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REVIEW

Respiratory Allergy: Hazard Identification and Risk Assessment[1]

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Various chemicals and proteins of industrial importance are known to cause respiratory allergy, with occupational asthma being the most important manifestation of the disease. This paper describes clinical syndromes, mechanisms associated with occupational respiratory hypersensitivity, and methods available currently for the prospective identification of potential respiratory allergens. Certain classes of chemicals are commonly associated with occupational respiratory allergy. There is insufficient information, however, to predict respiratory sensitization potential from analysis of structure alone, although reactivity with proteins is likely to be relevant. As yet there exist no fully validated or widely applied predictive methods or internationally harmonized guidelines. The most promising predictive animal methods are the mouse IgE test and guinea pig models. Work in mice has focused upon events occurring during the induction phase of sensitization following primary encounter with the test chemical. In contrast, guinea pig models have been used primarily to identify respiratory allergens (chemicals or proteins) as a function of elicitation reactions induced in previously sensitized animals. Given the possible serious health manifestations of respiratory allergy, early identification of respiratory sensitizers is urgently required. The two methods should, as a priority, be developed further and the production of a detailed protocol for these methods be undertaken to facilitate further validation. Together, this information will allow for two types of risk assessment associated with respiratory allergy: the risk that exposure to a material will (1) induce sensitization in an individual and (2) elicit allergic reactions in a previously sensitized individual. © 1994 Society of Toxicology.

[1] All authors were members of an ECETOC Task Force on Respiratory Allergy.

Respiratory sensitization is an immune state (typically, the presence of specific IgE antibodies) which is likely to result in symptoms of respiratory disease or distress when a particular allergen is inhaled. Respiratory allergy is the clinical manifestation of this state, with bronchial asthma and/or rhinitis (e.g., hay fever) constituting the most important manifestations.

For centuries exposure to various substances and chemicals in the workplace has resulted in respiratory sensitization and allergy. Baker's allergy, for instance, is a well-known example of occupational respiratory hypersensitivity, in which sensitized individuals exhibit symptoms of bronchial asthma or rhinitis following exposure to different kinds of flour (Musk *et al.*, 1989). The number of known allergens is increasing and includes both proteins and low-molecular-weight chemicals (Cullen *et al.*, 1990).

The immunologic reactions underlying the symptoms associated with respiratory allergy in sensitized individuals are fairly well characterized (Karol, 1989). In most cases the symptoms can be treated successfully. Much less is known, however, about the mechanisms which result in respiratory sensitization. While individuals already sensitized may exhibit symptoms following inhalation of extremely low concentrations of a respiratory allergen, little is known about the doses necessary for inducing sensitization, even with common allergens like pollens, house dust mite, or animal dander. Relatively high concentrations of an allergen, possibly in just one or a few exposures, are believed to be important in the induction stage (Karol, 1989). Little evidence supports the common view that repeated exposure to low doses is a prerequisite for sensitization (Karol and Thorne, 1988). The available evidence indicates that a variety of factors, both genetically determined and environmental, will influence predisposition for respiratory allergy (Karol *et al.*, 1993).

One of the most important issues in occupational allergy,

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therefore, is to determine the critical exposure conditions which result in sensitization and which will cause respiratory symptoms in previously sensitized individuals. Armed with such information industries involved in the production and handling of compounds with allergenic potential can take measures to prevent exposure of workers to levels which might otherwise lead to respiratory sensitization and allergy. This paper reviews what is presently known in the area of respiratory sensitization and allergy, investigates future needs, and has the following specific aims:

- review the syndromes and immunological mechanisms underlying respiratory sensitization and allergy,
- review animal and *in vitro* models currently available for predicting the potential of a substance to cause respiratory allergy and assess their present state of validation,
- recommend approaches to hazard identification and risk assessment in respiratory sensitization and allergy.

BACKGROUND

Allergic Respiratory Disease Can Take Several Forms

Hypersensitivity pneumonitis, or extrinsic allergic alveolitis, is a well known occupational disease. Many forms are known under names related to either the occupation or the responsible substance, i.e., farmers' lung, mushroom workers' lung, malt workers' lung, bird fanciers' disease, bagassosis, suberosis, and byssinosis. Most of them are relatively well-studied because of the possible serious long-term sequelae. Nearly all are due to organic dust, i.e., material of animal, vegetable, or microbial origin. For most of the chemical industry, hypersensitivity pneumonitis is rarely a cause of occupational disease. Allergic bronchopulmonary aspergillosis is another form of allergic respiratory disease. It is a condition caused by *Aspergillus* spores characterized by pulmonary eosinophilia (Slavin, 1983) and is rarely an occupational disease. For this reason these subjects are not discussed further in this paper.

Another form of respiratory disease is associated with asthma and/or rhinitis. This form of respiratory allergy can be caused both by high-molecular-weight substances, usually proteins, and by low-molecular-weight chemicals.

Occupational asthma caused by proteins is associated primarily with exposure to animal allergens and handling of protein powders. An example of the latter was asthma among workers in the detergent industry in the late sixties and early seventies, caused by exposure to dusty proteolytic enzyme powders. The occurrence of occupational allergy was controlled successfully by reducing the level of enzyme in the working environment with the introduction of nondusty enzyme products and by the implementation of appropriate containment and ventilation (Gilson *et al.*, 1976). Studies by Zetterstrom (1977) and Pepys *et al.* (1985) confirmed that the widespread use of nondusty enzymes in detergents has not caused respiratory allergy in consumers.

The best documented cases of occupational rhinitis are due to exposure to animal allergens (Rudolph *et al.*, 1980; Hunskaar and Fosse, 1993). There are few published data on occupational rhinitis resulting from exposure to low-molecular-weight chemicals (Chan-Yeung *et al.*, 1973). Presumably, occupational rhinitis is less well studied because of the relative lack of severity in the clinical symptoms compared with asthma and the difficulty in establishing the etiology.

Occupational asthma resulting from exposure to low-molecular-weight chemicals is of continued concern to the chemical industry (McGrath *et al.*, 1983). The best examples of chemicals which have the potential to sensitize include some isocyanates, reactive dyes, and organic acid anhydrides (Bardana and Andrach, 1983; Butcher *et al.*, 1989; Vandenplas *et al.*, 1993). For some applications of isocyanates, the problem has largely been solved by the development of "capped" isocyanates and isocyanates with very low vapor pressures. All published cases of respiratory allergy to low-molecular-weight chemicals are derived from occupational rather than consumer exposure.

Nevertheless, this form of allergic disease has received little attention in the past, to the extent that even the incidence and severity of the problem is not well known. Several factors may have contributed to this lack of attention:

- Much of the clinical attention was focused on the much more prevalent pneumoconioses (with and without tuberculosis), and the above-mentioned extrinsic allergic alveolitis, because of their serious long-term health consequences.
- Individual cases are easily lost in the large pool of "intrinsic" and "atopic" asthma patients; thereby, the diagnosis is often delayed, and the relationship between symptoms and exposure to a specific substance can easily be missed.
- There are only a few epidemiological studies (Diem *et al.*, 1982; Sequin *et al.*, 1987); in fact, with few exceptions, most "sensitizing" chemicals are classified as such on the basis of a limited number of clinical case studies, most of them without relevant exposure data. Levels and route of exposure required to induce respiratory sensitization are largely unknown.
- It is not always easy to correlate clinical and epidemiological data. While signs and symptoms in individuals sensitive to a given material may indicate a strongly allergenic substance, the percentage of exposed people that develop allergy may be quite low. To complicate matters further, not all individuals who appear to be sensitized, on the basis, for example, of specific IgE antibody, develop allergic disease and not all individuals with occupational asthma have detectable specific IgE antibody to the putative agent (Chan-Yeung and Lam, 1986).

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- Allergy, with its high species and individual specificity, was until recently regarded more as an aberration of an individual than as a hazard of a relevant chemical. Even in relatively recent textbooks on toxicology, allergic asthma is not even mentioned under the *toxic* responses of the respiratory system, and receives minor attention in the discussion of immune system dysfunction (Dean *et al.*, 1989; Dean and Murray, 1991).
- In contrast to contact hypersensitivity, the other major form of occupational allergy associated with exposure to chemicals, no fully validated models are available to identify hazard of respiratory sensitization and allergy.

With emerging evidence for the ability of some chemicals to cause respiratory allergy in humans, there is now a need to develop methods which will permit their prospective identification. Such candidate methods should be validated with chemicals of known and different allergenic potential, and with nonsensitizing materials.

DEFINITION OF RESPIRATORY SENSITIZATION AND ALLERGY

Respiratory sensitization is an immune status whereas respiratory allergy is a clinical manifestation. Respiratory sensitization results from an immune response to antigen (usually, but not exclusively, exogenous antigen), which may result in clinical hypersensitivity upon subsequent inhalation exposure to the same or similar antigen. An allergic response characteristically requires at least two encounters with antigen. Following first exposure the susceptible individual mounts a primary immune response which results in sensitization (the induction or sensitization phase). If the sensitized individual subsequently comes into contact with the same antigen a clinical allergic reaction may be provoked (the elicitation phase). Allergic reactions may be attributable to either antibody or cell-mediated immune responses. Acute allergic reactions in the respiratory tract induced by exposure to exogenous antigens are almost invariably associated with specific antibody responses, frequently, but not always, of the IgE class.

Asthma can be defined as a lung disease characterized by airways obstruction (which in most patients is reversible, either spontaneously or after treatment), airways inflammation, and increased airway responsiveness to a variety of stimuli (Sheffer, 1991).

Occupational asthma is defined as a respiratory disease characterized by variable bronchial obstruction and hyperreactivity caused by specific agents inhaled at work (Maestrelli *et al.*, 1992). Rhinitis is a disease that involves inflammation of the nasal mucosal membrane which is characterized by periods of nasal discharge, sneezing, and congestion that persist for an average of 30–60 min/day (Mygind and Weeke, 1983).

Pseudoallergic reactions clinically indistinguishable from true allergic reactions and occupational asthma caused or elicited by nonallergic mechanisms are outside the scope of this paper.

MECHANISMS UNDERLYING RESPIRATORY SENSITIZATION AND ALLERGY

Introduction

Acute allergic reactions in the respiratory tract resulting from exposure to exogenous antigens are usually, but not exclusively, effected by specific antibody. For ease two major classes of pulmonary allergic reactions to external antigens can be distinguished:

Hypersensitivity pneumonitis results from inflammatory reactions caused by short- or long-term intermittent exposure to certain protein antigens. Examples include farmer's lung caused by inhalation of *Saccharopolyspora recivirgula* (*Micropolyspora faeni*) antigens and cheese worker's lung caused by *Penicillin roqueforti* antigens. The disease is associated characteristically with the presence of antigen-precipitating IgG antibody. Pathogenesis may, however, also involve the activation of complement and cell-mediated immunity.

Allergic asthma and rhinitis are most commonly immediate-onset reactions (within 1 hr and often within minutes of exposure) and result from the local release of inflammatory mediators. Such reactions are frequently, but not always, effected by IgE antibody. Asthmatic reactions may also be persistent or have a late onset and it is possible here that other types of immune processes may play a part (O'Byrne *et al.*, 1987; Corrigan and Kay, 1992).

Cellular and Molecular Mechanisms

The most important event during the induction phase of respiratory sensitivity is the generation of an immune response, and in particular production of antibodies which are able to bind to tissue mast cells. In the past such antibodies have been designated cytotropic. It was recognized that two types of cytotropic antibody could be distinguished: homocytotropic and heterocytotropic. As these names imply, the former were found to bind only to mast cells of the same, or similar, species, while the latter proved not to be species-specific. Heterocytotropic antibodies are of the IgG class, in most species representing a separate subclass or isotype of IgG. Thus, for instance, in man IgG4 antibodies bind to mast cells, whereas antibodies of subclasses IgG1, IgG2, and IgG3 do not. IgE antibodies are the most important with regard to sensitization because of their high affinity for mast cell receptors for the Fc region of the IgE molecule (FcϵR). If during an immune response antigen-specific IgE antibodies are produced they will then "prime" mast cells in various tissues through interaction with FcϵR

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(Brostoff and Hall, 1989). At this point the individual is sensitized and capable of mounting a hypersensitivity reaction following subsequent exposure to the same or similar substance. If the individual sensitized in this way encounters allergen in the skin then inflammation (edema and erythema) will result from the release by local mast cells of bioactive mediators (the basis of skin prick tests). Alternatively, if the allergen is inhaled and encountered in the respiratory tract, then inflammation will occur at this site, as described below.

The critical event during the sensitization process is the development of mast cell binding antibody, and in particular IgE. In recent years much has been learned about the cellular and molecular events which initiate and control IgE responses. A number of suppressor mechanisms have been described which apparently inhibit IgE antibody production. Many of these remain ill-defined and their relevance to the induction and regulation of IgE responses under physiological conditions is uncertain (Katz, 1980; Ishizaka, 1982; Sorg, 1989).

More recently it has become apparent that cytokines play an important role in the regulation of antibody responses and in determining which isotypes of antibody are produced (Coffman *et al.*, 1988). Of particular relevance to the induction of allergic disease is the fact that certain cytokines reciprocally regulate IgE antibody production.

Interleukin 4 (IL-4) and interferon-γ (IFN-γ) exert important influences on regulation of IgE antibody. In mice, the initiation and maintenance of IgE responses is dependent upon the availability of IL-4 (Finkelman *et al.*, 1988b). In contrast, IFN-γ inhibits IgE production in the mouse (Finkelman *et al.*, 1988a). In humans IL-4 and IFN-γ also have similar reciprocal effects on IgE antibody (Del Prete *et al.*, 1988; Pene *et al.*, 1988; Romagnani *et al.*, 1989).

Interestingly, there exists a functional heterogeneity among $CD4^+$ T helper (T_H) cells, the class of T lymphocytes required for B lymphocytes to respond productively to antigen and develop into antibody-producing plasma cells. Mosmann *et al.* (1986) described two main populations of T_H cells in mice, designated T_{H1} and T_{H2}, which differ with respect to the spectrum of cytokines they produce following activation. Although both populations secrete interleukin 3 (IL-3) and granulocyte/macrophage colony stimulating factor, only T_{H1} cells produce interleukin 2 (IL-2), tumor necrosis factor β (TNF-β, lymphotoxin), and IFN-γ, and only T_{H2} cells produce interleukins 4, 5, 6, and 10 (IL-4, IL-5, IL-6, and IL-10) (Mosmann and Coffman, 1989). There is no doubt, therefore, that the differential activation of functional subpopulations of T_H cells and the consequential selective production of regulatory cytokines will have a significant impact on IgE responses and allergic sensitization. The development of respiratory sensitization will be favored by T_{H2} cell activation.

Heterogeneity among T_H cells is not restricted to the mouse. Recently, a similar functional dichotomy of human T_H cells has been confirmed (Romagnani, 1991), and there is emerging evidence that immediate-onset allergic reactions in man are associated with the selective activation of T_{H2}-type cells (Parronchi *et al.*, 1991).

Therefore, the nature of T cell activation and the relative availability of specific cytokines, particularly IL-4 and IFN-γ, in the immunological microenvironment will likely be of critical importance in determining the initiation of IgE responses. Conditions which favor the activation of T_{H2} cells and IL-4 production will facilitate IgE antibody responses and the development of respiratory sensitization. Variables which might affect the balance between T_H subpopulation responses include the nature of the antigen, the route and duration of exposure, and genetic predisposition.

IgE antibody induced by exposure to respiratory allergens will prime mast cells which are found throughout vascularized tissue. When the same antigen is encountered for a second time, then antibody primed mast cells may be induced to degranulate. The antigen associates with mast cell-bound IgE, cross-links the antibody, and causes membrane perturbation and the release of various inflammatory mediators. These are many and varied and include (1) vasoactive amines (such as histamine) which induce increased vasopermeability and exudation of plasma and edema, and (2) cyclo- and lipoxygenase products of arachidonic acid metabolism, some of which induce the contraction of smooth muscle. When these events occur in the respiratory tract of sensitized individuals, clinical signs ranging from wheezing and rhinitis to frank asthmatic reactions are manifest.

There is growing interest in the possibility that cell-mediated immune processes may play a role in the pathogenesis of asthma (Corrigan and Kay, 1992). Chronic inflammation plays an important role in asthma and is associated with the accumulation of leukocytes in the bronchial mucosa, the production of mucus, the destruction and sloughing of airway cells, and subepithelial fibrosis secondary to collagen deposition. Central to the development of bronchial inflammation and injury are T lymphocytes acting in concert with eosinophils. Cell-mediated immune reactions may be important in the development of late-phase respiratory reactions to allergens and in the longer-term development of asthma.

Role of Airway Hyperresponsiveness

Asthma is characterized by airway hyperresponsiveness, a condition manifested by an exaggerated bronchoconstrictor response to many physical changes and chemical and pharmacological stimuli (Boushey *et al.*, 1980). Asthma patients develop clinical symptoms after exposure to allergens, environmental irritants, viral infections, cold air, or

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exercise. Airway hyperresponsiveness also appears to be important in the pathogenesis of asthma, and is ubiquitous in the disease (Bleecker, 1985). Several mechanisms have been proposed to explain airway hyperresponsiveness in asthma, including airway inflammation, abnormalities in bronchial epithelial integrity, alteration in autonomic neural control of airways, changes in intrinsic bronchial smooth muscle function, and baseline airflow obstruction (Sheffer, 1991).

HAZARD IDENTIFICATION

Introduction

The prospective identification of chemicals and proteins which have the potential to induce respiratory sensitization is still in its infancy. There is, as yet, no widely applied or fully validated method available.

Although certain classes of chemicals such as acid anhydrides and diisocyanates are commonly associated with occupational respiratory allergy, there is presently insufficient information available to predict respiratory sensitization potential from analysis of structure alone. Some physicochemical characteristics and biological properties appear important correlates of respiratory sensitization. Reactivity with proteins and lipid solubility are likely to be relevant and it is probable that most, if not all, chemical respiratory allergens also have the potential to cause contact sensitization in experimental animal models. Thus, for example, two anhydrides, which are well known human respiratory sensitizers (trimellitic and phthalic anhydrides), have been found to be positive in both the guinea pig maximization test and the local lymph node assay (Basketter and Scholes, 1992). Various other chemical respiratory allergens have been positive in one or more assays for contact sensitizers (Kimber *et al.*, 1994). It is clear, however, that very few skin allergens have been found to induce respiratory sensitization. Effective use of structure–activity relationships for the prospective identification of respiratory sensitizers will not become a reality until more is known of this disease and the activity of different chemical classes established.

Two main approaches to hazard identification exist: the use of animal models and *in vitro* methods. In the case of animal models both mice and guinea pigs have been used. Work in mice has focused upon events occurring during the induction phase of sensitization following primary encounter with the test chemical. In contrast, guinea pig methods seek mainly to identify respiratory allergens as a function of elicitation reactions induced in previously sensitized animals.

Animal Models

Mouse Model

There has been only a single systematic attempt to develop a method for the prospective identification of chemical respiratory allergens in mice. In this method events occurring during the induction phase, rather than during the elicitation phase, of respiratory sensitization are measured.

This method has as its theoretical basis the fact that chemical allergens of different classes induce qualitatively different immune responses in mice. Dearman and Kimber (1991) and Dearman *et al.* (1991, 1992a) performed experiments with trimellitic anhydride (TMA) and 2,4-dinitrochlorobenzene (DNCB). TMA is a well known human respiratory allergen and a comparatively weak contact allergen. In contrast, although it is a strong contact sensitizer, DNCB apparently lacks the potential for respiratory sensitization. Although both chemicals are immunogenic in mice, they induce qualitatively different immune responses characteristic of differential activation of functional subpopulations of T helper cells (T_{H1} and T_{H2} cells). TMA induces responses characteristic of T_{H2} cell activation, including both an increase in the serum concentration of IgE and the appearance of hapten-specific IgE antibodies. DNCB was found to stimulate selectively T_{H1}-type responses and failed to cause either an increase in serum IgE concentration or the production of IgE anti-hapten antibody (Dearman and Kimber, 1991). Subsequent studies have revealed that exposure of mice to other known human respiratory allergens such as diphenylmethane diisocyanate (MDI) and phthalic anhydride (PA) also results in T_{H2}-type responses and IgE production (Dearman and Kimber, 1992; Dearman *et al.*, 1992b). Conversely, other chemicals which, while contact allergens, are known or suspected not to possess respiratory sensitizing potential (4-ethoxymethylene-2-phenyloxazol-5-one, oxazolone; dicyclohexylmethane-4,4-diisocyanate, HMDI, and isophorone diisocyanate, IPDI) induced responses characteristic of selective T_{H1} cell activation (Dearman and Kimber, 1992; Dearman *et al.*, 1992b).

Among several qualitative immune variables recorded, a consistent finding was that only those chemicals which have the potential to cause respiratory allergy in man provoke a significant increase in the serum concentration of IgE in mice, a consequence presumably of the production by activated T_{H2} cells of IL-4, an inducer of IgE production. Conversely, contact allergens which are considered to lack the potential to induce respiratory allergy in humans fail to cause a similar increase in mouse serum IgE concentration.

On the basis of these data, a novel predictive test method, the Mouse IgE Test, has been described wherein respiratory sensitizing potential is measured as a function of increases in the serum concentration of IgE following topical exposure of BALB/c strain mice to immunogenic concentrations of the test chemical (Dearman *et al.*, 1992b). At present this test is being validated further with a wider range of chemicals.

Guinea Pig Models for Elicitation of Respiratory Hypersensitivity

Guinea pig models for measuring both immediate- and delayed-onset respiratory hypersensitivity reactions have

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been described. Guinea pigs can be sensitized in such a way as to display respiratory reactions similar to those seen in human allergic disease. The guinea pig model of respiratory hypersensitivity has been claimed to bear a close mechanistic similarity to human asthma (Drazen, 1977). In the case of irritant chemicals (e.g., certain isocyanates), guinea pigs have been reported to respond in a similar way as observed in man regarding respiratory tract irritation, antibody production, and pulmonary sensitization (Karol, 1988). Therefore, the results of studies using this species can be considered a suitable basis for hazard assessment in humans. Of course, in order to assess the specific risk, additional parameters such as the actual exposure concentration and the likelihood of exposure to high concentrations must also be taken into account. However, there may be some concern regarding the difference between humans and guinea pigs with respect to the classes of antibodies responsible for respiratory hypersensitivity, insofar as IgG antibodies are of greater importance than IgE antibodies in immediate allergic reactions in the guinea pig. A positive correlation between induction dose and IgG levels has been demonstrated frequently in guinea pigs, but a clear association between elevated IgG titers and immediate-onset reactions has not always been found (Karol, 1988; Sarlo and Clark, 1992; Ritz *et al.*, 1993).

For many years, efforts have been directed toward development of an animal model for inhalation sensitization where exposure to foreign materials occurs via the respiratory tract. The first animal model for respiratory hypersensitivity using inhalation exposure for sensitization and elicitation was developed using natural products, such as dander, molds, and spores (Ratner *et al.*, 1927; Ratner, 1939). Using inhalation exposure, sensitization with natural products has been achieved also in several other animal species (Karol, 1981). In the guinea pig characteristic respiratory reactions attributable to respiratory hypersensitivity have been described, i.e., the immediate-onset and delayed-onset respiratory response, as well as the hyperreactive airway response due to respiratory tract irritation (Karol and Thorne, 1988). The pharmacological aspects of the immediate-type hypersensitivity in commonly used laboratory animals have been reviewed (Ahlstedt *et al.*, 1983; Fügner, 1985; Wanner and Abraham, 1982). The guinea pig models represent useful tools to study some pathophysiological aspects of respiratory hypersensitivity, as long as the limitations of the model are recognized.

Different guinea pig models were used to evaluate the relationship between exposure and elicitation of immediate- and delayed-onset responses of respiratory hypersensitivity to inhaled industrial chemicals (haptens), protein-conjugates of haptens, or naturally occurring proteins. Pharmacological stimuli (e.g., aerosolized histamine) have also been used to substantiate the findings observed during the hapten challenge.

As summarized in Table 1, sensitization to low-molecular-weight chemicals has been achieved via single or repeated inhalation exposures using the free chemical or the protein conjugate of the hapten. Sensitization by inhalation was a concentration-dependent rather than a dose (concentration × time)-dependent phenomenon (Karol, 1983; Pauluhn and Eben, 1991). Experimental evidence suggests that bronchial hyperreactivity may also be related to airway mucosal injury and concomitant-inflammatory reactions as a result of exposures to irritant concentrations of chemicals used for sensitization (Cibulas *et al.*, 1986).

The data summarized in Tables 1 and 2 have been selected to display the range of protocols which have been used to investigate allergic hypersensitivity. It must be emphasized that such lists are not comprehensive and do not necessarily include unsuccessful attempts to induce or elicit respiratory reactions.

As summarized in Table 2, sensitization to low-molecular-weight chemicals has been achieved via single or repeated intradermal or subcutaneous injections using the free chemical. Attempts have been made to standardize this animal model by using subcutaneous or intradermal routes of induction as the primary encounter with the test chemical (Botham *et al.*, 1989; Pauluhn and Eben, 1991; Sarlo and Clark, 1992). Induction by the dermal route minimizes the risk of nonspecific bronchial hyperreactivity induced during the sensitization phase, which complicates the interpretation of elicitation-induced alterations.

The common features of the guinea pig models described in Tables 1 and 2 are the measurement of elicitation reactions during or following inhalation challenge in an attempt, in most instances, to simulate conditions of human inhalation exposure and the ensuing respiratory hypersensitivity response. Through the use of these models, the importance of the exposure concentration in the elicitation of respiratory hypersensitivity has become apparent.

A cardinal feature of the asthmatic state is the presence of airway hyperreactivity recognized as responses to lower than normal amounts of inhaled histamine (Cibulas *et al.*, 1986; Griffiths-Johnson and Karol, 1991). However, respiratory effects caused by nonspecific irritation and respiratory effects resulting from challenge exposures to free chemical are physiologically indistinguishable. In the majority of experiments summarized in Tables 1 and 2, the onset of respiratory hypersensitivity response was "immediate" with reactions occurring either during or shortly after the challenge period. Although animals were monitored continuously up to 24 hr after challenge, only in very rare instances were delayed-onset or dual responses reported.

Parameters Monitored for the Detection of Respiratory Hypersensitivity

Breathing parameters. Breathing parameters that are useful in the quantitative evaluation of respiratory hyper-

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TABLE 1
Protocols Employed for Sensitization and Elicitation of Respiratory Hypersensitivity of Industrial Chemicals or Protein Conjugates of Industrial Chemicals in Guinea Pigs—Sensitization by Inhalation

Substance/exposure	Elicitation/challenge	Reference
2-Isocyanatoethyl methylacrylate-conjugate Exposure: 10 min/day, 5×/week, 23 days, except Day 15–19	Conjugate; immediate-onset responses after approx. 2 weeks	Mullin *et al.* (1983)
2-Isocyanatoethyl methacrylate-conjugate Exposure: 10 min/day 5×/week, 23 days, except Day 15–19	Hapten: delayed-onset responses (Day 12 and 23); conjugate: immediate-onset responses (Day 12–23)	Mullin *et al.* (1983)
p-Tolylisocyanate (TMI)-, hexylisocyanate (HMI)-conjugates Exposure: 10 min/day, 5×/week, up to 15 days	Conjugate: immediate-onset responses on Day 9 or 15, respectively	DeCeaurriz *et al.* (1987)
Hexylisocyanate (HMI)-conjugate Exposure: 10 min/day, 5×/week, up to 18 days	Conjugate: immediate-onset responses on Day 10 and thereafter	Karol *et al.* (1979)
Hexamethylenediisocyanate-trimer (HDI-trimer) Exposure: 3 hr/day, 5×/week, 5 days	Hapten (Week 3), conjugate (Week 4): neither immediate- nor delayed-onset responses	Pauluhn and Eben (1991)
Toluene diisocyanate (TDI) Exposure: 3 hr/day, 5×/week, 5 days or up to 70 days	Conjugate: immediate-onset responses	Karol (1983)
Toluene diisocyanate (TDI) Exposure: 3 hr/day, 5×/week, 5 days	Conjugate: immediate-onset response with the "Karol-conjugate" but no response with the "Botham-conjugate"	Botham *et al.* (1988)
Toluene diisocyanate (TDI) Exposure: 4 hr/day, 5 consecutive days	Increased doses of aerosolized histamine: positive immediate-onset response	Cibulas *et al.* (1986)
Toluene diisocyanate (TDI) Exposure: 3 hr/day, 5 consecutive days	Hapten (for 30 min, Days 15–22): no immediate-onset response	Karol (1980)
Toluene diisocyanate (TDI) Exposure: 3 hr/day, 5 consecutive days	Conjugate (Week 4): immediate-onset response	Sarlo and Clark (1992)
Trimellitic anhydride (TMA), dichlorotriazine reactive dye Exposure: 3 hr/day, 5 days	Conjugate: no immediate-onset response	Botham *et al.* (1988)
Diphenylmethane 4,4'diisocyanate (MDI) Exposure: 3 hr/day, up to 3 or 5 consecutive exposures	Hapten (Day 17, 24): delayed-onset responses, conjugate (Day 30): immediate- and delayed-onset responses	Karol and Thorne (1988)
p-Tolyl isocyanate (TMI) Exposure: 5 consecutive days: (I) Alum-aerosol (30 min), followed by different TMI exposures; (II) Same as (I) but without alum preexposure	Hapten: no immediate-onset response: conjugate: positive response. Responses in alum pretreated and non-pretreated animals did not differ	Karol (1980)
Phthalic anhydride (PA) Exposure: 3 hr/day, 5 consecutive days	Conjugate (Week 4): immediate-onset response	Sarlo and Clark (1992)
Azodicarbonamide Exposure: 6 hr/day, 5 days/week for 4 weeks	Hapten and histamine challenge (Week 4): neither challenge resulted in specific reactions	Gerlach *et al.* (1989)

sensitivity responses in animal models are breathing frequency, flow-volume loops, respiratory minute volume, inspiratory and expiratory times, peak expiratory flow rates, tidal volume, or plethysmographic pressure (Thorne and Karol, 1988; Pauluhn and Eben, 1991; Pauluhn and Mohr, 1994). The analysis of inspiratory and expiratory times as well as the presence of characteristic breathing patterns appears to be useful to distinguish between effects caused by irritation and hypersensitivity. However, these breathing parameters are all indirect indicators of bronchoconstriction and, in some cases, may occur in the absence of bronchoconstriction. This tradeoff is necessary in order to utilize unanesthetized animals. Two-chambered plethysmographic systems used for measuring common respiration parameters as well as specific airway conductance in noncannulated guinea pigs have also been used (Gerlach *et al.*, 1989).

The nature of changes in breathing frequency necessitates careful interpretation. It is widely recognized that chemicals may cause a decrease in breathing frequency by

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TABLE 2
Protocols Employed for Sensitization and Elicitation of Respiratory Hypersensitivity and Industrial Chemicals or Protein Conjugates of Industrial Chemicals in Guinea Pig—Sensitization by Intradermal/Subcutaneous Injection

Substance/exposure	Elicitation/challenge	Reference
Trimellitic anhydride (TMA), intradermal induction: 6× during 5 days	Hapten (Week 3): immediate-onset response, conjugate challenge (Week 4): immediate-onset response	Pauluhn and Eben (1991)
Trimellitic anhydride (TMA), single intradermal induction	Hapten and conjugate: immediate-onset response after both challenges	Botham *et al.* (1989)
Hexamethylene diisocyanate-trimer (HDI-timer), intradermal induction: 6× during 5 days	Hapten (Week 3), conjugate (Week 4): neither immediate-nor delayed-onset responses	Pauluhn and Eben (1991)
Toluene diisocyanate (TDI), subcutaneous induction: 2×/week for 4 consecutive weeks; Week 6: booster injection	Conjugate (Week 8, intratracheally): immediate-onset responses	Sarlo and Clark (1992)
Phthalic anhydride (PA) subcutaneous induction: 2×/week for 4 consecutive weeks; Week 6: booster injection	Conjugate (Week 8, intratracheally): immediate-onset responses	Sarlo and Clark (1992)

sensory irritation (e.g., TDI, TMI) or an increase in breathing frequency as a consequence of pulmonary irritation and stimulation of nerve receptors (e.g., MDI) (Karol, 1991). In guinea pigs, high concentrations of respiratory tract irritants can cause bronchoconstriction. To detect a characteristic immediate-onset response attributable to respiratory allergic hypersensitivity, the irritation response must be avoided by using subirritant concentrations of the free chemical, and by careful comparison of sensitized animals with control animals. In contrast, several studies have reported that challenge exposures using haptens require slightly irritant concentrations in order to elicit pulmonary responses (Tao *et al.*, 1991; Obta *et al.*, 1992; Pauluhn and Mohr, 1994). The confounding effect of respiratory irritation may be overcome when several breathing parameters are evaluated and when other parameters, such as acetylcholine provocation and eosinophil influx, are also measured. Eosinophil infiltration is a prominent feature of asthma and differentiates asthma from other inflammatory conditions of the airways (Dunn *et al.*, 1988; Gulbenkian *et al.*, 1990). Finally, experimental evidence suggests that the presence or absence of a characteristic immediate-onset breathing pattern not spontaneously occurring in control animals is of greater diagnostic validity than the evaluation of changes in respiratory rate alone (Pauluhn and Eben, 1991).

Antibodies. The induction of respiratory hypersensitivity is dependent on the induction of specific homocytotropic antibodies. Measurement of antibody, rather than pulmonary responses, has two potential advantages. First, from a practical point of view, serological measurements may provide evidence of respiratory sensitization potential without the need for inhalation challenge. Second, antibody responses may be more sensitive than respiratory reactions. That is, chemicals and proteins may induce the production of relevant antibodies at exposure concentrations below those which are required for measurable respiratory reactions following inhalation challenge.

Advantages and Disadvantages of the Mouse IgE Test and Guinea Pig Methods for Identification of Respiratory Allergens

Mouse IgE test. The advantages of the mouse IgE test are that:

- it uses fewer animals and does not depend upon the development of an adverse reaction;
- it is relatively rapid, cost-effective, and comparatively easy to perform;
- it is quantitative and relevant in that it is based upon measurement of IgE, the class of antibody known to effect immediate hypersensitivity reactions in humans;
- dose–response relationships at the induction phase can be measured. The relative antibody producing potential of chemicals can be determined and no-observable-effect levels (NOEL) for IgE antibodies established.

Disadvantages of the mouse IgE test are that:

- it is not at present suitable for measuring the respiratory sensitization potential of proteins;
- it cannot be used for evaluation of cross-reactivity between chemical respiratory allergens;
- it has not yet been formally validated.

Guinea pig methods. The advantages of the guinea pig methods described above are that:

- respiratory hypersensitivity is measured as a function of elicitation reactions during or following inhalation challenge exposure, the relevant route for the elicitation of pulmonary reactions in people;

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- elicitation reactions can be measured using a variety of breathing parameters;
- induction can be performed using a variety of exposure routes;
- dose/concentration response studies can be performed at both the induction and elicitation stages and no-observable-effect-levels determined;
- cross-reactivity between respiratory allergens can be investigated;
- the respiratory sensitization potential of proteins can be measured.

The disadvantages of guinea pig methods are that:

- they are time consuming and costly;
- they depend upon a number of test specific factors, such as specific antibody measurements and the generation of the challenge atmosphere;
- they require specialized personnel and inhalation laboratory facilities;
- effective elicitation of sensitization to chemicals by inhalation exposure may require the use of a hapten–protein conjugate;
- standardization of hapten–protein conjugates is difficult to achieve (A dependence of immediate-onset respiratory hypersensitivity response on the quality of the protein conjugate has been reported (Botham *et al.*, 1988). In addition, selection of the most appropriate subirritating inhalation challenge concentrations of free chemical is difficult and measurements of respiratory hypersensitivity may be confounded by respiratory irritancy.);
- previous encounters by inhalation exposure to the hapten may lower the threshold for nonspecific (irritation-induced) effects;
- there is no well-defined IgE antibody in guinea pigs, and standardized reagents for detailed analysis of serological responses are only now becoming available;
- they have not been formally validated.

In Vitro Methods

There are no standard validated *in vitro* methods for determining the potential of a chemical to cause respiratory sensitization. However, the potential of a low-molecular-weight chemical to interact with protein can be considered a prerequisite for allergenicity, since low-molecular-weight compounds are incomplete antigens (haptens) which require association with protein carriers to become immunogenic. Thus, attempts have been made to predict the immunogenicity of a chemical from its protein or peptide binding properties. A number of published studies evaluated the interaction between protein and chemicals known to cause occupational asthma, such as trimellitic anhydride, chloramine-T, β-lactam antibiotics, and isocyanates (Patterson *et al.*, 1978; Evans *et al.*, 1986; Edwards *et al.*, 1988; Jin and Karol, 1988). Using high-pressure liquid chromatography, Wass and Belin (1990) demonstrated that isocyanates, anhydrides, and chloramine-T reacted with a lysine-containing peptide, whereas simple acids, bases, and solvents did not. The ability of a low-molecular-weight chemical to bind to protein or peptides may therefore be useful as a prescreen for detecting potential respiratory sensitisers, especially for new chemicals or complex mixtures with unknown properties (Sarlo and Clark, 1992).

However, binding studies *in vitro* are not models for the induction of an immunologic response, a complex physiologic event involving the interaction of many cell types and cytokines. No *in vitro* model of respiratory sensitization exists which results in the production of antigen-specific homocytotropic antibodies capable of binding to tissue mast cells. An *in vitro* model would also need to address the problems of solubility, metabolism, and direct toxicity of potential respiratory sensitizers.

There are also no *in vitro* correlates to the immediate-onset reaction (elicitation phase of the immune response) typical of many respiratory sensitizers, since these assays would require a population of cells obtained from animals following prior exposure(s) to a potential sensitizing chemical. The problems associated with the site and availability of such cells, the timing of collection, and the solubility, composition, and inherent toxicity of the antigen have made these studies difficult to design and perform. Finally, the endpoints measurable *in vitro* which would correlate with endpoints observed during both immediate- and delayed-onset respiratory allergic reactions *in vivo* are not completely known, given the current state of knowledge of the causative factors in each of these reactions.

In conclusion, the use of *in vitro* assays to model the induction and elicitation phases of the immune responses in respiratory allergy rests in the future. As the mechanisms involved in respiratory sensitization became clearer, *in vitro* assays useful for predicting potential chemical sensitizers will no doubt be utilized.

Structure Activity Relationships (SAR)

The ability to predict the *in vivo* activity of a chemical based upon analysis of its structure has recently been examined in the area of respiratory sensitization. Agius *et al.* (1991) generated a structure–activity hypothesis from investigations comparing the structure and reactivity of substances known to cause occupational asthma with that of other related chemicals which were known not to cause occupational asthma. The ability of molecules to form multiple covalent, coordination, and hydrogen bonds was identified as an important predictor of occupational asthma. This hypotheses now needs to be tested and revised. A better understanding is also needed in the area of chemical–protein interactions, since the immunological response in-

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volved in respiratory sensitization may well be directed against chemical-induced conformational changes in carrier proteins such as albumin. Kochman *et al.* (1990), using Fourier transform infrared spectroscopy of guinea pig serum albumin following repeated inhalation of the known respiratory sensitizer TDI, demonstrated major alterations in the TDI alpha-helix content of albumin compared to control protein. These new conformational determinants may play an important role in the development of respiratory sensitization.

Recommendations for Identification of Respiratory Sensitization Hazard of Chemicals

It must be emphasized that there are as yet no fully validated or widely accepted methods for the prospective evaluation of respiratory sensitizing activity. However, based upon the evidence available we suggest the following process for hazard identification of chemical respiratory sensitization which is summarized in Fig. 1. It should be noted also that this process is unsuitable for assessment of proteins.

Preliminary Considerations

Necessarily the first step in any hazard identification process should include an examination of the physicochemical properties of the test material, particularly in the context of structure-activity relationships (Sarlo and Clark, 1992). With regard to identification of respiratory sensitization hazard, important considerations include the volatility of the chemical and the likelihood that aerosols or vapors will be present during manufacture or use. Certain classes of chemical (anhydrides, reactive dyes, and isocyanates, for instance) may signal particular concern.

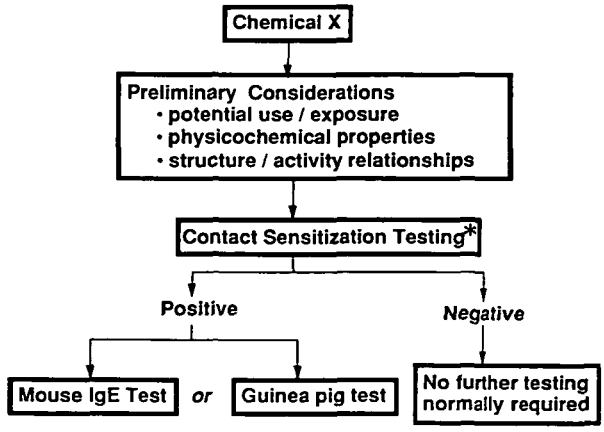

FIG. 1. Testing strategy for chemicals (not for proteins). *Respiratory sensitization testing may be necessary if it has been possible to assess contact sensitization potential only at low concentrations or if there is reason to suspect that physicochemical properties preclude skin penetration.

Skin Sensitization

In the case of chemicals it appears that only those materials which exhibit at least some potential to cause contact sensitization in experimental animals are able to induce respiratory allergy. We recommend therefore that an important step in the hazard identification process is the performance of a well-conducted standard predictive testing for skin sensitization activity (guinea pig maximization test, local lymph node assay, etc). Chemicals which lack the potential to cause contact sensitization can be classified also as lacking the ability to induce respiratory allergy and it is our recommendation that no further testing will usually be necessary. If, however, it has been possible to assess skin sensitization potential only at low concentrations, then respiratory sensitization testing may still be necessary. Additionally, if there is reason to suspect that physicochemical properties preclude skin penetration, then respiratory sensitization testing may also be necessary.

Respiratory Sensitization

If identification of the respiratory sensitization hazard of chemicals is then required there exist two options, neither of which is fully validated.

Mouse test. This test should be performed to the methods described in the relevant publications. It is our recommendation that each chemical is tested at at least three concentrations which can be selected on the basis of skin sensitization test data.

Guinea pig tests. It is our recommendation that protocols requiring inhalation sensitization or challenge with hapten–protein conjugates be avoided. The approach we favor is the use of intradermal or subcutaneous administration for sensitization. Challenge should be via the respiratory tract (inhalation exposure to aerosols is appropriate, and intratracheal administration has also been used successfully; in contrast, in most instances, elicitation of reactions by inhalation challenge of sensitized animals to irritant vapors has been unsuccessful and this approach may therefore be inappropriate.). Challenge-induced reactions should be measured as a function of changes in respiratory volume and rate and breathing pattern. We recommend also that test and control animals be examined for the presence of anti-hapten antibody. There is still no consensus regarding many detailed aspects of procedure and protocol and it is not possible presently to give clear recommendations with respect to a number of issues (e.g., length of time between sensitization and challenge, nature of conjugates, and which parameters to measure).

RISK ASSESSMENT IN RESPIRATORY ALLERGY

In all forms of chemical and protein induced allergic hypersensitivity there are two types of risk assessment.

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- The risk that exposure to a material will induce sensitization in an individual.
- The risk that exposure to a material will elicit allergic reactions in a previously sensitized individual.

In most, if not all, instances the dose of chemical required for initial sensitization will be different from and higher than that necessary to elicit respiratory hypersensitivity reactions in a previously sensitized individual.

One part of the risk assessment process relies upon evaluation of the potency of the material. In theory, the relative potential of a chemical to cause respiratory sensitization can be evaluated by the methods described previously. In the mouse IgE test the preferred approach would be to identify the minimum concentration of chemical necessary to cause a significant increase in the serum concentration of IgE. In the case of guinea pig tests the determination of the relative potential would require estimation of the amount of material capable of inducing a given level of sensitization as judged by either the stimulation of antibody production or the subsequent elicitation of respiratory reactions following inhalation exposure or intratracheal administration. Alternatively, an estimation of the NOEL for induction and elicitation could be made.

In practice such measurements would be of value only in terms of *relative* potency, that is, relative to the activity of a known chemical of a similar class for which occupational health data were available. For example, it might prove possible to evaluate whether a new chemical is more or less likely than an index chemical of related structure to cause occupational respiratory sensitization under similar conditions of exposure. If there exists an occupational exposure level for the index chemical, then such comparisons should provide one approach to determining safe working conditions for the new material.

Evaluation of the ability of a chemical to cause respiratory allergic reactions in a previously sensitized animal could be approached only by the use of guinea pig models which employ inhalation challenge. For human beings already sensitized to a given material, there is no consensus on the possibility of identifying a dose/concentration below which adverse effects are unlikely to occur.

The other aspect of risk assessment requires consideration of factors other than inherent potency. For respiratory sensitization and the elicitation of respiratory reactions in previously sensitized individuals this includes:

- extent, frequency, duration, and route of exposure (peak exposures may be particularly important);
- concomitant exposure (especially with potential adjuvants or irritants);
- genetic or acquired differences in susceptibility to sensitization via the skin (for chemicals) or the respiratory tract (for chemicals and proteins).

CONCLUSIONS

A number of chemicals and proteins of industrial importance are known to cause respiratory allergy. There are still several gaps in our understanding of the sequence of events through which chemical agents evoke an immune response leading to specific allergic clinical manifestations. Some kinds of exogenous chemical exposure appear more likely to evoke immune reaction than others. Likewise, some chemicals affecting the human immune system appear to manifest themselves preferentially in one immune response rather than another. For proteins, which are complete antigens, the main concern is in establishing the relative allergenic potential. For low-molecular-weight chemicals acting as a hapten, we need to know which chemical properties are important for its binding to the autologous carrier protein, and which chemical properties are responsible for the hapten–carrier complex behaving immunogenically.

The skin and the respiratory tract are the most important organs exposed to the environment and which can be affected by immune responses to chemical agents. Evidence is emerging that the skin also may be an important route for sensitization for respiratory allergy to chemicals (Nemery and Lenaerts, 1993). The relevant route of exposure for sensitization to protein respiratory allergens is almost invariably by inhalation.

It is also important that the large body of data pertaining to human disease is not ignored, even if it generally lacks an assessment of exposure and estimation of risk. Attempts have been made to derive structure activity relationships for a wide range of chemical agents which may cause allergic asthma (Agius *et al.*, 1991). Given the possible serious health manifestations of respiratory allergy, early identification of possible sensitizers is urgently required. In recent years significant advances have been made in our understanding of the mechanisms underlying respiratory allergy, although less progress has been made in understanding the factors predisposing to it. However, the overall increase in understanding has resulted in the development of new but as yet not fully validated predictive methods for the identification of chemicals and proteins that have the potential to cause respiratory sensitization. A multidisciplinary approach is essential to understand the ways in which chemicals interact with the immune system to produce allergies and similar manifestations (ECETOC, 1987). In addition to the use of animal models, more emphasis needs to be placed upon clinical and epidemiological studies, especially of workers, with emphasis on better qualitative and quantitative measures of exposure to specific chemicals in the relevant environment (Agius, 1992).

RECOMMENDATIONS

- For chemicals, the two methods which are the most promising with respect to predictive testing and which

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should, as a priority, be developed further, are the mouse IgE test and the guinea pig model with induction by intra- and/or subcutaneous injection followed by inhalation challenge with the free chemical. We recommend that for the identification of proteins with respiratory allergenic potential, a guinea pig model should be used.

- Further validation of the methods will be facilitated by the production of a detailed protocol. Occupational hygiene aspects and epidemiology will need to be included in the validation process.
- The further development of tiered approaches for hazard identification should be encouraged, which should include the development of SAR's.
- Since in theory both skin and respiratory sensitizing chemicals can induce sensitization through skin contact, one method of reducing sensitization from respiratory sensitizers is to control skin contact, which should be taken into account in setting occupational hygiene standards for these chemicals.

REFERENCES

Agius, R. M. (1992). Environmental chemicals and differential stimulation of immune response. *Clin. Exp Allergy* **22,** 183–185.

Agius, R. M., Nee, J., McGovern, B., and Robertson, A. (1991). Structural activity hypotheses in occupational asthma caused by low molecular weight substances. *Ann. Occup. Hyg* **35**(2), 129–137.

Ahlstedt, S., Smedegaerd, G., Nygren, H., and Bjoerksten, B. (1983). Immune responses in rats sensitized with aerosolized antigen. *Int. Archs. Allergy Appl. Immun.* **72,** 71–78.

Bardana, E. J., and Andrach, R. H. (1983). Occupational asthma due to low molecular weight agents used in the plastic and resin industry. *Eur. J. Respir. Dis.* **64,** 241–251.

Basketter, D. A., Scholes, E. W., Kimber, I., Botham, P. A., Hilton, J., Miller, K., Robbins, M. C., Harrison, P. T. C., and Waite, S. J. (1991). Interlaboratory evaluation of the local lymph node assay with 25 chemicals and comparison with guinea pig test data. *Toxicol. Methods* **1,** 30–43.

Basketter, D. A., and Scholes, E. W. (1992). Comparison of the local lymph node assay with the guinea-pig maximization test for the detection of a range of contact allergens. *Food Chem. Toxicol.* **30,** 65–69.

Bleecker, E. R. (1985). Airways reactivity and asthma: Significance and treatment. *J. Allergy Clin. Immunol.* **75,** 21–24.

Botham, P. A., Hext, P. M., Rattray, N. J., Walsh, S. T., and Woodcock, D. R. (1988). Sensitization of guinea pigs by inhalation exposure to low molecular weight chemicals. *Toxicol. Lett.* **41,** 159–173.

Botham, P. A., Rattray, N. J., Woodcock, D. R., Walsh, S. T., and Hext, P. M. (1989). The induction of respiratory allergy in guinea pigs following intradermal injection of trimellitic anhydride: A comparison with the response to 2,4-dinitrochlorobenzene. *Toxicol. Lett.* **47,** 25–39.

Boushey, H. A., Holtzman, M. J., Sheller, J. R., and Nadel, J. A. (1980). Bronchial hyperreactivity. *Am. Rev. Respir. Dis.* **121,** 389–413.

Brostoff, J., and Hall, T. (1989). Hypersensitivity—Type 1. In *Immunology,* 2nd ed. (I. M. Roit, J. Brostoff, and D. K. Malo, Eds.), Chap. 19. Gowen Medical Publishing, London.

Butcher, B. T., Bernstein, I. L., and Schwartz, H. J. (1989). Guidelines for the clinical evaluation of occupational asthma due to small molecular weight chemicals. *J. Allergy Clin. Immunol.* **84,** 834–838.

Chan-Yeung, M., Barton, G. M., MacLean, L., and Grzybowski, S. (1973). Occupational asthma and rhinitis due to western red cedar (Thuja plicata). *Am. Rev. Respir. Dis* **108,** 1094–1102.

Chan-Yeung, M., and Lam, S. (1986). Occupational asthma. *Am. Rev. Respir. Dis.* **133,** 686–703.

Cibulas, W., Murlas, C. G., Miller, M. L., Vinegar, A., Schmidt, D. J., McKay, R. T., Bernstein, L., and Brooks, S. M. (1986). Toluene diisocyanate-induced airway hypersensitivity and pathology in the guinea pig. *J. Allergy Clin. Immunol.* **77,** 828–834.

Coffman, R. L., Seymour, B. W. P., Lebman, D. A., Hiraki, D. D., Christiansen, J. A., Schrader, B., Cherwinski, H. M., Savelkoul, H. F. J., Finkelman, F. D., Bond, M. W., and Mosmann, T. R. (1988). The role of helper T cell products in mouse B cell differentiation and isotype regulation. *Immunol. Rev.* **102,** 5–28.

Corrigan, C. J., and Kay, A. B. (1992). T cells and eosinophils in the pathogenesis of asthma. *Immunol. Today* **13,** 501–507.

Cullen, M. R., Cherniak, M. G., and Rosenstock, L. (1990). Occupational medicine (Part 1). *N. Engl. J. Med.* **322,** 594–601.

De Ceaurriz, J., Ducos, P., Micillino, J.-C., Gaudin, R., and Cavelier, C. (1987). Guinea pig pulmonary response to sensitization by five preformed monoisocyanate-ovalbumin conjugates. *Toxicology* **43,** 93–101.

Dean, J. H., Cornacoff, J. B., Rosenthal, G. J., and Luster, M. I. (1989). Immune system: Evaluation of injury. In *Principles and Methods of Toxicology,* 2nd ed. (A. W. Hayes, Ed.), pp. 741–760. Raven Press, New York.

Dean, J. H., and Murray, M. T. (1991). Toxic responses of the immune system. In *Casarett and Doull's Toxicology: The Basic Science of Poisons,* 4th ed. (M. O. Amdur, J. Doull, and C. D. Klaassen, Eds.), pp. 282–333. Pergamon Press, New York.

Dearman, R. J., and Kimber, I. (1991). Differential stimulation of immune function by respiratory and contact chemical allergens. *Immunology* **72,** 563–570.

Dearman, R. J., and Kimber, I. (1992). Divergent immune responses to respiratory and contact chemical allergens: Antibody elicited by phthalic anhydride and oxazolone. *Clin. Exp. Allergy* **22,** 241–250.

Dearman, R. J., Hegarty, J. M., and Kimber, I. (1991). Inhalation exposure of mice to trimellitic anhydride induces both IgG and IgE anti-hapten antibody. *Int. Arch. Allergy Immunol.* **95,** 70–76.

Dearman, R. J., Mitchell, J. A., Basketter, D. A., and Kimber, I. (1992a). Differential ability of occupational chemical contact and respiratory allergens to cause immediate and delayed dermal hypersensitivity reactions in mice. *Int. Arch. Allergy Immunol.* **97,** 315–321.

Dearman, R. J., Spence, L. M., and Kimber, I. (1992b). Characterization of murine immune responses to allergenic diisocyanates. *Toxicol. Appl. Pharmacol* **112,** 190–197.

Dearman, R. J., Basketter, D. A., and Kimber, I. (1992c). Variable effects of chemical allergens on serum IgE concentration in mice. Preliminary evaluation of a novel approach to the identification of respiratory sensitizers. *J. Appl. Toxicol.* **12,** 317–323.

Del Prete, G. F., Maggi, E., Parronchi, P., Chretien, I., Tiri, A., Macchia, D., Ricci, M., Banchereau, F., De Vries, J., and Romagnani, S. (1988). IL-4 is an essential factor for the IgE synthesis induced *in vitro* by T cell clones and their supernatants. *J. Immunol.* **140,** 4193–4198.

Diem, J. E., Jones, R. N., Hendrick, D. J., Glindmeyer, H. W., Dharmarajan, V., Butcher, B. T., Salvaggio, J. E., and Weill, H. (1982). Five year longitudinal study of workers employed in a new toluene diiocyanate manufacturing plant. *Am. Rev. Respir. Dis.* **126,** 420–428.

Drazen, J. M. (1977). In *Asthma: Physiology, Immunopharmacology, and Treatment* (L. M. Lichtenstein and K. F. Austen, Eds.), Chap. 16. Academic Press, New York.

Dunn, C. J., Elliott, G. A., Oostveen, J. A., and Richards, I. M. (1988).

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Development of a prolonged eosinophil-rich inflammatory leukocyte infiltration in the guinea-pig asthmatic response to ovalbumin inhalation. *Am. Rev. Respir. Dis.* **137,** 541–547.

ECETOC (1987). Identification of immunotoxic effects of chemicals and assessment of their relevance to man. Monograph No. 10.

Edwards, R. G., Dewdney, J. M., Dobryanski, R. J., and Lee, D. (1988). Immunogenicity and allergenicity studies on two β-lactam structures, a clavam, clavulanic acid and a carbapenem: Structure activity relationships. *Int. Archs. Allergy Appl. Immun.* **85,** 184–189.

Evans, J. C., Jackson, S. K., Rowlands, C. C. and Barratt, M. D. (1986). Covalent binding of human serum albumin and ovalbumin by chloramine-T and chemical modification of the proteins. *Anal. Chim. Acta* **186,** 319–323.

Finkelman, F. D., Katona, I. M., Mosmann, T. R., and Coffman, R. L. (1988a) IFN-γ regulates the isotypes of Ig secreted during *in vivo* humoral immune responses. *J. Immunol.* **140,** 1022–1027.

Finkelman, F. D., Katona, I. M., Urgan, J. F., Holmes, J., Ohara, J., Tung, A. S., Sample, J. G., and Paul, W. E. (1988b). IL-4 is required to generate and sustain *in vivo* IgE responses. *J. Immunol.* **141,** 2335–2341.

Fügner, A. (1985). Pharmacological aspects of immediate hypersensitivity reaction *in vivo*. In *Pulmonary and Antiallergic Drugs* (J. P. Devlin, Ed.), pp. 123–190. Wiley, New York.

Gerlach, R. F., Medinsky, M. A., Hobbs, C. H., Bice, D. E., Bechtold, W. E., Cheng, Y.-S., Gillet, N. A., Birnbaum, L. S., and Mauderly, J. L. (1989). Effect of four-week repeated inhalation exposure to unconjugated azodicarbonamide on specific and non-specific airway sensitivity of the guinea pig. *J. Appl. Toxicol.* **9,** 145–153.

Gilson, J. P., Juniper, C. P., Martin, R. B., and Weill, H. (1976). Biological effects of proteolytic enzyme detergents. *Thorax* **31,** 621.

Griffiths-Johnson, D. A., and Karol, M. H. (1991). Validation of a non-invasive technique to assess development of airway hyperreactivity in an animal model of immunologic pulmonary hypersensitivity. *Toxicology* **65,** 283–294.

Gulbenkian, A. R., Fernandez, X. Kreutner, W., Minnicozzi, M., Watnick, A. S., Kung, T., and Egan, R. W. (1990). Anaphylactic challenge causes eosinophil accumulation in bronchoalveolar lavage fluid of guinea pigs. *Am. Rev. Respir. Dis.* **142,** 680–685.

Hunskaar, S., and Fosse, R. T. (1993). Allergy to laboratory mice and rats: A review of its prevention, management, and treatment. *Lab. Animals* **27,** 206–221.

Ishizaka, K. (1982). Regulation of the IgE antibody response. In *Progress in Allergy,* Vol. 32. Karger, Basel.

Jin, R., and Karol, M. (1988). Intra- and intermolecular reactions of 4,4'-diisocyanatodiphenylmethane with human serum albumin. *Chem. Res. Toxicol.* **1,** 281–287.

Karol, M. H. (1980). Study of guinea pig and human antibodies to toluene diisocyanate. *Am. Rev. Respir. Dis.* **122,** 965–970.

Karol, M. H. (1981). Immunologic response of the respiratory system to industrial chemicals. In *Proceedings of the Inhalation Toxicology and Technology* (B. K. S. Leong, Ed.), pp. 233–246. Ann Arbor Science Publications, Ann Arbor, MI.

Karol, M. H. (1983). Concentration-dependent immunologic response to toluene di-isocyanate (TDI) following inhalation exposure. *Toxicol. Appl. Pharmacol.* **68,** 229–241.

Karol, M. H. (1988). The development of an animal model for TDI asthma. *Bull. Eur. Physiopathol. Respir.* **23,** 571.

Karol, M. H. (1989). Immunologic responses of the lung to inhaled toxicants. In *Concepts in Inhalation Toxicology* (R. O. McClellan and R. F. Henderson, Eds.), pp. 403–413. Hemisphere, New York.

Karol, M. H. (1991). Allergic reactions to indoor air pollutants. *Environ. Health Perspect.* **95,** 45–51.

Karol, M. H., and Thorne, P. S. (1988). Pulmonary hypersensitivity and hyperreactivity: Implications for assessing allergic responses. In *Toxicology of the Lung* (D. E. Gardner, J. D. Crapo, and E. J. Massaro, Eds.), pp. 427–448. Raven Press, New York.

Karol, M. H., Hauth, B. A., and Alarie, Y. (1979). Pulmonary hypersensitivity to hexyl isocyanateovalbumin aerosol in guinea pigs. *Toxicol. Appl. Pharmacol.* **51,** 73–80.

Karol, M. H., Griffiths-Johnson, D. A., and Skoner, D. P. (1993). Chemically induced pulmonary hypersensitivity, airway hyperreactivity, and asthma. In *Toxicology of the Lung,* 2nd ed. (D. E. Gardiner, J. D. Crapo, and E. J. Massaro, Eds.), pp. 417–434. Raven Press, New York.

Katz, D. H. (1980). Recent studies on the regulation of IgE antibody synthesis in experimental animals and man. *Immunology* **41,** 1–24.

Kimber, I., Dearman, R., Scholes, E., and Basketter, D. (1994). Local lymph node assay: Development and applications. *Toxicology,* in press.

Kochman, S., Lefebvre, S., Bernard, J., Maujean, M., Cazabat, A., Lavaud, F., and Manfait, M. (1990). Toulene diisocyanate-induced conformational changes of serum albumin: A study on repeated inhalation in guinea pigs. *Tox. Lett.* **50,** 165–171.

Maestrelli, P. (1992). Subcommittee on 'Occupational Allergy' of the European Academy of Allergology and Clinical Immunology. Guidelines for the diagnosis of occupational asthma. *Clin. Exp. Allergy* **22,** 103–108.

McGrath, K. G., Zeiss, C. R., and Patterson, R. (1983). Allergic reactions to industrial chemical. *Clin. Immunol. Rev.* **2,** 1–58.

Mosmann, T. R., Cherwinski, H., Bond, M. W., Giedlin, M. A., and Coffman, R. L. (1986). Two types of murine helper T cell clone. 1. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* **136,** 2348–2357.

Mosmann, T. R., and Coffman, R. L. (1989). Heterogeneity of cytokine secretion patterns and function of helper T cells. *Adv. Immunol.* **46,** 111–147.

Mullin, L. S., Wood, C. K., and Krivanek, N. D. (1983). Guinea pig respiratory response to isocyanates. *Toxicol. Appl. Pharmacol.* **71,** 113–122.

Musk, A. W., Venables, K. M., Crook, B., Nunn, A. J., Hawkins, R., Crook, G. D. W., Graneek, B. J., and Tee, R. D. (1989). Respiratory symptoms, lung function and sensitisation to flour in a British battery. *Br. J. Indust. Med.* **46,** 636–642.

Mygind, N., and Weeke, B. (1983). Allergic and nonallergic rhinitis. In *Allergy, Principles and Practice* (E. Middleton, C. E. Reed, and E. F. Ellis, Eds.), 2nd ed., p. 1101. Mosby, St. Louis/Toronto.

Nemery, B., and Lenaerts, L. (1993). Exposure to methylene diphenyl diioscyanate in coal mines. *Lancet* **341,** 318.

Obata, H., Tao, Y., Kido, M., Nagata, N., Tanaka, I., and Kuroiwa, A. (1992). Guinea-pig model of immunologic asthma induced by inhalation of trimellitic anhydride. *Am. Rev. Respir. Dis.* **146,** 1553–1558.

O'Byrne, P. M., Dolovich, J., and Hargreave, F. E. (1987). Late asthmatic responses. *Am. Rev. Respir. Dis.* **136,** 740–751.

Parronchi, P., Macchia, D., Piccinni, P-P., Biswas, P., Simonelli, C., Maggi, E., Ricci, M., Ansari, A. A. and Romagnani, S. (1991). Allergen- and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production. *Proc. Natl. Acad. Sci. USA* **881,** 4538–4542.

Patterson, R., Zeiss, C. R., Roberts, M., Pruzanski, J. J., Wolkonsky, P., and Chacon, R. (1978). Human antihapten antibodies in trimellitic anhydride inhalation reactions. *J. Clin. Invest.* **62,** 971–978.

Pauluhn, J., and Mohr, U. (1994). Assessment of respiratory hypersensitivity in guinea-pigs sensitized to diphenylmethane-4,4'-diisocyanate

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(MDI) and challenged with MDI, acetylcholine or MDI-albumin conjugate. *Toxicology*, in press.

Pauluhn, J., and Eben, A. (1991). Validation of a non-invasive technique to assess immediate or delayed onset of airway hypersensitivity in guinea pigs. *J. Appl. Toxicol.* **11**(6), 423–431.

Pene, J., Rousset, F., Priere, F., Chretien, I., Paliard, X., Banchereau, J., Spits, H., and De Vries, J. E. (1988). IgE production by normal human B cells induced by alloreactive T cell clones is mediated by IL-4 and suppressed by IFN-γ. *J. Immunol.* **141,** 1218–1224.

Pepys, J., Mitchell, J., Hawkins, R., and Malo, J. L. (1985). A longitudinal study of possible allergy to enzyme detergents. *Clin. All.* **15,** 101–115.

Ratner, B. (1939). Experimental asthma. *Am. J. Dis. Child.* **58,** 699–733.

Ratner, B., Jackson, H. C., and Gruehl, H. L. (1927). Respiratory anaphylaxis. Sensitization, shock, bronchial asthma and death induced in the guinea pig by nasal inhalation of dry horse dander. *Am. J. Dis. Child* **34,** 23–52.

Ritz, H., Evans, B., Bruce, R., Fletcher, E., Fisher, G., and Sarlo, K. (1993). Respiratory and immunological responses of guinea pigs to enzyme-containing detergents: A comparison of intratracheal and inhalation modes of exposure. *Fundam. Appl. Toxicol.* **21,** 31–37.

Romagnani, S. (1991). Human T_{H1} and T_{H2} subsets: Doubt no more. *Immunol. Today* **12,** 256–257.

Romagnani, S., Del Prete, G., Maggi, E., Parronchi, P., Tiri, A., Macchia, D., Giudizi, M. G., Almerigogna, F. and Ricci, M. (1989). Role of interleukins in induction and regulation of human IgE synthesis. *Clin. Immunol. Immunopathol.* **50,** S13–S23.

Rudolph, R., Kunkel, G., Diller, G., and Baumgarten, C. (1980). On a case of respiratory allergy caused by sensitization against elephant dandruff. *Allergologie* **3,** 34–36.

Sarlo, K., and Clark, E. D. (1992). A tier approach for evaluating the respiratory allergenicity of low molecular weight chemicals. *Fundam. Appl. Toxicol.* **18,** 107–114.

Sequin, P., Allart, A., Cartier, A., and Malo, J. L. (1987). Prevalence of occupational asthma in spray painters exposed to several types of isocyanates, including polymethylene polyphenylisocyanate. *J. Occup. Med.* **29,** 340.

Sheffer, A. L. (1991). Definition and diagnosis. *J. Allergy Clin. Immunol.* **88** (3, part 2), 427–438.

Slavin, R. G., (1983). Allergic bronchopulmonary aspergillosis. In *Allergy, Principles and Practice,* 2nd ed. (E. Middleton, Jr., C. E. Reed, and E. F. Ellis, Eds.), p 1076. Mosby, St. Louis/Toronto.

Sorg, C. (1989). Cytokines regulating the allergic response. *Cytokines,* Vol. 2. Karger, Basel.

Tao, Y., Sugiura, T., Nakamura, H., Kido, M., Tanaka, I., and Kuroiwa, A. (1991). Experimental lung injury induced by trimellitic anhydride inhalation on guinea-pigs. *Int. Arch. Allergy Appl. Immunol.* **96,** 119–127.

Thorne, P. S., and Karol, M. H. (1988). Assessment of airway reactivity in guinea pigs: Comparison of methods employing whole body plethysmography. *Toxicology* **52,** 141–163.

Vandenplas, O., Malo, J.-L., Saetta, M., Mapp, C. E., and Fabbri, L. M. (1993). Occupational asthma and extrinsic alveolitis due to isocyanates: Current status and perspectives. *Br. J. Ind. Med.* **50,** 213–228.

Wanner, A., and Abraham, W. M. (1982). Experimental models of asthma. *Lung* **160,** 231–243.

Wass, U., and Belin, L. (1990). An *in vitro* method for predicting sensitizing properties of inhaled chemicals. *Scand. J. Work. Environ. Health* **16,** 208–214.

Zetterstrom, O. (1977). Challenge and exposure test reactions to enzyme in detergents in subjects sensitized to subtilisin. *Clin. Allergy* **7,** 355.

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