

# Asthma Induced by Isocyanates: a Model of IgE-independent Asthma

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**Abstract.** Developments in the understanding of causes and natural history of asthma induced by isocyanates may allow improved preventive strategies for occupational asthma (OA), and may also lead to improved understanding of mechanisms involved in IgE-independent nonoccupational asthma. Studies of genetic markers in OA induced by isocyanates suggest that HLA class II genes, glutathione S-transferase and NAT1 genotypes may predispose to development of this type of OA. Specific IgE antibodies against isocyanates are not always found in subjects with OA caused by isocyanates, leading most researchers to consider this type of OA, as a model of IgE-independent asthma. Evidence for cell-mediated immunity in OA induced by isocyanates has been provided by bronchoalveolar lavage, bronchial biopsy and induced sputum studies. The pathology of this type of asthma is similar to that of nonoccupational asthma, with cells such as eosinophils and T lymphocytes that exhibit signs of activation, and with thickening of the reticular layer of the basement membrane. Animal studies have shown that isocyanate asthma is driven primarily by CD4+ T cells and is dependent upon the expression of Th2 cytokines. However, animal models are not always reflective of human responses. OA induced by isocyanates similarly to nonoccupational asthma, is a multifactorial condition, and it is likely that complex gene-environment interactions play a role. Better understanding of these interactions is important for affected workers, and also has potential relevance for nonoccupational asthma.

**Keywords:** isocyanates, asthma, genetics, environment, exposure

## Introduction

OA has been suggested as a model of adult-onset asthma (1, 2). The wide interest is justified because OA is estimated to account for approximately 15% of the adult asthmatic population (3). In spite of the recognized harmful health effects of isocyanates, they are widely used in several industrialized countries and are the leading cause of OA. Thus, isocyanate-induced asthma is one of the most studied models of OA (4). The mechanisms by which these chemicals can induce host sensitization and asthma development remain unclear. Available information indicates that a classical IgE-dependent hypersensitivity mechanism occurs for OA induced by high-molecular-weight (HMW) agents, while for most low-molecular-weight (LMW)

agents, such as isocyanates, the presence of specific IgE antibodies has been documented in only a small subset of affected workers (5). The absence of demonstrable specific IgE antibodies in subjects with OA induced by LMW agents has led to speculation about IgE-independent immunological or even nonimmunological mechanisms (6). An area of current interest in isocyanate asthma is the possibility that this type of OA may be driven by T-cell recognition of isocyanates or isocyanate derivatives (7). To date, several lines of evidence indicate that cell-mediated immunity (8, 9) and airway epithelium (4) are involved in the pathogenesis of OA induced by exposure to isocyanates.

A number of recent publications have provided further understanding of OA induced by exposure to

isocyanates and findings will be grouped under the following headings: animal studies of sensitization and asthma, genetic studies, airway inflammation and remodeling, outcome, conclusions.

### Animal studies of sensitization and asthma

Experimental animal models of isocyanate asthma have been described in review articles (10, 11) and in several original studies (12-20). A common finding of these models is the demonstration of an immunological basis for the disease, including the involvement of T-helper type 1/2 responses as well as CD4 and CD8 T cells (21). Sensitized animals develop a specific IgG and IgE antibody response, airway hyper-responsiveness and inflammation accompanied by increased expression of cytokines, similar to human disease (22, 23). Moreover, dermal or respiratory exposure may be important in the workplace for the development of sensitization, and pulmonary pathology associated with isocyanates can vary depending upon the exposure (i.e. low-level subchronic or high-dose acute inhalation) (23). Another important finding is that sensitization can occur through subchronic inhalation of vapor-phase diisocyanate at levels as low as 20 ppb. Even if animal models are not always reflective of human responses, they provide increased understanding of sensitization to LMW agents, such as isocyanates.

### Genetic studies

The study of gene-environment interactions is facilitated in OA because it is possible to give precise occupational case definitions, to estimate exposure to occupational sensitizers, and comparisons can be made between affected workers and healthy workers with similar exposures (24).

Data obtained in studies on isocyanate asthma indicate that major histocompatibility complex class II proteins are important factors for the specificity of the response to isocyanates (25). HLA class II molecules are highly polymorphic and therefore plausible candidate genes that influence the development of a specif-

ic immunologic response. DQA1\*0104 and DQB1\*0503 confers susceptibility to asthma induced by toluene diisocyanate (TDI), whereas DQB1\*0501 offers protection (26). However, one study did not find associations between isocyanate asthma and HLA-DR or HLA-DQ alleles, but in this study, accurate phenotypes of the subjects included in the study were not provided (27). By contrast, HLA class I antigens and tumor necrosis factor  $\alpha$ -308G are not associated with either susceptibility or protection to the development of TDI-induced asthma (28).

A feature of isocyanate asthma is the development of persistent airway inflammation (29). Oxidative stress is a key component of this inflammation (30). The superfamily of glutathione-S transferase (GST) is critical for protecting cells from oxidative stress products. Individuals may differ in their ability to deal with an oxidant burden, and such differences are in part genetically determined (31).

Evidence of the involvement of GST has been provided for workers exposed to TDI for 10 years or more. The frequency of the *GSTP1 Val/Val* genotype was lower in asthmatic and hyperresponsive subjects, and the protective effect of homozygosity for the *GSTP1\*Val* allele increases in proportion to the duration of exposure to TDI (32). Therefore, subjects lacking this genotype may develop ongoing airway inflammation and consequent airway remodeling, leading to chronic asthma symptoms.

Recently, it has also been shown that the N-acetyltransferase (NAT1) slow acetylator genotype posed an increased risk of isocyanate asthma (33, 34). Any association between a genetic marker and risk of disease is relevant if findings of the study have been replicated. Asthma is a complex disease, and genetic susceptibility is one of the factors involved in this condition (35).

### Airway inflammation and remodeling

The airway inflammation process is similar in IgE-dependent and IgE-independent asthma (36). In the airways, inflammatory cells are increased in number and activated, resulting in the secretion of proinflammatory mediators and proteins (37, 38) with

harmful effects on epithelial cells. Along with these findings, the thickening of the reticular basement membrane has been described (7). In isocyanate asthma, the majority of T cells obtained from bronchial biopsy specimens of TDI asthmatics showed the CD8 phenotype and produced interferon- $\gamma$  (IFN- $\gamma$ ) and interleukin-5 (IL-5) (39).

A reasonable index of airway inflammation is eosinophilia, and eosinophil counts in sputum are useful in OA induced by isocyanates (40). However, it is still unclear whether changes in sputum eosinophil count relate to airway hyper-responsiveness. Other studies have described an increase in neutrophils in sputum of subjects with isocyanate asthma (41, 42), but their role should be further delineated. Non-invasive methods of assessing airway inflammation including analysis of induced sputum have been recently reviewed (43). The conclusions are that this non-invasive method is a valid and reproducible tool for studying airway inflammation.

It is almost universally accepted that airway inflammation is present in both OA and non-occupational asthma, whereas the role of airway remodelling in the disease remains controversial. It provides an explanation for many conditions observed in asthmatics (44). However, for some investigators, the thickening of the airway wall protects against airway narrowing and attenuates airway reactivity in subjects with asthma (45). It is also unclear whether, in adult asthma, airway inflammation occurs first and remodelling later. In isocyanate asthma, 6 to 21 months after cessation of exposure, the thickening of reticular layer of the basement membrane, even if reduced, is still present, and it is therefore a marker of long-lasting structural changes of the airway wall (46).

Available data indicate that both airway inflammation and airway remodelling are present in OA induced by isocyanates, but further research is needed to establish their role in causing obstruction, airway hyper-responsiveness and chronic asthma.

## Outcome

Numerous follow-up studies have shown that subjects with isocyanate asthma are left with perma-

nent disability, even after cessation of exposure (47). Improvement does continue over long time, although at a slower rate than in the first two years after cessation of exposure. Important determinants of improvement are the total duration of exposure, the duration of symptoms, the severity of asthma, baseline lung volumes and the degree of airway hyper-responsiveness at the time of diagnosis. Most subjects with OA retain specific bronchial reactivity to the sensitizing agent (HMW agent) even two or more years after cessation of exposure (48). Similarly, in OA induced by isocyanates, after removal from exposure, specific bronchial reactivity has been described (49, 50). One of the hypotheses for the persistence of OA after the end of exposure is the presence of airway inflammation that can be documented even years after cessation of exposure (51, 52). A recent study that involved induced sputum has shown an increase of eosinophils, neutrophils, and their product myeloperoxidase, and the neutrophil chemoattractant, IL-8, in subjects with persistent airway hyper-responsiveness many years after the last exposure to both HMW and LMW agents (53). Authors concluded that airway inflammation is the cause of persisting airway hyper-responsiveness and asthmatic symptoms. Because sputum was not examined before cessation of exposure, the starting degree of airway inflammation is lacking. The persistence of airway inflammation in the absence of re-exposure to the offending agent appears self-sustained. Recent data indicate that, in isocyanate asthma, exposure to TDI can augment cytocheratin-19 expression from the bronchial epithelial cell, which may involve immune responses as an autoantigen to induce airway inflammation in this common type of OA (54).

## Conclusions

The pathogenesis of OA induced by LMW agents remains controversial. Several lines of evidence indicate that isocyanate-induced asthma is a multifactorial disease involving genetic susceptibility, the immune system, and airway epithelium. To elucidate the mechanisms of OA induced by isocyanates, further research is needed on the molecular interactions between these chemicals and human airway proteins.

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