

Recent developments in diisocyanate asthma

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Despite recent advances in our understanding of diisocyanate-induced asthma, this disease remains a perplexing phenomenon. Studies reported in the past year have focused on: (i) diisocyanate antigens; (ii) the role of airway and skin epithelium; (iii) human immune responses to exposure; (iv) neurogenic pathways; and (v) genetic factors that may confer susceptibility. These studies support the hypothesis that diisocyanate asthma results from the host immune response to these chemicals, and may represent a mixed T helper type 1/2 response. A better understanding of the pathogenesis of diisocyanate asthma should facilitate the development of better diagnostic tests and strategies for disease surveillance and intervention. *Curr Opin Allergy Clin Immunol* 1:169–175. © 2001 Lippincott

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Abbreviations

HDI	hexamethylene diisocyanate
HLA	human leukocyte antigen
MDI	diphenyl-methane diisocyanate
TDI	toluene diisocyanate

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Introduction

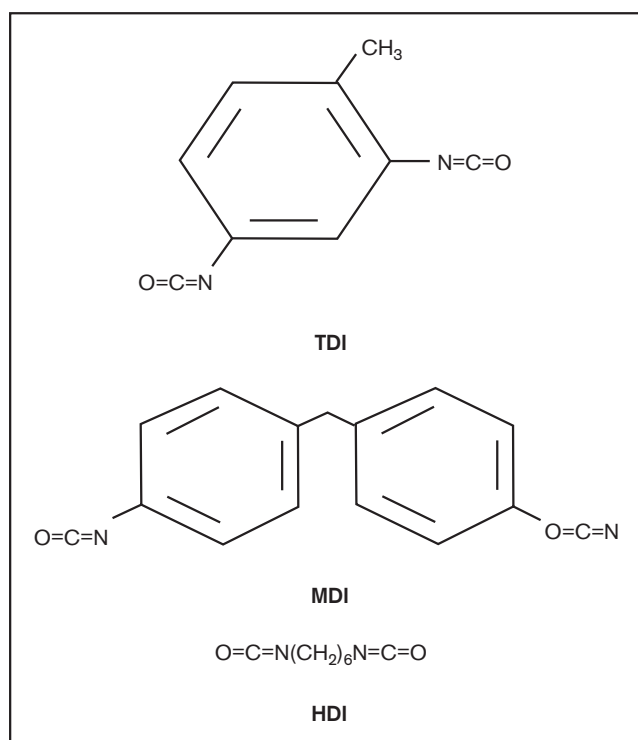
Diisocyanates are one of the most commonly identified causes of occupational asthma [1•–5•,6••,7•,8–11]. The production, use and exposure to diisocyanates have risen steadily since their commercial potential in generating polyurethane-based products was realized in the late 1930s [12]. The three most commonly used diisocyanates, toluene diisocyanate (TDI), diphenyl-methane diisocyanate (MDI) and hexamethylene diisocyanate (HDI) are shown in Fig. 1. Polymers of these common diisocyanates, with much lower vapor pressure, have been used more recently but these can also cause asthma. Estimates of the prevalence of diisocyanate asthma among exposed workers range between 5 and 15% [5•,13–15]. Early identification of diisocyanate asthma is critical because diisocyanate-induced asthma often persists even after removal from exposure, as highlighted in recent reports [7•,16–18]. Exposure is difficult to eliminate in end-use settings in which use is sporadic, widespread and difficult to control [5•,19,20]. A report this year reinforced the fact that asthma can occur even at low concentrations, emphasizing the need for early detection [4•]. The early identification of individuals with diisocyanate asthma is complicated by the lack of simple diagnostic or screening tests, uncertainty regarding risk factors, and a poor understanding of the mechanisms by which diisocyanates cause asthma [9,21–23].

Clinical similarities between diisocyanate-induced asthma and asthma caused by more common allergens, such as dust mite or cat dander, suggest that similar immunopathogenic mechanisms may be involved [24–28]. However, attempts to elucidate the pathogenesis of diisocyanate-induced asthma have been hampered by the unusual reactivity of these chemicals [29] and the limitations of the currently utilized animal models. Despite these limitations, significant insights have recently been gained through characterization of the human response to exposure [24–27]. Animal studies have provided further evidence of immune-mediated pathogenic mechanisms [30–34]. Such studies, along with our current understanding of asthma pathogenesis induced by other ‘low-molecular weight’ compounds have led to the model of diisocyanate asthma shown in Fig. 2 [28,35]. The key components of this model are discussed in this review, with particular attention to recent developments published in the past year.

Diisocyanate allergenicity

Diisocyanates differ from common non-occupational causes of asthma such as dust mite antigens or cat

Figure 1. Chemical structure of commonly used diisocyanates



TDI, Toluene diisocyanate; MDI, diphenyl-methane diisocyanate; HDI, hexamethylene diisocyanate.

dander, which are generally large protein molecules with well-described, and in some instances molecularly cloned, antigenic epitopes [28,29]. Diisocyanates are much smaller in size but possess a unique inherent ability to react directly with functional groups present on human proteins [36,37,38••]. The bivalency of diisocyanates may contribute to their allergenicity by forming 'neo-epitopes' that arise from the cross-linking of individual proteins [33]. Diisocyanates may also cross-link different proteins leading to aggregation, and thereby act as an adjuvant. Interestingly, many other low molecular weight chemicals that cause asthma are polyfunctional, including aliphatic or cyclic amines, dicarboxylic acid anhydrides and dialdehydes [39•]. Diisocyanates are also unique in that, unlike large protein allergens, they may be shuttled through the body in a reactive form via glutathione, and subsequently conjugate to proteins distant to the primary exposure site [40]. Alternatively, metabolic activation of diisocyanates could occur, as in allergic reactions to other low molecular weight compounds such as paraphenylene diamine or sulfamethoxazole [41,42]. Finally, the direct toxic effects of diisocyanates, as highlighted in recent studies [43•,44•,45,46•], may also contribute to their sensitizing potential.

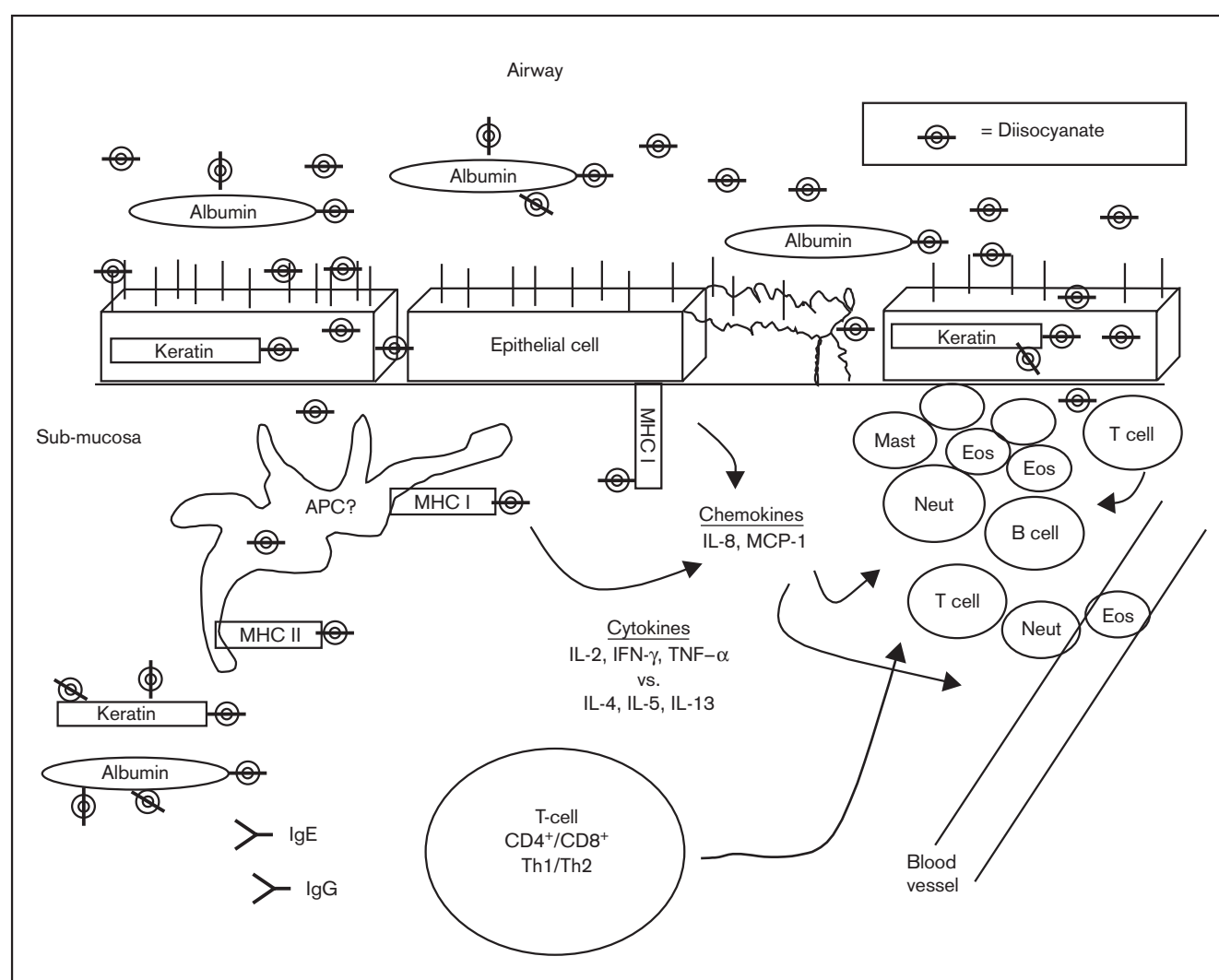
Exposure to highly reactive diisocyanates probably results in their conjugation to human proteins and the formation of several biologically important antigens *in vivo*. Albumin has been the primary protein studied as a carrier for diisocyanates in most immunological studies to date [47–49,50••]. Such studies indirectly support the potential of albumin to serve as a carrier for diisocyanate *in vivo*. De Marzo *et al.* [51•] recently demonstrated in rats that TDI may become conjugated to serum albumin after exposure and cause changes in 'acute phase response' proteins. Direct evidence for albumin conjugation with diisocyanate in the airways after inhalation exposure in humans was recently reported by Wisniewski *et al.* [38••]. In addition, other proteins in the airway and skin epithelium have been shown to become conjugated with diisocyanate after exposure *in vitro* and *in vivo*, including keratin, actin, and tubulin [38••,52]. Hemoglobin conjugation with MDI has also been reported by Sabbioni *et al.* [53•], in studies that characterized these adducts as potential biomarkers of exposure.

What forms of diisocyanates act as antigens that drive airway inflammation and hyperreactivity remains unclear. The identification and characterization of biologically relevant diisocyanate antigens is the key to understanding the pathogenesis and development of *in vitro* diagnostic assays. It is likely that a limited number of human proteins are the predominant targets of diisocyanate conjugation, on the basis of the biology of exposure and studies reported to date [37,38••,47,52]. However, it is possible that different antigenic forms of diisocyanates may predominate in different patients or exposure settings.

Diisocyanate/airway epithelium interactions

Active participation of the airway epithelium in asthma pathogenesis is an emerging concept recently reviewed by Holgate *et al.* [54••], who presented evidence supporting a dynamic interaction between airway epithelium, lung mesenchyme and the immune system. In diisocyanate-induced asthma, the interaction between diisocyanates and the human airway epithelium is probably 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 [26,37,38••,52,55••]. Lange *et al.* [55••] recently identified intracellular S-glutathionyl adducts in murine lung *in vivo* and human broncho-epithelial cells *in vitro* after exposure to TDI. In previous studies [52], the authors had demonstrated a colocalization of TDI with tubulin on the cilia of exposed differentiated human airway epithelial cells. Wisniewski *et al.* [38••] recently identified specific human airway and skin epithelial cell proteins that become conjugated with HDI *in vitro* and *in vivo*, including keratins as mentioned

Figure 2. Hypothetical model of the immunopathogenesis of diisocyanate asthma



The key aspects of the pathogenic cascade leading to the development of diisocyanate asthma are depicted on the basis of the growing body of knowledge regarding this disease. The primary event in this process is probably the conjugation of diisocyanates with normal human proteins in the airway, which include albumin and possibly other recently identified epithelial cell proteins. The antigenic epitopes resulting from the interactions of diisocyanates with these proteins and the subsequent steps leading to airway inflammation remain poorly characterized. Specific chemokines, including IL-8 and monocyte chemoattractant protein-1 (MCP-1) have been associated with diisocyanate asthma as well as neutrophil involvement. The role of diisocyanate-specific antibodies in pathogenesis remains questionable. The cell types that take up, process and present diisocyanates to the immune system and the specific T cells that may be involved are unknown. Genetic risk factors have not been extensively studied, but specific human leukocyte antigen haplotypes may predispose certain individuals. The role of diisocyanate toxicity and epithelial cell damage in the development of diisocyanate asthma remains to be evaluated. APC, Antigen-presenting cells; EOS, eosinophils; MHC, major histocompatibility complex; TNF- α , tumor necrosis factor alpha.

above. HDI-exposed airway epithelial cell protein preparations have previously been shown to specifically induce the proliferation of lymphocytes from diisocyanate asthmatic individuals [37].

Further studies on the toxicity of diisocyanates on airway epithelium have also been reported in the past year. MDI and HDI prepolymers were shown to induce airway epithelial barrier dysfunction, which was partly

associated with glutathione levels [56*,57*]. Specific effects of TDI on the expression of lung cytochrome P450 enzymes have also been documented [58*].

Such recent studies build upon previous reports of diisocyanate effects on airway epithelial cells, in particular the potentiation of cytokine production and T cell proliferation [59]. 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 respiratory tract sensitization to diisocyanates has been well-documented in animal models [60–62]. In the past year, several studies have begun to address this important issue in humans. Petsonk *et al.* [6••] recently reported an increased risk of diisocyanate asthma among MDI workers with evidence of previous skin exposure. Liu *et al.* [63•] recently demonstrated that substantial skin exposure occurs in typical end-use settings of diisocyanate, namely autobody shops. Studies by Wisniewski *et al.* [38••] demonstrating keratins as a common target for diisocyanate conjugation in both lung and skin, defined a structural link that could underlie airway sensitization via skin exposure. A novel anti-inflammatory drug, RPR 73401, was recently found to be topically effective in reducing TDI skin allergy in a murine model, further highlighting the potential role of the skin in immune sensitization to diisocyanates [64••]. Together such studies suggest that skin exposure may be an important route of diisocyanate sensitization in human workers.

Immune responses in diisocyanate asthmatic individuals

Previous studies focused on characterizing the immune response to diisocyanates and the resulting airway inflammation that occurs in diisocyanate asthmatic individuals. Additional progress has been made in the past year, including further studies on antibody responses to diisocyanate-albumin conjugates as well as the potential role of neutrophils and the chemokine IL-8 in diisocyanate asthma pathogenesis.

Airway inflammation, similar to that observed in common atopic asthma, is a prominent characteristic of diisocyanate asthma. Increased activated T cells (predominantly CD4 cells) have been described in the airways of diisocyanate asthmatic individuals, as well as increased levels of cytokines produced by T helper type 2 cells (IL-4, IL-5) [24–28]. Other immune responses reported in diisocyanate asthmatic individuals include diisocyanate-specific antibodies, lymphocyte proliferative responses or cytokine/chemokine production [6••,50••,65]. Of note is the fact that many of the studies found direct or indirect evidence of mixed T helper type 1/2-like responses to diisocyanates rather than a clear-cut T helper type 2-like response as observed for many common environmental allergens [65–67].

Diisocyanate-specific serum IgE or IgG antibodies were previously associated with diisocyanate asthma, but can also be found in some exposed non-asthmatic workers [68–70]. Two studies this year by Petsonk *et al.* [6••] and

by Park *et al.* [50••] further demonstrated that diisocyanate-specific IgE, when present, was highly predictive of diisocyanate asthma. However, many patients with diisocyanate asthma have normal total IgE levels and no detectable diisocyanate-specific IgE, prompting the notion that diisocyanate asthma pathogenesis may involve mechanisms distinct from those operative in atopic asthma [71•,72,73]. The recent study by Park *et al.* [50••] also demonstrated a strong association between TDI-specific serum IgG and TDI-induced asthma. These findings differ from previous reports on diisocyanate-exposed workers, in which the presence of diisocyanate-specific IgG correlated with exposure but not necessarily asthma [68–70,74].

Neutrophilia and IL-8 production were previously associated with diisocyanate asthma [24,65,75,76]. Studies over the past year further supported the potential role of neutrophils in the pathogenesis of diisocyanate asthma [77•]. Neutrophil activation after TDI bronchial airway challenge was specifically noted in subjects with diisocyanate asthma. Increased levels of IL-8, a key chemokine in inducing neutrophil recruitment and activation, were reported in induced sputum samples from diisocyanate asthmatic individuals compared with exposed controls. Diisocyanate-induced IL-8 production by peripheral blood mononuclear cells was previously linked to diisocyanate asthma [24,65,75,76].

Diisocyanates are thus clearly immunogenic compounds that have the capacity to elicit airway inflammation and a wide array of immune responses, including both T helper types 1 and 2, as depicted in Fig. 2. Further characterization of these immune responses in larger populations of diisocyanate asthmatic individuals and exposed asymptomatic workers should identify those components of the immune response central to pathogenesis.

Neurogenic mechanisms

Neuro-immune interactions in the lung remain incompletely defined, but were previously suggested to play a role in animal models of TDI asthma [78–80]. In the past year, Hunter *et al.* [81•] demonstrated TDI-induced increases in substance P in sensory neurons at the molecular level in a rat model. Increased messenger RNA levels for substance P were preceded by an influx of inflammatory cells, particularly neutrophils. Hunter *et al.* [81•] suggested that TDI-induced substance P production may 'result from inflammatory mediators known to enhance substance P production ... such as TNF- α ', a cytokine previously linked to diisocyanate asthma [65].

Genetic susceptibility

It is likely that specific genetic factors account for individual differences in susceptibility to diisocyanate-induced asthma. The few reports to date that have

investigated genetics markers in diisocyanate asthma have been restricted to human leukocyte antigen (HLA) II allele studies and have yielded conflicting data [82–85]. In the past year, increased frequencies of HLA DQA1 and DQB1 alleles in diisocyanate asthma were reported [86•]. The linkage of diisocyanate asthma to HLA genes has important implications for our understanding of diisocyanate asthma pathogenesis, given the well-described role of HLA gene products in antigen presentation. Further studies to identify genetic risk factors for diisocyanate asthma offer potential in screening and targeted surveillance strategies, and may help provide key insights into pathogenic mechanisms.

Conclusion

In the past year, research has added new insights into diisocyanate asthma, including: (i) the identification of diisocyanate-conjugated proteins that may act as antigens; (ii) the potential role of epithelium (both airway and skin) in sensitization and subsequent pathogenesis; (iii) immune responses that characterize diisocyanate asthmatic individuals; (iv) neurogenic pathways; and (v) genetic factors that may confer susceptibility. Together, such studies support the concept that diisocyanate asthma results from the host immune response to these chemicals, and may represent a mixed T helper type 1/2-like response.

Despite these recent advances in understanding the immunogenic pathways that lead to diisocyanate asthma, a number of key questions persist. The biologically relevant 'diisocyanate antigen(s)' that stimulates the immune system and initiates or perpetuates airway inflammation and hyperreactivity is still unclear. The specific cell types that directly respond to diisocyanates have not been fully characterized, and the role of antigen-presenting cells, including lung and skin dendritic cells, monocyte/macrophages, or possibly airway epithelial cells, is uncertain. Which T cell subsets (i.e. CD4 versus CD8, T helper types 1/2) are directly involved in pathogenesis also remains unclear. Elucidation of these critical features of diisocyanate asthma will facilitate the development of bioassays for the diagnosis of diisocyanate sensitivity and asthma, strategies for surveillance and identification of susceptible individuals, and immunotherapies.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

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- 2 Carlton GN, England EC. Exposures to 1,6-hexamethylene diisocyanate during polyurethane spray painting in the US Air Force. *Appl Occup Environ Hyg* 2000; 15:705–712.

Improved techniques to reduce worker exposures to isocyanates are given, including standing in upwind orientation, limiting paint application to one worker at a time; and limiting spray gun air cap pressures to reduce paint overspray.

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These findings suggest that workers exposed to MDI are at a higher risk of asthma in comparison with TDI-exposed workers and control subjects.

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Asthma can occur at low concentrations of isocyanates, but even at low concentrations, the higher the exposure the greater the risk. In contrast with other studies, smoking and atopy seemed to increase the odds of occupational asthma caused by isocyanates, but did not affect the estimate of risk associated with exposure.

- 5 Ott MG, Klees JE, Poche SL. Respiratory health surveillance in a toluene diisocyanate production unit, 1967–97: clinical observations and lung function analyses. *Occup Environ Med* 2000; 57:43–52.

This retrospective study of 313 workers in a TDI production unit, over a 30 year period, characterized irritant and allergic airway responses and assessed changes in forced vital capacity and forced expiratory volume in 1 s relative to exposure to TDI. Occurrences of both asthma induced by TDI and irritant airway responses caused by exposure to TDI were found in this cohort, but there was no relationship between the cumulative exposure to TDI and irreversible airflow obstruction as assessed by spirometry.

- 6 Petsonk EL, Wang ML, Lewis DM, et al. Asthma-like symptoms in wood product plant workers exposed to methylene diphenyl diisocyanate. *Chest* 2000; 118:1183–1193.

Respiratory health in workers at a new wood products manufacturing plant, designed and operated with a goal of minimizing worker exposures to MDI, was evaluated. Working around and cleaning up liquid MDI represented a significant risk for asthma-like symptoms, which were associated with specific IgE to MDI-albumin, but not with skin prick tests to common aeroallergens. The data also suggested that skin exposure to liquid MDI may pose a significant risk factor for developing MDI asthma.

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The results confirm the poor medical outcome of diisocyanate-induced asthma; the persistence of symptoms and unspecific bronchial reactivity were pronounced in TDI-induced asthma. A more favourable outcome was associated with IgE mediation and HDI inducement. However, it is possible that the presence of IgE may lead to early recognition of sensitization and hence better prognosis.

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