Cell and molecular biology of chemical allergy

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Objective: The objective of this review is to provide current approaches to gain increased understanding of the molecular basis of chemical allergenicity. Chemical allergy refers to an allergic reaction to a low molecular weight agent (ie, <1 kD). The symptoms and pathology of chemical asthma resemble those of allergy to larger sized agents, such as pollens, weeds, and danders. The differences relate to mechanisms of disease. To stimulate an immune response, low molecular weight chemicals function as haptens and bind to carrier macromolecules. This article focuses on the chemical reactions and physicochemical characteristics of chemical allergens.

Data Sources: Data were obtained from published clinical reports and from the Documentation of Threshold Limit Values (1998) published by the American Congress of Governmental Industrial Hygienists.

Results: In vitro studies indicate the stoichiometric reaction of some chemical allergens with glutathione and the subsequent transfer of the allergen from glutathione to other nucleophiles. Computer-generated structure-activity relationship models have been developed for chemicals that induce respiratory allergy. The models, based on physicochemical properties of the agents, have high sensitivity and specificity.

Conclusions: The structure-activity relationship model suggests that chemical binding is the essential feature of chemical allergens. Their in vivo reactions with thiols may result in glutathione deficiency with consequent alteration in cellular reduction-oxidation (redox) status, release of cytokines, and promotion of the T helper cell 2 phenotype. Prevention of permanent disease is dependent on periodic medical surveillance of affected workers. When detected early, the disease can frequently be reversed.

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INTRODUCTION

A number of low molecular weight (LMW) chemicals (ie, <1,000 D) are known to cause respiratory sensitization. Contact with these chemicals frequently occurs in the workplace. Symptoms include wheezing, tightness in the chest, coughing, and shortness of breath.[1] Inflammation of the airways, if present, is characterized by activated lymphocytes and eosinophils in the bronchial mucosa.[2] Airway hyperreactivity to nonspecific agents further characterizes the disease.

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The symptoms and pathology of chemical asthma resemble those of allergy to larger sized natural agents, such as pollens, weeds, and danders. Differences between chemical allergy and allergy to environmental agents relate to mechanisms of disease. Immunologic factors seem to be important in the pathogenesis of chemical asthma. In contrast to allergy because of high molecular weight agents, atopy is not a risk factor for developing chemical allergy. Immunoglobulin (Ig)E is *infrequently* detected in chemical asthma.[3] LMW allergens are either inherently chemically reactive or are metabolized in vivo into chemically reactive species. They function as haptens and bind to carrier macromolecules to initiate immunologic stimulation. The chemistry of haptenation may provide insight into the molecular mechanism(s) of allergenicity of LMW chemicals.

Chemical Sensitizers Are Haptens

LMW sensitizers are typically electrophiles or proelectrophiles capable of reaction with hydroxyl, amino, and thiol functionalities on proteins. For many, the identity of the macromolecular target(s) to which binding results in sensitization is unknown. Studies with two diisocyanate allergens, toluene diisocyanate and hexamethylene diisocyanate (HDI), have indicated a rapid reaction of each under physiologic conditions with glutathione.[4] Further, in the presence of certain peptides, the adducts are transferred from glutathione to nucleophilic sites on the peptides, suggesting the possibility of regeneration of the reactive chemicals and binding to targets distant from the initial site of reaction.[4]

Another consequence of chemical binding to thiols may be development of glutathione deficiency. Alteration of cellular redox potential is known to affect numerous physiologic and pathophysiologic processes, including activation of MAP kinase, induction of cytokine expression,[5] and promotion of the T helper cell 2 phenotype.[6] In human bronchial epithelial cells, sensitivity to tumor necrosis factor α is inversely correlated with cellular redox state.[5]

The identity and immunologic activity of chemically adducted proteins has received recent interest. Epithelial proteins, adducted after contact with HDI, were found to stimulate proliferation of lymphocytes from HDI-asthmatic patients, but not those from HDI-exposed nonasthmatics nor from atopics with non-HDI–induced asthma.[7] Toluene diisocyanate was found to associate with ciliary tubulin of human airway epithelial cells.[8] Tubulin, a subunit protein of microtubules, possesses numerous sulfhydryl moieties

Table 1. Criteria for Acceptance of Chemicals Into Database

General
Chemicals included in the model had to be:
1. Tested or identified as pure substances (*i.e.*, no mixtures)
2. Nonmetal-containing organics
Sensitizers
Data published in a clinical report
Patient underwent inhalative challenge with the chemical
Response was a decrease in FEV1 $\geq$ 20% occurring within 24 hours of provocation challenge
Nonsensitizers
Chemical in high volume production for many years[14]
No reports of respiratory sensitization cited in ACGIH documentation of TLVs[14]

and is essential to cytoskeletal-derived signal transduction.

Structure-Activity Relationship (SAR) Models

SAR models have provided valuable insight into mechanisms of many toxicities, including mutagenesis, carcinogenesis, and reproductive toxicity.[9] We have described SAR models for chemically induced respiratory hypersensitivity.[10–12] The models were derived from a database of chemicals known to cause respiratory sensitivity. The database of active chemicals consisted of 40 chemicals identified as sensitizers by reports in the clinical literature. Because our modeling technique requires a comparison between active and inactive chemicals, and non-sensitizers would not be listed in clinical reports, we initially generated a database of "inactive" respiratory sensitizers by assuming that chemicals inactive in causing human dermal sensitization would also be inactive in causing respiratory sensitization. Forty such chemicals were randomly selected from human patch-testing data and combined with

Table 2. Physicochemical Parameters Examined for Each Chemical

Electronic
Dipole moment
Hansen dispersion
Hansen polarity
Hansen hydrogen binding
Sum of partial positive charges
Sum of partial negative charges
Highest occupied molecular orbital (HOMO)
Lowest unoccupied molecular orbital (LUMO)
Transport
Water solubility
Hydrophilic-lipophilic balance
Percent hydrophilic surface area
Solubility parameter
Mean water of hydration
Log octanol–water partition coefficient (log P)
Steric
Molecular weight
Molecular volume
Density
Surface area
Molar refractivity

Table 3. Database for Human Respiratory Sensitization

Active sensitizers	Inactive sensitizers
6-Amino penicillanic acid	Acrolein
7-Aminocephalosporanic acid	Adiponitrile
Ampicillin	Amyl acetate
Azocarbamide	Benzene
Benzylpenicillin	Biphenyl
Brilliant orange GR	Bromacil
Carminic acid	*N*-Butylamine
Cephalexin	Chlordane
Chlorhexidine	Chlorobenzene
Dichlorvos	Chlorodifluoromethane
Dimethyl ethanolamine	Chloromethyl methyl ether
Diphenyl methane-4,4′-diisocyanate	2-Chlorotoluene
Epigallocatechin gallate	Chlorpyrifos
Ethanolamine	Chrysene
Ethyl cyanoacrylate	Cumene
Ethylenediamine	Cyclohexane
Fenthion	Cyclonite
Hexamethylene diisocyanate	Cyclopentadiene
Iso-nonanoyl oxybenzene sulphonate	Dibutyl phthalate
Isophorone diisocyanate	Dicrotophos
Maleic anhydride	Diethanolamine
Methyl-2-cyanoacrylate	Dioxathion
Methyldopa	Diuron
1,5-Naphthalene diisocyanate	Ethanolamine
2-(*N*-benzyl-*N*-tertbutylamino)-4′-hydroxy-3′-hydroxymethyl	Ethylene glycol monoethyl ether
Phenylglycine acid chloride	Glycerol
Phthalic anhydride	*N*-Heptane
Piperacillin	Hexane
Piperazine	Isophorone
Plicatic acid	Methacrylic acid
Reactive orange 3R	Methyl methacrylate
Rifafix red BBN	Methyl *n*-amyl ketone
Rifazol black GR	Methylal
Tetrachloroisophthalonitrile	Methylcyclohexane
Tetrachlorophthalic anhydride	Naphthalene
2,4-Toluene diisocyanate	Nitrobenzene
2,6-Toluene diisocyanate	*N*-Pentane
Triethylenetetramine	Phenol
Trimellitic anhydride	Toluene

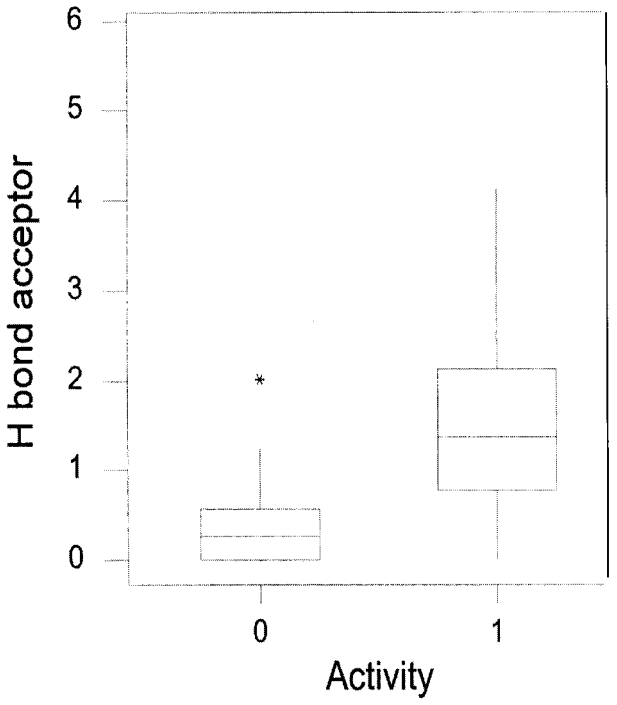

Figure 1. Box and whiskers plot of H bond acceptor values for sensitizers (1) and non-sensitizers (0). * $P < 0.05$.

the 40 active respiratory sensitizers for generation of SAR models for respiratory sensitization. Using internal validation, the models showed high sensitivity, (ie, ability to accurately predict the activity of chemical sensitizers), and specificity (ability to accurately predict the inactivity of non-sensitizers). However, they also predicted a higher that expected percentage of random chemicals to be active. Assuming that the high prediction (we estimate <20% are positive) resulted from the nature of the inactive set of chemicals, an alternate source of inactive chemicals was selected.

This report describes the new criteria for selection of chemicals, the database that was developed, and the model based on physicochemical (PC) properties of the agents. The performance of this model and the PC parameters found to be associated with respiratory hypersensitivity are presented. Last, the mechanistic interpretation of the model is discussed.

MATERIALS AND METHODS

Database

Criteria for selection of chemicals into the database are listed in Table 1.

SAR Model

The molecular modeling and calculation of PC parameters were performed using Molecular Modeling Pro (version 1.44; Window Chem Software, North Fairfield, CA). Individual three-dimensional structures were developed, and rigid conformational analysis was performed to identify low-energy molecular conformations. Nineteen parameters (Table 2) were calculated for each chemical from the energy-minimized structures.

Test of the Model

One hundred chemicals were randomly selected from a list of 10,000 chemicals representative of those in the environment. The list is consistent with categories of chemicals generated by the National Academy of Sciences.

RESULTS

The database developed for modeling of respiratory sensitization is provided in Table 3. Prominent among the active sensitizers in the database are several diisocyanates, acid anhydrides, and reactive dyes. Metallochemicals, although recognized as having sensitizing activity, are not included because many PC values can not be determined for these agents.

Nineteen parameters were calculated for each chemical in the database. As indicated in Table 2, the parameters encompassed electronic, transport, and steric properties. The parameters that best distinguished the active from the inactive set of chemicals, as determined from linear discriminant analysis, were Hansen polarity and hydrogen bond acceptance. Figures 1 and 2 illustrate the discrimination achieved by linear analysis between positive and negative sets with each of these parameters. In both cases, the active chemicals had higher values than the inactives suggesting that chemical binding is a major characteristic distinguishing pulmonary-sensitizing chemicals from pulmonary non-sensitzers.

An internal validation exercise was conducted to evaluate the power of the model. Results (Table 4) indicated a sensitivity of 0.850 and a specificity of 0.744. Importantly, when asked to predict the activity of 100 random chemicals, the SAR model predicted 19% to be positive.

A comparison of the current model with one previously developed is provided in Table 5. Reactivity characteristics are the discriminating parameters in the current model, whereas transport factors were prominent discriminators in the previous model. This major difference reflects the source of the negative sensitizers. As indicated in Table 5, in the original model the negative set was derived from patch-testing data, whereas that in the current model reflects chemicals encountered via the inhalation route. The basis for discrimination of active from inactive *airborne* chemicals is the potential of the

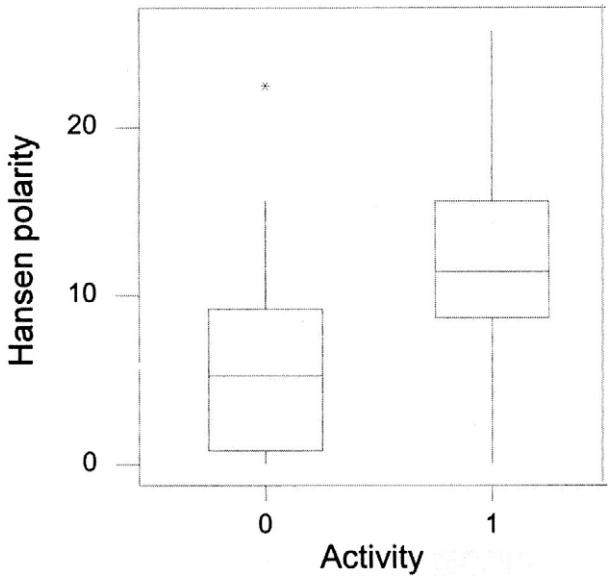

Figure 2. Box and whiskers plot of Hansen polarity values for sensitizers (1) and non-sensitizers (0). * $P < 0.05$.

active set of chemicals to *bind* to other molecules.

DISCUSSION

Understanding the cellular and molecular events underlying chemical allergy is essential to protection of at-risk individuals. The requirement that chemicals associate with, or bind to, carrier molecules for initiation of sensitization[15] highlights the importance of chemical reactivity to this process.

The SAR model described here has reinforced appreciation of the need to understand the chemical reactions underlying the sensitization process. The model is derived from human data. The inactive group of chemicals represents compounds that have been in high volume production for many years and, although present in the atmosphere, have not been reported to cause respiratory sensitization. The parameters identified by the model indicate that chemical binding is important for sensitization.

SAR models can be used for prediction of activity of untested chemicals as well as for gaining mechanistic understanding of respiratory sensitization. With recognition of the recent worldwide increase in the prevalence of asthma, it is particularly important to consider diverse approaches to understand the basis for increased disease. One hypothesis for the increase is exposure of the population to newly produced synthetic chemicals that are allergenic. The SAR model provides an opportunity to evaluate the hypothesis. Further, computerized testing of the chemicals is simple, rapid, and inexpensive.

Table 4. Internal Validation and Prediction of Activity of 100 Random Chemicals

Model parameters	Sensitivity	Specificity	Prediction of unknowns*
Hansen polarity Hydrogen bond acceptance	0.850	0.744	19%

* 100 random chemicals (representative of chemicals in the environment) were submitted to the model for prediction of activity. The model predicted 19% to be active.

Table 5. Comparison of SAR Models Based on PC Properties of the Chemicals

This model	Previous model*
Database	
40 sensitizers	40 sensitizers
40 negatives derived from airborne industrial chemicals	40 negatives derived from dermal non-sensitizers
Physicochemical parameters that discriminate positive from negative	
Hansen polarity	% Hydrophilic surface area
Hydrogen bond acceptor	Hydrophilic-lipophilic balance
	Hydrogen bond acceptor

* Described in refs. 11 and 13.

CONCLUSION

The recent explosion of information regarding molecular factors and pathways involved in allergic asthma have suggested new avenues for both diagnosis and treatment. It is anticipated that SAR models will contribute additional insight into this critical problem and result in novel approaches to clinical investigations.

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