

Diisocyanate sensitization and antibody production

Diisocyanates are very reactive low molecular weight chemicals that are widely used in industry in many parts of the world in many different chemical compounds, especially in the manufacture of polyurethane foam and spray paints. They are the most common cause of occupational asthma (OA) in Quebec,¹ Ontario,² Great Britain,³ and the US.⁴ In a recent review of Ontario Workers' Compensation Board claims for OA, exposure to isocyanates was identified in 30% of claims submitted and in 58% of claims accepted.² Isocyanates have occasionally caused urticaria and anaphylaxis, and symptoms of rhinitis² (although not typically associated with sneezing, itching, or conjunctivitis) commonly accompany isocyanate-induced OA. Hypersensitivity pneumonitis has also been described in small numbers of patients, most commonly associated with diphenylmethane diisocyanate (MDI) exposure.⁵

Isocyanate-induced OA generally arises from sensitization to isocyanates. There is a latent period of exposure after which a sensitized individual will have asthma triggered by even extremely low exposure levels of isocyanates, often below the detection level of monitoring equipment. Less commonly, isocyanates can cause irritant-induced asthma⁶ by accidental exposure to a high airborne concentration of isocyanates (eg, from a spill of a volatile isocyanate such as toluene diisocyanate [TDI] or hexamethylene diisocyanate [HDI]). Usually these patients with irritant-induced asthma will not have an exacerbation of their asthma by subsequent low-level exposure to isocyanates and therefore can usually continue in the same occupation, but occasionally the high-level exposure will also result in sensitization.⁷

Sensitization to isocyanates does not occur to monoisocyanates (with one $-N=C=O$ group), but require at least 2 functional $-N=C=O$ groups per monomer (ie, a diisocyanate), and this sensitization is often very type specific. For example, a worker can be sensitized to TDI and not to MDI or HDI, although there have been patients reported who appear to have cross-reactivity in their response to different diisocyanates. There can be a specific inhalation response to one isomeric form (eg, 2,4-TDI and not 2,6-TDI⁸), and there can be sensitization to a prepolymer of TDI and not the monomer.⁹ Approximately 55% or fewer exposed workers become sensi-

Abbreviations used

HDI: Hexamethylene diisocyanate
HSA: Human serum albumin
MDI: Diphenylmethane diisocyanate
OA: Occupational asthma
TDI: Toluene diisocyanate

tized. These features, in addition to the latent period and subsequent response to very low exposure levels, suggest an allergic mechanism of response. However, the absence of an association with typical symptoms of allergic rhinitis and the frequent finding of an isolated late asthmatic response on history or on specific challenge testing would be unusual for an IgE-mediated response. In addition, the mean latent period before sensitization is shorter than that for IgE-mediated sensitization to high molecular weight workplace allergens.

At present, the role of an IgE-mediated allergic response in the pathogenesis of sensitization to isocyanates is unclear. Although some patients have a predominantly neutrophilic airway response, an increase in total and activated eosinophils and increased CD25⁺ cells (presumed activated T lymphocytes) have been described in bronchial biopsy specimens from both patients with isocyanate-induced OA and those with atopic asthma, suggesting some common pathogenic features.¹⁰

Nonantibody-mediated proposed mechanisms for isocyanate sensitization have been well reviewed by Fabbri et al.¹¹ They include possible neurogenic inflammatory airway mechanisms and/or antigen activation of T cells and cytokine release from CD8⁺ T cells in genetically predisposed individuals, leading to an eosinophilic airway inflammatory response by means of IL-5 production. In addition, histamine-releasing factors have been characterized by Lummus et al.¹² Although the mechanism of response is of great interest and importance, a readily measurable specific immunologic marker, if such exists, would be of most use in clinical diagnosis.

Because diisocyanates are low molecular weight molecules (except when polymerized into large molecules), they would be expected to act as haptens, requiring a carrier protein to be antigenic. Although serum IgE antibodies to conjugates of isocyanates with human serum albumin (HSA) have been identified, these have been present in a minority of patients with isocyanate-related OA (about 20%) and in exposed but asymptomatic workers.^{13,14} It has been shown that the antibodies are more commonly demonstrated to diisocyanates conjugated

with HSA than to conjugates with BSA, IgA, IgG, or polylysine.¹⁵ Animal studies, mainly in a guinea pig model, have shown the ability to produce sensitization to isocyanates by dermal contact or inhalation and an associated production of specific IgE antibodies to diisocyanates. Animal studies have also shown that the very reactive inhaled diisocyanates can become bound to airway proteins, and it is possible that the current relative lack of identified specific antibodies in human serologic studies of OA may relate in part to lack of identification of the relevant diisocyanate/carrier conjugate and neoantigen for use in testing. There are other technical difficulties in such antibody assays. The results of antibody determination by RAST with sera from sensitized patients has been shown to be highly dependent on the degree of isocyanate substitution in the conjugate. In one study workers with isocyanate-induced OA reacted optimally to conjugates bound with 10 or less isocyanate molecules per molecule of HSA.¹⁵ A recent article¹⁶ found heterogeneous binding patterns of individuals sensitized to TDI by using HSA conjugated with TDI over different preparation times, with higher binding after 5 minutes of conjugation than after 30 minutes. The authors also found that use of a monoisocyanate for conjugation was less effective than use of a diisocyanate by using sera from sensitized patients, but they found a high degree of antibody specificity for the type of isocyanate (eg, TDI vs MDI vs HDI).

Tee et al¹⁴ recently reported positive specific IgE antibodies to diisocyanates (as defined by a RAST score of at least 2) in 20% of patients being investigated for OA and in 28% of the 46 patients with a positive provocation test response to isocyanates. Increasing the positive cutoff for RAST score to 3 increased the specificity to 100%, but only 9 (20%) of the confirmed isocyanate-sensitized patients achieved this response. Of note, the authors did find cross-reactivity between RAST responses to different diisocyanates in 8 subjects, which was contrary to the findings of Wass and Belin¹⁵ and Son et al.¹⁶ Therefore the reliable performance of these assays is dependent on many variables and at present is not clinically useful.

In some studies of OA, IgG antibodies have been found to relate better than IgE antibodies to the results of specific inhalation challenge tests with isocyanates, particularly with MDI.¹³ However, there is again relatively low specificity for the finding of specific IgG antibodies to isocyanates, and it has been suggested that they are more reflective of exposure than disease.¹⁷ As might be expected, higher levels of specific IgG antibodies to isocyanates have been demonstrated in patients with hypersensitivity pneumonitis caused by isocyanates, although in addition, perhaps surprisingly, these patients also demonstrated IgE antibodies.⁵

The article in this issue by Aul et al¹⁸ addresses the isocyanate-type specificity of MDI-HSA antibodies in 4 patients with MDI-induced OA and 1 patient with MDI-induced hypersensitivity pneumonitis compared with 8 control patients sensitized to other isocyanates. They

found good specificity for MDI-HSA antibody production in MDI-sensitized patients compared with control subjects with one interesting exception—a TDI-sensitized patient who responded equally to HSA alone and MDI-HSA. Other studies have also illustrated that such a response has to be considered when interpreting results. Aul et al¹⁸ found that higher levels of IgG antibodies could be measured by using the MDI polymer (containing 50% monomer) compared with the monomer alone. These findings, although limited by the relatively small sample size, again illustrate the complexity of antibody responses to such reactive haptens and the need for more research in this area to develop clinical tests.

There is a real need for a sensitive and specific laboratory test for clinically relevant isocyanate sensitization. The diagnosis is often made circumstantially on the basis of documented asthma which appears while working with isocyanates. Serial peak expiratory flow monitoring with paired methacholine challenges at the end of a working week and after a period off work usually can provide objective documentation of OA, but if there are several potential sensitizers in the work area, they will not distinguish the specific cause.¹⁹ In addition, there can be confounding factors that need to be considered in the interpretation of these tests. Laboratory chamber challenges with isocyanates are difficult to control adequately, are time-consuming, and also may result in false-positive or false-negative responses.¹⁹ We have reported that approximately 50% of compensation claims accepted for isocyanate-induced OA did not have definitive evidence for the diagnosis.² It is likely that at least some patients with coincidental onset of asthma unrelated to isocyanates are diagnosed with OA and unnecessarily leave their job possibly without receiving adequate assessment for other asthma triggers. Thus a specific in vitro laboratory test would be extremely useful in contributing to the certainty of diagnosis.

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