induced by toluene diisocyanate. *Am Rev Respir Dis*, 1987, 136, 36–42.
8. Boschetto P, Fabbri LM, Zocca E, Milani GF, Pivirotto F, Dal Vecchio A, Plebani M, Mapp CE. – Prednisone inhibits late asthmatic reactions and airway inflammation induced by toluene diisocyanate in sensitized subjects. *J Allergy Clin Immunol*, 1987, 80, 261–267.
9. Fabbri LM, Di Giacomo R, Dal Vecchio L, Zocca E, De Marzo N, Maestrelli P, Mapp CE. – Prednisone, indomethacin and airway responsiveness in toluene diisocyanate sensitized subjects. *Bull Eur Physiopathol Respir*, 1985, 21, 421–426.
10. Mapp CE, Boschetto P, Dal Vecchio L, Crescioli S, De Marzo N, Paleari D, Fabbri LM. – Protective effect of antiasthma drugs on late asthmatic reaction an increased responsiveness induced by toluene diisocyanate in sensitized subjects. *Am Rev Respir Dis*, 1987, 136, 1403–1407.
11. De Marzo N, Fabbri LM, Crescioli S, Plebani M, Tognella S, R, Mapp CE. – Dose dependent inhibitory effect of inhaled beclomethasone on late asthmatic reactions and increased airway responsiveness to methacholine induced by toluene diisocyanate in sensitized subjects. *Pulmonary Pharmacology*, 1988, 1, 15–20.
12. Tossin L, De Marzo N, Crescioli S, Mapp CE, Fabbri LM, Boschetto P. – Ketotifen does not inhibit asthmatic reactions induced by toluene diisocyanate in sensitized subjects. *Clin Exper Allergy*, 1989, 19, 177–182.
13. Paggiaro PL, Bacci E, Talini D, Dente FL, Rossi O, Pulerà N, Fabbri LM, Giuntini C. – Atropine does not inhibit late asthmatic responses induced by toluene diisocyanate in sensitized subjects. *Am Rev Respir Dis*, 1987, 136, 1237–1241.
14. Zocca E, Fabbri LM, Boschetto P et al. – LTB_4 and late asthmatic reactions induced by TDI. *J Appl Physiol*, 1990, in press.

Bronchoalveolar lavage in allergic asthma

G.A. Rossi, E. Crimi, S. Lantero, P. Gianiorio, V. Brusasco

Bronchoalveolar lavage (BAL) has improved knowledge of the defence mechanisms of the human lung and the inflammatory and immune mediated changes involved in the pathogenesis of diseases of the pulmonary parenchyma [1]. In addition, it has been used in disorders of the airways such as allergic occupational and intrinsic asthma and chronic bronchitis, in which the bronchial components of lavage may produce more useful information than the alveolar components. Fluid containing mainly components from the airways compartment can be obtained by bronchial lavage (BL).

In bronchial asthma, inflammation is thought to play a major role in perpetuating bronchial hyperreactivity and obstruction. Clinical and morphological observations on biopsy or autopsy specimens support this hypothesis but the relative importance of the various inflammatory cells and the mediators released by them is not established.

In patients with allergic asthma, BL and BAL have made it possible to study: 1) the cell populations, antibodies and mediators present in the bronchial tree which may contribute to airway hyperreactivity and inflammation; 2) the mechanisms of allergic asthmatic response after bronchial exposure to the sensitizing allergen; 3) the action and efficacy of drugs.

Inflammatory cells present in the asthmatic lung vary with stage, severity and type of disease. In mild asthmatics studied during disease quiescence BAL showed a mild increase in % eosinophils and neutrophils [2, 3]. In BL from asymptomatic patients, CRIMI *et al.* [4] found increased albumin and specific IgE's, correlating well with the results of bronchial challenge. These data suggest that airways inflammation with increased alveolar capillary permeability is present in asthmatics even at a time of quiescence. Mast cells, which are believed to play a major role in response to allergen, were found in BAL of mild asthmatics during clinically asymptomatic periods [5–8]. Also, the level of histamine in BAL of asthmatics was higher than in BAL of controls [7] and correlated with bronchial hyperresponsiveness [6, 7]. However, RANKIN *et al.* [9] found that levels of histamine in BAL of asthmatics did not differ from controls or correlate with mast cell or basophil count. WENZEL *et al.* [10] showed that pulmonary activation of mast cells occurs after allergen challenge in subjects with atopy and asthma and, to a lesser degree, in those with atopy alone.

Mast cells do not, therefore, seem to be constantly involved in patients with day-to-day asthma but are probably recruited in response to allergen inhalation or other stimuli. Conflicting results may depend on the stage and severity of disease. When bronchial hyperresponsiveness was systematically determined a significant negative correlation was found between methacholine PC_{20} and % mast cells, eosinophils and epithelial cells recovered from BAL.

The combination of bronchial provocation test (BPT) with BL and/or BAL provides new insights into events following allergen inhalation. Exposure to specific antigen causes increased permeability of bronchial mucosa resulting in visual evidence of oedema and migration of more proteins into the bronchial lumen [11]. METZLER *et al.* [12] showed an increased % neutrophils and eosinophils in BAL within 4 h of BPT, whilst eosinophils alone were still increased 24 h later; all but one patient developed a dual response to bronchial challenge. DE MONCHY *et al.* [13] showed an increased % eosinophils

I Divisione di Pneumologia, Ospedale S. Martino, Genova, and Istituto Di Medicina Dello Sport, Università Di Genova, Italy.

within 6–7 h of BPT only in patients who developed a dual asthmatic response, whilst no differences were found between patients with isolated early asthmatic reaction and normals.

We found higher levels of specific IgE's 72 h after inhalation challenge in BL of patents who developed a dual response than in BL of patients with an isolated early response. However, the % neutrophils and eosinophils in these two groups was no different and desquamative epithelial cells were similarly elevated in BL from both.

These findings indicate that airway inflammation occurs after experimental allergen exposure, resulting in epithelial damage both in patients with dual reaction and those with isolated early reaction. A more pronounced, transient cellular influx into the lung is probably responsible for the development of the late asthmatic reaction. It also seems that epithelial cells are a target of the inflammatory reaction which follows allergen inhalation.

Macrophages appeared suddenly after local bronchial challenge with a further increase 48 h later [14]. Electron microscopy showed highly activated cells, evidenced by ruffled membranes, vesicles, cytoplasmic projections and phagocytosed mast cell granules [14]. After specific IgE antigen-antibody complex stimulation alveolar macrophages from asthmatics release PAF-acether, which has recently been proposed as an important mediator of inflammation and bronchoconstriction [15]. Involvement of macrophages in the response to local bronchial provocation was observed by TONNEL *et al.* [16] who found an increased level of β-glucuronidase after challenge.

Does a cellular mechanism make a patient more likely to develop a late asthmatic reaction to allergen exposure? Using BAL in patients with previously documented dual asthmatic response METZGER *et al.* [14] showed, 48 h after the immediate response, not only an increased number of neutrophils and eosinophils but also of T-lymphocytes. T-cells were still elevated 48 h later, with a high proportion of helper T-cells. Conversely, in patients with an isolated early asthmatic reaction GONZALES *et al.* [17] found an increased proportion of suppressor T-cells. These findings suggest that T-lymphocytes play a key role in development of the late asthmatic reaction, possibly by modulating B-lymphocytes to produce and release antigen specific IgE's.

Lavage techniques may provide information on the relationships between cells and mediator release by allowing quantitation of soluble components secreted by inflammatory cells in the bronchial lumen. The presence of a mediator in BAL or BL is not necessarily correlated with the presence of the cells which produce it, because of the time lag between cell influx and mediator release at the site of inflammation. MURRAY *et al.* [18] recorded the release of a major mast cell mediator into BAL fluid after local allergen challenge. Nine minutes after challenge a 150 fold mean rise in PGD_2 and an increased concentration of 15-HETE and β-glucuronidase were observed. Changes between control and post-challenge levels of 5-HETE, LTB_4, LTC_4, LTD_4 and LTE_4 were not detected. Leukotrienes B_4, D_4 and E_4 were observed by CHAN-YEUNG *et al.* [19] in BAL from patients with red cedar asthma 2 h after bronchial challenge. An increased concentration of eosinophil cationic protein to albumin ratio in BAL from patients who underwent bronchial challenge has also been reported [12]; again the highest concentrations of this protein were observed for patients with a dual response. GRAVELYN *et al.* [20] studied the release of mediators after local exposure to hyperosmolar stimulus and observed a significant increase in the lavage concentration of histamine, PGD_2 and PGF_2 alpha.

Possible repetitive evaluation of inflammatory cells and their products in the lungs makes the BAL technique useful in understanding the action of anti-asthmatic drugs. DIAZ *et al.* [21] showed that sodium cromoglycate suppressed local accumulation of eosinophils and reduced levels of specific IgE antibodies in allergic asthmatics treated for 28 days. BOSCHETTO *et al.* [22] showed the efficacy of prednisone in preventing the late asthmatic reaction and airway inflammation induced by toluene diisocyanate.

Few studies have evaluated the complications of BL and BAL techniques. Some have reported BAL-induced bronchospasm [23, 24] whilst others observed no significant deterioration of pulmonary function even when BAL was performed after local allergen provocation [14, 25]. We found no significant changes of sensitivity to methacholine in patients with isolated early response or dual response who underwent BL and BAL 72 h after bronchial challenge.

Studies using a combination of BPT and BAL may provide useful information on the pathophysiology of allergic asthma. They should be designed to clarify the complex relationships between the inflammatory cells and their mediators and between these mediators, tissue damage and bronchial constriction. The role of non-specific stimuli and of specific and non-specific pharmacologic antagonists should also be evaluated.

References

1. Rossi GA. – Bronchoalveolar lavage in the investigation of disorders of the lower respiratory tract. *Eur J Respir Dis*, 1986, 69, 293–315.
2. Godard P, Aubas P, Calvayrac P, *et al.* – Endoscopie et lavage bronchiolo-alvéolaire chez l'asthmatique allergique. *Nouv Press Méd*, 1981, 10, 3141–3148.
3. Godard P, Terral C, Aubas P, *et al.* – Intérêt diagnostic et physiopatologique du lavage bronchoalveolaire chez l'asthmatique. *In*: Bronches de l'asthmatique: 7th International Congress, F.B. Michel ed., Masson, New York and Paris, 1982, pp. 49–53.
4. Crimi E, Scordamaglia A, Crimi P, Zupo S, Barocci S. – Total and specific IgE in serum, bronchial lavage and bronchoalveolar lavage of asthmatic patients. *Allergy*, 1983, 38, 553–559.
5. Tomioka M, Ida S, Shindoh Y, Ishihara T, Takishima T. – Mast cells in bronchoalveolar lumen of patients with bronchial asthma. *Am Rev Respir Dis*, 1984, 129, 1000–1005.
6. Flint KC, Leung KBP, Hudspith BN, Brostoff J, Pearce FL, Johnson NMcI. – Bronchoalveolar mast cells

in extrinsic asthma: a mechanism for the initiation of antigen specific bronchoconstriction. *Br Med J*, 1985, 291, 923–926.
7. Casale TB, Wood D, Richerson HB, Trapp S, Metzger WJ, Zavala D, Hunninghake GW. – Elevated bronchoalveolar lavage fluid histamine levels in allergic asthmatics are associated with methacholine hyperresponsiveness. *J Clin Invest*, 1987, 79, 1197–1203.
8. Kirby JG, Hargreave FE, Gleich GJ, O'Byrne PM. – Bronchoalveolar cell profiles of asthmatic and nonasthmatic subjects. *Am Rev Respir Dis*, 1987, 136, 379–383.
9. Rankin JA, Kaliner M, Raynolds HY. – Histamine levels in bronchoalveolar lavage from patients with asthma, sarcoidosis and idiopathic fibrosis. *J Allergy Clin Immunol*, 1987, 79, 371–377.
10. Wenzel SE, Fowler AA, Schwartz LB. – Activation of pulmonary mast cells by bronchoalveolar allergen challenge. *Am Rev Respir Dis*, 1988, 137, 1002–1008.
11. Fick RB, Metzger WJ, Moseley PL, Richerson HB, Hunninghake GW. – Increased bronchovascular permeability following allergen exposure in asthmatics. *J Appl Physiol*, 1987, 63, 1147–1155.
12. Metzger WJ, Richerson HB, Worden K, Monick M, Hunninghake GW. – Bronchoalveolar lavage of asthmatic patients following allergen bronchoprovocation. *Chest*, 1986, 89, 477–483.
13. De Monchy JGR, Kauffman HF, Venge P, Koeter GH, Jansen HM, Sluiter HJ, De Vries K. – Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. *Am Rev Respir Dis*, 1985, 131, 373–376.
14. Metzger WJ, Zavala D, Richerson HB, Moseley P, Iwamota P, Monick M, Sjoerdsma K, Hunningake GW. – Local allergen challenge and bronchoalveolar lavage of allergic asthmatic lungs. *Am Rev Respir Dis*, 1987, 131, 433–440.
15. Arnoux B, Duval D, Benveniste J. – Release of platelet-activating factor (PAF-acether) from alveolar macrophages by the calcium ionophore A23187 and phagocytosis. *Eur J Clin Invest*, 1980, 6, 437–441.
16. Tonnel AB, Gosset P, Joseph M, Fourier G, Capron A. Stimulation of alveolar macrophages in asthmatic patients after local provocation. *Lancet*, 1983, i, 1406–1409.
17. Gonzales MC, Diaz P, Galleguillos FR, Ancic P, Cromwell O, Kay AB. – Allergen-induced recruitment of bronchoalveolar helper (OKT4) and suppressor (OKT8) T-cells in asthma.
18. Murray JJ, Tonnel AB, Brash AR, *et al.* – Release of prostaglandin D_2 into human airways during acute antigen challenge. *N Engl J Med*, 1986, 315, 800–804.
19. Chan-Yeung M, Salari M, Lam S. – Role of leukotrienes in bronchial asthma. *Chest*, 1986, 89, 500A.
20. Gravelyn TR, Pan PM, Eschenbacher WL. – Mediator release in an isolated airway segment in subjects with asthma. *Am Rev Respir Dis*, 1988, 137, 641–646.
21. Diaz P, Galleguillos FR, Gonzales MC, Pantin CFA, Kay AB. – Bronchoalveolar lavage in asthma: the effect of disodium cromoglycate (cromolyn) on leucocyte counts, immunoglobulins and complement. *J Allergy Clin Immunol*, 1984, 74, 41–48.
22. Boschetto P, Fabbri LM, Zocca E, Milan G, Pivirotto F, Dal Vecchio A, Plebani M, Mapp CE. – Prednisone inhibits late asthmatic reactions and airway inflammation induced by toluene diisocyanate in sensitized subjects. *J Allergy Clin Immunol*, 1987, 80, 261–267.
23. Rosenow EC, Andersen HA. – Bronchoscopically induced bronchospasm. *Chest*, 1976, 75, 565–566.
24. Kelly C, Hendrick D, Walters H. – The effect of bronchoalveolar lavage on bronchial responsiveness in patients with airflow obstruction. *Chest*, 1988, 93, 325–328.
25. Ancic P, Diaz P, Galleguillos F. – Pulmonary function changes after bronchoalveolar lavage in asthmatic patients. *Br J Dis Chest*, 1984, 78, 261.

Role of bronchoalveolar lavage in the investigation of cell-mediated defence mechanisms against lung cancer

M. Spatafora, A.M. Merendino, G. Chiappara, M. Melis, D. Volpes, V. Bellia, G. Bonsignore

Little information is available on the applicability and reliability of bronchoalveolar lavage (BAL) in the evaluation of local cellular defence mechanisms against lung cancer (LC). LC is frequently located in the large airways, whilst BAL allows recovery of cells from the alveolar spaces. Important functional differences may exist between cells obtained by BAL and immunologically competent cells isolated after disaggregation of whole lung tissue specimens, thus BAL does not always sample the pulmonary cell population correctly.

Alveolar macrophages (AM) are thought to play an important role in the host defence mechanism against LC by releasing a variety of cytotoxic and cytostatic substances and by functioning as accessory cells for lymphocytes (LY). The importance of AM is demonstrated by the fact that over 90% of cells recovered from the alveoli by BAL and over one third of cells isolated after disaggregation of LC surgical specimens belong to the monocyte macrophage lineage [1]. These were shown to exert specific cytotoxic activity against autologous LC cells but not against non-malignant tumour targets, hence their toxicity could be related to tumour antigen driven specific responses [2].

Investigations into the differentiation of blood monocytes into AM and association to modifications of tumour killing properties have led to conflicting results. AM from smoking and nonsmoking normals and from patients with LC were shown to be more cytotoxic than autologous blood monocytes for various tumour cell lines (including squamous LC) with no differences between study groups [1]. In contrast, Bordignon *et al.* reported

Istituto di Fisiopatologia Respiratoria and Istituto di Medicina Generale e Pneumologia, Palermo, Italy.