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Experimental approaches to evaluate respiratory allergy in animal models

Jürgen Pauluhn[a,*], Ulrich Mohr[b]

[a]*Institute of Toxicology, BAYER HealthCare, Building No. 514, Wuppertal 42096, Germany*
[b]*Fraunhofer Institute of Toxicology and Experimental Medicine (FhG ITEM), Hanover, Germany*

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Abstract

Asthma is defined as a chronic disease of the entire lung and asthma attacks may either be immediate, delayed or dual in onset. Allergic asthma is a complex chronic inflammatory disease of the airways and its etiology is multifactorial. It involves the recruitment and activation of many inflammatory and structural cells, all of which release mediators that result in typical pathological changes of asthma. A wealth of clinical and experimental data suggests that allergic asthma is due to an aberrant lung immune response mediated through T-helper type 2 (Th2) cells and associated cytokine-signaling pathways. The pathology of asthma is associated with reversible narrowing of airways, associated with prominent features that involve structural changes in the airway walls and extracellular matrix remodeling including abnormalities of bronchial smooth muscle, eosinophilic inflammation of the bronchial wall, hyperplasia and hypertrophy of mucous glands. The primary objective of respiratory allergy tests is to determine whether a low-molecular-weight chemical (hapten) or high-molecular-weight compound (antigen) exhibits sensitizing properties to the respiratory tract. This may range from reactions occurring in the nose (allergic rhinitis), in the bronchial airways (i.e., allergic bronchitis, asthma) or alveoli (e.g., hypersensitivity pneumonitis). Current assays utilize several phases, viz. an induction phase, which includes multiple exposures to the test compound (sensitization) via the respiratory tract (e.g., by intranasal or intratracheal instillations), by inhalation exposures or by dermal contact, and a single or multiple challenge or elicitation phase. The challenge can either be with the chemical (hapten), the homologous protein conjugate of the hapten or the antigen. The choice depends both on the irritant potency and the physical form (vapor, aerosol) of the hapten. The appropriate selection of concentrations (dosages) both for the induction and elicitation of respiratory allergy appears to be paramount for the outcome of test. Endpoints to characterize positive response range from the induction of immunoglobulins, cytokine or lymphokine patterns in serum (or the lung) to (patho-)physiological reactions typifying asthma. None of the currently applied animal models duplicate all features of human asthma. Accordingly, the specific pros and cons of the selected animal model, including protocol variables, animal species and strain selected, must be interpreted cautiously in order to arrive at a meaningful extrapolation for humans.

Keywords: Animal models; Respiratory allergy; Respiratory function; Lung remodeling; Bronchoconstriction

*Corresponding author. Tel.: +49 202 368909; fax: +49 202 364589.
E-mail address: juergen.pauluhn@bayerhealthcare.com (J. Pauluhn).

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Introduction

Allergic asthma is a complex chronic inflammatory disease of the airways and its etiology is multifactorial. It involves the recruitment and activation of many inflammatory and structural cells, all of which release inflammatory mediators that result in typical pathological changes of asthma (Barnes et al., 1998; O'Byrne and Postma, 1999). Several features of asthma can suitably be investigated in animal models. These features include cellular infiltrations in the lung, antigen-specific IgE production, and a predominant T-helper type 2 (Th2) immune response characterized by elevations in the levels of typical cytokines seen upon allergen (hapten) sensitization and challenge. The mechanisms that control CD4+ T lymphocyte polarization to allergenic Th2 phenotypes are incompletely understood but seem to involve genetic predispositions, local factors such as pre-existing cytokine concentrations and inflammation, and antigenic factors that include potency, dose, frequency and duration of exposure. The number of mediators involved in the sensitization process to an allergen and/or the development of a chronic inflammatory process in the mucosa of the lower airways, including airway remodeling, tends to confer an image of overwhelming complexity (Henderson et al., 2002; Leigh et al., 2004; Pauwels et al., 1997). Airway hyperreactivity is defined as an exaggerated acute obstructive response of the airways to one or more non-specific stimuli, often associated with airway epithelial damage and disruption, a common feature of even mild asthma (Laitinen et al., 1985). Increased hyperresponsiveness may also be associated with direct exposure of sensory nerve endings (Barnes, 1986) or by the loss of enzymes which metabolize sensory neuropeptides (Frossard et al., 1989; Barnes et al., 1991). Neurotrophins are important mediators between the (systemic) immune system and the local nervous system (Carr et al., 2001). Rather than having unique actions on immune cells, the neurotrophins often act in concert with known immune-regulating factors. They are produced locally during the allergic reaction and serve as amplifiers for Th2 effector functions and thus play an important role in the development of inflammation and airway hyperresponsiveness (Braun et al., 2000). This demonstrates that the microenvironment adjacent to the site of injury and/or sensitization and challenge may be important for the progression of disease. Commonly, animals are sensitized by two successive occasions, followed by challenge with the antigen (or hapten). This primary allergen challenge results in an asthmatic phenotype. However, to more closely resemble the human disease, secondary allergen challenges, after prolonged gaps, are often used. The route, method and dose of allergen exposure also determine the phenotype of the allergen response (Kannan and Deshpande, 2003). The response to short-term, high-level exposures cause different airway lesions when compared low-level, chronic antigen challenge. Also the methods used to assess immediate or delayed bronchoconstriction, the kind and extent of airway inflammation and tissue remodeling have an impact on the outcome of studies involving allergen (hapten)-sensitized animal models of asthma.

The emphasis of this paper is to review methodologies suitable to identify respiratory allergens for the purpose of hazard identification in animal models currently used in toxicology. This includes an analysis how the test design, i.e., route, dose, frequency of dosing, timing of challenge exposures with the hapten or antigen and the endpoint selected, can affect the outcome of study. The validity and the limitations of the various approaches are discussed.

Mechanisms leading to an asthmatic phenotype

The agents causing respiratory allergy may be present as a gas, aerosol or represent mixture of a volatile hapten partitioned with the aerosol phase. For irritant chemicals it is as yet not clear, for instance, whether induced airway hyperresponsiveness is a dose-dependent phenomenon or whether a brief high-level exposure plays a more important role. High-level exposure to irritant agents may cause airway hyperreactivity considered to be different from typical occupational asthma because of its rapid onset, specific relationship to a single environmental exposure, and no apparent pre-existing period of sensitization to occur with the apparent lack of an allergic or immunologic etiology. Hence, this illness is termed reactive airways dysfunction syndrome, or RADS. Mechanisms to explain the development of RADS focus on the toxic effects of the irritant exposure on the airways and may be attributed to neurogenic effect (Brooks et al., 1985).

The asthma phenotype is characterized by a chronic airway inflammation and inflammatory mediator cell products that orchestrate the disease. Mediator interaction may also occur by "priming" of inflammatory cells, leading then to augmented release of secondary mediators. This implies some uncertainty as to whether exogenously administered agents exert similar effects when the exposure is direct (by inhalation) or via systemic routes. To date, more than 50 different mediators have been identified in asthma, although, at the same time, current gene technology identifies an ever increasing range of molecules that could be involved in the sensitization process to an allergen and/or the development of a chronic inflammatory process in the mucosa of the lower airways. This evolution tends to confer an image of overwhelming complexity. The intricate interaction of structural and mobile cell