

REVIEW

Investigative use of fiberoptic bronchoscopy for local airway challenge in asthma

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ABSTRACT: Local airway challenge has advantages over inhalation bronchial challenge as the response of the airway can be restricted and directly observed. It has been safely performed in subjects with mild or moderate asthma, either by the direct instillation of challenge solution to the selected segmental airways via a bronchoscope, or delivered to an airway segment isolated with a double-balloon catheter. However, these techniques carry potential complications, such as generalized wheeze, and due care is required in selection of subjects. Most investigators have used the method for studying the airway events following allergen challenge. Others have studied the airway changes following challenge with non-allergen provocation agents, such as hypertonic saline, adenosine 5'-monophosphate and cold dry air. The method has helped to define changes in the inflammatory cells and mediators in relation to early and late airway responses to allergen. Similarly, study of airway events following local challenge with hypertonic solution has provided useful knowledge in understanding the mechanisms of exercise-induced asthma.

With more experience and an improved margin of safety, it will be possible to study local changes in airway physiology following local airway challenge. Finally, the techniques also have potential use for studying the airway events following provocation with a wide range of agents of potential relevance to the pathogenesis of asthma.

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Investigative fiberoptic bronchoscopy for obtaining bronchoalveolar lavage (BAL) and bronchial biopsies in asthma has proved to be a useful tool in the characterization of cellular, epithelial and immunopathological changes in asthmatic airways. Local airway challenge (LAC) via fiberoptic bronchoscopy was a natural extension of its use in the study of airway changes following bronchoprovocation. Initially, bronchoprovocation was performed by the inhaled route, comparing the lavage and bronchial biopsies obtained before and after challenge. This usually required two bronchoscopies, with the second on the same day or after a varying interval on subsequent days. Predetermined doses of inhaled allergen were given to achieve a predicted airway response of a 15-20% fall in baseline forced expiratory volume in one second (FEV₁). However, it was often difficult to achieve this exact response. Introduction of the bronchoscope followed by BAL and mucosal bronchial biopsies, in the setting of generalized bronchoconstriction induced by aerosol inhalation challenge, carried the risk of an acute asthma attack and, therefore, the procedure could only be performed safely in patients with mild disease.

Local airway challenge of selected bronchi via the fiberoptic bronchoscope has been a great improvement over the inhaled aerosol challenge for the study of endobronchial events, as the airway response could be restricted to segmental airways and a control challenge could be performed at

the same bronchoscopy [1]. The method also allows direct observation of any airway changes following the local challenge. Improvements in the margin of safety, have led to the increasing use of this method for the study of airway events at various time-points after LAC, and also study of the airways in moderately severe asthmatics. Initially, local airway challenge was used to study airway changes following allergen challenge. However, the success of the method with allergen has prompted the study of airway events following local challenge with hypertonic saline, in the investigation of the mechanisms of exercise-induced asthma.

In this paper, we review the use of local airway challenge in asthma, technical and safety considerations, challenge agents used, methods of delivering challenge, assessment of the airway response, and use of local airway challenge (LAC) in understanding immunohistopathological changes in provoked asthma.

Technical considerations

Rigid versus fiberoptic bronchoscopy

Most investigators use a fiberoptic bronchoscope under light sedation when performing a LAC. However, some investigators have preferred the use of a rigid bronchoscope under local anaesthesia, as it allows a larger biopsy

to be obtained. It has been suggested that the artefactual damage to epithelial structure in the biopsies obtained with a rigid bronchoscope is less than that produced with the smaller biopsies obtained *via* the fiberoptic bronchoscope [2]. Local airway challenge *via* fiberoptic bronchoscope was initially performed after endotracheal intubation under light sedation [3]. Fiberoptic bronchoscopy in asthma has also been performed under general anaesthesia, with endotracheal intubation and mechanical ventilation [4]. Under these controlled conditions, more time is available to undertake endobronchial procedures.

However, rigid bronchoscopy has the potential to cause considerable discomfort to the patient, and a general anaesthetic carries the added risk of morbidity and mortality. Furthermore, most respiratory physicians have acquired the majority of their skill and experience with the procedure of fiberoptic bronchoscopy. Endotracheal intubation for fiberoptic bronchoscopy under light sedation offers no additional advantage over direct bronchoscopy, and is likely to cause more discomfort to the patients, unless heavy sedation is employed. Although fiberoptic bronchoscopy under general anaesthesia and mechanical ventilation with intubation may provide controlled conditions for LAC and endobronchial procedure, a general anaesthetic is likely to alter the airway response to the provocation agent. We feel that the use of rigid bronchoscopy or fiberoptic bronchoscopy with endotracheal intubation under light sedation or general anaesthetic in asthma should be restricted to those research projects in which it can provide additional information over the more routine use of a fiberoptic bronchoscopy.

Additional devices for local airway challenge

Single lumen catheter. Local challenge to the bronchial mucosa can be delivered by wedging the bronchoscope into a selected airway and instilling the challenge solution through the instrument channel of the bronchoscope [3]. However, it is preferable to use a long plastic catheter, inserted through the instrument channel of the bronchoscope, to instil challenge solution. It allows a precise delivery of the solution by positioning the catheter tip into the relevant airway, and it also helps to prevent contamination of the instrument channel with the challenge solution. Aerosol can also be delivered using such a catheter. However, the small diameter and long length of the catheter (0.3×105 cm) make it difficult for aerosol particles to travel through the length of the tubing without impaction on the wall of the catheter, with reduced delivery of aerosol particles at the tip. To overcome these difficulties, we have developed a technique to deliver aerosol particles of $2-3 \mu\text{m}$ mass median diameter at the tip of bronchoscope at a rate of $172 \text{ mg}\cdot\text{min}^{-1}$ [5]. An ultrasonic nebulizer (Pulmosonic, model 2511, DeVilbiss, USA) was used to generate the aerosols of normal and hypertonic saline. The outlet port of the nebulizer was connected to the catheter, passed through the instrument channel of bronchoscope, until it emerged at the distal end (fig 1a). The aerosol was generated by the nebulizer, with a priming volume of 5 ml, and directed down the catheter by an airflow of $200 \text{ ml}\cdot\text{min}^{-1}$ (fig 1b).

Double-balloon catheter. ESCHENBACHER and GRAVELYN [6] described a technique of isolating an airway segment using a double-balloon catheter, thereby enabling challenge and sampling by lavage from an isolated airway segment. The double-balloon catheter was a modification of a pulmonary artery catheter, measuring 110 cm in length and 0.2 cm in diameter. The two balloons are located at the distal end of the catheter, separated by 2-4 cm. Each balloon can be inflated with up to 2.5 ml of air. The inter-balloon segment has channels for infusion and withdrawal of fluid (fig. 2).

Drug premedication

It is common practice to use an inhaled β_2 -agonist and systemic atropine as a premedication, lignocaine for surface anaesthesia of upper airways, and light sedation to facilitate bronchoscopy in asthmatics. However, some of these drugs can inhibit bronchoconstrictor response to local challenge and it is, therefore, important to select the appropriate premedication for bronchoscopy, depending upon the type of study. For example, ipratropium bromide should be substituted for the β_2 -agonists for studying the effect of local challenge on mast cell mediator release, since it is known that the latter class of the bronchodilators inhibit stimulus related mast cell activation and subsequent mediator release [8]. Moreover, if the effect of premedication

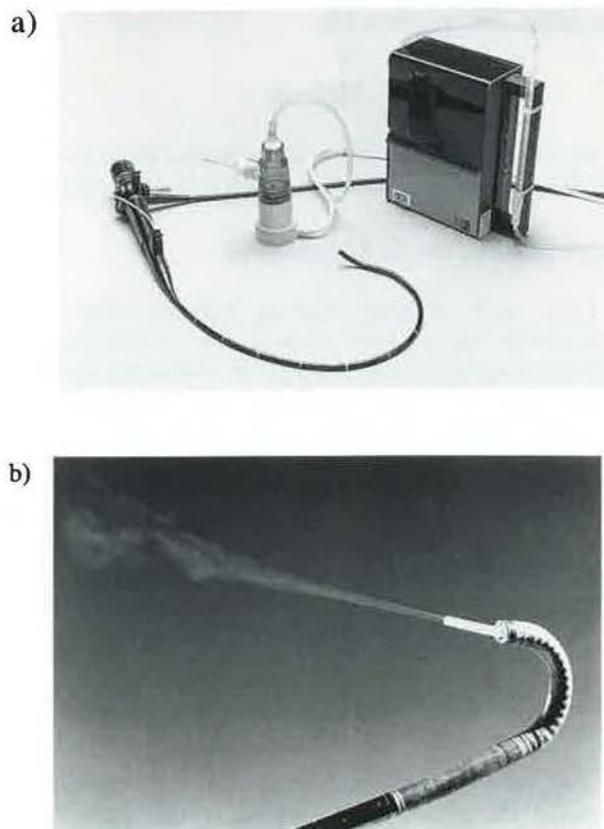


Fig. 1. - a) ultrasonic nebulizer outlet-port connected to the catheter, passed through the instrument channel of the fiberoptic bronchoscope for local aerosol delivery; b) aerosol emerging from the catheter tip at the distal end of bronchoscope.

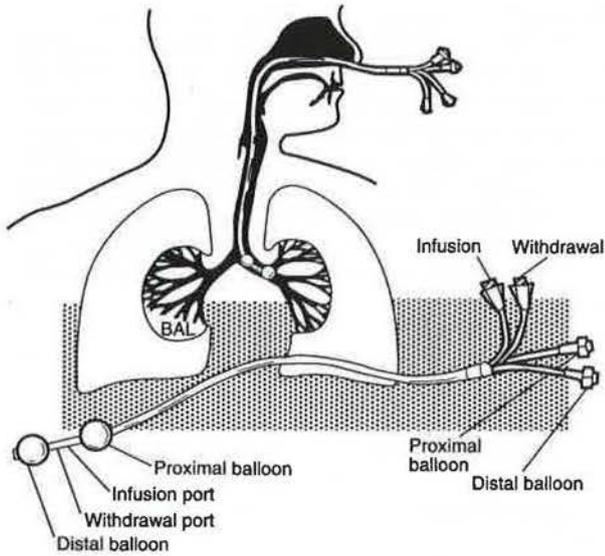


Fig. 2. - Double-balloon catheter for isolated airway segment challenge and lavage. (Reproduced with permission from [7]).

drugs, such as ipratropium bromide, atropine and lignocaine, on the local airway response is not known, then it can be assessed beforehand by determining the effect of the drug on inhalation challenge, in order that the appropriate dose for local challenge can be chosen, so as not to interfere with the provocation response [9].

Methods

Local airway challenge can be performed either through the fiberoptic bronchoscope or a double-balloon catheter.

Segmental LAC via the bronchoscope

Local airway challenge, either by instillation of the solution through the instrument channel of the bronchoscope or through a catheter passed through the instrument channel, allows inspection of the challenged areas of airways for mucosal changes, narrowing of the lumen, and to obtain BAL and bronchial biopsies. Furthermore, a control challenge followed by BAL and bronchial biopsies can be performed in another segmental airway at the same bronchoscopy [3]. Most investigators have used this method to study the early and late airway response following allergen challenge, by obtaining BAL for inflammatory mediators and cells at various time-points after the challenge.

LAC of an isolated airway segment using a double-balloon catheter

This has been performed by inserting a double-balloon catheter into the left main bronchus (LMB) under direct bronchoscopic vision [6]. The distal end of the catheter is placed into the LMB, so that the distal balloon is just proximal to the bifurcation of the LMB, and the proximal balloon is just distal to the main carina. An airway segment

of approximately 2 cm in size is isolated and used for sham challenge, followed by hypertonic challenge. Isolated airway segment lavage (IASL) is obtained after each challenge (fig. 2). Others have applied a slight modification of this technique, using a single balloon catheter to isolate the proximal airway segment of the LMB and performing local challenge and IASL with the patient in sitting position [10]. Recently, a further modification of this technique has allowed a smaller size double-balloon catheter to be inserted through the instrument channel of the bronchoscope for isolating segmental bronchi [11]. One advantage of this method is that only the isolated airway segment receives the challenge and is usually restricted to studies involving measurement of mediators. Higher levels of relevant mediators are obtained with IASL when compared to BAL, since the airway fluid is not diluted by lavage recovered from distal non-challenged airways and alveoli [7]. However, one limitation of the technique is that it requires the complete occlusion of the main bronchus to isolate an airway. This carries a significant risk not only of oxygen desaturation, but also lung collapse, dyspnoea and discomfort from insertion of the inflated balloon. In addition, the irritation of bronchial mucosa with the balloon catheter itself causes mediator release [12]. Also, the technique does not allow for direct visualization of the challenged airways to observe airway narrowing and other mucosal changes, nor does it provide an opportunity for obtaining mucosal bronchial biopsies from the challenged site.

The provocation agents used for LAC can be broadly categorized on the basis of allergenicity- 1) allergen - house dust mite, mixed grass pollen, ragweed, *etc*; 2) nonallergen - hypertonic saline, adenosine 5'-monophosphate and cold dry air. So far, most studies have employed allergen for LAC, with a few studies using hypertonic saline. However, it is theoretically possible to use any of the provocative agents for LAC, which induce bronchoconstriction when inhaled.

There is still a limited experience with dose-response studies using LAC in asthma. At the time of writing, the dose of the challenge agent has been determined by the provocation dose of the challenge agent required to achieve a 20% fall in FEV₁ from baseline (PD₂₀FEV₁), obtained from an inhaled bronchial challenge or from the concentration of allergen required to produce positive skin test response. Ideally, the dose of the provocation agents for LAC should be determined by performing dose-response studies for each subject. Such studies with LAC have been successfully performed in anaesthetized dogs, in which incremental doses of LAC with hypertonic aerosol have been administered, with measurements of collateral resistance via an additional catheter placed in the airways [13]. To some extent, it has been possible to perform a dose-response study in subjects with rhinitis by performing LAC of three different segmental bronchi with low, medium and high doses of the allergen [14]. However, in asthma these additional dose-response studies are likely to compromise the safety. Since the experience with LAC in asthma is still quite limited, repeated challenges and objective measurement of the airway response by placement of an additional device, for example for the measurement of collateral resistance, could be risky. However, without dose-response

studies, the difficulty of knowing the exact dose of the provocation agent for LAC exists, since it is not always possible to extrapolate the dose for LAC from inhaled bronchial challenges or skin test responses. This difficulty can be overcome, to some extent, by observing the visual response of the airways for local bronchoconstriction after instillation of increasing doses of the challenge agent. However, the assessment of local visible bronchoconstriction with bronchoscopy is difficult to quantitate, and only medium size airways can be visualized with the bronchoscope. In future studies and with more experience with LAC, it may be possible to quantitate the response by measuring airway resistance with the catheter used for instillation of challenge solution.

Risks and safety

The safety of the subjects is of paramount importance, and cannot not be jeopardized for the sake of completing a research protocol for obtaining scientific data. The available experience indicates that BAL and bronchial biopsies increase the risk associated with bronchoscopy alone. Local airway challenge is likely to increase these risks, and may lead to the following additional problems:

1. Generalized airway narrowing, if there is a failure to localize the challenge to the selected site and if the dose for LAC is not selected accurately.
2. Systemic reactions due to absorption of the allergen. In most studies, LAC has been performed by the instillation of the solution through the bronchoscope and the allergen has been one of the most common challenge agent used. In some studies, the allergen solution was instilled in serial small aliquots of 5–10 ml each, to achieve local response. This required a variable amount of allergen solution from 10–50 ml. The excess allergen absorbed not only carries the risk of inducing generalized bronchoconstriction, but also absorption of allergen from alveoli carries the risk of systemic reactions.
3. Infection from the challenge solution.
4. The technique of IASL for LAC requires placement of the double-balloon catheter in the major airways and complete occlusion of the airway for about 30 min. Although no major complications have been reported with this technique, it carries the potential complications of lung collapse, hypoxaemia and airway trauma.

Some of these risks can be reduced by the following measures, in addition to those suggested by recent workshop guidelines for investigative bronchoscopy in asthma [15].

1. *Restricted use - for subjects with mild-moderate asthma only.* Although some of the studies have shown that investigative bronchoscopy can be safely performed in severe asthmatics, its use for LAC should be restricted to patients with mild to moderate asthma. This may be defined as: asthma controlled with low dose inhaled corticosteroids, FEV₁ >70%, peak flow variability of <20–30%, and nonspecific bronchial hyperresponsiveness to histamine or methacholine of >1 mg·ml⁻¹.

2. *Premedication with a bronchodilator.* Workshop guidelines suggest that premedication for bronchoscopy may be

omitted, depending upon the research project [15]. In our experience, omitting a bronchodilator (ipratropium bromide), with or without atropine, induces generalized bronchoconstriction [5]. Therefore, we suggest the use of either salbutamol, ipratropium bromide or aminophylline, depending on the nature of the research project. The bronchodilator which least affects airway response to inhaled challenge may be used in the premedication.

3. *Use of the smallest effective dose challenge with an aerosol method of delivery.* Local airway challenge with an aerosol may help to reduce the problems associated with absorption of excess amounts of instilled allergen solution. We have shown that a much smaller dose of hypertonic saline aerosol, 172 mg, is more effective in achieving a local response than instillation of 10–20 ml of hypertonic saline solution [5]. Local airway challenge with an aerosol probably leads to more effective contact between the airway mucosa and hypertonic saline, thus providing an effective stimulus in a smaller dose.

Applications of Local Airway Challenge (Table 1)

The use of LAC has been confined mainly to the study of airway events following provocation with allergen and hypertonic challenge. LAC with allergen has helped to characterize the early airway response (EAR) as predominantly due to cellular activation and mediator release, and the late airway response (LAR) as due to infiltration of inflammatory cells contributing to the increase in bronchial hyperreactivity.

Airway events following local challenge with allergen

Mediators. LAC with allergen has proved useful in demonstrating mast cell degranulation immediately following the challenge shown by release of the preformed mast cell mediators, histamine and tryptase [14, 16, 17, 24], as well as increased levels of the cyclooxygenase metabolites, prostaglandin (PG)D₂ and thromboxane [16, 17, 20] and lipoxigenase metabolites of arachidonic acid, such as leukotriene C₄ [18, 19]. The technique has also been used to demonstrate kinin release during the EAR to allergen [20].

Cellular changes. Most studies during EAR following LAC with allergen have reported minimal or no changes in the absolute number of inflammatory cells in the airways [14, 17, 20, 22]. METZGER *et al.* [3] reported some increase in lymphocytes and macrophages in the lavage during EAR following LAC with allergen, and important cellular changes on electronmicroscopy of lavaged cells showing changes suggestive of activation (degranulation) of mast cells, eosinophils and macrophages. However, during EAR we have found a reduction in the total number of T-cells recovered in BAL from allergen challenged site and decrease in the CD4:CD8 ratio, suggesting selective entrapment of CD4 cells within the lung, probably through upregulation of adhesion molecules [22]. Apart from these changes in BAL during EAR, we have also examined bronchial biopsies obtained from allergen- and saline-challenged bronchial segments by immunohistochemical

Table 1. - Studies using local airway challenge to investigate cellular and mediator changes in asthma

Studies and subjects	Local airway challenge	Bronchoalveolar lavage	
		Early	Late
Allergen			
MEITZGER <i>et al.</i> [3] (1987) 11 asthma 6 normal	Ragweed, Alternaria and cat saliva, in serial 5 ml aliquots to achieve local visible response	BAL within 10 min; mast cell and eosinophil degranulation. Increase in macrophage number and activation	After 48 and 96 h; persistent mast cell and eosinophil degranulation. Increase in neutrophils, eosinophils and T-lymphocytes
MURRAY <i>et al.</i> [16] (1986) 5 asthma	<i>D. pteronyssinus</i> in dose of 10 µg	BAL within 5 min; increase in PGD ₂	
WENZEL <i>et al.</i> [17] (1989) 11 asthma 15 non-asthma	Ragweed, Alternaria, cat dander and grass, in 5 ml saline, in dose based on skin response	BAL after 5 min; increase in histamine PGD ₂ and thromboxane	
WENZEL <i>et al.</i> [18] (1990) 14 asthma 10 non-asthma	As above	BAL after 5 min; increase in LTC ₄	
MIADONNA <i>et al.</i> [19] (1990) 8 asthma 3 rhinitis	<i>D. pteronyssinus</i>	BAL after 5 and 15 min; increase in histamine, PGD ₂ and LTC ₄	
LIU <i>et al.</i> [20] (1991) 10 asthma 7 normal	Ragweed, in 5 ml aliquot of 100–300 pnu·ml ⁻¹ . 1,000 pnu·ml ⁻¹ in normal subjects	BAL within 5 min; increase in histamine, PGD ₂ , PGF _{2α} , thromboxane, kinins. No change in cells numbers and permeability	After 19h; persistent elevation of histamine and PGD ₂ , with increase in PGE ₂ , PGF _{2α} , kinins, albumin and urea. Increase in eosinophils basophils, and lymphocytes
SEDGWICK <i>et al.</i> [14] (1992) 6 rhinitis 5 normal	Ragweed, in low, medium and high dose (based on PD ₂₀), in 10 ml saline, in three different airway segments	BAL after 12 min; increase in histamine and tryptase, no cellular response	After 48 h increase in eosinophils eosinophilic granular proteins and IL-5. Also, increase in LTC ₄
GEORAS <i>et al.</i> [21] (1992) eosinophils 14 rhinitis/and asthma	Ragweed, <i>D. farinae</i> and grass, in 5 ml saline, in dose 100 pnu·ml ⁻¹ in most subjects		After 18 h, increase in and basophils. Granulocytes; increase expression of CD11b and decrease in L-selectin and VLA-4 expression
GRATZIOU <i>et al.</i> [22] (1992) 13 asthma 10 normal	<i>D. pteronyssinus</i> , in 20 ml saline, in dose based on skin response	10 min after challenge, loss of CD4+ cells mostly of CD4, with decrease in CD4:CD8. No change in T-cell activation (IL-2 R and HLA-DR)	
MONTEFORT <i>et al.</i> [23] (1993) 6 asthma	<i>D. pteronyssinus</i> , in 20 ml saline, in dose based on skin response	Bronchial biopsies at 6 h; increased eosinophils, mast cells, CD3+ lymphocytes. Increased endothelial ICAM-1 and E-selectin	
Non-allergen			
GRAVELYN <i>et al.</i> [12] (1988) 6 asthma 12 non-asthma	Hyperosmolar challenge of isolated airway segment (IAS)	Immediate IAS lavage: increase in histamine, PGD ₂ and thromboxane. Mediator release with balloon catheter	
MAXWELL <i>et al.</i> [9] (1990) 5 asthma 6 normal	Hypertonic challenge of isolated airway segment (IAS)	Immediate IAS lavage: increase in histamine	
MAKKER and HOLGATE [8] (1993) 18 asthma	Hypertonic saline by instillation and aerosol to achieve local bronchoconstrictor response	BAL within 5 min followed by bronchial biopsies. Correlation between BAL histamine and PD ₂₀ hypertonic saline response	

PD₂₀: provocative dose producing a 20% fall in forced expiratory volume in one second; BAL: bronchoalveolar lavage; PGD₂: prostaglandin D₂; LTC₄: leukotriene C₄; PGD₂, E₂ and PGF_{2α}: prostaglandins D₂, E₂ and F_{2α}; IL-2: interleukin-2; HLA-DR: human leucocyte antigen-DR; ICAM-1: intercellular adhesion molecule-1; VLA-4: very late activation antigen.

and electronmicroscopic analysis [25]. The immunohistochemical staining of the bronchial biopsies showed no difference in the number of any leucocyte subset or mast cells between the active and control sites. However, electronmicroscopic examination did demonstrate a greater degree of mast cell degranulation in the biopsies from the allergen-challenged site compared to the control site. The studies of airway cellular changes during EAR to allergen have helped to focus on the role of T-cells and macrophages as orchestrating the airway response to allergen. Local airway challenge has also been very informative in determining cellular changes during LAR. METZGER *et al.* [3] demonstrated impressive cellular changes 48 h and 72 h after LAC with allergen. BAL collected from the challenged site at 48 h post-allergen showed an increase in neutrophils, T-lymphocytes and eosinophils, with the latter two cells subsets persisting at these high levels for up to the 96 h [3]. LIU *et al.* [17] studied airway events 19 h following LAC with ragweed, and demonstrated that post-challenge BAL from the stimulated site contained an increased number of eosinophils, basophils and lymphocytes. An increase in neutrophils was deemed to be nonspecific, as a similar increase in these polymorphonuclear granulocytes was observed at the sham-challenged site. This highlights one of the problems associated with the instrumentation of the airways. An increase in local microvascular permeability, as demonstrated by raised albumin levels, was a further marker used as evidence of local allergen-induced inflammatory response.

The same group recently published data from a similarly-challenged group of subjects, in which they studied the expression of the integrins very late activation antigen (VLA)-4 and CD11b/18, and the selectin, L-selectin, on peripheral blood and BAL granulocytes [21]. The levels of CD11b/18 were increased on the surface of granulocytes collected in the BAL from the antigen-challenged segment, when compared to those within peripheral blood, whilst the reverse occurred for L-selectin. This suggests that the granulocytes making their way to the bronchial lumen from the circulation were ones with a higher level of expression of the β_2 -integrins, and that they had shed their surface L-selectin on transmigration into the bronchial submucosa. Neutrophils showed similar changes in the saline-challenged segments, thus implying that the changes noted on this type of granulocyte were not allergen-specific. VLA-4 levels were only analysed on eosinophils from BAL and peripheral blood in two subjects, and no significant difference in expression was noted between the two compartments. Interestingly, levels of soluble endothelial leucocyte adhesion molecule (ELAM)-1 were also increased in BAL from the allergen-challenged sites, when compared to the saline control site, an observation that has not been made with the soluble adhesion molecule measured in peripheral blood. In a study by SEDGWICK *et al.* [14], segmental endobronchial ragweed challenges on six sensitized seasonal rhinitics showed a dose-related increase in eosinophils, the levels of eosinophilic arginine-rich proteins and interleukin-5 in the BAL 48 h after challenge. At this time-point, there was also an increase from baseline of eosinophil membrane expression of intercellular adhesion molecule (ICAM)-1 and levels of soluble ICAM-1 in the

BAL [26]. Recently, in a group of six asthmatics undergoing allergen challenge and biopsied 6 h later, we observed a large allergen-induced increase in the bronchial submucosal number of eosinophils, neutrophils, mast cells and CD3+ lymphocytes, and also a significant difference from the control site in the endothelial expression of ICAM-1, E-selectin, but not VCAM-1, together with a parallel increase in lymphocyte function associated antigen (LFA)-1+ cells [23]. In most of these patients the segmental challenge induced a significant decrease in FEV₁ and an increase in bronchial hyperreactivity 24 h later, which returned to normal by 1 week.

Local airway challenge with hypertonic saline in exercise-induced asthma (EIA)

It has been suggested that exercise-induced bronchoconstriction is due to the hypertonicity of airway lining fluid, consequent upon the high rate of evaporative water loss during exercise [27]. Since, it is difficult to study endobronchial events immediately following exercise-induced bronchoconstriction, LAC with hypertonic saline has been used in the investigation of the mechanisms of EIA.

GRAVELYN *et al.* [12] studied the mediator release following hypertonic saline challenge in an isolated airway segment. The isolated airway segment lavage (IASL) showed a rise in histamine. Similarly, MAXWELL *et al.* [10] using a slight modification of the above technique, also showed a mast mediator release following local hypertonic challenge. However, with the IASL technique the catheter alone also caused mediator release, due to its irritant effect.

Recently, we have studied the airway events following LAC with hypertonic saline *via* the bronchoscope in subjects with EIA [5]. We have performed LAC with an aerosol of hypertonic saline in addition to instillation, and obtained BAL and mucosal bronchial biopsies immediately after the challenge. With this method, we were able to observe the airways for visible local bronchoconstriction and perform control challenge in another segmental airway at the same bronchoscopy. We found that the aerosol was more effective in inducing local bronchoconstrictor response, and better tolerated when compared to instillation of hypertonic saline solution. The BAL level of histamine correlated with airway responsiveness to inhaled hypertonic saline challenge. Following local challenge with hypertonic saline, some subjects demonstrated a rise in histamine in BAL, but the overall levels were not significantly different between the control and challenged sites. We have also observed extensive mast cell degranulation in the bronchial biopsies from both control and challenged sites, with no difference between the two. The method also allowed us to study the epithelial changes in the asthmatic airway following hypertonic challenge, and we found extensive epithelial damage, with only one-fifth of the bronchial mucosa being covered with intact epithelium.

Concluding remarks

Local airway challenge has proved to be a safe and useful technique to study airway events following allergen

and hypertonic challenge. So far, its use has been confined to studying cellular and mediator changes in asthma. However, the technique has potential for use in exploring the role of cytokines *in vivo*, and endobronchial physiological changes following local airway challenge. Its use could be extended to the study of airway events following other precipitating factors in asthma, and to investigate the effect of anti-asthma drugs at cellular, cytokine and receptor levels.

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