Objective monitoring of nasal airway inflammation in rhinitis

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Allergic rhinitis is an inflammatory nasal disorder in which a range of different cells participates. A variety of approaches has been used to monitor nasal inflammation objectively to investigate disease processes and to evaluate the effect of therapeutic intervention. These approaches include nasal lavage, nasal cytology, and nasal biopsy, together with the more recently established measurement of nasal nitric oxide (NO) concentration. Although all provide information about nasal mucosal inflammation, the extent of information that can be obtained by each approach, the ease of sampling, and the complexity of sample handling differ. Such considerations influence the choice of approach when measurement of nasal inflammation is to be an objective outcome parameter in a clinical trial. In addition, the choice of approach is also determined by the questions or hypotheses that are to be addressed.

Nasal lavage is simple and rapid to perform, is well tolerated, and provides a sample that can provide information about luminal cell recruitment, cell activation, and plasma protein extravasation. Nasal cytology involves sampling and recovering mucosal surface cells. It is also easy to perform and is well tolerated in general, although some find that the procedure causes a transient unpleasant sensation. A differential cell count from the sample provides information about relative cell populations. Both nasal lavage and nasal cytology are readily applicable to clinical trials. Nasal cytology sample handling is easier, but nasal lavage offers the advantage of providing considerably greater information from the sample.

Nasal biopsy is a considerably more invasive procedure and requires expertise not only in tissue sampling but also in biopsy processing. Therefore, it is applicable only in specialist centers. However, nasal biopsy is the only sampling technique that directly informs about tissue cellular events, although these may be implied, in part from the other sampling approaches. Tissue specimens can be used to evaluate both protein and gene expression.

Measurement of nasal NO involves expensive equipment but provides an instantaneous result, unlike the other approaches, all of which require sample processing and analysis.

Recommendations for standardization of measurement have been made, and measures are considered in part to reflect allergic inflammation within the nasal mucosa. The limitations of nasal NO are that it reflects only a certain aspect of allergic mucosal inflammation, and that because a proportion of nasally measured NO is derived from the sinuses under normal circumstances, nasal NO is not specific for nasal disease. The high contribution from the sinus mucosa limits the discriminatory ability of nasal NO to reflect nasal tissue–specific alterations.

The incorporation of measures of nasal inflammation in clinical trials has distinguished anti-inflammatory therapy from symptomatic therapy and has the potential to provide information about the efficacy of novel therapies for allergic rhinitis. (J Allergy Clin Immunol 2005;115:S414-41.)

Key words: *Rhinitis, allergic inflammation, lavage, cytology, biopsy, exhaled nitric oxide, immunohistochemistry, in situ hybridization, mast cells, eosinophils, epithelial cells, T-cells, plasma protein exudation*

Allergic rhinitis is a nasal inflammatory disorder in which a range of different cells participates.[1] This abnormal airway inflammation involves activation and tissue recruitment of both structural cells and infiltrating

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Disclosure of potential conflict of interest: P. H. Howarth has been an advisory board member for AstraZeneca, GlaxoSmithKline, Sanofi-Aventis, and Altana. C. G. A. Persson—none disclosed. E. O. Meltzer has consultant arrangements with Alcon, Altana, AstraZeneca, Sanofi Aventis, Dey, Genentech, GSK, Merck, Pfizer, Rigel, Schering, and Wyeth; is employed with the Allergy and Asthma Medical Group and Research Center, San Diego, Calif; and is a member of the Speakers' Bureau for Sanofi Aventis, Genentech, GSK, Merck, Pfizer, Schering, and UCB. M. R. Jacobson—none disclosed. S. R. Durham has consultant arrangements with AstraZeneca, GSK, Aventis, UCB, and ALK-Abelló; receives grants or research support from GSK and ALK-Abelló; and is a member of the Speakers' Bureau for GSK, UCB, Schering Plough, and ALK-Abelló. P. E. Silkoff is a full time employee of AstraZeneca.

Received for publication November 15, 2004; revised December 9, 2004; accepted for publication December 13, 2004.

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0091-6749/$30.00

doi:10.1016/j.jaci.2004.12.1134

Abbreviations used

ATS: American Thoracic Society
ECP: Eosinophil cationic protein
EPX: Eosinophil peroxidase
GMA: Glycol methacrylate
GM-CSF: Granulocyte/macrophage colony stimulating factor
H: Histamine
HPF: High power field
ICAM-1: Intercellular adhesion molecule 1
IL: Interleukin
iNOS: Inducible NO synthase
ISH: In situ hybridization
LT: Leukotriene
MIP: Macrophage inflammatory protein
NARES: Nonallergic rhinitis with eosinophilia syndrome
NO: Nitric oxide
PAR: Perennial allergic rhinitis
PG: Prostaglandin
RANTES: Regulated on activation normally T-cell expressed and secreted
SAR: Seasonal allergic rhinitis
TAME: Tosyl-arginine methyl ester

leukocytes. The tissue recruitment of eosinophils is a hallmark of untreated allergic inflammation, and mast cell activation is prominent in clinical disease expression. The tissue recruitment and retention of the eosinophil leucocyte, in contrast with the neutrophil leukocyte, is a reflection of the local release of T_H2-orientated cytokines, in particular from activated tissue T lymphocytes and mast cells, and the consequent endothelial cell activation, with the selective upregulation of specific leucocyte endothelial adhesion molecules. This cytokine release also favors epithelial cell activation and the directed migration, under chemotactic stimulation, of cells toward the airway lumen. Mediators released from activated cells, such as mast cells, basophils, and eosinophils, can account for symptom expression because of their effects on sensory nerves, glandular components, and the nasal vasculature.[1]

A range of approaches has been applied to the monitoring of nasal airway inflammation, such as nasal lavage to recover lumenal mediators and cells, nasal luminal and surface epithelial cytologic examination, nasal biopsy to explore tissue cellular events, and, more recently, measurement of the nitric oxide (NO) in nasal exhaled air as an indirect marker of epithelial cell interactions in allergic inflammation. In many circumstances, different approaches are complementary, because each may provide information relating to separate aspects of the allergic inflammatory process. The monitoring of nasal airway inflammation in clinical trials provides insight into the mechanism of action of the therapeutic intervention, and the choice of approach depends on the questions to be addressed and the practical applicability of the selected methods of sampling to the trial situation. Nasal lavage and cytology are relatively simple to perform, whereas nasal biopsy sampling is more invasive. However, only the last will directly inform about a range of mucosal tissue cellular events. Whichever approach is used, it is critical that the sampling technique and the sample processing are tightly regulated and consistent to ensure reliability of results. The different approaches to the monitoring of nasal airway inflammation and their practical applicability are assessed with these considerations in mind.

NASAL LAVAGE

The introduction of fluid into the nasal cavity and its recovery after a predetermined dwell time has been used to investigate nasal mucosal and intralumenal events in rhinitis. This process is termed *nasal lavage*. The recovered fluid can be evaluated for soluble factors to evaluate changes in cell activation, glandular secretion, and vascular permeability. Centrifugation of this recovered fluid to obtain a cell pellet enables a microscope slide to be made to evaluate the cellular content of the nasal lining fluid.

Nasal lavage has been used to investigate nasal lumenal events in naturally occurring seasonal allergic rhinitis (SAR), perennial allergic rhinitis (PAR),[2-9] and infective rhinitis[10,11] to gain insight into the pathophysiologic processes underlying clinical disease expression. Because this technique is relatively noninvasive, is easy to perform, and is repeatable over relatively short periods, it has also been widely used as a research tool to gain a greater understanding of rhinitis. Through this technique, insight has been gained into the mechanisms underlying the early and delayed nasal responses to intranasal allergen challenge.[12-16] Similarly, nasal lavage has been undertaken before and after nasal challenge with a range of stimuli, including histamine,[17-19] bradykinin,[19-21] capsaicin,[20-22] methacholine,[23,24] and substance P,[25] to gain insight into their effects on the nasal vasculature, glandular secretion, and cell recruitment and activation. Nasal insufflation with chemokines, such as IL-8,[26] eotaxin,[27] and regulated on activation normally T-cell expressed and secreted (RANTES),[28] has also been used to evaluate the effect and time course of their effect on cell recruitment into the nasal lumen. Studies have also been undertaken to investigate the effects of non-IgE-related stimuli, such as hyperosmolar challenge, aspirin, and sodium metabisulfite, on mediator release within the nose,[29-32] and with or without therapeutic intervention, to gain insight into the magnitude of effect and mechanism of action of differing pharmacologic modalities on cell recruitment, cell activation, and induced vascular permeability.[33-41]

Although these approaches have been widely used in the limited research setting, they have been less widely applied as a means of objectively monitoring nasal disease in the clinical trial setting. The use of nasal lavage to