
Recent developments in diisocyanate asthma

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Objective: To summarize the latest experimental findings on diisocyanate asthma and discuss the impact of these data on our understanding of disease pathogenesis and diagnosis.

Data Sources: The literature reviewed includes articles from PubMed (National Library of Medicine) published within the last 3 years (1999–2001). In addition, pertinent older references are discussed to provide a historical perspective and background.

Study Selection: The data discussed were chosen to highlight key concepts relevant to diisocyanate asthma pathogenesis and are grouped accordingly.

Results: In many ways, diisocyanate-induced asthma mirrors allergic asthma caused by other stimuli; however, the immune-mediated pathways believed to be central to the disease have been difficult to define. Recent studies on the human immune response to diisocyanates provide additional evidence supportive of an immune basis for pathogenesis but also highlight well-recognized differences between diisocyanate asthma and common atopic asthma. Studies on the antigenic form of diisocyanates and their interaction with epithelial tissues provide new insights that may help explain these apparent immunologic differences. Genetic factors that influence disease have begun to be identified but remain poorly characterized. Associations of particular major histocompatibility complex class II alleles with diisocyanate asthma further fuel the hypothesis that immune-dependent mechanisms underlie pathogenesis, whereas associations of glutathione S-transferase polymorphisms (in conjunction with recent studies defining the effects of diisocyanates on thiol-redox homeostasis) may implicate additional antigen-independent mechanisms. Long-term follow-up studies of diisocyanate asthma patients have confirmed the prognostic value of early removal of symptomatic patients from exposure and highlight the need for effective diagnostic tests of sensitivity and susceptibility.

Conclusions: Diisocyanate-induced asthma appears to be a multifactorial disease involving the immune system, airway epithelium, and genetic factors. The potential long-term adverse effects of diisocyanate exposure in sensitized patients underscore the need for further studies to elucidate the pathogenesis of this disease and identify biomarkers for sensitization and susceptibility.

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INTRODUCTION

Diisocyanates, a leading cause of occupational asthma worldwide, are low-molecular-weight, cross-linking chemicals with significant sensitizing potential.¹ The 3 predominant diisocyanates in use today are diphenylmethane diisocyanate (MDI), toluene diisocyanate (TDI), and hexamethylene diisocyanate (HDI; Fig 1). Numerous occupations involve risk of isocyanate exposure, including polyurethane foam makers, plastic molders, upholstery and furniture workers, spray painters, wire coating workers, rubber workers, insulation installers, and workers in foundries and transportation equipment manufacturing. The clinical condition caused by diisocyanates closely resembles that caused by common environmental allergens (eg, cat, dust), suggesting that similar mechanisms involving antigen-mediated airway inflammation are central to disease.^{2–4} However, attempts to elucidate the immunogenic cascade elicited by diisocyanates have proven challenging due to uncertainty regarding the natural carrier protein(s) for these chemicals in vivo and the lack of a well established animal model that replicates the natural

course of human disease.^{5–10} Failure to overcome these challenges obscures our current conception of diisocyanate asthma pathogenesis and modern approaches to disease surveillance and prevention.

We performed a PubMed (Library of Medicine) search of articles published within the last 3 years (1999–2001) to summarize the latest experimental findings on diisocyanate asthma and discuss the impact of these data on our understanding of disease pathogenesis and diagnosis. In addition, pertinent older references are discussed to provide a historical perspective and background. The data discussed were chosen to highlight key concepts relevant to diisocyanate asthma pathogenesis and are grouped accordingly.

CLINICAL COURSE OF DIISOCYANATE ASTHMA

Diisocyanate asthma usually develops following a significant lag (months to years) between the time of first exposure and the onset of symptoms, during which time it is believed that systemic immune sensitization develops.¹¹ Once sensitization occurs, respiratory tract exposure to low levels of diisocyanates, below the Occupational Safety and Health Administration-mandated limits, can induce early, late, or dual-phase asthmatic attacks.^{12,13} Lung pathology in diisocyanate asthmatic patients typically involves significant inflammatory infiltrates in the airway mucosa, which in some cases are eosinophilic in composition and in other cases may be more neutrophilic.^{2,14} Clinical diagnosis can be positively con-

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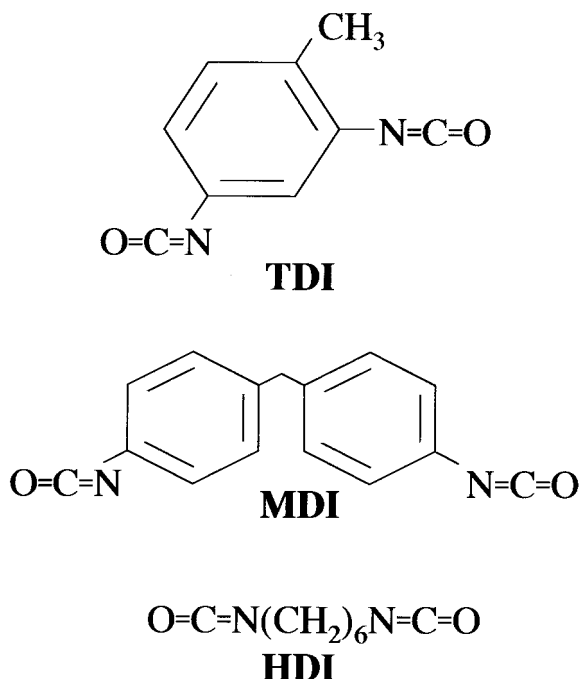


Figure 1. Chemical structure of the predominately used diisocyanates. TDI indicates toluene diisocyanate; MDI, diphenylmethane diisocyanate; and HDI, hexamethylene diisocyanate.

firmed through specific inhalational challenge; however, calibrated and validated diisocyanate exposure chambers are not readily available in most parts of the world. In practice, diagnosis usually depends on documentation of bronchial hyperreactivity and a positive association of symptoms and physiologic changes with exposure.¹⁵ Prognosis improves with prompt removal from exposure, but bronchial hyperreactivity frequently persists away from exposure. Continued exposure may lead to long-lasting nonspecific bronchial hyperreactivity.¹⁶

RECENT DEVELOPMENTS IN DIISOCYANATE-INDUCED ASTHMA

In the past several years, new findings relevant to diisocyanate-induced asthma have emerged through research on several different fronts, aided by the use of recently developed technologies and reagents. Six interrelated foci of research discussed herein include (1) the human immune response to exposure, (2) diisocyanate antigens, (3) airway epithelium, (4) skin exposure, (5) genetic factors, and (6) long-term health effects of exposure. These topics will be summarized briefly in historical perspective, concluding with the most recently published findings.

Immune Responses in Diisocyanate Asthmatic Patients

Studies characterizing diisocyanate immunogenicity and airway inflammation in diisocyanate asthmatic patients support the hypothesis that immune mechanisms underlie diisocyanate asthma.^{1-4,9,11,14,15} Systemic immune sensitization is evi-

denced by diisocyanate-specific antibodies, lymphocyte proliferative responses, or cytokine and chemokine production.¹⁷⁻²⁰ In the airways of diisocyanate asthmatic patients, inflammation similar to that observed in common atopic asthma is a prominent characteristic. Activated T cells (predominantly CD4⁺) and increased levels of cytokines produced by T_H2 cells (interleukin [IL]-4, IL-5) have been reported in a limited number of diisocyanate asthma patients studied to date.^{2-4,21,22}

Despite similarities between common environmental allergen-induced asthma and diisocyanate-induced asthma, certain differences have also been reported. Perhaps most striking is the conspicuous absence of diisocyanate-specific IgE in many diisocyanate asthmatic patients and the lack of association with atopy.²³⁻³⁵ The characteristics and magnitude of lymphocyte responses to diisocyanate stimulation in vitro also differ from those reported for common environmental allergens. In contrast to the clearcut T_H2-like responses and robust proliferation elicited by common allergens such as dust mite or cat dander, lymphocytes from diisocyanate-sensitive workers may respond with a mixed T_H1/T_H2-like response and only modest proliferation when stimulated with diisocyanate in vitro.^{19,20,36} Subtle differences also exist in the airway inflammation of diisocyanate asthmatic patients, which may contain relatively more neutrophils and less eosinophils compared with common atopic asthma and reflect the differences noted in in vitro studies.^{2,14,37,38}

Several recent studies have further investigated antibody responses in diisocyanate-exposed workers with and without asthma.^{29,39-41} These studies have confirmed that, when present, diisocyanate-specific IgE is highly predictive of diisocyanate asthma.^{29,41} However, consistent with previous reports, most patients with diisocyanate asthma had normal total IgE levels and no detectable diisocyanate-specific IgE. One unique finding of a study by Park et al²⁹ was a strong association between TDI-specific serum IgG and TDI-induced asthma. However, these findings differ from previous reports on diisocyanate-exposed workers, where the presence of diisocyanate-specific IgG correlates with exposure but not necessarily asthma.^{25,34,35} Recent findings by Redlich et al⁴² highlight the prevalence of diisocyanate-specific IgG responses in otherwise healthy HDI-exposed workers, indicating that immune responsiveness, without asthma, occurs in a substantial proportion of exposed workers. Longitudinal studies of such workforce populations should help determine if immune-responsive workers are at risk for asthma with continued exposure or alternatively if specific anti-isocyanate responses may play a protective role.

The differences in the airway inflammation of diisocyanate asthmatic patients compared with common atopic asthmatic patients, as described herein, may reflect differences in the immune mechanisms central to pathogenesis. The most striking differences in the limited number of diisocyanate asthmatic patients studied to date are relative increases in neutrophil influx and IL-8 production.^{3,14,38} Recent clinical studies by Park et al³⁷ further support the potential role of neutrophils in the pathogenesis of diisocyanate asthma. Neu-

trophil activation and increased levels of IL-8 (a chemokine that induces neutrophil recruitment and activation) were noted in diisocyanate asthmatic patients compared with exposed controls by analyzing induced sputum samples following TDI exposure. Together with studies by Lummus et al²⁰ that highlight diisocyanate-induced IL-8 production by peripheral blood mononuclear cells from diisocyanate asthmatic patients in vitro, these data suggest fundamental differences in the inflammatory response elicited by diisocyanates compared with common aeroallergens.

Thus, diisocyanates are immunogenic compounds that have the capacity to elicit airway inflammation and an array of immune responses. Further characterization of these immune responses in larger populations of diisocyanate asthmatic patients and exposed nonasthmatic workers are needed to identify those components of the immune response central to pathogenesis. In pursuing such investigations, especially in vitro immunology studies, a better understanding of the “antigenic” form of diisocyanates is essential.

Diisocyanate Antigens

Understanding the antigenic form of diisocyanates is key to elucidating the pathogenesis of diisocyanate-induced asthma and the development of diagnostic assays. Most human studies to date have used diisocyanate-conjugated albumin adducts as antigens for immunoassays, including the radioallergen sorbent test, enzyme-linked immunosorbent assay, in vitro lymphocyte proliferation and cytokine tests, and skin prick tests. Using diisocyanate-albumin antigens in these assays, a poor correlation has often been found between positive assay indices and asthma.^{1,15,19,23–27,29,35,43} These findings are in contrast to common atopic asthma, in which elevated antigen-specific IgE and T_H2 cell responses correlate with sensitization and/or disease.^{44,45} Two hypotheses have been postulated to explain this discrepancy: diisocyanate-induced asthma pathogenesis is distinct from that occurring in common atopic asthma or diisocyanate albumin is not the biologically relevant antigen central to disease pathogenesis.

The routine use of albumin as a carrier protein for diisocyanates likely arose from convenience (high-quality, purified human serum albumin is readily available commercially); however, in retrospect, albumin was probably a fortuitous choice. Recent studies suggest that albumin is one of a limited number of preferred targets for diisocyanate conjugation in vivo under occupational exposure conditions. Albumin is the single HDI conjugated protein detected in the airway fluid of HDI-exposed humans using sensitive immunologic assays based on a novel HDI-specific antisera.^{10,46} This finding is consistent with previously reported animal studies that used similar immunologic approaches and radioisotopic tracing methods.^{47,48} HDI albumin has also been found to act as an effective antigen in a recently reported murine model of HDI asthma that involves eosinophilic airway inflammation similar to that observed in human patients.⁵

The conjugation of albumin with diisocyanate in exposed human airways is not surprising given that albumin is the

most abundant protein in airway fluid. Recent data from our laboratory further highlight the role of albumin as a carrier for diisocyanate in vivo and support previous studies that suggest a crucial role for albumin in immune recognition of diisocyanates. As shown in Figure 2, although HDI can bind to numerous human proteins, only human albumin acts as an effective carrier for recognition by human IgG from diisocyanate asthmatic patients. Furthermore, bovine albumin, which is more than 87% homologous to human albumin, is not an effective substitute. Similar data have been previously reported with regard to IgE antibodies from diisocyanate asthmatic patients.^{32,49} Together these findings suggest that specific sites on human albumin may be preferred targets for diisocyanate reactivity, at least with regard to recognition by the humoral arm of the immune system.

Thus, diisocyanate conjugated albumin represents at least one antigenic form of diisocyanates that can be found in the airways of exposed workers and is specifically recognized by the humoral arm of the immune system. However, controversy still exists regarding whether diisocyanate-albumin adducts are truly important antigens in diisocyanate asthma pathogenesis or merely an epiphenomenon of exposure and increased immune activity in the airways of sensitive individuals. Given that biologically relevant diisocyanate antigens may form the basis of effective diagnostic tests for sensitivity, further studies are needed to address this important issue, including the possibility that other proteins (eg, airway epithelial) may also participate in pathogenesis.

Airway Epithelium

As early as 1990, Mattoli et al⁵⁰ described the capacity of TDI to stimulate human bronchial epithelial cells to produce factors that potentiate T-cell proliferation. Nonetheless, the role of the airway epithelium in the pathogenesis of diisocyanate asthma has been largely ignored until recently, despite the fact that it is a major site of exposure. Active participation of the airway epithelium in asthma pathogenesis in general is an emerging concept recently reviewed by Holgate et al,⁵¹ who presented evidence that supported a dynamic interaction between airway epithelium, lung mesenchyme, and the immune

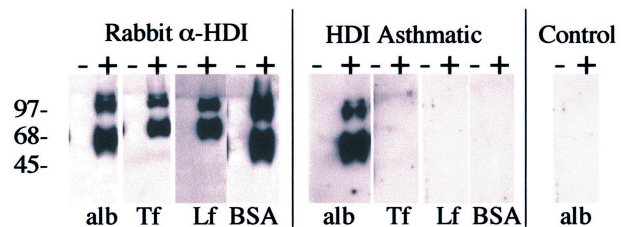


Figure 2. Importance of albumin as the carrier protein for recognition of hexamethylene diisocyanate (HDI) by human IgG. Different proteins were unconjugated (–) or conjugated with HDI (+) and analyzed by Western blot for specific IgG binding with polyclonal α-HDI rabbit serum, serum from a representative HDI asthmatic patient, or unexposed control subject. alb indicates human albumin; Tf, human transferrin; Lf, human lactoferrin; and BSA, bovine serum albumin.

system. New studies providing clues as to how the airway epithelium might participate in the development of diisocyanate asthma are summarized herein.

The interaction between diisocyanates and human airway epithelium is likely a primary event in pathogenesis given the anatomy of the air/lung interface and the reactivity of diisocyanates. Mounting evidence suggests that diisocyanates bind to airway epithelial cell proteins and are taken up by these cells.^{8,10,22,52,53} Lange et al⁵² identified intracellular S-glutathionyl TDI adducts in murine lung in vivo and human bronchoepithelial cells in vitro after exposure. In earlier studies, these authors had demonstrated colocalization of TDI with tubulin on cilia of exposed differentiated human airway epithelial cells.⁵³ Recently, specific human airway epithelial cell proteins that become conjugated with HDI in vitro and in vivo have been identified, including keratin 18.¹⁰ HDI-exposed airway epithelial cell protein preparations have previously been shown to specifically induce proliferation of lymphocytes from diisocyanate asthmatic patients.⁸ Together these data suggest the potential for airway epithelial cell proteins to act as carriers for diisocyanate in vivo, which could help explain some of the unique characteristics of diisocyanate asthma compared with common atopic asthma. A similar phenomenon has recently been described for another occupational allergen, trimellitic anhydride, suggesting that airway epithelial cell protein conjugation may represent a fundamental process in the pathogenesis of asthma induced by reactive chemicals.^{54,55}

New insights into the specific functional effects of diisocyanates on airway epithelial cells have emerged through several lines of study using diverse methods. In animal toxicity studies, MDI and HDI prepolymers were shown to induce airway epithelial barrier dysfunction, which was partially dependent on glutathione levels.^{56–59} Specific effects of TDI on the expression of lung cytochrome P450 enzymes was documented by Pons et al,⁶⁰ suggesting that diisocyanates may alter lung metabolic activity. More recently, several independent studies have suggested that diisocyanate exposure may alter thiol-redox homeostasis in the airway. Lantz et al⁶¹ reported that TDI exposure reduces intracellular glutathione levels in normal bronchial epithelial cells in vitro using a biochemical fluorescence assay to measure thiol levels in living cells. Elms et al⁶² have shown that diisocyanates can induce reactive oxygen species and hydrogen peroxide production by monocytic cells in vitro. With the aid of microarray technology, our laboratory has shown that HDI exposure increases airway epithelial cell messenger RNA expression of thioredoxin reductase, an essential enzyme in maintaining thiol-redox homeostasis, and an oxidant-induced dioldehydrogenase previously shown to be highly susceptible to HDI conjugation in vitro.⁶³ Given that thiol-redox homeostasis is tightly coupled to inflammatory signal transduction pathways,^{64,65} these studies suggest the potential for diisocyanates to modulate the host immune response, via airway epithelial cells, through an antigen receptor-independent mechanism.

Thus, recent studies have expanded on previous reports that described diisocyanate's effects on airway epithelial cells and identified specific responses that may be linked to airway inflammation. Together these data suggest that the airway epithelium is not simply a passive structural barrier but plays an active role in the induction of diisocyanate asthma.

Skin Exposure

The potential role of skin exposure in the development of diisocyanate sensitivity has recently drawn attention from several investigators. Three new studies in animal models have reported additional evidence describing the reciprocity of the skin and airway epithelium in sensitization and elicitation of inflammatory responses following diisocyanate exposure. Ebino et al⁶⁶ have shown that isolated airway exposure to TDI results in skin sensitization. Studies by Matheson et al⁷ have shown that subcutaneous injection of TDI can lead to nasal and tracheal inflammation following intranasal challenge. Herrick et al⁵ have demonstrated that epicutaneous HDI exposure (occluded patch) of mice followed by airway challenge with HDI-albumin antigens results in eosinophilic airway inflammation strikingly similar to that observed in human isocyanate asthmatic patients.

In human studies, recent data have described the ubiquity of skin exposure in diisocyanate workers and identified skin exposure as a potential risk factor for MDI asthma. Liu et al⁶⁷ quantitated diisocyanate skin exposure in auto body shops, a predominant end-use setting for HDI, while Petsonk et al⁴¹ reported an increased prevalence of diisocyanate asthma among workers with evidence of substantial MDI skin exposure. The major skin proteins susceptible to HDI conjugation following skin exposure of humans in vivo have recently been identified as keratins, structurally related to the predominate HDI-susceptible protein (keratin 18) in the airway epithelium.¹⁰

Thus, substantial data implicate the skin as a potential route of sensitization to diisocyanates. Although the potential mechanisms by which skin exposure might result in respiratory tract sensitization remain unclear, diisocyanate conjugated keratin may provide an antigenic structural link between these different epithelia.

Genetic Susceptibility Factors

Genetic factors likely contribute to diisocyanate asthma pathogenesis, given individual differences in susceptibility following similar occupational exposures. The relatively small population size of most diisocyanate asthma studies has limited large-scale genomic approaches to identifying candidate diisocyanate asthma genes. The few genetic studies reported to date have focused on polymorphisms within major histocompatibility complex (MHC) class II genes on chromosome 6, a locus that has been recently linked to asthma in certain ethnic populations through genomewide screens⁶⁸ and in association with specific stimuli, including acidic anhydrides and ragweed.^{69,70} In diisocyanate asthma, a significant association of certain HLA-DQ alleles (DQB1*0503 in particular), but not others, provides evidence that further bolsters the im-

mune-mediated hypothesis regarding pathogenesis.^{71,72} Important insights could be gained by linking MHC class II genes with diisocyanate asthma given the crucial role of these cell surface proteins in antigen presentation to CD4⁺ T cells, which are postulated to be central to asthma pathogenesis in general. However, other investigators have failed to verify HLA associations with diisocyanate asthma or focused on different genes (eg, metabolic enzymes).^{33,73} Piirila et al⁷³ recently demonstrated an association of certain glutathione S-transferase haplotypes, particularly involving polymorphisms in the glutathione S-transferase M1 gene, with diisocyanate asthma and lack of diisocyanate-specific IgE. The same glutathione S-transferase M1 polymorphisms have been associated with an increased risk for atopic asthma in another recent study.⁷⁴ Most recently, Mapp et al⁷⁵ showed that specific alleles of the P1 class of glutathione S-transferase confer a protective effect against TDI. These genetic associations of glutathione S-transferase polymorphisms and isocyanate asthma are particularly intriguing in light of recent studies describing the effects of diisocyanates on airway thiol-redox homeostasis, which can affect signal transduction pathways involved in inflammation.^{64,65}

Thus, although our current understanding of diisocyanate asthma genetics remains rudimentary, candidate genes that may modify pathogenesis have begun to be identified. Given that asthma is likely a multifactorial disease, several different genes or haplotypes may contribute to pathogenesis.

Long-term Follow-up

Several studies to date have documented long-lasting non-specific bronchial hyperreactivity in diisocyanate asthma patients that may persist despite cessation of exposure. A recent report by Piirila et al¹⁶ further highlights this important fact, documenting the poor medical outcome of patients with diisocyanate-induced asthma. One interesting finding of these studies was the observation that workers with diisocyanate-specific IgE fared better than those without. The significance of this finding remains unclear, although the authors speculate that the presence of diisocyanate-specific IgE may have allowed these patients to be identified earlier in the course of their disease and promptly removed from exposure, hence a more favorable prognosis. Further studies are needed to address this potentially important observation given the long-term health benefits of early disease recognition. Despite the fact that diisocyanate-specific IgE antibodies are present in only a small percentage of diisocyanate asthmatic patients, their positive predictive value for asthma and the feasibility of routinely monitoring workers IgE levels may provide an effective strategy to help identify at least a fraction of the at risk population.

CONCLUSION

Diisocyanate-induced asthma shares many characteristics with asthma caused by other more common environmental allergens. However, considerable uncertainty remains regarding the antigen and the T-cell types (T_H1 vs T_H2, CD4, CD8)

that orchestrate the inflammatory responses to exposure. Recent studies have suggested that airway epithelium may play an active role in modulating airway inflammation and molecular approaches have begun to identify candidate genetic risk factors for diisocyanate asthma. Further studies to elucidate the mechanisms that lead to diisocyanate-induced asthma promise to yield better methods for preventive, therapeutic, and diagnostic interventions.

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