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REVIEW

# Assessing the Potential to Induce Respiratory Hypersensitivity

Michael P. Holsapple,*,[1] David Jones,† Thomas T. Kawabata,‡ Ian Kimber,§ Kathy Sarlo,¶ MaryJane K. Selgrade,‖ Jui Shah,|||,[2] and Michael R. Woolhiser|||

*ILSI Health and Environmental Sciences Institute, Washington, DC; †UK Medicines and Healthcare Products Regulatory Agency, London, U.K.; ‡Pfizer, Inc., Groton, Connecticut; §Syngenta Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K.; ¶The Procter & Gamble Company, Cincinnati, Ohio; ‖U.S. Environmental Protection Agency, Research Triangle Park, North Carolina; |||U.S. Food and Drug Administration, Rockville, Maryland; and |||The Dow Chemical Company, Midland, Michigan

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**Acute and repeat dose inhalation studies have been an important part of the safety assessment of drugs, chemicals, and other products throughout the world for many years. It is known that damage to the respiratory tract can be triggered either by nonspecific irritation or by specific immune-mediated pathogenesis, and it is acknowledged that traditional inhalation studies are not designed to address fully the impact of the latter. It is also recognized that different types of immune-mediated responses can be triggered by different classes of compounds and that some immune reactions in the lung are life threatening. As such, it is important to understand as fully as possible the basis for the immune-mediated damage to the lung in order to characterize adequately the risks of individual chemicals or proteins. It is against this background that a review of the methods used to assess the potential for immune-mediated respiratory hypersensitivity was conducted. The primary objectives of this review are to discuss appropriate methods for identifying and characterizing respiratory hypersensitivity hazards and risks; and to identify key data gaps and related research needs with respect to respiratory hypersensitivity testing. The following working definition of respiratory hypersensitivity was formulated: a hypersensitivity response in the respiratory tract precipitated by a specific immune response, mediated by multiple mechanisms, including IgE antibody. Because of the importance played by various classes of compounds, the subsequent sections of this review will consider protein-specific, chemical-specific, and drug-specific aspects of respiratory hypersensitivity.**

*Key Words:* **respiratory toxicology—respiratory sensitization; immunotoxicology—chemical allergy; immunotoxicology—protein allergy.**

The respiratory tract has been long recognized as an important target organ in the safety assessment of drugs and chemicals, as well as protein- or peptide-based products. Indeed, acute and repeat dose inhalation studies have been an important part of guideline studies throughout the world for many years. Hypersensitivity (allergy) is defined as humoral or cellular immune responses to an otherwise innocuous antigen, which can lead to tissue damage (Janeway *et al.*, 2005a). Hypersensitivity responses have been divided into several types based on the immunologic mechanisms involved. Although any of these types of immune mediated injury can occur in the lung as a result of chemical exposure, those that cause rhinitis and asthma via IgE- and Th2-cell-mediated responses are of particular concern. A number of factors have recently contributed to an increase in the attention focused on hypersensitivity in the respiratory tract as a target organ for safety assessment. There has been an increase in asthma among the general population from the mid-1960's to the mid-1990's prompting investigations into cause and prevention (Devereux, 2003). One study indicated that occupational asthma has surpassed traditional dust disorders to become the most commonly reported occupational lung disease (Petsonk, 2002). Clinical investigators estimate that up to 20% of adult onset asthma is caused by occupational factors and that roughly 90% of these cases involve immunological mechanisms (Mapp, 2005). Another consideration is that our knowledge of the biology of the lung as it relates to the cellular and molecular responses to damage is increasing. Moreover, it is known that damage to the respiratory tract can be triggered either by nonspecific irritation or by specific immune-mediated mechanisms, and it is

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[1] To whom correspondence should be addressed at One Thomas Circle, N.W. Ninth Floor, Washington, DC 20005-5802. Fax: (202) 659-3617. E-mail: mholsapple@ilsi.org.

[2] Present address: Division of Allergy, Immunology and Transplantation, National Institute of Allergy and Infectious Diseases, National Institute of Health, Bethesda, MD.

acknowledged that traditional inhalation studies are not designed to address fully the impact of the latter.

The Immunotoxicology Technical Committee (ITC) of the International Life Sciences Institute (ILSI) Health and Environmental Sciences Institute (HESI) organized two activities centered on the state-of-the-science of testing methods to identify proteins and chemicals that pose the risk of immune-mediated respiratory hypersensitivity. First, an expert roundtable discussion was convened in May 2003, and attracted ~40 participants from the U.S. and Europe representing primarily industry (50%) and government (35%). Second, a two-day international workshop was organized in June 2004, and attracted ~75 participants again representing mostly industry (45%) and government (38%). The format for this workshop comprised a combination of plenary lectures, after which a series of predetermined questions were addressed. The plenary lectures were intended to provide a foundation of appropriate background information by discussing the clinical aspects of respiratory hypersensitivity, and the state-of-the-science of a variety of animal models used to determine the potential of proteins or chemicals to cause immunological sensitization of the respiratory tract. The questions were intended to provide a framework for discussion by the participants, and are presented in Table 1. These two activities, especially the later workshop provided the foundation for this review.

At the outset of the workshop, the participants agreed to focus on hypersensitivity reactions that cause rhinitis and asthma via IgE- and Th2-cell-mediated responses for reasons noted above. The participants acknowledged that there are other types of immune-mediated hypersensitivity that can occur in the lung; however in the interest of time (the workshop) and space (this review), these were not part of the discussion. The workshop was structured to address various classes of compounds and the subsequent sections of this review will consider protein-specific, chemical-specific, and drug-specific aspects of respiratory hypersensitivity.

The specific objective of this review is to address the current state-of-the-science associated with respiratory hypersensitivity, as defined above, and in the context of the following questions:

- What are the appropriate methods for identifying and characterizing respiratory hypersensitivity hazards and risks?
- What are the key data gaps and related research needs with respect to respiratory hypersensitivity testing?

**TABLE 1**
**Respiratory Hypersensitivity Workshop Discussion Questions**

1. How should respiratory hypersensitivity be defined?
2. What distinguishes chemical or drug respiratory sensitization from protein allergy?
3. Is there evidence for several mechanisms of action for the induction of respiratory hypersensitivity across compound classes?
4. What is the current understanding of thresholds for induction of respiratory sensitization and elicitation of respiratory hypersensitivity?
5. Under what circumstances would respiratory hypersensitivity testing be required?
6. What methods are currently available for identification and characterization of chemicals, peptides and proteins that have the potential of inducing respiratory hypersensitivity?
7. To what extent have currently available methods been evaluated?
8. In the development and selection of new methods, what are the most appropriate parameters to consider?

## OVERVIEW OF MECHANISMS OF RESPIRATORY HYPERSENSITIVITY WITH A FOCUS ON IGE- AND TH2-MEDIATED IMMUNE RESPONSES

Respiratory hypersensitivity can be induced by both high molecular weight (HWM) and low molecular weight (LMW) allergens. The HMW allergens are generally proteins while the LMW allergens are reactive chemicals and certain drugs. Like other immunogenic compounds, the HMW allergens are large enough to be recognized by the immune system. In contrast, the LMW chemicals and drugs function like haptens, by binding to a larger carrier molecule to become immunogenic. The immune response can be directed either to the chemical or to the chemical-carrier moiety.

The development of respiratory allergy to protein and chemical allergens occurs in two phases: induction and elicitation. During induction, the allergen is engulfed by antigen-presenting cells (usually dendritic cells), processed into peptides that are presented by CD4+ T cells via the MHC Class II complex. T cells recognize linear sequences as epitopes. Cells with receptors that recognize epitopes in context with class II molecules differentiate into Th2 cells via a series of events starting with the activation of the transcription factor GATA-3 (Ray and Cohn, 1999). B cells also have receptors that can recognize the allergen. The B cell receptors can recognize linear sequences or three-dimensional structural regions. The Th2 cells secrete cytokines such as IL4 and IL13 that help ''push'' B cells to undergo class switching to increase production of IgE antibody that binds to specific epitopes. The IgE antibody can stay in circulation and/or bind to the surface of cells, including tissue mast cells or circulating basophils. Re-exposure to the protein leads to binding of the protein to pre-formed IgE antibody on the surface of these cells leading to elicitation. This interaction sends a signal to the cell to degranulate and release pro-inflammatory mediators (e.g., histamine, prostaglandins) that cause the symptoms of immediate-type hypersensitivity that may range from mild rhinitis to anaphylactic shock. The immediate symptoms in the respiratory tract include rhinitis, bronchoconstriction, and asthma. Between 2 and 8 h after the immediate response, some individuals (usually asthmatics) experience a more severe and

prolonged late phase reaction that is characterized by mucus hypersecretion, bronchoconstriction, airway hyperresponsiveness to a variety of nonspecific stimuli, and airway inflammation characterized by infiltration of eosinophils. This later response is not IgE-mediated, but is thought to involve Th2 lymphocytes. In some cases, these reactions lead to chronic inflammation in the tissue (frequently characterized by an accumulation of eosinophils) characteristic of chronic asthma (Gelfand, 2004). In addition to the greater role played by Th2-mediated processes in the late phase when compared to IgE, it is also known that for certain LMW chemicals (e.g., toluene diisocyanate and plicatic acid), the development of IgE antibody may not be the only immune mechanism responsible for respiratory allergy. An inflammatory response dominated by Th2 lymphocytes may also contribute to the disease.

## PROTEINS AND RESPIRATORY ALLERGY

IgE antibody-mediated immunological responses are responsible for a major category of respiratory allergic responses described for proteins. While the basic biology of IgE antibody mediated allergic responses to proteins has been described, much is yet to be understood about the mechanisms of allergic responses. The development of an IgE-antibody response to a protein is dependent upon a Th2 dominant immune response (Mossmann *et al.*, 1986; Mossmann and Coffman, 1987). In humans, the Th2 dominant response can lead to the development of protein specific IgE antibody, IgG4 antibody or both types of antibody, and eosiniphilic cell-mediated inflammation (Adamko *et al.*, 2005; Ostroukhouva and Ray, 2005). While the presence of IgE antibody does not equate to disease, it does increase the risk for development of allergy symptoms.

Inhalation exposure to a protein allergen is the most effective route for induction of IgE antibody. A small percent of the population can develop IgE antibody to certain proteins via the GI tract (e.g., food allergens). Very little data exist to show that induction of IgE antibody to protein allergens can occur via the skin; conventional wisdom dictates that this is an unlikely route of exposure for induction of allergic antibody because proteins do not readily penetrate through the intact skin. Experimental studies conducted in mice showed that a Th2 dominated immune response to ovalbumin was generated upon extended and occluded exposure to this protein via compromised skin (Spergel *et al.,* 1998). A similar observation was made for natural rubber latex proteins (Meade and Woolhiser, 2002). Elicitation of allergy symptoms in the airways can occur via inhalation or gut exposure (Spergel and Fiedler, 2005). Skin contact with an allergen can elicit local skin symptoms but very little evidence for respiratory symptoms. Whether or not a combination of exposures can alter thresholds for induction of IgE or elicitation of allergic symptoms needs to be better understood for risk assessment.

Diagnosis of IgE-mediated respiratory allergy to proteins is usually conducted via an understanding of exposure and clinical history, measurement of lung function, protein specific IgE antibody (e.g., skin tests, serology) and in certain cases, provocation tests (European Academy of Allergology and Clinical Immunology, 1989; Campo *et al.*, 2004). As noted above, although the presence of IgE antibody does not always equate to allergy symptoms, having specific IgE antibody can raise the risk of developing allergy symptoms upon re-exposure to the protein. Investigators have tried to use IgE levels as a predictor for risk of food allergy but there are no absolute relationships between the levels of IgE antibody and symptoms, especially for respiratory allergens (Sampson, 2001). Investigators recognize that there are thresholds of exposure for both induction of IgE antibody and elicitation of allergy symptoms but the data are not clear as to whether the threshold for elicitation is higher or lower than the threshold for induction, or how to deal with cumulative exposures. Current dogma dictates that the threshold of exposure for elicitation of symptoms is lower than the threshold of exposure for induction of IgE antibody. However, prospective monitoring of induction and elicitation of respiratory allergy to enzymes used in the detergent industry suggest the opposite (Sarlo and Kirchner, 2002). Continued study of the relationships between exposure and occupational allergy should shed light on the question of thresholds. It is currently difficult to identify thresholds for allergy since these can vary among individuals. The best effort would be to define thresholds on a population basis. In some cases, regulatory agencies have sought to prevent induction of allergy based on a body of epidemiological studies (usually occupational exposures) that included at least one prospective study that contained a NOAEL (no-observed-adverse-effect-level). Whether the development of low levels of IgE antibody is problematic needs to be understood. Focused prospective clinical work (e.g., occupational studies, clinical trials) assessing the IgE response (level, affinity, epitope binding), exposure and the absence or presence of allergic symptoms will help to address these questions. Appropriate animal models would also be useful for studying the relationship between exposure dose, IgE levels, pulmonary inflammation, and bronchoconstriction.

Not all proteins will induce an IgE antibody response and not all individuals will develop IgE antibody responses to those proteins that function as allergens. Remarkably, little is known about the structure-activity relationships (SAR) of proteins to allergy (Huby *et al.*, 2000; Stewart and Thompson, 1996). Allergens are derived from multiple sources such as microbes, plants, and animals (invertebrates and vertebrates). Many allergens have hydrolytic activity but this is not a universal feature of all allergens. Stability in gastric fluids may be an important feature for food allergens but stability of respiratory allergens has not been well studied. Limited data on a few allergens indicate that they can affect the local environment via interactions with airway epithelial cells and dendritic cells to help skew towards a Th2 response (Asokananthan *et al.*, 2002;

Ritz *et al.,* 2004). Additional information on how allergens interact with airway cells (including immune cells) will help to identify unique features of allergens. In addition, not enough information on the T-cell and B-cell epitopes of allergens exist to be able to use sequence and structure information to predict allergenicity. Several on-line allergen databases are available for searching sequence similarities between proteins and known allergens (Mari, 2005), however uniform criteria for entering proteins as allergens into these databases and uniform search strategies do not exist. Sequence and/or structural similarities between a protein of interest and an allergen may indicate cross-reactivity but this should be confirmed by serology. Some allergens, referred to as ''pan allergens,'' induce IgE antibodies that can recognize epitopes on other proteins. A comprehensive database on allergen sequence, structure, epitopes, function, pan allergen properties, and other pertinent information will be crucial to the development of valid bioinformatics tools for protein allergy.

Multiple animal models (guinea pig, rat, and mouse) have been used to study protein allergy but very few, if any, have been validated via rigorous interlaboratory testing as predictive models to identify a protein as an allergen in addition to its allergenic potency. Only a handful of protein allergens have been studied in this regard. Measurement of protein specific antibody (IgE and IgG1) is the primary parameter for assessing potency in these systems. For detergent enzymes, the allergenic potency data (relative to a known allergen) has been used to develop exposure guidelines for occupational settings (Robinson *et al.*, 1998). None of these models have been formally validated for hazard identification and risk assessment. Consensus among investigators in this field is that these models need further development and none can be used as currently designed to address all concerns of protein allergy. In addition, there needs to be agreement on how to define allergenic potency—Is it based on antibody response, symptom scores or some other endpoint relevant to respiratory hypersensitivity? Numerous factors need to be considered when refining existing models or developing *in vivo* models for protein allergy: route of exposure, frequency of exposure, use of adjuvants, dose limitations, species variability (mice, rats, and humans), and strain (intra-species) variability, availability of positive and negative control proteins and standard methods for measuring antibody (or other immunological endpoints). A thorough review of existing models and data will help to focus future efforts on refinement and development of models for hazard identification and estimates of potency.

There is growing interest in the lung as a route for drug delivery and these same issues exist when assessing the potential allergenicity of inhaled protein drugs. Standard repeated-dose inhalation toxicity studies are used to support clinical trials with drugs to be administered by the respiratory route. Assessment of clinical signs consistent with allergy (e.g., anaphylaxis) or histopathological findings consistent with allergic inflammation can be an indicator that the protein drug can cause a Th2-dominant respiratory hypersensitivity. Finding an anti-drug antibody response consistent with a Th2-response (e.g., IgE and IgG1 in mice) would be consistent with clinical and histopathological signs of Th2-dominant respiratory hypersensitivity. These findings can dictate caution or evaluation of the immune response during clinical studies. This situation may be different from the one where IgG antibody is found. The inhalation toxicity studies can also be used to assess potential hypersensitivity pneumonitis (e.g., Farmer's lung disease) where T-cell (CD4+ and CD8+) involvement in the generation of granulomas and the ensuing inflammation are now believed to be the dominant mechanism as compared to IgG precipitation antibodies (Ando *et al.*, 1999). An anti-drug antibody response would need to be evaluated in detail to determine if it was related to the clinical or histopathological signs from the toxicity test. Measurement of the anti-drug antibody response in most preclinical studies is primarily used to explain potential changes in toxicity, pharmacokinetics and pharmacodynamics observed in the toxicity study. On the other hand, measurement of anti-drug responses in preclinical studies is not useful in predicting immunogenicity in humans. As we gain more preclinical and clinical experience with inhaled protein drugs, preclinical *in vitro* or *in vivo* methods that predict hypersensitivity responses in humans may be developed in the future.

## CHEMICALS AND RESPIRATORY ALLERGY

It is generally accepted that there are four basic mechanisms that lead to allergic hypersensitivity (Janeway *et al.*, 2005b), and evidence shows that chemicals can cause allergic disease in the lung based on each of these mechanisms (Kirschner, 2002). In addition, more than one of these responses may be involved in respiratory responses to a chemical allergen. Of particular concern are chemicals that cause rhinitis and asthma via IgE- and Th2-cell mediated responses, briefly considered in this section.

Chemical respiratory allergy is an important health issue for a number of reasons. First, respiratory allergy to chemicals, characterized by rhinitis and/or asthma, is associated with high levels of morbidity, and even mortality. Second, it is usually manifest in an occupational setting where allergic sensitization results from exposure to chemicals in the workplace. The occurrence of immune-meditated occupational asthma is frequently associated with financial and social consequences. Third, although some important progress has been made, the lack of rigorous interlaboratory studies have resulted in a lack of widely accepted, formally validated, methods for the identification and characterization of chemical respiratory allergens.

Although much is known about the initiation and regulation of allergic responses, and of the pathogenesis of asthma, our understanding is far from complete. Respiratory allergy and

immune-mediated occupational asthma, resulting from exposure to chemicals, present additional challenges and issues. The first of these is the identification of the relevant effector mechanisms. There is no doubt that in the acquisition and expression of respiratory allergy to proteins, and in other forms of atopic allergic disease, IgE antibody plays an important role. However, the relevance of IgE antibody for the development of chemical respiratory allergy is more contentious. Although there is evidence that all known chemical respiratory allergens (including diisocyanates, acid anhydrides, some reactive dyes, and platinum salts) induce specific IgE in some symptomatic subjects, other subjects do not exhibit this response, particularly in allergy and asthma associated with diisocyanates (Bernstein *et al.*, 2002; Cartier *et al.*, 1989; Park *et al.*, 1999). This is a matter of debate, partially due to technical limitations involved in the synthesis of hapten conjugates and immunoassay methodology that can hamper the accurate identification of chemical-specific IgE antibody (Park *et al.*, 2001). As noted above, a chemical ''hapten'' must bind to a ''carrier'' protein to elicit an immune response. The requirement for haptenation is a major distinction from a protein- or peptide-induced IgE response.

The available evidence suggests that although the specific IgE antibody can be causally associated with chemical respiratory allergy in some instances, IgE-independent, immunological pathogenesis may exist that permit the acquisition of respiratory sensitization to chemical allergens. For example, it has been suggested that the presence of specific IgG can be (more) closely associated with positive bronchoprovocation tests from asthmatic patients (Park *et al.*, 2002). However, the absence in some studies of a strong correlation between symptoms of respiratory hypersensitivity and plasma IgE antibody may be attributable to one or more of a number of artifacts, including the limitations mentioned above. If such proves to be the case then the relationship between IgE antibody and chemical respiratory allergy may be stronger than is sometimes suggested (Kimber and Dearman, 2002). Whether or not a universal mandatory role exists for IgE antibody in respiratory allergy to chemicals, there is growing evidence that sensitization is associated with development of a selective Th2 immune response (Kimber and Dearman, 1999).

Another area of uncertainty and debate relates to relevant routes of exposure. It is commonly assumed that allergic sensitization of the respiratory tract necessarily results from inhalation exposure to the inducing allergen (Bernstein and Malo, 1999). Although this might usually be the case for sensitization to protein allergens, there is reason to speculate that effective acquisition of respiratory sensitization to chemical allergens might also result from other routes of exposure, including dermal contact, an important distinction between chemicals and proteins (Klink and Meade, 2003; Sailstad *et al.,* 2003). The argument is that skin exposure to a sufficient amount of a relevant chemical allergen will induce an immune response of the quantity and quality (Th2 selective—possibly with or without IgE antibody) necessary to cause systemic sensitization, including sensitization of the respiratory tract. This issue reaches beyond academic interest, in that the development of accurate risk assessments and of effective risk management strategies are dependent upon an understanding of the likely risks associated with different routes of exposure (Kimber and Dearman, 2002).

Against this background there is a need to have available approaches for the identification and characterization of chemicals that have the potential to cause respiratory allergy, manifested as rhinitis and asthma. Initiatives in this area focused initially on the use of models (usually guinea pig models) in which sensitizing activity was measured as a function of inhalation challenge-induced pulmonary reactions in previously sensitized animals (Karol, 1994). These models varied in utility, and were found to have a number of drawbacks and limitations, among these being cost and technical complexity. As a result there has been interest in developing alternative strategies for hazard identification, usually employing either rats, or in particular, mice (Dearman and Kimber, 1999).

Among the better characterized approaches are those that are based upon evaluation of immune responses induced by chemical allergens. One such strategy is the mouse IgE test in which respiratory sensitizing chemicals are defined by their ability to stimulate an increase in the serum concentration of total IgE following dermal exposure (Dearman *et al.*, 1998; Hilton *et al.*, 1996). A similar approach evaluates the local production of IgE in draining lymph nodes following dermal chemical exposure by quantitating IgE bound to CD23 on B cells using flow cytometry (Manetz and Meade, 1999). Another method, known as cytokine profiling, instead identifies respiratory sensitizing potential on the basis of induced cytokine secretion. Studies by Dearman and co-workers have shown that chemical respiratory allergens stimulate cytokine expression patterns reflective of preferential Th2 immune responses (e.g., interleukin-4 [IL-4], IL-5, IL-10 and IL-13) as opposed to a profile associated with Th1 cells (e.g., IL-2 and interferon gamma (IFN-$\gamma$) (Dearman and Kimber, 2001; Dearman *et al.*, 2002, 2003)). Variants of the approach to cytokine profiling have been described (Plitnick *et al.*, 2002, 2003; Van Och *et al.*, 2002). Both the mouse IgE test and cytokine profiling show promise, but neither of these approaches has yet been validated formally in rigorous interlaboratory studies.

In both the mouse IgE test and cytokine profiling, the strategy is to identify chemical respiratory allergens, and to distinguish these from other chemical allergens (contact allergens) that are not associated with sensitization of the respiratory tract. This approach raises an interesting question about the relationship between contact and respiratory allergens and about the opportunities that may exist for a tiered testing strategy.

A method that has been validated for the identification of contact sensitizing hazard in a number of interlaboratory studies is the mouse local lymph node assay (LLNA) (Kimber

*et al.*, 2002). While the mouse LLNA is now accepted as a method to identify contact sensitization potential, it is apparent that the LLNA is also able to identify chemical respiratory allergens even though some of these chemicals are not usually associated with clinical allergic contact dermatitis. These observations raise the possibility that the LLNA could be considered as the first step in a safety evaluation process for allergenic potential (as a method for identifying sensitizing potential per se) with other methods being deployed subsequently if a need existed to distinguish between skin and respiratory sensitizing activity. Such an approach would of course be predicated on an assumption that a negative result in the LLNA is indicative of the lack of respiratory sensitizing activity, as well as skin sensitizing activity. Such a hierarchical approach might be viable, but has not yet been tested formally (Dearman *et al.*, 2003).

## DRUGS AND RESPIRATORY ALLERGY

As noted above for HMW protein drugs, there is increased interest in the lung as the organ of delivery, and it is important that preclinical studies evaluate the potential of LMW therapies to produce hypersensitivity reactions in the lung. Immune responses against the drug during clinical trials, or once marketed, may lead to adverse hypersensitivity reactions and immediate, Type 1 hypersensitivity reactions are particularly concerning since that may lead to anaphylaxis and/or shock.

Respiratory hypersensitivity testing of drugs is constrained by the same issues discussed above for chemical and protein allergens. Issues, proposed mechanisms for hypersensitivity reactions and testing methods described previously for chemical respiratory allergens apply to LMW drugs. Likewise, much of the section on protein respiratory allergy applies to HMW protein drugs administered via the respiratory route. The primary difference between drugs and non-drug chemicals is based on how the data is used for the risk assessment process and how the data will impact the clinical studies.

Standard preclinical repeated-dose inhalation toxicity studies in two species and testing in a contact hypersensitivity model are the current recommended approaches to identifying potential respiratory sensitizers for drugs to be administered by the respiratory route (U.S. Food and Drug Administration [FDA] and Medicines and Healthcare products Regulatory Agency [MHRA]). For the standard inhalation toxicity studies, a comprehensive histopathological evaluation of the respiratory tract is very important. Because most known respiratory chemical allergens are also irritating, a warning signal (e.g., inflammation, eosinophil or lymphocyte infiltration) would be detected in inhalation toxicity studies. This may account for the extremely low incidence of LMW drugs that produce respiratory hypersensitivity reactions. It also would be expected that Type IV sensitizers would also produce histopathological changes that would be detected in inhalation toxicity studies, but this has not been evaluated. Another approach is to add a satellite group to a repeated dose rat study that would be allowed to enter into a recovery phase (no drug exposure), rechallenged and assessed by histopathology. This approach may be advantageous since it can be added to an ongoing study. However, it has not been extensively used and should not be considered a validated approach at this time. Other indicators of respiratory hypersensitivity, such as plethysmography, may also be incorporated into standard toxicity studies as appropriate. The use of plethysmography is discussed further below.

The mouse LLNA or other contact hypersensitivity models (e.g., Guinea pig maximization test or Buehler test) is the recommended screen for respiratory drugs, as proposed for the chemical allergens. Testing for contact hypersensitivity will not test for Type I respiratory sensitizers but it will (1) identify compounds with high protein reactivity and ability to form haptens (required steps to the generation of Type I responses), and (2) identify compounds that may produce a Type IV response in the respiratory tract. Although there are no known drugs that produce Type IV reactions with inhalation exposure, there is a theoretical safety concern since Type IV reactions in the lung have been observed with other chemicals in humans (e.g., beryllium). There are also concerns, however, that a contact hypersensitivity model may not be able to detect sensitizers that require metabolic activation. If a positive response is observed, the relative potency may be determined from LLNA studies to determine relative risk. The best method to assess potency has not been established; but the EC3 (Estimated Concentration required to give a stimulation index of '3') approach from the LLNA appears to be promising (Kimber *et al.*, 2002).

The FDA Immunotoxicology guidance document also recommends that the tiered approach of Sarlo and Clark (1992) be used as a means to evaluate the potential for a compound to produce respiratory sensitization. This can be an alternative to the contact hypersensitivity model or a follow-on study if a positive contact hypersensitivity response is observed. The initial tier of this method is based on evaluating the relative reactivity of the test material toward proteins (Gauggel *et al.*, 1993). The last tier of the ''Sarlo method'' is the Karol guinea pig whole body plethysmography method (1994) also recommended by the FDA guidelines. However, the Karol method has been tested with only a few classes of compounds (e.g., diisocyanates and anhydrides), and is not considered to be well validated. Moreover, plethysmography in guinea pigs, rats, and mice can be technically challenging and labor intensive. In addition, there is some controversy over interpretation of measurements made from whole body plethysmography and whether these measurements truly reflect changes in airway mechanics (Lundblad *et al.*, 2002; Mitzner and Tankersley, 2003).

If the drug is found to be positive in a contact hypersensitivity test then follow-on studies may be needed. To test for the potential to produce a respiratory hypersensitivity reaction

(Type I, IgE), follow-on studies such as the mouse cytokine profiling method (Dearman and Kimber, 2001; Plitnick *et al.*, 2002, 2003) or mouse IgE method (Dearman *et al.*, 1998) could be used as discussed above in the chemical allergens section. However, as indicated previously, these methods have not been formally validated. Moreover, it is important to note that different approaches to cytokine profiling have generated different results. At least one study has demonstrated that a strong LLNA response (i.e., much greater than an EC3 value that is an accepted indicator of a positive response for contact sensitivity) is needed to have a measurable response using cytokine profiling (Van Och *et al.*, 2002).

During the 2004 workshop, participants from the FDA and MHRA recommended that the most direct approach for respiratory hypersensitivity testing is the combination of standard inhalation toxicity studies and the LLNA or some type of contact hypersensitivity testing. Indeed, contact hypersensitivity studies must be conducted before multi-dose clinical studies, and at least some type of local tolerance test (e.g., inhalation toxicity studies) must be done before the first in human studies. If a contact hypersensitivity response is observed, follow-on studies may be needed to better assess potential risk; but this does not necessarily mean that drug development must be discontinued. The assessment of risk should be handled on a case-by-case basis. A variety of factors such as the incidence and severity of the contact hypersensitivity reaction, relative potency (EC3 from a LLNA), results from preclinical inhalation toxicity studies, drug indication, doses used in relation to clinical exposures, and clinical plans for monitoring for respiratory hypersensitivity should be taken into consideration. Guidance on a path forward with compounds that have a positive contact hypersensitivity response is not clear since the FDA and MHRA have only seen negative contact hypersensitivity results to-date for inhaled drugs submitted for clinical trials.

## KEY DATA GAPS AND RESEARCH NEEDS

A number of key data gaps have been identified above. The objective of this section of the review is to summarize the gaps and to offer some suggestions for approaches to fill the gaps. Because the focus of this review is on methods of respiratory hypersensitivity testing, most of the gaps concern methodology. Because the workshop that provided a critical foundation for this discussion emphasized the need for improved animal testing, that will be the emphasis here, as well. However, in light of efforts to find alternatives to animal models, applications of *in vitro* and/or in silico work will also be highlighted as possible ways forward to advance our predictive capability. Finally, we will attempt to highlight the critical areas of basic biology that are important areas of future research.

The research gaps for protein-specific respiratory hypersensitivity are summarized in Table 2. The first gap focuses on the development of valid databases for SAR for protein allergy. The path forward for setting priorities for research in respiratory hypersensitivity should include work on allergen databases so that investigators have a first tool to screen proteins for potential allergy. These databases should be built using uniform criteria for entering proteins and for the subsequent searches. There should be a way to foster collaborations and linkages among the various groups. In conjunction with this effort, there are needs to improve the identification of epitopes and to develop better methods to assess cross-reactivity among proteins. Conducting basic research to determine the reasons why certain compounds induce IgE reactions and Th2-dominant immune responses is needed to build the in silico models, especially for inhaled protein drugs. In silico prediction of peptide binding to MHC class II molecules may be a way forward to predicting immunogenicity and potentially allergenicity. The second gap is to develop more predictive animal models. As noted above, several models involving multiple species including guinea pigs, rats and mice have been studied; but few have been well-characterized especially across multiple labs. The next priority is to adapt existing animal models so that they can have predictive usefulness beyond the small families of proteins that have been tested. A critical review of all the data generated in the various guinea pig, mouse and rat models using different protein allergens is needed so that some rational thought

**TABLE 2**
**Research Gaps for Protein-Specific Respiratory Hypersensitivity**

| Key research gap | Approach to fill gap |
|---|---|
| 1. SAR for protein allergy. | 1a. Build allergen databases using uniform criteria for entering proteins into the database + uniform criteria for searches.<br>1b. Identification of epitopes.<br>1c. Methods to assess cross-reactions.<br>1d. In silico prediction of peptide binding to MHC class II. |
| 2. Predictive animal models. | 2a. Expand testing in existing guinea pig, mouse, or rat models of protein allergy.<br>2b. Validation of histopathology from inhalation tox studies.<br>2c. Evaluate utility of transgenic mice to predict immunogenticity and/or allergenicity in humans.<br>2d. Conduct more basic research to determine the reasons why certain compounds produce IgE reactions or T-cell reactions that may result in respiratory hypersensitivity reactions. |
| 3. IgE and risk of disease. | 3a. Develop robust measures of IgE for non-drug and drug proteins.<br>3b. Assess clinical tests relevance of IgE to disease—assess vs. IgE level to predict % responders in a population (see limited work in food allergy area). |

contribute to moving the right models to the next level. In addition, the animal models should be applied to assess matrix effects in the immune response to chemicals and proteins. As discussed for protein drugs, greater use should be made of the histopathology from inhalation toxicity studies, and indeed, this approach should be validated for its potential to predict the hazard of respiratory sensitization potential. Two additional points need to be emphasized in the context of improving our predictive models for protein allergy. Greater attention is warranted to develop *in vitro* cell-based assays that can be used to identify potential allergenicity. It is recognized that the animal work will need to be done first since *in vivo* data will be needed to help validate the *in vitro* systems. Greater attention should also be focused on developing a rational approach to the use of genomic and proteomic tools. The final gap deals with improving our understanding of the relationship between the presence of IgE antibody and the risk of disease. The approach to this gap would include robust measurements of IgE and of clinical tests for disease. The ultimate objective would be to assess the IgE levels to predict the % responders to respiratory hypersensitivity in a population in an analogous way to the limited work that has been performed in the area of food allergy. Ultimately, a partnership between investigators running multi-center allergy epidemiology studies is needed to provide information on thresholds for sensitization and disease, how IgE levels relate to risk of symptoms, etc. The clinical data will help identify the different susceptibility factors associated with the development of allergy to proteins. This should include individual and population factors, as well as environmental factors.

The research gaps for chemical-specific respiratory hypersensitivity are summarized in Table 3. As described above for proteins, the first research need for chemicals is to increase our knowledge of the SAR for the ability of chemicals to cause sensitization of the respiratory tract; and to understand better the structural features that distinguish chemical respiratory allergens from contact allergens. In the past, some attempts have been made with a relatively small number of chemicals to define relevant SAR; but none of these previous models have been validated and are not widely accepted. As such, it should be recognized that SAR models for respiratory allergy are in their infancy and their development is severely constrained by the small numbers of chemicals that have been implicated as respiratory allergens. The second gap is the need to understand in greater detail the mechanisms through which chemicals are able to induce sensitization of the respiratory tract and the role played by IgE antibody in chemical-induced allergic responses. The approach to this gap will require experimental studies in appropriate animal models, combined with appropriate clinical investigations into the kinetics and immune pathogenesis of occupational asthma and rhinitis due to chemicals. Finally, there is a need to define the optimal approach for the identification and characterization of chemical respiratory allergens. As discussed above, one laboratory has developed a promising approach to cytokine profiling that appears to provide a sensitive and selective method (Dearman and Kimber, 2001; Dearman *et al.*, 2002, 2003). Variants of this method have been described also, but with somewhat mixed results (Plitnick *et al.*, 2002, 2003; Van Och *et al.*, 2002). The requirement now is for a formal and properly managed inter-laboratory trial of this method along the lines of the successful validation efforts associated with the murine LLNA. One such initiative with cytokine profiling is about to be implemented in Europe.

As noted above, there are similarities between LMW drugs and chemically induced respiratory allergy, and between HMW drugs and protein-induced respiratory allergy; and this is the case for the key research gaps, as well. The research gaps for LMW drugs are summarized in Table 4 and begin with an emphasis on SAR. Specifically, it is suggested that a reasonable starting point would be to build off the existing SAR datasets

**TABLE 3**
**Research Gaps for Chemical-Specific Respiratory Hypersensitivity**

| Key research gap | Approach to fill gap |
|---|---|
| 1. SAR for chemical allergy. | 1a. Understand mechanism(s) for respiratory hypersensitivity and identify distinctive characteristics of respiratory allergic immune responses.<br>1b. Continue to build databases of sensitization until chemicals can be clearly identified as respiratory allergens. |
| 2. Better understanding of mechanisms for sensitization. | 2. Experimental studies in animals combined with clinical investigations. |
| 3. Fully characterize cytokine profiling as an approach for hazard identification. | 3. Properly managed and tiered inter-laboratory collaboration. |

**TABLE 4**
**Research Gaps for Drug-Specific Respiratory Hypersensitivity (Low Molecular-Weight Drugs)**

| Key research gap | Approach to fill gap |
|---|---|
| 1. SAR with pharmaceuticals. | 1. Build off of existing SAR datasets for contact allergens. |
| 2. Validation of contact hypersensitivity testing for respiratory allergens. Understanding of the significance of weak to moderate contact hypersensitivity responses in preclinical models. | 2. Preclinical inhalation testing of weak to moderate contact allergens. |
| 3. Understanding why certain drugs result in IgE response. | 3. Basic research and modeling on chemically induced IgE responses in animal models. |
| 4. Predictive models for IgE-mediated hypersensitivity. | 4. Conduct studies to better understand why certain chemicals produce IgE responses. |
| 5. Monitoring for IgE reactions in humans. | 5. Develop more robust measures of specific IgE response. |

for contact sensitizers. Because of the nature of the drug development process and the ability to expose humans, the relationship between contact and respiratory sensitizers was emphasized in the second gap. Specifically, the validation of contact hypersensitivity testing for respiratory allergens must be extended in order to understand the significance of weak to moderate contact hypersensitivity responses in preclinical models. The approach to this gap would include the preclinical inhalation testing of weak to moderate contact allergens. Not surprisingly, the remaining gaps in this section are to improve our understanding of why certain drugs cause an IgE antibody response, to emphasize the need for more predictive animal models and to improve our ability to monitor IgE reactions in humans by more robust measures of the IgE response.

As captured in this review, progress has been made in terms of developing methods to identify and characterize the potential to cause respiratory sensitization. However, there are still a number of challenges and, as noted above, a number of data gaps that the scientific community needs to address.

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