybridized with 50% formamide and 2×standard saline citrate (SSC). Nonspecific binding was removed by posthybridization washing under high-stringency conditions, subsequent treatment with ribnuclease (RNase) and the use of iodoacetamide,

N-ethylmaleimide and dithiothreitol. Hybridization signals were visualized by standard autoradiography. The absence of hybridization signals with the sense probe or following pre-treatment of the tissues with RNase ensured the specificity of the signal.

Analysis

Slides were randomly encoded and read by one observer blinded to the identity of the slides. For each animal, one lung explant was fixed from which one slice underwent *in situ* hybridization. IL-2, IL-4 and IFN-γ mRNA positive cells were counted in the airway submucosa from 2–3 airways. Cell counts were carried out by applying a 0.115 mm × 0.115 mm counting grid around the circumference of each airway, and the counts expressed as the number of positive cells per millimetre of airway basement membrane.

Values for cytokine mRNA expression obtained between "responder" and "nonresponder" allergen sensitized rats, BSA challenged allergen-sensitized rats and sham-sensitized rats were analyzed by a Kruskal-Wallis nonparametric analysis of variance (ANOVA) and *post hoc* Mann-Whitney U-tests. The data obtained in allergen-sensitized rats, pre- and postallergen, were analysed using a paired Wilcoxon sign rank test. Correlation coefficients were calculated using a Pearson chi-squared goodness-of-fit test with a subsequent Bonferroni correction (SyStat v5.1, Systat, Evanston, IL, USA). A p-value of less than 0.05 was considered statistically significant.

Results

Late responses

In lung explants from OVA-challenged rats, changes in airway luminal area indicative of LARs (>11% of the baseline value) were observed in six of 11 animals, a prevalence similar to previous studies in this model [11]. In contrast, LARs were not detected in the remainder of the allergen-sensitized animals, those challenged with BSA, or the sham-sensitized animals.

Baseline cytokine expression

There was a striking difference in expression of IF'N-γ mRNA between animals with (responders) and without (nonresponders) LARs (fig. 1). Nonresponders had several fold more IFN-γ mRNA-positive cells prior to OVA challenge compared to responders ($p<0.005$). Indeed, there was a complete separation between these two groups. Similarly, the number of IL-2 mRNA positive cells was increased among the nonresponders compared to the responders, although not to the same degree ($p<0.05$).

Cytokine mRNA expression following antigen challenge

The number of IL-4 mRNA-positive cells increased significantly following OVA challenge in the explants of responders (fig. 2; $p<0.05$). Although there was a tendency

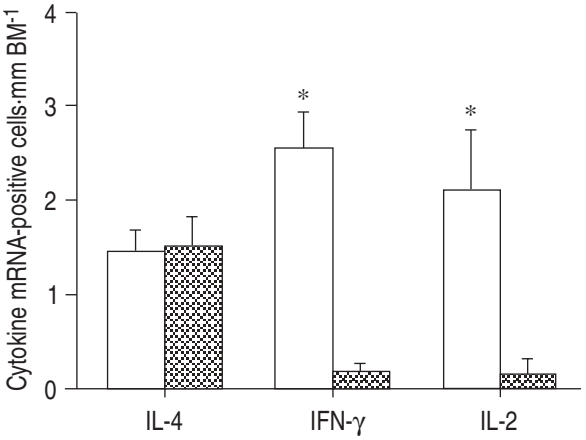

Fig. 1. – Baseline cytokine levels for interleukin (IL)-4, interferon (IFN)-γ and IL-2 in explanted airways of sensitized Brown-Norway (BN) rats prior to antigen challenge. Bars represent the mean number of cells expressing cytokine messenger ribonucleic acid (mRNA) per millimetre of basement membrane and the error bar indicates the upper range of the SEM. A significantly greater number of cells expressed IFN-γ and IL-2 mRNA in sensitized lung explants not undergoing allergen-induced late asthmatic responses (LARs) (▭ ; $p<0.05$; n=5) compared to explants exhibiting LARs (n=6; ▩). BM: basement membrane.

toward a similar increase among nonresponders in that three out of five animals exhibited an increase in IL-4 mRNA expression, this failed to reach statistical significance ($p>0.05$). While there were no statistically significant changes in IFN-γ mRNA-positive cells following allergen exposure in either responders or nonresponders ($p>0.05$), in four out of five nonresponders IFN-γ mRNA expression either decreased or remained unchanged. Allergen challenge elicited a significant reduction in the numbers of cells expressing IL-2 mRNA in nonresponders ($p<0.01$), which was not observed in explants exhibiting LARs.

In explants which did not demonstrate LARs, the numbers of cells expressing IL-4 mRNA were similar in OVA-sensitized animals 6 h after either OVA or BSA challenge (table 1). In contrast, explants from sham-sensitized rats expressed significantly less IL-4 mRNA following OVA challenge than "responder" allergen-sensitized animals ($p<0.05$). Following OVA challenge, there were approximately fivefold more cells expressing IFN-γ mRNA in explants from "nonresponder" compared to "responder" rats ($p<0$ 05). Similarly, "nonresponder" allergen-sensitized animals expressed significantly more IFN-γ mRNA following OVA challenge than BSA-challenged explants from allergen-sensitized rats and OVA-challenged sham-sensitized animals ($p<0.05$). There were no significant differences in IL-2 mRNA expression between the various groups of rat lung explants ($p<0.05$).

Cytokine expression and constriction

When comparing the physiological changes in airway luminal area with the numbers of cells expressing cytokine mRNA, there was a significant correlation between the expression of IFN-γ mRNA prior to allergen challenge and the magnitude of the LAR (fig. 3; $r^2=0.57$). For the expression of IL-2 mRNA following antigen challenge, a positive association with the magnitude of the LAR was observed ($r^2=0.60$). In contrast, there was no

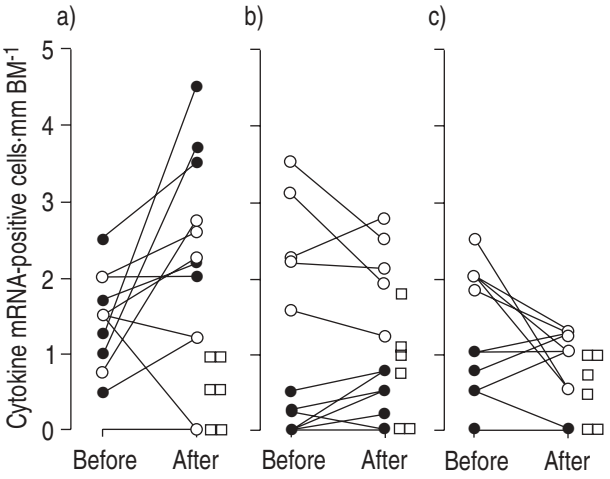

Fig. 2. – Changes in mRNA for: a) IL-4, b) IFN-γ and c) IL-2 in explanted airways before and after allergen challenge. Filled circles represent mean data points from lung explants that underwent late-onset airway responses 6 h after allergen challenge. Open circles represent mean values from lung explants not exhibiting LARs. Open squares represent the mean values from sham-sensitized animals (n=6). Following allergen challenge, there was a significant increase in IL-4 mRNA expression ($p<0.05$) in explants from sensitized rats which exhibited LARs (n=6). In lung explants not undergoing LARs (n=5), there was a significant reduction the numbers of cells expressing IL-2 mRNA after allergen challenge ($p<0.05$). For definitions see legend to figure 1.

Table 1. – Results of cytokine messenger ribonucleic acid (mRNA) expression 6 h after ovalbumin (OVA) or bovine serum albumin (BSA) challenge in explants from antigen or sham-sensitized animals

	mRNA		
	IL-4	IL-2	IFN-γ
OVA-challenged "responders" (n=6)	2.85 (1.2–4.5)	0.75 (0–1.25)	0.45+ (0–0.75)
OVA-challenged "nonresponders" (n=5)	1.76 (0–2.75)	0.89 (0.5–1.25)	2.09 (1.2–2.75)
BSA-challenged OVA-sensitized (n=4)	1.54 (1–2.5)	0.75 (0–1.5)	0.5+ (0–1.5)
OVA-challenged sham-sensitized (n=6)	0.60* (0–1)	0.81 (0–1)	0.78+ (0–1.8)

Values are expressed as numbers of positive cells per millimetre of basement membrane and presented as mean with range in parenthesis. *: $p<0.05$ *versus* OVA-challenged "responders"; +: $p<0.05$ *versus* OVA-challenged "nonresponders". IL: interleukin; IFN: interferon.

association between IL-4 mRNA expression either prior to or after allergen challenge and the change in luminal area at 6 h.

Discussion

This study was aimed at investigating the relationship between the development of LARs in lung explants from sensitized BN rats and the local cytokine milieu. Our findings demonstrated that LARs were attenuated in rat lung explants with high baseline levels of IL-2 and IFN-γ mRNA, and that expression of IL-4 mRNA was temporally associated with the development of these LARs.

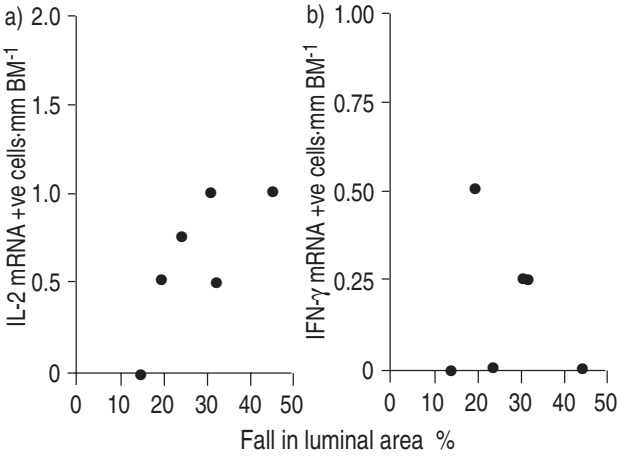

Fig. 3. – Correlations between the mean fall in luminal area from baseline 6 h after allergen challenge in sensitized rat lung explants exhibiting LARs, and the changes in cytokine mRNA for a) IL-2 ($r^2=0.60$) and b) IFN-γ ($r^2=0.57$). The magnitude of the LAR is the mean value of usually 2–3 determinations. There were no significant correlations between the change in airway luminal area and IL-4 mRNA expression ($p>0.05$). +ve: positive. For further definitions, see legend to figure 1.

Together with previous observations that LARs in sensitized rat lung explants are accompanied by an increase in mRNA for IL-5 and immunoreactivity for major basic protein (MBP) [9], these observations point to the importance of the local cytokine milieu as a determining factor in the subsequent response to allergen in sensitized rat lung explants.

The explant LAR model is an attractive tool for studying this process as it permits the simultaneous investigation of physiological changes and the local inflammatory milieu in the absence of *de novo* inflammatory cell recruitment [11]. Expression of cytokines and the associated activation of inflammatory cells is, thus, dependent on the immunological cell population present in the airway at the time when the lungs are harvested and the influence of cellular recruitment from the blood and capillary leak is obviated. One further advantage of studying the BN rat model, is that LARs are not invariably seen using either *in vivo* [12] or *in vitro* [11] techniques. This heterogeneity permits us to investigate the inflammatory and cytokine profile related to late-onset changes in airway luminal area.

LARs were not evident in lung explants from sensitized rats challenged with BSA or in OVA- challenged explants from sham-sensitized rats. Consistent with this observation was the finding that the profile of IL-2 and IL-4 mRNA expression in these two groups of control animals was similar to that seen following OVA challenge in the "nonresponder" rats. These results suggest that the cytokine response with respect to the development of LARs is antigen-specific and dependent upon the presence of antigen during sensitization.

Although the factors that contribute to the development of LARs in animal models and human asthma remain to be elucidated, genetic and environmental influences, as well as the immunological response following sensitization, may play a role. It is unlikely that the heterogeneity in expression of LARs that we observed is simply a failure of the animals to be appropriately sensitized since this observation appears to be constant in the hands of different

investigators and over a considerable time period. In OVA-sensitized BN rats, LARs appear to be dependent at least in part on the production of leukotriene D_4 (LTD_4) [11] and to be closely associated with the expression of IL-5 and MBP at the level of the individual airways [9]. In addition, the prevalence of the LAR has been shown to vary according to the particular inbred strain of rat used [13]. Sprague-Dawley rats with relatively high numbers of circulating CD8+ T-cells exhibit few or no LARs, but can be made to exhibit them by administration of anti-CD8 antibodies [14]. Similar "suppressor" activity of CD8+ cells on the LAR has been described in the blood of sensitized BN rats [15]. It is also possible that release of mast cell mediators subsequent to allergen challenge may be an important factor in the development of LARs, since there is evidence *in vivo* that the magnitude of the early response in the BN rat correlates with that of the late response [15]. The relationship between the different cell types present, their phenotype with regard to cytokine expression and the development of the LAR remains to be elucidated.

In the present study we focused on the relationship between expression of LARs and local cytokine expression. The most striking findings were the relatively high levels of expression of IL-2 and IFN-γ mRNA in "nonresponding" animals. In particular, there was no overlap between "nonresponders" and "responders" with regard to the expression of IFN-γ mRNA either prior to or following antigen exposure. This suggests that, in this animal model at least, the prevalence of LARs is dependent on local baseline expression of these cytokines, presumably as a result of the sensitization protocol.

The roles of IFN-γ and IL-2 in the pathophysiology of asthma are unclear at present. Compared to normal individuals, levels of IFN-γ mRNA are generally either decreased or unchanged in bronchial biopsies and bronchoalveolar lavage fluid (BALF) from allergic asthmatics [3, 12, 16, 17]. These observations may reflect the prominent expression, within the airways of asthmatic individuals, of IL-4, a cytokine that has been shown to reciprocally inhibit IFN-γ production from T-cell clones [18]. Whilst a direct role for IFN-γ in modulating airway function remains to be elucidated, it has been shown that therapeutic strategies which limit the development of LARs in asthma, such as steroids [19] and immunotherapy [20], have been shown to promote the expression of IFN-γ mRNA [21, 22].

Increased or unchanged numbers of cells expressing IL-2 mRNA have been reported in atopic asthma [3, 12, 17] when compared to normal controls. While this cytokine was originally described as being "Th1-type", its presence may reflect a general activation phenotype of T-lymphocytes or the specific activation of CD8+ T-cells, which has been reported in non-allergic asthma [18]. Whether the cellular source of IL-2 mRNA is CD8+ lymphocytes, which have been shown to attenuate the development of the LAR in sensitized BN rats *in vivo* [14], remains to be established.

Allergen challenge in this model was associated with increased numbers of IL-4 mRNA-positive cells compared to baseline. Although this was most clear-cut among responders, IL-4 also increased in three of five nonresponders. These findings are consistent with previous studies that suggest the importance of this cytokine in allergen-induced late phase inflammatory responses [10, 23]. Unlike IL-5 [9], however increases in local IL-4 mRNA were seen even in explants without LARs. This suggests that whereas IL-4 may somehow contribute to *in vitro* late allergic bronchoconstriction, it is not central to this phenomenon. In an intact organism, IL-4 may play a much more central role. For example, it may act on the local B-cell population to induce allergen-specific immunoglobulin isotype switching to favour IgE production [7], and induce VCAM-1 expression on endothelial cells facilitating eosinophil recruitment [10]. Indeed, there is a trend for levels of antigen-specific IgE to be higher in individuals exhibiting dual asthmatic responses [24], and for eosinophils to be present in increased numbers 3–4 h after allergen challenge [25, 26]. Furthermore, soluble VCAM levels in BALF following allergen challenge have been strongly correlated to IL-4 and IL-5 levels and the presence of eosinophils within the airways [10].

It is of interest that in nonresponders this tendency toward an increase in the numbers of IL-4 mRNA-positive cells was associated with the suggestions of a decrease in the numbers of cells expressing IFN-γ and IL-2 mRNA. No decrease was seen among responders who started with very low IFN-γ and IL-2 levels at baseline. These findings are consistent with the notion that antigen-induced local production of IL-4 acts to reduce "Th1-type" cytokine expression [27].

Although we did not perform localization of cytokine mRNA to determine the phenotype of cells expressing these cytokines, within the airways mast cells, eosinophils and T-lymphocytes are all potential sources of IL-4 [6, 28]. Recently, it has been demonstrated that IL-4 mRNA expression is elevated following IgE-dependent activation of human lung mast cells and basophils [29, 30]. This acute time course of mRNA synthesis in human mast cells following IgE-dependent stimulation may suggest a similar cellular source in our tissue explants. The majority of cells expressing IL-2 and IFN-γ mRNA in asthmatic and normal bronchial biopsies are CD3-positive T-lymphocytes [4]. While the cellular sources of these cytokines in our explant model remain to be elucidated, it is possible that these are CD8-positive T-lymphocytes. Indeed, the presence of these cells has been associated with an attenuation of the LAR in sensitized rats [22].

In summary, we have demonstrated that late-onset allergic constrictor responses in isolated Brown-Norway rat lung explants are associated with a low baseline expression of interferon-γ and interleukin-2 messenger ribonucleic acid. The prevalence of *in vitro* late response in this model was closely tied to the baseline expression of interferon-γ and interleukin-2 messenger ribonucleic acid. Furthermore, we have observed that there is a local increase in interleukin-4 messenger ribonucleic acid following antigen challenge. These results suggest that the local inflammatory milieu within the airways dictates the events that occur as a result of allergen exposure, and that the balance of cytokines plays an intrinsic role.

Acknowledgements: The rat cDNA probes for IL-2, IL-4 and IFN-γ were generous gifts from P.H. van der Meide, Medisch Biologisch Laboratorium, TNO, the Netherlands. The authors would like to thank Z. Yasruel, C. Dolman and E. Schotman for their invaluable technical assistance.

References

1. Walker C, Kaegi MK, Braun P, Blaser K. Activated T-cells and eosinophilia in bronchoalveolar lavages from subjects with asthma correlated with disease severity. *J Allergy Clin Immunol* 1991; 88: 935–942.
2. Azzawi M, Bradley B, Jeffery PK, *et al*. Identification of activated T-lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. *Am Rev Respir Dis* 1990; 142: 1407–1413.
3. Hamid Q, Azzawi M, Ying S, *et al*. Expression of mRNA for interleukin-5 in mucosal bronchial biopsies from asthma. *J Clin Invest* 1991; 87: 1541–1546.
4. Ying S, Durham SR, Corrigan CJ, Hamid Q, Kay AB. Phenotype of cells expressing mRNA for Th2-type (interleukin-4 and interleukin-5) and Th1-type (interleukin-2 and interferon-γ) cytokines in bronchoalveolar lavage and bronchial biopsies from atopic asthmatic and normal control subjects. *Am J Respir Cell Mol Biol* 1995; 12: 477–487.
5. Clutterbuck EJ, Hirst EMA, Sanderson CJ. Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6 and GM-CSF. *Blood* 1989; 73: 1504–1512.
6. Yamaguchi Y, Hayashi Y, Sugama Y, *et al*. Highly purified murine interleukin-5 (IL-5) stimulates eosinophil function and prolongs *in vitro* survival. *J Exp Med* 1988; 167: 1737–1742.
7. Del Prete G, Maggi E, Parronchi P, *et al*. IL-4 is an essential co-factor for the IgE synthesis induced *in vitro* by human T-cell clones and their supernatants. *J Immunol* 1988; 140: 4193–4198.
8. Ohnishi T, Kita H, Weiler D, *et al*. IL-5 is the predominant eosinophil-active cytokine in the antigen-induced pulmonary late-phase reaction. *Am Rev Respir Dis* 1993; 147: 901–907.
9. Eidelman DH, Minshall E, Dandurand RJ, *et al*. Evidence for major basic protein immunoreactivity and IL-5 gene activation during the late phase response in explanted airways. *Am J Respir Cell Mol Biol* 1996; 15: 582–589.
10. Zangrilli JG, Shaver JR, Cirelli RA, *et al*. sVCAM-1 levels after segemental antigen challenge correlate with eosinophil influx, IL-4 and IL-5 production, and the late phase response. *Am J Respir Crit Care Med* 1995; 151: 1346–1353.
11. Dandurand RJ, Wang CG, Laberge S, Martin JG, Eidelman DH. *In vitro* allergic bronchoconstriction in the Brown Norway rat. *Am J Respir Crit Care Med* 1994; 149: 1499–1505.
12. Robinson D, Hamid Q, Bentley A, Ying S, Kay AB, Durham SR. Activation of CD4+ T-cells, increased Th2-type cytokine mRNA expression and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in patients with atopic asthma. *J Allergy Clin Immunol* 1993; 92: 313–324.
13. Eidelman DH, Bellofiore S, Martin JG. Late airway responses to antigen challenge in sensitized inbred rats. *Am Rev Respir Dis* 1988; 137(5): 1033–1037.
14. Olivenstein R, Renzi PM, Yang JP, *et al*. Depletion of OX-8 lymphocytes from the blood and airways using monoclonal antibodies enhances the late airway response in rats. *J Clin Invest* 1993; 92(3): 1477–1482.
15. Waserman S, Olivenstein R, Renzi P, Xu LJ, Martin JG. The relationship between late asthmatic responses and antigen-specific immunoglobulin. *J Allergy Clin Immunol* 1992; 90: 661–669.
16. Bentley AM, Meng Q, Robinson DS, Hamid Q, Kay AB, Durham SR. Increases in activated T-lymphocytes, eosinophils and cytokine mRNA expression for interleukin-5, granulocyte-macrophage colony-stimulating factor in bronchial biopsies after allergen inhalation challenge in atopic asthma. *Am J Respir Cell Mol Biol* 1993; 8: 35–42.
17. Walker C, Bode E, Boer L, Hansel TT, Blaser K, Virchow J-C. Allergic and nonallergic asthmatics have distinct patterns of T-cell activation and cytokine production in peripheral blood and bronchoalveolar lavage. *Am Rev Respir Dis* 1992; 146: 109–115.
18. Pene J, Rousset F, Briere F, *et al*. IgE production by normal human lymphocytes is induced by interleukin-4 and suppressed by interferons γ and α and prostaglandin E_2. *Proc Natl Acad Sci USA* 1988; 85: 6880–6884.
19. Cockcroft DW, Murdock KY. Comparative effects of inhaled salbutamol, sodium cromoglycate, and beclomethasone diproprionate on allergen-induced early asthmatic responses, late asthmatic responses, and increased bronchial responsiveness to histamine. *J Allergy Clin Immunol* 1987; 79: 734–740.
20. Van Bever HP, Stevens WJ. Evolution of the late asthmatic response during immunotherapy and after stopping immunotherapy. *J Allergy Clin Immunol* 1990; 86: 141–146.
21. Robinson DS, Hamid Q, Ying S, *et al*. Prednisolone treatment in asthma is associated with modulation of bronchoalveolar lavage cell interleukin-4, interleukin-5 and interferon-gamma cytokine gene expression. *Am Rev Respir Dis* 1993; 148: 401–408.
22. Varney VA, Hamid Q, Bentley AM, Ying S, Kay AB, Durham SR. Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. *J Clin Invest* 1993; 92: 313–324.
23. Kay AB, Ying S, Varney V, *et al*. Messenger RNA expression of the cytokine gene cluster, interleukin-3 (IL-3), IL-4, IL-5 and granulocyte/macrophage colony-stimulating factor, in allergen induced late-phase cutaneous reactions in atopic subjects. *J Exp Med* 1991; 173: 775–778.
24. Aalbers R, Kauffman HF, Vrugt B, *et al*. Bronchial lavage and bronchoalveolar lavage in allergen-induced single early and dual asthmatic responders. *Am Rev Respir Dis* 1993; 147: 76–81.
25. De Monchy JGR, Kauffman HF, Venge P, *et al*. Bronchoalveolar eosinophilial during allergen-induced late asthmatic reactions. *Am Rev Respir Dis* 1985; 131: 373–376.
26. Rossi GA, Crimi E, Lantero S, *et al*. Late phase asthmatic reaction to inhaled allergen is associated with early recruitment of eosinophils in the airways. *Am Rev Respir Dis* 1991; 144: 379–383.
27. Maggi E, Parronchi P, Manetti R, *et al*. Reciprocal regulatory effects of IFN-γ and IL-4 on the *in vitro* development of Th1 and Th2 clones. *J Immunol* 1992; 148: 2142–2147.
28. Bradding P, Roberts JA, Britten KM, *et al*. Interleukin-4, -5 and -6 and tumor necrosis factor-α in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am J Respir Cell Mol Biol* 1994; 10: 471–480.
29. Okayama Y, Petit-Frere C, Kassel O, *et al*. IgE-dependent expression of mRNA for IL-4 and IL-5 in human lung mast cells. *J Immunol* 1995; 155(4): 1796–1808.
30. MacGlashan DW, White JM, Huang S-K, Ono SJ, Schroeder JT, Lichtenstein LM. Secretion of IL-4 from human basophils: the relationship between IL-4 mRNA and protein in resting and stimulated basophils. *J Immunol* 1994; 152: 3006–3016.