

EDITORIAL

Nasal testing for novel anti-inflammatory agents

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The nose is much more accessible than the airways to assess the effects of anti-inflammatory therapy. Hence, it is possible to obtain repeated samples of nasal exudates and mucosa cells before and after nasal allergen challenge (NAC) in a relatively non-invasive way by techniques such as nasal lavage, filter paper, and nasal brushing and scraping. A comprehensive review of the extensive clinical research experience with these nasal methodologies has recently been published by Howarth et al. [1]. Nasal samples can then be analysed by novel semi-automated analytical methods to assess chemokines and cytokines, inflammatory mediators, mRNA, and transcription factors. Inhaled allergen challenge commonly calls a profound decrease in forced expiratory volume in 1 s, while the nasal symptoms that follow NAC are generally mild. Furthermore, it is much easier to recruit potential subjects with allergic rhinitis (AR) because of grass pollen outside the hayfever season, rather than subjects with a dual early and late reaction to an inhaled allergen. Following NAC it is possible to measure symptoms, use acoustic rhinomanometry, and measure levels of cells and mediators to evaluate new drugs for AR and asthma [2–4].

It has long been recognized that there is a strong functional and immunological relationship between the nose and bronchi [5, 6], especially in terms of infiltrating leucocytes and inflammatory mediators when comparing AR and allergic asthma [7]. The upper and lower airways have related respiratory epithelium and similar responses to allergen challenge. Indeed, AR and asthma commonly coexist [8], as allergy is a systemic disorder that can affect various organs within the unified immune system [9, 10]. This is in line with the WHO Initiative on Allergic Rhinitis and its Impact on Asthma (ARIA) stressing the concept of a single airway disease [11]. However, the nasal model involves a different vasculature to that in the airways, while the bronchi have added airway smooth muscle. At a pathological level, the extent of nasal remodelling in AR seems to be much less than that in the bronchi of asthmatic patients [12, 13].

There is strong evidence that allergen-reactive type 2 T helper (Th2) cells play an important role in the induction and maintenance of the allergic inflammatory cascade [14]. Cytokines and chemokines produced by Th2 cells (IL-4, IL-5, IL-9, and IL-13) may be pivotal to the pathophysiology of allergic disorders involving production of IgE, recruitment and activation of mast cells and eosinophils, mucus hypersecretion, subepithelial fibrosis, and tissue remodelling. Several studies have demonstrated significant expression of various cytokines and chemokines in inflammatory cells at sites of nasal allergic inflammation [15–19]. Excessive production of IL-5 and IL-13 may be critical to the allergic response [14, 20].

Maintenance treatment with topical steroids exerts a range of anti-inflammatory nasal effects on production of eotaxin [21], RANTES, MIP-1 α , IL-8, IL-1 β [17], TNF- α [22], and IL-5 [23]. Topical allergen challenge increases the levels of mucosal mRNA of IL-5 and IL-13 [19, 23] but nasal cytokines and chemokines may be produced at low concentration in nasal secretions, and may be undetectable when using conventional ELISAs. A single dose of topical corticosteroid has been shown to reduce levels of granulocyte macrophage-colony stimulating factor and IL-5 detected by absorption with filter paper following nasal challenge with grass pollen in AR [23, 24].

In order to sample nasal exudates for allergic inflammatory mediators, the classical methods of nasal lavage are those described by Naclerio et al. [25], the nasal pool method of Greiff et al. [26], and the use of a Foley's catheter by Grünberg et al. [27]. Lavage is performed with saline at volumes between 1 and 10 mL. The repeatability and validity of different nasal lavage methods have been compared [28]. An important demonstration of the utility of this methodology for assessing therapy was the demonstration that pre-treatment with topical corticosteroids causes inhibition of release of histamine, kinins, and symptoms after NAC [29]. Peptidyl leukotrienes are also released [30] and there is a later increase in histamine during the late nasal reaction [31]. More recently, increases in IL-5 and eotaxin have been detected in nasal lavage fluid in the early and late reactions following NAC [21, 32]. In this edition of *Clinical and Experimental Allergy*, Rami Salib, Laurie Lau, and Peter Howarth demonstrate elegantly that nasal lavage levels of eotaxin-1 (CCL11) are elevated in symptomatic AR compared with controls [33].

Tommy Sim and colleagues have developed the use of filter paper strips, which are placed on the turbinates to absorb nasal secretions [34]. The nasal filter paper method has the advantage of directly sampling nasal secretions that are less diluted and can therefore pick up protein signals, which are below the detection limits of nasal lavage. The matrix or filter paper method has been used to measure chemokines and cytokines after NAC [17, 24, 35, 36]. However, it should be noted that nasal lavage probably represents an extracellular signal, while nasal sampling by absorption into filter paper strips probably represents both an intracellular and extracellular signal, as cells that adhere to the surface of the filter paper may lyse and release their intracellular contents.

Cells samples may be obtained from the nasal mucosal using small nylon dental flossing brushes which are gently rotated over the epithelium; then the attached cells are dislodged in balanced salt solution [37, 38]. It has been demonstrated that nasal brushing can be used as an

alternative to nasal biopsy [39], while nasal brush supernatants can be analysed for cytokine release [40]. An alternative method is to use nasal mucosal scrapings using a plastic curette (Rhino-probeTM, Arlington Scientific, Springville, UT, USA) [38, 41–43]. Nasal brushing and scraping causes some discomfort but does not require local anaesthesia. Nasal biopsy is generally performed from the lower edge of the inferior turbinate by a specialist. This is a traumatic procedure that requires careful local anaesthesia, but a specimen of mucosal epithelium with basement membrane and submucosal tissue is obtained [44–48]. High-quality 2.5 mm biopsies can be taken under direct vision with nasal biopsy forceps [49]. One advantage of the nasal biopsy technique is the ability to study T and B lymphocyte responses, because lymphocytes tend to compartmentalize to tissue rather than migrate into the nasal lumen. Nasal biopsy also affords the potential to culture T cells directly from the nasal compartment for the purpose of functional analysis in response to various stimulants/inhibitors [1].

Human nasal epithelial *in vitro* cell cultures have proved useful during drug discovery and development, and have been the subject of a recent review by Dimova et al. [50]. IL-8 and GRO- α produced by cultured nasal epithelial cells are at functional levels [51]. Collagen is an important cell support matrix to establish a differentiated primary cell culture system that is functionally stable for *in vitro* nasal studies [52].

Microarray technology has exciting potential for research into mechanisms of allergy and lung disease [53, 54]. Gene profiling has been performed on nasal cells in AR [55], in skin lesions from atopic dermatitis and psoriasis [56], and in asthma [57, 58]. Microarray techniques have also been used to profile cigarette smoke- and hydrogen peroxide-induced genes in a human bronchial epithelial cell line [59]. In addition, gene expression has been studied in terms of antioxidants-related genes in bronchial epithelium of cigarette smokers [60] and also in lung tissue from patients with chronic obstructive pulmonary disease (COPD) [61, 62]. Nasal epithelial cells both unstimulated and after challenge represent an interesting tissue source to study in allergy, asthma, and COPD.

In 1985, a pollen challenge environment was described by Davies that gave grass pollen at specified levels of grains/m³ to individuals [63]. The Vienna Challenge Chamber (VCC) developed by Friedrich Horak and colleagues [64–67] is a controlled experimental allergen exposure unit that can challenge up to 20 individuals under controlled and reproducible conditions, and that has proved useful in the assessment of new therapies for AR. The Environmental Exposure Unit (EEU) was developed by James Day and associates [68–70] in Kingston, Ontario, and adapted for allergy research, and has a capacity for up to 160 subjects. Data from environmental chamber studies are acceptable within the latest draft guidance for clinical testing from the FDA [71].

Attenuation of airway responses to inhaled allergen has been the classical model to detect effects of novel potential anti-inflammatory asthma therapy [72, 73]. Indeed inhaled allergen challenge was used in early studies to demonstrate the potential of systemic anti-IgE therapy [74, 75]. Inhaled steroids are especially potent, even at single doses, in inhibiting the late asthmatic reaction (LAR) following inhaled allergen challenge [76]. The use of bolus dose allergen challenge for repeated tests in the same patient is a safe and

validated method to administer inhaled allergen in clinical trials with valid responses when compared with incremental dose allergen challenge [77, 78].

Anti-IgE treatment with the monoclonal antibody omalizumab represents the first licensed biotechnology agent for allergic disease [79]. Systemic omalizumab has efficacy on the LAR following inhaled allergen challenge [74, 75], and has recently been shown to inhibit NAC responses [80]. Drugs that target chemokines and their receptors may also be tested on allergen challenge methodologies [74, 81, 82]. There is also a range of novel agents to target allergen-specific Th2 cells and their products [4, 83–85], as well as allergen immunotherapy and adjuvants [86, 87]. NAC is an elegant model that is increasingly used to assess both topical and systemic new therapies because of features such as ease of recruitment, reproducibility, possibility for multiple crossover designs, safety of challenge, and potential for repeat sampling of a range of mediators.

A major challenge is to develop phase IIa clinical pharmacology designs that can be used to assess anti-inflammatory drugs being developed for COPD. The nasal and bronchial mucosa of smokers with COPD have been demonstrated to be infiltrated with CD8⁺ T lymphocytes and neutrophils [88]. This corresponds to the presence of nasal symptoms during exacerbations of COPD [89, 90], and sputum and nasal levels of IL-8 are correlated in COPD [91, 92]. Nasal epithelial and inflammatory responses to ozone exposure have been much studied [93–101]. Indeed COPD is characterized by chronic inflammation with an imbalance in oxidant/antioxidant levels [102, 103], and systemic inflammation may trigger local exercise-induced oxidative stress [104]. Following exposure to histamine as a vasodilator, the influence of IL-8 challenge has been studied in atopic and non-atopic subjects [105], and cigarette smoke has been shown to induce IL-8 release from human bronchial epithelial cells [106]. Bacterial lipopolysaccharide or endotoxin is an active component of cigarette smoke [92, 107], and nasal challenge with LPS has been studied in a variety of different models [108–111]. In atopic subjects nasal LPS increases the percent of eosinophils in lavage fluid [112].

Use of nasal challenge models coupled to sensitive biomarkers and clinical endpoints can readily be used to establish clinical efficacy in small-scale studies. Novel therapies have the potential to selectively inhibit various cell types and particular mediators involved in diverse inflammatory diseases. We believe that NAC has advantages over inhaled allergen challenge: easy recruitment, safety, repeat non-invasive sampling. In the future this will ensure that nasal challenge will play a growing role in clinical pharmacology assessment of anti-inflammatory therapy. Direct analysis of nasal epithelial cells has great potential in terms of translational research. Major efforts are ongoing to develop nasal challenge models to mimic the inflammation found in COPD, which can be used in clinical pharmacology assessment of new anti-inflammatory drugs.

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