Eur Respir J 2002; 20: Suppl. 37, 51s–55s
DOI: 10.1183/09031936.02.00005102

European Respiratory Journal
ISSN 0904-1850

REPORT OF WORKING GROUP 8

Future directions

Leader of the Working Group: A.M. Vignola*,#

Members of the Working Group: S.I. Rennard¶, F.E. Hargreave+, J.V. Fahy§,f, M.R. Bonsignore*, R. Djukanović**, P.J. Sterk##

The identification of biomarkers that allow early diagnosis, monitoring and optimisation of therapy of lung diseases is one of the most ambitious goals in respiratory medicine. At present, routine diagnosis and management of asthma and chronic obstructive pulmonary disease (COPD) are based on clinical/pulmonary function parameters even though it is appreciated that both asthma and COPD are syndromes comprising a spectrum of diseases. As a specialty, respiratory medicine should aim to achieve characterisation at least as good as has been possible for chronic liver and renal diseases.

The simplest definition of a biomarker is a molecule that indicates, or is associated with, an alteration in physiology. A more practical definition of a biomarker is a molecule that is of clinical utility. In this sense, the biomarker should specifically and with enough sensitivity reflect a disease state. As such it could be used for diagnosis as well as disease monitoring during and after completion of therapy.

The induced sputum technique allows sampling of the airways in a noninvasive fashion and thus offers a unique opportunity to identify biomarkers of potential clinical utility in respiratory medicine. It is hoped that, in the future, induced sputum will provide clinicians with useful markers which may be used routinely to perform a more accurate and, ideally, rapid determination of disease phenotypes in many lung diseases. For example, this would be extremely useful in asthma and COPD, two diseases which represent a major health and socioeconomic burden. Better characterisation is also needed since both asthma and COPD show a great degree of clinical heterogeneity, which must impact on responses to treatment. Moreover, the clinical picture in asthma and COPD frequently overlaps; patients with COPD may show some features of asthma, and many asthmatics are also smokers, thereby presenting with features of COPD. Such large heterogeneity and overlap highlight the need to accurately determine the phenotype of patients suffering from asthma and/or COPD. In addition, accurate phenotypic determination is crucial for a better understanding of relationships between biological markers and clinical outcomes, as well as the efficacy of novel therapeutic strategies.

Development of clinically useful sputum markers in asthma and chronic obstructive pulmonary disease

The ability to differentiate groups or subgroups of patients sharing similar biological patterns on clinical grounds was greatly limited before the advent of non- or minimally invasive techniques for assessing airway inflammation. In this respect, the use of the induced sputum technique as a noninvasive and reproducible technique for sampling the airways is a major improvement. The availability of useful sputum markers would allow better characterisation of the severity of airways diseases, assessment of disease control, assessment or prediction of the response to various treatments (*e.g.* corticosteroids and antibodies directed against immunoglobulin E), and the development of strategies to select phenotypes and/or subphenotypes of patients showing specific biological and clinical patterns. Ideally, it would be desirable to have markers which identify individuals who have not yet developed overt clinical disease, or have minimal symptoms (*e.g.* simple chronic bronchitis) but are at risk of developing clinically relevant disease. This would lead to a major shift in emphasis from treatment to prevention.

As with all public health interventions, effectiveness will be increased if interventions target the group at greatest risk of developing an ongoing clinically important illness. For this purpose, it is necessary to be able to predict which patients with mild disease (in particular, children) will go on to develop severe illness. In asthma, atopy and airways hyperresponsiveness measured during childhood have a relatively high sensitivity and specificity for this outcome and, as a result, a high positive likelihood ratio for predicting the presence of asthmatic symptoms in later life.

*Cattedra di Malattie Respiratoire, University of Palermo, Palermo, Italy. #Istituto di Fisiopatologia Respiratoria CNR, Palermo, Italy. ¶Dept of Internal Medicine, University of Nebraska Medical Center, Omaha, NE, USA. +Asthma Research Group, Firestone Institute for Respiratory Health, McMaster University, Hamilton, Ontario, Canada. §Pulmonary and Critical Care Division, Dept of Medicine. fCardiovascular Research Institute, University of California at San Francisco, San Francisco, CA, USA. **Southampton University General Hospital, Southampton, UK. ##Leiden University Medical Centre, Leiden, the Netherlands.

Correspondence: A.M. Vignola, Cattedra di Malattie Respiratoire, University of Palermo, Palermo, Italy. Fax: 39 091 6882165. E-mail: vignola.am@iol.it

Received: April 4 2002; Accepted: April 16 2002

However, it remains difficult to predict which of those children will relapse after a period free of illness, continue to be symptomatic or grow up to be nonsymptomatic as adults. Thus, it is still necessary to search for factors to improve the specificity of screening tests so that a group who are likely to have an ongoing and clinically important illness can be targeted. In the future, these factors may be represented by sputum markers which, in combination with other clinical and functional parameters, could significantly improve the ability to identify high-risk patients.

It is also hoped that the induced sputum technique will provide a simple and cost-effective tool for monitoring airway inflammation in the clinical setting, an approach which was precluded by previous techniques, such as bronchial biopsy and bronchoalveolar lavage (BAL). In addition, since the induced sputum technique enables regular monitoring of inflammation, it will be of great help in assessing the anti-inflammatory potential of new treatments.

The induced sputum technique and other lung diseases

Although the induced sputum technique has been widely used in asthma and COPD, it can also be extremely helpful in improving knowledge of the pathogenesis of other lung diseases, such as lung cancer, infectious diseases and parenchymal diseases. With regard to lung cancer, identification of early (or pre-) lung cancer in smokers is considered the best strategy for preventing this disease. Effective early detection of any disease requires that three essential criteria be met: 1) there must be a preclinical phase of the disease; 2) the technology to detect the disease in its preclinical stage must be available; and 3) this must open a window of opportunity for effective intervention as soon as the disease is discovered.

There is ample evidence of a prolonged preclinical phase in lung cancer. Clones of endobronchial cell populations accumulate genetic mutations, leading to a progressively more malignant and ultimately invasive malignant state. Neither the critical number of mutations, critical combinations of mutations nor a necessary order of events (if such an order exists) are known at this time. Elucidating these issues is one of the most exciting areas of current research because of the potential prospect that such understanding may lead to better detection and interventions.

Sputum cytological examination has been shown, in several studies, to lead to detection of lung cancer at an earlier stage, resulting in an improved 5-yr survival rate [1–3]. How this will combine with other approaches remains to be seen. Monoclonal antibody detection, fluorescence bronchoscopy and low-dose spiral computed tomography all increase diagnostic sensitivity and improve the ability to localise early-stage lesions. The ultimate goal of improving long-term survival in lung cancer will be achieved only when cancer can be detected in its early stages and lesions localised in large numbers of patients who can be cured. Early studies have demonstrated that molecular techniques can detect tumour cells in BAL fluid [4]. Recent studies of sputum specimens and clinical data linking specimens to lung cancer outcomes may allow determination of a molecular diagnosis of cancer several years before its clinical presentation [5–11]. This has been possible using tests to evaluate altered gene expression, including specific oncogene activation and tumour suppressor gene deletion, as well as genomic instability and abnormal methylation. Such studies clearly indicate that good sputum samples should permit performance of complicated genetic analysis, providing further impetus for considering the induced sputum technique as a tool for lung cancer screening. Utilising these new techniques and improving the definition of high-risk groups may improve the success and cost-effectiveness of early detection based on sputum cytology.

Pulmonary parenchymal diseases

To date, BAL has been the method of choice for studying parenchymal diseases. Recently, a few studies have employed sputum induction to investigate inflammation in patients with sarcoidosis in whom endobronchial, peribronchial and parenchymal inflammation is present. The CD4:CD8 ratio and levels of tumour necrosis factor-α in induced sputum have been found to be correlated strongly with those in BAL fluid either before or after treatment [12], suggesting that sputum may be a good noninvasive substitute for certain parameters in BAL fluid in patients with sarcoidosis [6, 13, 14]. Clearly, more work needs to be done in this and other interstitial lung diseases, not least in those that are related to occupation.

Can induced sputum be used in daily practice at the general practitioner level?

In clinical practice, assessment of airways inflammation and the effects of medication is difficult. Subjective assessment of symptoms is always difficult and has often been found to be unsatisfactory for monitoring asthma severity. Although the regular use of peak flow measurements has been shown to improve asthma control, peak flow rates or diurnal peak flow variability are not always predictive of a relapse [4, 7, 15, 16]. Measurement of levels of exhaled gases such as nitric oxide may be useful [17], but more data are needed to fully evaluate the importance of these markers in assessing airways inflammation in asthma, especially since NO can be produced in large amounts in paranasal sinuses [9, 18] and the stomach [10, 18].

When considering use of the induced sputum technique in clinical practice, a number of aspects need to be considered. 1) Can sputum induction be easily carried out by untrained personnel (safety and cost-effectiveness) and in general practice? 2) Is sputum induction sufficiently reproducible when carried out by untrained personnel? 3) Inflammatory markers have been shown to follow (or predict) airways inflammation and its response to treatment. However, the individual patient's response may vary

widely (this is the case for serum eosinophil cationic protein concentrations). Thus prospective studies showing the cost-effectiveness of sputum induction are required.

Although it is potentially meaningful and practical to measure inflammatory markers (cells or their secretory products) in specialist clinics [11, 19–21], the technique of sputum induction does not at present lend itself to routine use by general practitioners. For this to become possible and cost-effective, technical simplification of sputum induction and processing (using rapid automated methods) is needed. This will have to coincide with clear indication that adding markers of airway inflammation as guides in asthma therapy is useful. In this respect, longitudinal studies are needed in order to elucidate which parameter is the most useful in guiding asthma management.

Induced sputum and respiratory physiology: traditional issues in respiratory mechanics and gas exchange revisited

The past 15–20 yrs have seen a shift in emphasis in the field of respiratory medicine away from respiratory physiology to cell biology and genetics. Until now, little consideration has been given to the possible contribution of airway and pulmonary inflammatory cells to traditional aspects of respiratory physiology. However, this new research direction may yield important results, and a better understanding of the role of cells in physiological processes could be useful in identifying new targets for treatment of pathological conditions. For example, if inflammatory cells in the airways were found to contribute to the pathogenesis of exercise-induced dyspnoea in COPD patients, new and improved therapeutic approaches could be used in pulmonary rehabilitation. Similarly, the finding of cellular mechanisms contributing to exercise-induced hypoxaemia in athletes may shed light on different pathophysiological mechanisms for exercise-induced hypoxaemia in both elite athletes and patients with pulmonary diseases.

The induced sputum technique has been only occasionally used to study athletes of different disciplines at rest, mostly in sports in which asthmatic symptoms may be frequent (skiers) but also in situations in which airway inflammation could be causally related to special environmental conditions (skiing and swimming). The inflammatory cells found in induced sputum obtained at rest from athletes of different disciplines show variable patterns: high percentages of lymphocytes have been reported in "ski asthma" [22], whereas eosinophil and neutrophil numbers were increased in otherwise asymptomatic swimmers [23] and runners [24], respectively. Much less is known about the effects of acute and intense exercise at the airway level, with very recent data in runners suggesting an influx of neutrophils into the airways after a marathon [25]. These data are intriguing because the inflammatory pattern caused by the marathon includes changes in adhesion molecules on the neutrophil surface and increased exhaled NO but no clear signs of neutrophil activation in sputum or changes in pulmonary function after the marathon. Therefore, it may be speculated that exercise could modulate both the number and degree of activation of airway resident cells. Furthermore, differences in the way in which airway cells respond to exercise may be related to age, the age at which regular physical activity was started or the intensity of regular training. If these hypotheses are correct, studies of airway cells after exercise may open the way to a better understanding of the physiological role of different inflammatory cells in sedentary subjects and athletes. In addition, these studies may point to potential mechanisms by which exercise-based rehabilitation programmes may exert their positive effects in patients with pulmonary disease.

Similarly, the occurrence of exercise-induced hypoxaemia in elite athletes has been repeatedly documented, but no studies to date have examined whether inflammatory cells may be involved in its pathogenesis. Indeed, the development of an inflammatory process is a potential culprit, as systemic release of histamine was found to be associated with exercise-induced hypoxaemia in runners [26]. Exercise-induced asthma is another physiological puzzle, still lacking a definitive solution. Future studies, using the induced sputum and/or other techniques, aimed at assessing pulmonary inflammation (*i.e.* exhaled NO determination) may help to identify new and yet unknown interactions between inflammatory cells and changes in pulmonary mechanics and/or gas exchange in the lungs. These new perspectives might not only be important for the science of pulmonary physiology but also relevant to the care of pulmonary patients. For example, changes in induced sputum after an exercise stress test might be important in defining the COPD or asthma phenotype (see above), allowing tailoring of the therapeutic approach according to the pathophysiological features of the disease in each patient.

From *in vitro* cell culture to functional genomics

Induced sputum is potentially an important source of several airway cell types for use *in vitro*. Following induced sputum processing, the viability of these cells is usually high, and, because of this, these cells may be cultured *in vitro*. The induced sputum technique may, therefore, represent a useful and noninvasive tool for isolating macrophages, neutrophils and eosinophils from the airways of healthy and diseased subjects. The ability to establish short- and long-term *in vitro* cultures with purified cell types would enable the evaluation of their functional and phenotypic characteristics. In addition, as the functional state of a cell reflects very closely the expressed genes of that cell, and the latter can be used to define cell type, stage of development and responses to stimuli, cell cultures obtained from induced sputum samples may allow functional genomics studies to be performed. This would expand the scope of biological investigation from a single gene to studying all simultaneously expressed genes in a systematic fashion.

Deoxyribonucleic acid (DNA) arrays are revolutionising the analysis of gene expression and single

nucleotide polymorphisms in genomic DNA. Currently, the expression of 10–15% of human genes can be analysed simultaneously in a single experiment using complementary DNA or the oligonucleotide-based format of DNA array. Alternatively, smaller DNA arrays with a limited number of selected genes, such as cytokines, growth factors or transcription factors, can be used. In concordance with the Human Genome Project, in a few years, DNA arrays may permit analysis of the expression of the whole human genome. This is likely to have great impact on basic research, drug development and diagnostics. It will be important to characterise pathogenetic mechanisms of asthma and COPD at the level of gene expression so that new therapeutic strategies can be identified. With the aid of DNA array technology, it should be possible to identify multiple simultaneous transcriptional events contributing to airway inflammation [27]. In order to maximise the information that can be deduced from functional genomics (and particularly by use of microarrays), it is preferable to work with a single cell type. For most *in vitro* experiments this is not a problem and it is possible to ask well-defined questions using well characterised systems (*e.g.* what genes are expressed in a given cell line in response to a particular cytokine). However, when dealing with clinical specimens, it may not be possible to obtain a pure cell population; in this case, care must be taken to correctly interpret the information from the array as this can be influenced by shifts in the relative numbers of different cell types.

From *in vitro* cell culture to proteomics

Proteomics has recently been proposed as a new approach for identifying novel biomarkers. Defined as the protein complement of the genome, the proteome represents a varied and dynamic repertoire of molecules that in many ways dictates the functional form that is taken by the genome. The importance of proteomics reflects the central role that proteins play in establishing the biological phenotype in health and disease. Moreover, proteins constitute the vast majority of drug targets against which pharmaceutical drug design processes are initiated. By studying interrelationships between proteins that occur in health and disease and following drug treatment, proteomics may be used to determine the pathophysiological basis of disease and enable study of the mechanistic basis of drug action and toxicity. Proteomics is also an effective means of identifying biomarkers that have the potential to improve decision making by taking into account drug efficacy and safety issues based on data derived from the study of key tissues and the discovery and appropriate utilisation of biomarkers.

Large amounts of proteins present in sputum play important roles in either normal lung physiology or the pathophysiology of lung diseases. The protein content of sputum is more concentrated than that of BAL or nasal lavage fluid, for which proteomic studies have already been conducted [28]. Furthermore, the induced sputum technique samples more proximal airways than BAL and the protein changes are, therefore, probably more relevant to airway diseases. It is, therefore, likely that proteomics will soon be increasingly used on sputum samples to evaluate the roles of different proteins in health and disease. In addition, the induced sputum technique may also be combined with protein chip technology. Proteomic technologies targeted at the mapping of proteins involved in tissue repair and destruction may allow better characterisation of the extent of airway remodelling in each patient and the identification of markers potentially useful for the early detection of lung cancer.

When applied to induced sputum, proteomics is a promising approach from a therapeutic standpoint. Indeed, some anticancer or anti-inflammatory strategies are directed against proteases that facilitate several steps in cancer progression or lung function decline, both of which would be useful in the management of patients with COPD. Continuing refinement of techniques and methodologies to determine the abundance and state of proteins *in vivo* holds great promise for future studies in respiratory medicine.

Key points

1) The induced sputum technique allows access to specific cell types from the airways, which may change the diagnosis and monitoring of patients with inflammatory airways diseases. 2) This may allow the characterisation of phenotypes of asthma and chronic obstructive pulmonary disease, which is relevant for identifying therapeutic targets. 3) The value of induced sputum in the early detection of airway and parenchymal diseases needs to be established. 4) The induced sputum technique may permit detailed genetic analysis for lung cancer screening. 5) There is a need to establish whether sputum induction is feasible, safe and cost-effective at the general practitioner level. 6) Induced sputum could be used in studies of functional genomics and proteomics.

References

1. Sato M, Saito Y, Usuda K, Takahashi S, Sagawa M, Fujimura S. Occult lung cancer beyond bronchoscopic visibility in sputum-cytology positive patients. *Lung Cancer* 1998; 20: 17–24.
2. Kennedy TC, Miller Y, Prindiville S. Screening for lung cancer revisited and the role of sputum cytology and fluorescence bronchoscopy in a high-risk group. *Chest* 2000; 117 (Suppl. 1): 72S–79S.
3. Bechtel JJ, Petty TL, Saccomanno G. Five year survival and later outcome of patients with X-ray occult lung cancer detected by sputum cytology. *Lung Cancer* 2000; 30: 1–7.
4. Ahrendt SA, Chow JT, Xu LH, *et al.* Molecular detection of tumor cells in bronchoalveolar lavage fluid from patients with early stage lung cancer. *J Natl Cancer Inst* 1999; 91: 332–339.
5. Tan DF, Huberman JA, Hyland A, *et al.* MCM2 - a promising marker for premalignant lesions of the lung: a cohort study. *BMC Cancer* 2001; 1: 6.

6. Lacroix J, Becker HD, Woerner SM, Rittgen W, Drings P, von Knebel Doeberitz M. Sensitive detection of rare cancer cells in sputum and peripheral blood samples of patients with lung cancer by preproGRP-specific RT-PCR. *Int J Cancer* 2001; 92: 1–8.
7. Mulshine JL, De Luca LM, Dedrick RL, Tockman MS, Webster R, Placke ME. Considerations in developing successful, population-based molecular screening and prevention of lung cancer. *Cancer* 2000; 89: Suppl. 11, 2465–2467.
8. Wiest JS, Franklin WA, Drabkin H, Gemmill R, Sidransky D, Anderson MW. Genetic markers for early detection of lung cancer and outcome measures for response to chemoprevention. *J Cell Biochem* 1997; 67: Suppl. 28–29, 64–73.
9. Marchetti A, Buttitta F, Carnicelli V, *et al.* Enriched SSCP: a highly sensitive method for the detection of unknown mutations. Application to the molecular diagnosis of lung cancer in sputum samples. *Diagn Mol Pathol* 1997; 6: 185–191.
10. Miozzo M, Sozzi G, Musso K, *et al.* Microsatellite alterations in bronchial and sputum specimens of lung cancer patients. *Cancer Res* 1996; 56: 2285–2288.
11. Zhou J, Jensen SM, Steinberg SM, Mulshine JL, Linnoila RI. Expression of early lung cancer detection marker p31 in neoplastic and non-neoplastic respiratory epithelium. *Lung Cancer* 1996; 14: 85–97.
12. Moodley YP, Dorasamy T, Venketasamy S, Naicker V, Lalloo UG. Correlation of CD4:CD8 ratio and tumour necrosis factor (TNF)α levels in induced sputum with bronchoalveolar lavage fluid in pulmonary sarcoidosis. *Thorax* 2000; 55: 696–699.
13. D'Ippolito R, Chetta A, Olivieri D. Role of induced sputum in interstitial lung disease. *Eur Respir J* 2000; 16: 573–574.
14. Fireman E. Induced sputum: opening a new window to the lung. *Sarcoidosis Vasc Diffuse Lung Dis* 2001; 18: 263–271.
15. Saetta M, Thiene G, Crescioli S, Fabbri LM. Fatal asthma in a young patient with severe bronchial hyperresponsiveness but stable peak flow records. *Eur Respir J* 1989; 2: 1008–1012.
16. Gibson PG, Wong BJ, Hepperle MJ, *et al.* A research method to induce and examine a mild exacerbation of asthma by withdrawal of inhaled corticos1teroid. *Clin Exp Allergy* 1992; 22: 525–532.
17. Kharitonov SA, Yates D, Robbins RA, Logan-Sinclair R, Shinebourne EA, Barnes PJ. Increased nitric oxide in exhaled air of asthmatic patients. *Lancet* 1994; 343: 133–135.
18. Lundberg JO, Rinder J, Weitzberg E, Lundberg JM, Alving K. Nasally exhaled nitric oxide in humans originates mainly in the paranasal sinuses. *Acta Physiol Scand* 1994; 152: 431–432.
19. Hargreave FE, Dolovich J. Nonspecific bronchial responsiveness. *Chest* 1982; 82: S22–S23.
20. Hargreave FE, Wong BJ, Popov T, Dolovich J. Non-invasive methods to examine the anti-inflammatory effects of drugs. *Agents Actions Suppl* 1993; 43: 291–295.
21. Hargreave FE, Popov T, Kidney J, Dolovich J. Sputum measurements to assess airway inflammation in asthma. *Allergy* 1993; 48: 81–83 [discussion 84–86].
22. Karjalainen EM, Laitinen A, Sue-Chu M, Altraja A, Bjermer L, Laitinen LA. Evidence of airway inflammation and remodeling in ski athletes with and without bronchial hyperresponsiveness to methacholine. *Am J Respir Crit Care Med* 2000; 161: 2086–2091.
23. Helenius IJ, Rytila P, Metso T, Haahtela T, Venge P, Tikkanen HO. Respiratory symptoms, bronchial responsiveness, and cellular characteristics of induced sputum in elite swimmers. *Allergy* 1998; 53: 346–352.
24. Langdeau JB, Turcotte H, Bowie DM, Jobin J, Desgagne P, Boulet LP. Airway hyperresponsiveness in elite athletes. *Am J Respir Crit Care Med* 2000; 161: 1479–1484.
25. Bonsignore MR, Morici G, Riccobono L, *et al.* Airway inflammation in nonasthmatic amateur runners. *Am J Physiol Lung Cell Mol Physiol* 2001; 281: L668–L676.
26. Anselme F, Caillaud C, Couret I, Rossi M, Prefaut C. Histamine and exercise-induced hypoxemia in highly trained athletes. *J Appl Physiol* 1994; 76: 127–132.
27. Davies DE, Polosa R, Puddicombe SM, Richter A, Holgate ST. The epidermal growth factor receptor and its ligand family: their potential role in repair and remodelling in asthma. *Allergy* 1999; 54: 771–783.
28. Lindahl M, Svartz J, Tagesson C. Demonstration of different forms of the anti-inflammatory proteins lipocortin-1 and Clara cell protein-16 in human nasal and bronchoalveolar lavage fluids. *Electrophoresis* 1999; 20: 881–890.