Investigative Bronchoprovocation and Bronchoscopy in Airway Diseases

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Rationale: Basic and clinical research strategies used for many lung diseases have depended on volunteer subjects undergoing bronchoscopy to establish access to the airways to collect biological specimens and tissue, perhaps with added bronchoprovocation in asthma syndromes. These procedures have yielded a wealth of important scientific information. Since the last critical review more than a decade ago, some of the techniques and applications have changed, and untoward events have occurred, raising safety concerns and increasing institutional review scrutiny.

Objectives and Methods: To reappraise these investigational methods in the context of current knowledge, the National Heart, Lung, and Blood Institute and the National Institute of Allergy and Infectious Diseases of the National Institutes of Health convened a working group to review these procedures used for airway disease research, emphasizing asthma and chronic obstructive pulmonary disease.

Main Results: The group reaffirmed the scientific importance of investigative bronchoscopy and bronchoprovocation, even as less invasive technologies evolve. The group also considered the safety of bronchoscopy and bronchoprovocation with methacholine and antigen to be acceptable for volunteer subjects and patients, but stressed the need to monitor this closely and to emphasize proper training of participating medical research personnel. Issues were raised about vulnerable volunteers, especially children who need surrogates for informed consent.

Conclusion: This review of investigative bronchoscopy and bronchoprovocation could serve as the basis for future guidelines for the use of these procedures in the United States.

Keywords: airway hyperresponsiveness; asthma; bronchoalveolar lavage; chronic obstructive pulmonary disease; lidocaine; methacholine; segmental allergen challenge

RATIONALE FOR WORKSHOP

Bronchoprovocation and fiberoptic bronchoscopy have become an integral part of research involving human subjects, especially

for the study of chronic pulmonary illnesses such as asthma, chronic obstructive pulmonary disease (COPD), and interstitial lung diseases. These investigative approaches have facilitated the acquisition of quantitative biological data that have advanced our understanding of disease mechanisms and now form the basis of many of the current concepts in pathogenesis, diagnosis, and treatment. Although considerable insight into the mechanisms and regulation of airway hyperresponsiveness (AHR) and inflammation has been gained from animal models, knowledge about the uniqueness of these processes in human airway diseases can only be studied and obtained in patients. Experience has found that the risk-benefit ratios of bronchoscopy and bronchoprovocation are relatively low, rendering these current procedures feasible for research involving patients and healthy control subjects, including children in special situations. Investigators, funding agencies, pharmaceutical companies, and regulatory organizations now accept bronchoprovocation and bronchoscopy as useful scientific tools and rely on the research generated by these procedures to determine mechanisms of altered lung function in humans.

Although investigative bronchoprovocation and bronchoscopy previously have been reviewed, there is now a need to revisit the application of these procedures to research. First, the most recent committee reviews were published over a decade ago (1, 2), and other helpful articles that addressed bronchoscopy and bronchoalveolar lavage (BAL) in adults and children with airway disease (3–6) did not focus on investigative uses. Second, the procedures have become more standardized, and the experience gained has been expanded so that a current review and reassessment of techniques for these procedures and their applications will facilitate the comparison of research findings among different laboratories. Third, there are a growing number of investigators who include bronchoscopy, bronchoprovocation, or both in their research protocols such that an update on the indications, techniques, and safety of these popular investigative tools is both timely and instructive. Finally, ethical considerations, regulatory requirements, and institutional review board diligence may not have been adequately covered in previous reviews, necessitating an assessment of such issues.

On July 25 and 26, 2003, the National Heart, Lung, and Blood Institute and the National Institute of Allergy and Infectious Diseases convened a workshop bringing together clinical investigators, research support staff, ethicists, and representatives of federal agencies for the purpose of discussing and evaluating the use of bronchoprovocation and bronchoscopy in airway disease research. The goals of the workshop were as follows: (1) review the experience and status of bronchoprovocation and bronchoscopy in the study of asthma and COPD; (2) evaluate and provide

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a rationale for using these investigative tools in preference over less invasive methodologies; (3) review the technical requirements, ethical implications, and regulatory aspects of the procedures where necessary; and (4) offer broad directives and suggestions, but not formal recommendations, for their use and safe application in human research.

This article is intended to review the state of the art of investigative bronchoprovocation and bronchoscopy at the time of the workshop and thus provide information that might be useful for clinical investigators, research sponsors, and research volunteers, and to highlight any related issues or topics that might need future consideration. Subsequent publications, some of which are included in this report, have added support to the workshop's conclusions.

The workshop was not intended to formulate consensus-based guidelines for investigative bronchoprovocation and bronchoscopy but rather to summarize the scientific contribution and safety of these tools that could be used for future guideline development in the United States.

BRONCHOPROVOCATION IN ADULTS: APPLICATIONS AND CONTRIBUTIONS

AHR, an exaggerated bronchoconstrictor response to a variety of stimuli, is a prominent characteristic of asthma and also found in COPD, cystic fibrosis, and allergic rhinitis. Although the mechanisms underlying AHR are not fully understood, it is believed to result, at least in part, from airway inflammation. Bronchoprovocation is a well-established method to detect and quantify AHR and to obtain insights into the mechanisms associated with this pathophysiologic abnormality, particularly when assessed in conjunction with procedures such as bronchoscopy and mucosal biopsy.

Pharmacologic agents, including acetylcholine, methacholine, histamine, cysteinyl leukotrienes, prostaglandins, and adenosine 5'-monophosphate, and physical stimuli such as exercise and isocapnic hyperventilation with cold, dry air, have been used to detect, quantify, and characterize nonspecific AHR in asthma. Experience has indicated that AHR varies with the clinical severity of asthma and, largely based on the observation that antiinflammatory therapy can reduce AHR measures of airway responsiveness, has been used as an indirect physiologic marker of airway inflammation (7). However, it is important to appreciate the potential differences that may arise from values of airway responsiveness to direct airway smooth muscle constrictors like methacholine versus responses that may follow more indirect stimuli like cold air or adenosine 5'-monophosphate.

Inhalation of allergens by allergic patients, with or without asthma, is often used to define mechanisms underlying the development of airway inflammation. Such insight has been aided by the differences between the immediate bronchospastic response to allergen and the development of the late allergic response, which is characterized by airway inflammation and enhanced airway responsiveness (8). Inflammatory cell function and phenotype may be altered by allergen challenge to modulate allergic inflammation, to provide insight into altered airway function and thus facilitate correlations among the cells and mediators of this complex inflammatory process and altered pulmonary physiology. However, insights into the complex mechanisms of the late phases are also undergoing reevaluation, as well as the clinical significance of treatments that affect this component of the airway response to antigen.

Insight into Disease Mechanisms with Bronchoprovocation

The basic techniques and applications of bronchoprovocation, both for testing nonspecific airway responsiveness and specific

allergen challenge, have been well defined (9). The challenges include provocative agents that induce bronchoconstriction directly or indirectly by the release of spasmogens from airway cells (10). An alternative to allergen/antigen bronchoprovocation, either through whole lung aerosol challenge or bronchoscopic segmental allergen challenges (*see below*), is natural seasonal allergen exposure in allergic individuals with well-defined seasonal rhinitis or asthma (11). In contrast to laboratory challenge procedures, seasonal exposures cannot be precisely controlled. One additional recent modification to antigen provocation has been the use of repetitive, low-dose airway challenges, in lieu of a single dose, to investigate possible enhancement or tolerogenic mechanisms involved in the modulation of allergic airway inflammation (12).

AHR to methacholine correlates in a general way with symptoms and severity of the disease (7) and is a risk factor for progressive airflow obstruction and an accelerated rate of decline in FEV₁ in smokers (13). Increased airway responsiveness after allergen inhalation parallels the subsequent inflammatory reaction, suggesting that the associated allergen-induced inflammation has direct effects on mechanisms of airway responsiveness (14); the observation that allergen avoidance can decrease AHR is consistent with this concept (15). Inhaled glucocorticosteroids reduce airway inflammation and to some extent bronchial responsiveness, further supporting the linkage between these two processes (14). Certain therapeutic agents, such as inhaled glucocorticosteroids and leukotriene receptor antagonists, have differential effects on the early and late response seen after allergen challenge, suggesting selective mechanisms of action (16, 17). Furthermore, late-phase airway responses to allergen inhalation are associated with inflammatory markers that are reflected both in the circulation and the airways somewhat differently, suggesting distinct and interactive effects in these two systemic compartments.

A number of physiologic factors characteristic of asthma have either been defined or explored using techniques of bronchoprovocation. These include the "excessive" airway closure that is characteristic of asthma (i.e., lack of a plateau on the methacholine dose-response curve). In addition, there appears to be a lack of regulatory mechanisms to restore airway caliber after bronchoconstriction (i.e., the effect of a deep breath), and the loss of airway-parenchymal interdependence (18). Moreover, when used appropriately, bronchial challenge studies have been a helpful tool for developing therapy. That is, pharmacologic intervention of the early and particularly the late allergic response after allergen challenge have successfully predicted that both leukotriene D receptor antagonism (17) and blocking IgE with a specific antibody (19) would be useful for the treatment of asthma. Conversely, studies with allergen challenge have also successfully predicted that platelet-activating factor antagonism would not be clinically beneficial in asthma treatment (20). Therefore, the collective experience with bronchoprovocation in humans using nonspecific stimuli or allergens has provided important *in vivo* information about the pathogenesis and pathophysiology of asthma that also has contributed directly to the development of new therapeutic strategies and underlying association between inflammation and AHR.

Safety

The availability of a U.S. Food and Drug Administration (FDA)-approved preparation of methacholine (Provocholine, Methapharma) (21) provides a well-defined agent for clinical and research use that has now largely precluded the need for histamine in the United States. Bronchoprovocation with methacholine has been standardized and is considered to be acceptably safe when established procedures are followed. The most important

TABLE 1. CONTRIBUTIONS OF RESEARCH BRONCHOSCOPY TO THE UNDERSTANDING OF ASTHMA AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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- Identification of inflammatory cells and mediators associated with asthma (3, 12, 40, 41)
 - Characterization of Th1 versus Th2 lymphocyte-directed inflammatory response in asthma (12, 40, 41)
 - Differential inflammatory features in different clinical phenotypes of asthma (3, 12, 37, 38, 48, 73)
 - Insight into patterns of airway remodeling (73)
 - Characteristics of the cellular, cytokine, and chemokine responses to allergen challenge (50, 51, 53, 54, 58, 59)
 - Effects of asthma therapy on features and characteristics of airway inflammation (32, 66, 69)
 - Characteristics of airway inflammation in childhood asthma (85, 86, 90–92)
 - Evidence for parenchymal involvement of inflammation in asthma (62, 69)
 - Description of inflammatory cellular and cytokine profiles in chronic obstructive pulmonary disease (44–47)
-

contraindication to performing this and other whole lung inhalational tests is a low baseline FEV₁, usually considered to be below 70% predicted, as noted in the manufacturer's package insert for Provocoline. However, one study has reported that methacholine testing can be performed safely in subjects with FEV₁ values ranging between 22 and 59% of predicted (22), and several clinical networks and studies have used methacholine bronchoprovocation safely in subjects with severe asthma (22) and COPD (23). However, the safety experience in such patients is still limited, and the magnitude of airflow obstruction under which this procedure can be used safely will need further evaluation; the data may be available through the Severe Asthma Research Program.*

For the other above-mentioned pharmacologic agents and physical stimuli used to assess nonspecific airway responsiveness, the methodologies have been less well standardized, and there is less experience with their safety profile (24–26). Likewise, the safety of inhalational challenge with allergens, approved by the FDA for human use as skin-test reagents but not for lung challenge studies, is less well established. However, serious adverse events have not been reported (27).

In summary, research bronchoprovocation appears to carry a low risk of untoward effects. However, the proven safety record of investigative methacholine challenge should not be extrapolated to other less frequently used provocative agents for which the same high level of experience is lacking. The introduction of new agents to bronchoprovocation protocols will require that safety, as well as biological relevance, be established before such agents are applied on a wider scale. Two tragedies involving research subjects have underscored the importance of these safety issues (28, 29). Because improving and monitoring the protection of research subjects is a high priority in clinical research (28), further details about preclinical data requirements as well as other ethical concerns are provided in Appendices E1 and E2 in the online supplement.

BRONCHOSCOPY IN ADULTS: APPLICATIONS AND CONTRIBUTIONS

BAL (30–32), endobronchial brush or forceps biopsy, and trans-bronchial biopsy (33) have emerged as the most widely used invasive research tools to assess inflammation and tissue remodeling in airway and interstitial lung diseases (Table 1) (34). Endo-

bronchial biopsy specimens can also be prepared as explants for further study (35).

Investigative bronchoscopy and related procedures provide clinically relevant *in vivo* information on the pathophysiology of asthma (36–43) and COPD (44–47). For example, profiles of infiltrating inflammatory cells and markers of their level of activation have been reported to correlate with physiologic parameters in asthma (42, 47–49) and COPD (44, 46, 47). Thus, much of our recent knowledge about the histopathology of mild asthma, the similarities and differences of airways in patients with allergic rhinitis versus those with allergic asthma, and the histologic subtypes of severe asthma in patients has come from bronchoscopic studies. Because the complexity of these inflammatory processes and the attendant changes in tissue architecture cannot be fully reproduced in animal models, research bronchoscopy in humans has been required to provide direction and insight into mechanisms of airway disease not available through other means.

Bronchoscopy can be used in conjunction with allergen challenge to correlate changes in pulmonary function with inflammatory cell recruitment into the airways (50, 51). Allergen can be delivered by aerosolization into the whole lung or via instillation through the bronchoscope into an isolated airway (i.e., segmental allergen challenge) (52–55). Segmental allergen challenge tends to better localize the site of allergen delivery, and higher doses of allergen can be used to induce greater localized inflammation with less overall bronchoconstriction. In addition, multiple segments of the airways can be challenged at the same time with different doses of antigen, or pharmacologic agents can be added in an attempt to block the *in situ* inflammatory response.

The limitations of segmental allergen challenge include inter- and intrasegment variabilities in the inflammatory response to allergen and the fact that this model does not necessarily mimic allergen-induced asthma exacerbations (43).

Research BAL and bronchoscopic biopsy procedures have not been standardized, and the reproducibility of reported findings often is not known. For example, it is not always clear how well a specific airway biopsy specimen reflects the histology of other airways and what the level of histologic similarity is between multiple biopsies in asthma and COPD. The ideal number of biopsies recommended in the previous workshop was 3 (1), but several studies have obtained 9 to 10 endobronchial biopsies at the same procedure without complications (56, 57).

Insight into Disease Mechanisms

In asthma, biological events associated with the initiation, propagation, and resolution of an acute inflammatory reaction in the airways have begun to be clarified with this research approach (51, 58, 59). Those studies were performed in the context of an acute allergen challenge, although events after a mechanical injury have also begun to be explored. The role of infectious agents, including viruses (e.g., rhinovirus) and atypical pathogens (i.e.,

* This program, sponsored by the Division of Lung Diseases, National Heart, Lung, and Blood Institute, and initiated in September 2003, supports a collaborative multicenter study in humans to investigate the mechanistic basis for severe asthma and to identify novel targets for potential therapeutic intervention. The goals are to reduce morbidity and mortality in patients with severe asthma and to lessen the substantial health and economic burden attributable to this disorder.

Mycoplasma pneumoniae and *Chlamydia pneumoniae*), in the pathogenesis and exacerbations of asthma has been studied using bronchoscopic methods (60, 61). Direct measurements of peripheral airway resistance and responsiveness as well as the characterization of airway surface liquid in thermally induced asthma also have been investigated by bronchoscopy (62–64). Moreover, the application of specialized techniques, such as bronchoscopic endobronchial ultrasonography, has been important for some of these findings (65). Finally, using appropriate clinical protocols designed to take into account intersubject variability and intrasubject reproducibility of airway sampling (54), bronchoscopic techniques have provided direct measurement of the effect of different drugs and therapy on airway inflammation and airway structural changes that occur in asthma and COPD (32, 66, 67). Recently, transbronchial lung biopsy has been performed in patients with asthma and demonstrated a significant inflammatory component in the lung periphery (42). Thus, the use of bronchoscopy in airway research has had broad application and has provided significant new information, especially on the nature of airway tissue inflammation in airway disease (68–70).

Safety

A number of reports have suggested that bronchoscopy, including BAL and bronchial forceps and brush biopsy, can be safely performed for research purposes in patients with asthma and COPD (34, 71–74). Repeated procedures have also been reported to be safe, at least in patients with mild asthma (59). Experience is more limited with transbronchial lung biopsy (42); thus far, serious adverse events have not been reported. However, because a death has been reported in a normal subject undergoing research bronchoscopy (28, 75), safety considerations generally need to be constantly reassessed (Table 2).

Bronchoscopy. Previous guidelines have suggested that an FEV₁ less than 60% constitutes a contraindication to performing research bronchoscopy (1). However, bronchoscopy in adults with asthma has been performed safely when the FEV₁ is lower, such as less than 50% predicted post-bronchodilator, and in patients with COPD (30, 72, 76) when the FEV₁ is less than 25% predicted prebronchodilator. Furthermore, in a single report, Martin and colleagues (22) demonstrated that bronchoscopy was safely performed in subjects with asthma with an FEV₁ less than 30% predicted. However, more experience needs to be gained before research bronchoscopy can be assumed to be safe in patients with such severe airflow obstruction. Premedication with atropine and bronchodilators can be given, or omitted, depending on the procedures to be performed (34) and the number of bronchoscopies a research subject may safely undergo over time.

Segmental allergen challenge. Segmental allergen challenge in patients with allergic airway disease is generally well tolerated. There have been reports of increased AHR, wheezing, and decrements in lung function in relationship to this procedure (76); however, the changes in AHR can be more pronounced when the segmental challenge is followed by BAL and biopsy, and has lasted up to 72 hours (76). Jarjour and colleagues (77) reported no significant difference in lung function measured 2 hours after a segmental allergen challenge in subjects with asthma as compared with subjects with allergic rhinitis. When BAL and biopsy were performed after whole lung allergen challenge, no further change in AHR was documented (78). Thus, the experience to date suggests that these challenge procedures are well tolerated.

Topical anesthesia. Topical anesthesia is needed for bronchoscopy and can be a source of increased risk. Previously, an upper dose limit of lidocaine of 400 mg was recommended (1), but a recent report suggested 600 mg or 9 mg/kg as a safe limit for adults (79). Despite these recommendations, a safe upper limit for topical lidocaine used in bronchoscopy has not been firmly established,

and instillation of lidocaine into the airways is not an FDA-approved route of administration. It has been suggested that the death of the above-mentioned volunteer subject undergoing bronchoscopy could have been related to lidocaine usage (28).

BAL. This is also generally well tolerated, although changes in lung function have been reported (76). BAL does not significantly alter airway responsiveness, airflow limitation, and/or airway inflammation when BAL is incorporated into other procedures, such as bronchial biopsy, and segmental or whole lung allergen challenge (71, 76–78, 80). Nonetheless, cough, wheezing, and post-bronchoscopy fever have been associated with BAL. Hypoxemia is less common, particularly because the use of supplemental oxygen has become a standard practice (80).

Bronchial biopsy. Bronchial forceps biopsy has become a routine investigative procedure, and a number of studies have addressed subject safety, often in the setting of combined biopsy and BAL. Djukanovic and colleagues (71) showed that endobronchial biopsy (up to four biopsies taken) with a BAL volume of 160 ml in 20 subjects with mild to moderate asthma (FEV₁ values of $88 \pm 18\%$ predicted) resulted in a fall in FEV₁ of $26 \pm 17\%$, which correlated with AHR before the procedure. Healthy control subjects were also assessed, and their mean decrement in FEV₁ was $9 \pm 4.7\%$. In addition, hypoxemia was found in asthma (mean fall of oxygen saturation, 3%; range, 1–17%) but not in normal control subjects. This report differs from that of Van Vyve and colleagues (80) who studied 50 subjects with asthma and 25 control subjects undergoing bronchoscopy with four endobronchial biopsies and a BAL volume of 250 ml. These investigators did not administer a β_2 -agonist before the procedure, but the subjects with asthma received it after the procedure; no supplemental oxygen was administered, unless an oxygen desaturation of less than 80% for more than 1 minute was observed. Decrements in arterial oxygen saturation were observed to a similar extent in subjects with asthma and control subjects, with a mean decrease in oxygen saturation of approximately 4 to 5%; the decrease in FEV₁ was similar in subjects with asthma (20% mean) and healthy control subjects (17% mean) (80).

Although these studies suggest that bronchoscopy induces transient changes in airway function and gas exchange in healthy subjects and subjects with asthma, asthma control, as determined by peak expiratory flow rate, symptom score, and medication use, appears not to be lost after bronchoscopy involving biopsy and BAL (56).

The safety of bronchoscopy with endobronchial biopsy and BAL also has been assessed in COPD by Hattotuwa and colleagues (72). Fifty-seven patients with COPD whose FEV₁ ranged from 25 to 75% predicted (mean, 44.5% predicted) underwent either bronchoscopy with endobronchial biopsy and BAL (68 procedures) or endobronchial biopsy alone (30 procedures). Eleven patients had mild disease, 28 were considered moderate, and 18 were considered severe according to British Thoracic Society guidelines (81); all were considered to have stable disease. In these 98 procedures, five adverse events occurred, including bronchospasm that required hospitalization (one subject), pneumothorax (one subject with severe disease), and hemoptysis (three subjects, but no hospitalization was required). The overall incidence of adverse events requiring hospital treatment was 2 and 3.1% for minor hemoptysis.

Information about the safety of transbronchial biopsy in asthma is limited and has been obtained primarily from studies at one center in the United States involving 49 subjects undergoing 72 bronchoscopies (42, 57, 82). The procedure was deemed safe by those investigators, although one subject experienced a 10% pneumothorax, which resolved with supplemental oxygen (42).

Airway brushing. Airway brushing during bronchoscopy is

TABLE 2. SAFETY OF RESEARCH BRONCHOSCOPY IN OBSTRUCTIVE LUNG DISEASE

Reference	No. Subjects	Diagnosis	FEV ₁ (% predicted; range if available)	Adverse Events	Interpretation/Comments
Chetta and coworkers (111)	13, asthma (age 19–41 yr)	Asthma	Asthma: 102.1 ± 16.6%	Change in PEFR: Asthma: 23.1 ± 13.9% fall	The fall in PEFR was proportional to PC ₂₀ .
	8, control subjects (age 22–29 yr)	Control subjects	Control subjects: not listed	Control: 7.8 ± 8.2% fall	
Djukanovic and coworkers (71)	Bronchoscopy, BAL, biopsy 20 subjects (age 19–68 yr)	Asthma	88.3 ± 18.1% (55.9–114.3%)	All recovered in 120 min One procedure terminated during BAL. Both asthma and control subjects had falls in FEV ₁ .	The fall in FEV ₁ in association with bronchoscopy was inversely related to PC ₂₀ .
	8 atopic subjects without asthma		98.5 ± 8.8% (89.0–113%)		
	8 control subjects		107.3 ± 7.1% (96.3–116.2%)		
Elston and coworkers (74)	159 subjects (age 18–52 yr) 273 bronchoscopies	Asthma	53–120% predicted	34/273: 4, pleuritic pain 14, increased asthma 9, flulike illness	Most adverse events were associated with BAL.
Hattotuwa and coworkers (72)	57 subjects bronchoscopy, BAL + biopsy	COPD: 11, mild 28, moderate 18, severe	25–74%	5 (2% required hospitalization)	One episode of hospitalization was for severe bronchospasm.
Humbert and coworkers (56)	Study A: 21 subjects (age 18–32 yr) BAL + biopsy	Asthma	62–129%	Significant fall in PEFR at 15 min post-procedure. Returned to baseline by 30 min post-procedure.	
	Study B: 35 subjects (age 18–55 yr) BAL + biopsy	Asthma: 15 asthma, 20 control subjects	Asthma: 55–106% Control subjects: 81–123%	Similar fall in PEFR in asthma and normal subjects	
Krug and coworkers (76)	59 subjects (age 31 ± 8 yr) Segmental allergen challenge	Asthma	See next column	Segmental allergen challenge alone: Fall in FEV ₁ (2 h post-challenge) 97.6 ± 13.9% to 83.4 ± 21.7% predicted Segmental allergen challenge, BAL + biopsy: 101.8 ± 14.2% to 78.5 ± 13.6% predicted. Both returned to near baseline by 24 h.	
Payne and coworkers (68)	48 children: 38 with flexible bronchoscopy and 10 with rigid bronchoscopy; age 4–17 yr	Asthma (difficult to control)	44–104%	1, flexible bronchoscopy, desaturation. 2, rigid bronchoscopy, bronchospasm, and desaturation.	The complications were greater in the control group.
	35 nonasthmatic children (age 5–15 yr)	Control	Not stated	2, fever. 17/35 had complications.	
Romagnoli and coworkers (83)	25 subjects (age 23–75 yr) Bronchial brushings	Asthma	45–121%	No adverse events	Bronchial brushings were well tolerated.
Van Vyve and coworkers (80)	50 subjects (age 18–76 yr)	Asthma	37–107% predicted	Bronchoscopy, BAL, biopsy: evaluated pre- and 5 min post-procedure. There was a fall in arterial O ₂ , FEV ₁ , and FVC, but changes not related to the severity of asthma.	Changes in lung function occurred in control and asthma, and in both to a significant degree. The relative changes were slightly greater in asthma.

Definition of abbreviations: BAL = bronchoalveolar lavage; COPD = chronic obstructive pulmonary disease; PEFR = peak expiratory flow rate.

generally considered safe and well tolerated (83). Bleeding can occur but is rare, and cough is self-limited. Although the previous workshop (1) recommended a maximum of three brushings per bronchoscopy, recent studies have reported that subjects can tolerate more extensive brushings, with up to 24 brushings performed during a single bronchoscopy (84).

BRONCHOPROVOCATION AND RESEARCH BRONCHOSCOPY IN CHILDREN: APPLICATIONS AND CONTRIBUTIONS

Fiberoptic bronchoscopy is routine in children and is used to evaluate stridor and recurrent pneumonia and exclude foreign bodies or infectious etiologies, and is an established, useful pro-

cedure in clinical medicine (85, 86). For example, Godfrey and colleagues (87) evaluated 200 consecutive bronchoscopies in children and found that bronchoscopy yielded abnormal findings in approximately two-thirds of the cases. Information from these procedures contributed to clinical management in approximately 90% of patients.

Because concepts of asthma pathophysiology and inflammation in adults cannot be extrapolated to children (74, 88, 89), research fiberoptic bronchoscopy in combination with bronchoprovocation has been recently extended to children and adolescents. However, only a limited understanding exists about the underlying pathology of wheezing phenotypes in children (90). BAL analysis in children with asthma or persistent wheezing has suggested that there is an increase of eosinophils and neutrophils

TABLE 3. CONCLUSIONS OF THE WORKSHOP

- Past experience with bronchoprovocation and fiberoptic bronchoscopy has established these techniques as invaluable research tools in basic and clinical investigations of asthma and COPD.
- In experienced hands, research bronchoprovocation and bronchoscopy generally are safe and well-tolerated procedures in adults.
- Segmental antigen challenge performed through the bronchoscope provides selective advantages over total airway challenge in the study of asthma pathogenesis and treatment.
- Comprehensive safety information on bronchoprovocation with inhaled agents is limited to methacholine and allergens.
- Important information on airway inflammation, remodeling, and dysfunction can be obtained with fiberoptic bronchoscopy for which adequate surrogate methods, such as analysis of exhaled breath condensate or expectorated sputum, or lung imaging, are not currently available.
- More information is needed on the safety of research bronchoprovocation and bronchoscopy in children and adolescents, and in patients with severe asthma.
- Although generally accepted guidelines exist for clinical bronchoprovocation and bronchoscopy in children and adults, uniform procedural standards for these techniques in the research setting have not been fully established.

Definition of abbreviation: COPD = chronic obstructive pulmonary disease.

compared with normal control subjects and those with chronic cough (90, 91). Furthermore, bronchoscopic studies and lavage fluid analyses suggest that a pure virus-associated wheeze is not just an eosinophilic disease in children (89, 92).

Safety

Although bronchoprovocation can be performed with acceptable safety in children, as noted in several large studies, including the Childhood Asthma Management Program (93), information on applications and safety of research bronchoscopy thus far has been derived primarily from clinical experience. Diagnostic flexible bronchoscopy in children has become routine for the evaluation of stridor and pulmonary infections. Bush and Pohunek (94) demonstrated with 278 endobronchial biopsies obtained from children that there were no complications other than minor bleeding. Another study evaluated the safety of bronchoscopy in severe or difficult-to-control asthma as determined by medication requirements (68). This 3-year, prospective, observational study was conducted in two tertiary pediatric respiratory centers specializing in the management of severe asthma. Bronchoscopy was performed in 38 children with mild to severe asthma, with FEV₁ values that ranged from 44 to 104% predicted, and in 35 nonasthmatic control subjects; rigid bronchoscopy was performed in 10 children, after a course of prednisolone. Perioperative complications occurred in one patient undergoing flexible bronchoscopy (oxygen desaturation) and two undergoing rigid bronchoscopy (desaturation and bronchospasm); four patients with asthma did report an increase in symptoms 1 week after bronchoscopy.

The largest published study with pediatric bronchoscopy is by de Blic and colleagues (6), who described their experiences in 1,328 pediatric patients undergoing bronchoscopy in a clinical setting. Of these patients, 3% had the diagnosis of asthma, and an additional 30% had airway disease (recurrent wheezy bronchitis, persistent cough, or bronchiectasis). Most of the procedures (92.8%) were performed in conscious patients with sedation, and 7.2% were performed under deep sedation with an endoscopic facemask. Minor, expected complications consisting of cough and epistaxis occurred in 46 of 1,328 subjects. Major complications were rare ($n = 22$) and included oxygen desaturation less than 90% ($n = 16$), coughing ($n = 4$), bronchospasm ($n = 1$), and pneumothorax ($n = 1$). Thus, the authors concluded that flexible bronchoscopy is a safe procedure in children, with complications occurring in less than 2% of the procedures.

The risk of the procedure lies not just with the bronchoscopy but also with deep sedation and/or the anesthesia. Therefore, if the child is being anesthetized for another purpose, it is legiti-

mate to seek consent for a BAL and/or an endobronchial biopsy, provided these are obtained by an experienced bronchoscopist and no contraindication exists (i.e., coagulopathy or respiratory compromise from a lung disease). The additional use of lavage specimens for research purposes in children with asthma, recurrent wheeze, or cough has also been reported (91, 95). To understand the biology of the developing airways, information from normal control subjects would be optimal to compare with subjects with asthma. In infants with cystic fibrosis, BAL analysis has suggested that inflammation and infection occur early in life (96–98). Furthermore a study demonstrated that BAL in healthy children undergoing elective surgery was safe (90).

ALTERNATIVES TO INVESTIGATIVE BRONCHOPROVOCATION AND BRONCHOSCOPY

Because of the importance of airway inflammation in the pathogenesis of asthma and COPD, investigators have sought to develop techniques to assess airway inflammation by noninvasive approaches, such as analysis of induced sputum (99, 100), exhaled gases, and breath condensates (101). The utility of these alternative research tools is increasingly being recognized (30, 102). For example, the distribution of inflammatory cells found in induced sputum correlates reasonably well with the cell spectrum obtained by lavage, particularly with respect to eosinophils (103). Likewise, the exhaled level of nitric oxide appears to be a sensitive indicator of airway inflammation and decreases rapidly with the administration of glucocorticosteroids, including low-dose inhaled glucocorticosteroids (104). Recently, lung imaging with hyperpolarized helium and computed tomographic scanning has provided quantitative data on some aspects of airway remodeling (105). However, a major limitation of currently available noninvasive alternatives to bronchoscopy is their failure to provide tissue specimens for histologic, immunologic, and molecular analyses. In addition, the wide variety of detachable lung cells found in expectorated sputum limits their utility for *ex vivo* studies.

NEED FOR NEW STANDARDS FOR INVESTIGATIVE BRONCHOPROVOCATION AND BRONCHOSCOPY

In discussing research applications of bronchoprovocation and bronchoscopy, the workshop participants suggested that previous recommendations may have to be revisited and expanded. More procedures are being coupled together; use of existing agents to challenge the airways has been modified; and new agents are likely to be introduced. Evidence to support continued use of research modalities, as discussed, was substantiated by published

TABLE 4. RECOMMENDATIONS OF THE WORKSHOP

- Given the major contributions that research bronchoprovocation and bronchoscopy have provided to the current understanding of the development and treatment of asthma and COPD, it is recommended that these procedures continue to be considered important research tools by investigators, sponsors, and regulatory agencies.
- The scope of research bronchoscopy should be expanded to include new technologies, children, individuals with severe airway disease, and new diseases.
- The scientific community is challenged with the task of developing and updating minimum requirements for safe bronchoprovocation and bronchoscopy in the research setting.

For definition of abbreviation, see Table 3.

citations and by personal experience of the participants, many of whom are leaders in this field of respiratory research. The procedures reviewed were judged to be acceptably safe for normal subjects and patients, as documented in published reports. But it is also apparent that this field of airway research is dynamic and growing such that a framework for considering new adaptations or incorporating new pharmacologic agents or allergens in research protocols might be needed. Therefore, in addition to the general conclusions and suggestions offered by workshop participants (Tables 3 and 4), detailed information about the regulatory requirements for the unapproved use of an approved drug or the use of a new drug or substance would be helpful to include for reference. Thus, information was prepared by the participants from the Division of Pulmonary and Allergy Drug Products of the FDA (Appendix E1).

The tradition of using volunteer normal subjects and patients for medical research is well established and is essential for advancing new knowledge about diseases, and this has been accompanied by a constantly evolving and challenging range of ethical issues. In particular, as new indications arise for the investigation of younger subjects with airway diseases, who need to be well protected and usually require surrogate consent, special ethical considerations must be evaluated. Perhaps the creation of a registry to track the safety and outcome of volunteer subjects should be considered. Participants in the workshop strongly endorsed the continuation of clinical research in airway diseases, but some cogent ethical issues were raised that were beyond the scope of the workshop. Some of these are presented for reference in Appendix E2.

CONCLUSIONS AND SUGGESTIONS

Workshop participants concluded that bronchoprovocation and research bronchoscopy were among the important technical developments in pulmonary research during the past 25 years, and have contributed significant insights about the pathogenesis of asthma and COPD (Table 3). Continued use of these procedures was affirmed by the workshop, including the introduction of novel techniques as they become available (Table 4).

These procedures will continue to provide further opportunities for longitudinal observations, including studies of the natural history of disease, an area of particular importance that should include infants and children at high risk for the development of asthma. As new targets for therapy are identified through gene profiling and other applications of genomic and proteomic research, the study of airway-associated cells and tissues from subjects with asthma and control subjects *ex vivo* will be important in defining the roles of specific mediators, cytokines, and chemokines, as well as neural and inflammatory pathways. Furthermore, the overall safety profile of these research procedures has been favorable, albeit with some precautions and limitations acknowledged.

In the future, imaging modalities, such as virtual bronchos-

copy, may either replace bronchoscopy (for some indications) or provide complementary information. These approaches likely will include ultrasound techniques and transbronchial lung biopsies. In addition, nasal brushing or lavage is an alternative to airway tissue obtained in a less invasive manner (106). Airway mapping to identify segmental or airway generational differences relevant to airway pathophysiology, including temperature, oxygen tension, concentration of gas phase molecules, such as nitric oxide and pH, is also promising (107–109). Finally, evanescent spectroscopy, microarray gene analysis, and a variety of other *in vivo* and *ex vivo* assay techniques will enhance the benefit of fiberoptic bronchoscopy as a research tool.

Expanded use of research bronchoscopy in pediatric subjects is promising and should lead to a better understanding of the complex airway processes that occur early in life. Although clinical phenotypes have been described in early childhood for transient or early-onset wheezing and persistent wheezing, which can reduce lung function, there is still little understanding of the underlying pathophysiology of these disorders (110). The application of investigative bronchoscopy in children is still in its early stages and poses special opportunities and requirements before this area of study is fully explored (Appendix E2).

Conflict of Interest Statement: W.W.B. has received consultancy fees for the past 3 years from the following companies, with a total consultancy fee for these 3 years as indicated: Bristol Myers Squibb (\$2,000), Dynavax (\$3,000), Hoffman LaRoche (\$2,000), Schering (\$3,000, 2002–2003), and Fujisawa (\$3,000). He has also served on advisory boards in various capacities over the past 3 years (2001–2003) with the following reimbursements: GlaxoSmithKline (GSK; \$8,500), Aventis (\$2,000), Schering (\$4,000), Pfizer (\$4,000, 2004), and AstraZeneca (\$2,000). He has also received honorarium for speaking or other educational activities in the past 3 years for Merck (\$7,000, 2003), GSK (\$2,500, 2003), and Aventis (\$2,500, 2003). He has received industry-sponsored support for research from GSK (\$750,000, 2002 and 2003) and for participation in multicenter trials: Fujisawa (\$250,000, 2002 and 2003), GSK (\$500,000, 2001–2003), Aventis (\$200,000, 2001–2003), Hoffman LaRoche (\$120,000, 2002), Genentech/Novartis (\$100,000 in 2002/2003), and Merck (\$100,000, 2003). A.W. serves on the GSK COPD Global Expert Panel and received \$6,000 in honoraria over the past 3 years. He was also the principal investigator on the GSK academic research grant (airway blood flow in COPD). K.A. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. H.Y.R. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. B.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.K. is a consultant for Genentech (\$3,000/year for 2002–2004) and Merck (\$2,000/year for 2001–2004). She has been reimbursed by Genentech (\$8,000/year for 2002–2004), Merck (\$5,000/year for 2001–2004), Novartis (\$3,000/year for 2002–2004), and GSK (\$2,500/year for 2001–2003) as a speaker. She was also the principal investigator on a grant sponsored by Genentech from 2000–2003, with the total grant award of \$5,300. R.J.L. is a member of the Bioethics Committee of Eli Lilly Corporation; for this he received \$5,000 in 2004, \$8,194 in 2002, and \$4,475 in 2001. S.P.P. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. E.J.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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