

Genetic markers for occupational asthma

Key words: Occupational asthma, genetics, isocyanates, toluene diisocyanate, asthma

Occupational asthma is common and relatively undiagnosed. It is now considered the most common respiratory lung disorder in the developed world, partly because dust control measures have reduced the incidence of coal workers' pneumoconiosis and other dust-induced fibrotic conditions and partly because of the widespread use of chemicals and organic agents in the light industries that have replaced the rust belt heavy industries of the early and mid-20th century.

Of all of the chemicals associated with occupational asthma, isocyanates are by far the most frequently cited. The most common culprit is toluene diisocyanate (TDI), a key component in the manufacture of polyurethanes, which are found in adhesives, foams, and surface coatings and especially in the 2-pack paints used in vehicle repair shops and many other industrial painting applications.¹ Hexamethylene diisocyanate and diphenylmethane diisocyanate are also respiratory sensitizers, though their higher vapor point makes exposure to them easier to control, except in high-temperature applications.

A considerable effort has been made to understand the mechanisms of isocyanate asthma. In occupational asthma that is associated with high-molecular-weight allergens, specific IgE seems to be the critical element in sensitization; in contrast, it is less clear whether allergen-specific IgE really plays an important role in TDI asthma. It is certainly possible to demonstrate IgE directed against TDI-albumin conjugates in a proportion of patients with TDI asthma, but TDI-IgE is by no means universal.²⁻⁴ Indeed, a recent analysis of patients presenting to the Royal Brompton Hospital, London, over a period of 23 years suggested that TDI-IgE might be a specific but not very sensitive marker of the condition.⁵

As in other forms of asthma, inflammatory changes are found in the airways of patients with TDI asthma, along with eosinophils, T cells, and collagen deposition as well as the typical range of cytokines associated with asthma.⁶⁻⁸

Abbreviations used

GSTP1: π Class glutathione-S-transferase locus

TDI: Toluene diisocyanate

When T-cell clones were derived from the airways of patients with TDI asthma, the majority were CD8⁺ and expressed IL-5 and IFN- γ ,⁹ suggesting similarities with the T-cell response seen in patients with atopic asthma.¹⁰ However, when patients with TDI asthma underwent bronchial challenge, they showed a more marked neutrophil response⁸ than would be expected in corresponding pollen or house dust mite challenges.¹¹ Although a variety of nonallergic mechanisms have also been proposed to explain TDI asthma, these fail to account for the typical immunologic features of the condition, with its latent interval between exposure and the onset of asthma.

Clinically, isocyanate asthma remains a difficult condition to manage. There are usually difficulties in making a diagnosis, and even after the condition has been diagnosed, the prognosis is not as good as one might imagine. In terms of long-term outcome, fewer than one half of patients diagnosed with isocyanate asthma will go into full remission; of those who do not remit, one half will show partial improvement and the remainder will continue to have ongoing symptoms and/or long-term requirements for prophylactic therapy despite complete avoidance of exposure.¹² There thus appears to be a clinical spectrum: some patients show complete dependence on the inciting agent, and in others the TDI appears to trigger a condition that is subsequently independent of TDI. This clinical heterogeneity suggests that there might well be several mechanisms at work, the final outcome depending on which mechanisms are dominant in any given individual.

Several potential markers of susceptibility have been described. Specific IgE against isocyanates has been found in 0% to 50% of cases, depending on the location where the study was performed and the type of isocyanate conjugate used to detect IgE. In a recent study using 3 TDI-human serum albumin conjugates, IgE responses were found in a minority of patients but did not discriminate between those who were asthmatic and those who were exposed but asymptomatic. In contrast, TDI-IgG responses were more closely associated with the bronchoprovocation test response. By no means did all of those with positive bronchoprovocation responses show increased amounts of TDI-IgG; concentrations of TDI-albumin-specific IgG above the level seen in non-exposed subjects were found only in those with positive bronchial challenge results.¹³ TDI-specific IgG was also a predictor of poor long-term prognosis, an increased

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proportion of patients showing TDI-IgG in the group of those who did not improve in comparison with those who improved or remitted completely. Virtually none of those with TDI-albumin IgE were found in the nonimproving group,¹³ suggesting that this group of subjects belongs to the category of those in whom TDI triggers asthma but does not set in train the process that leads to ongoing TDI-independent asthma.

More recently, evidence has been presented that isocyanates might form conjugates with human lung epithelial cell proteins, 3 distinct proteins (with apparent molecular weights of 47, 71, and 91 kD) being targeted in particular.¹⁴ This work should make us question the validity of previous analyses of serologic responses to TDI, inasmuch as we do not know for certain the precise nature of the conjugates that are formed in vivo and it seems highly plausible that epithelial cell proteins might be targeted.

As well as forming conjugates with human proteins, TDI imposes an oxidative stress at the epithelial surface. There are data indicating that isocyanate exposure induces intracellular hydrogen peroxide production and ICAM-1 expression on cultured mononuclear cells¹⁵; the investigators suggested that the production of reactive oxygen species by monocytes at the site of exposure to an isocyanate might contribute to tissue damage at the site of inflammation and augment neutrophil infiltration, leading to further oxidative stress. Moreover, it is possible that this production of hydrogen peroxide upregulates ICAM-1 expression, which in turn might potentiate the infiltration and adhesion of cells at the site of exposure. These data support the concept that defects in antioxidant defenses could contribute to the susceptibility of development of TDI-induced asthma.

Several strands of evidence suggest that asthma is associated with oxidative stress, not least because of the recruitment of inflammatory cells that use oxidative killing mechanisms.¹⁶ Levels of antioxidants in epithelial lining fluid are reduced in asthma, which is consistent with the presence of oxidation but also implies a reduced resistance to further oxidative threats.¹⁷ Inability to deal with oxidative stress might lead to increased ability of the isocyanates to bind to host proteins and form hapten-carrier conjugates, whereas the oxidation might have direct effects on airways inflammation and neurogenic mechanisms that might contribute the chronicity of TDI asthma in some subjects. In other words, this oxidative capacity of isocyanates might influence both the likelihood of becoming sensitized and the likelihood that asthma will persist with continuing exposure.

In an article in this month's issue of the Journal, Mapp et al¹⁸ report on a relationship between TDI asthma and allelic variants of the glutathione-S-transferase enzyme, which serves as an important protector of cells from oxidative stress products, including lipid and DNA peroxides. The π class glutathione-S-transferase locus (*GSTP1*) gene product is thought to provide more than 90% of the glutathione-S-transferase activity in the lung,¹⁹ and *GSTP1* is also of interest because its chromosomal location at 11q13 is close to other markers associated with

asthma. Previous work from the same authors has shown an association between *GSTP1* phenotypes and the risk of allergic asthma.²⁰ Interestingly, the protective *Val*¹⁰⁵/*Val*¹⁰⁵ phenotype was also associated with a reduced risk of being atopic, suggesting that a reduced ability to cope with oxidative stress might enhance the risk of becoming sensitized as well as the risk of asthma.

The present article presents data indicating that TDI asthma is also associated with the *Ile*¹⁰⁵/*Ile*¹⁰⁵ *GSTP1* variant. The CI for the relative risk of TDI asthma was wide, and when the whole group was considered, the result was not statistically significant; however, when those with long-term exposure were considered in isolation, a 4-fold relative risk of increased airways hyperresponsiveness emerged in the TDI asthma group. This must be compared with the 9-fold reduction in relative risk of asthma associated with the *Val*¹⁰⁵/*Val*¹⁰⁵ *GSTP1* phenotype in the previous work. The present article by Mapp et al¹⁸ provides some useful support with respect to the previous observation as well as suggesting that this allelic variation might contribute to the risks of occupational asthma.

There are a few problems with the study—in particular, the relatively small size of the control sample. Given that TDI asthma is a relatively low-prevalence condition, there were enough probands (131), but the number of exposed, unaffected controls was only 39, which reduces the precision of any estimates of relative risk and makes it difficult to know whether the association seen here is the same as the risk identified in atopic asthma or whether there is an additional component relating to TDI. There are also some issues about the timing of assessment of asthma severity. Ideally, severity should have been assessed at onset or, failing that, at the time of diagnosis. In studies of occupational asthma, there are always some difficulties in this area, because it can take several years for the diagnostic penny to drop. This means that even at diagnosis these patients are heterogeneous as regards duration of disease. Those with milder disease and those whose disease remits are more likely to be lost to follow up. Severity can thus become a proxy measure of persistence. It is of course vitally important to understand the factors that determine persistence as well, and this can be assessed by addressing the association between *GSTP1* phenotype and asthma symptom severity or the degree of airway hyperresponsiveness to methacholine (as assessed after 10 years of follow-up); that question, however, is different from the matter of assessing determinants of initial severity.

Although the numbers were low and statistical significance was not attained, one aspect of the subanalysis deserves further mention. Because the frequency of the *Val*¹⁰⁵/*Val*¹⁰⁵ phenotype appeared to be lower in subjects who developed asthma after 10 years, one could argue that the critical role of the *GSTP1* phenotype is not so much in the determination of susceptibility to sensitization as in the long-term deleterious effects of asthma. It follows that exposure intensity or other forms of genetic susceptibility might be more important in those patients who develop TDI asthma after short-term exposure.

In conclusion, this interesting piece of work supports the possible role of *GSTP1* as a determinant of asthma in general as well as providing some insight into the mechanisms of TDI asthma. Occupational asthma remains a challenge for clinical scientists, both in its own right as an important clinical condition and as a model through which we might seek to understand other forms of asthma. More use should be made of this natural model, which allows us to assess aspects of asthma that are difficult to address with regard to the comparable period for the development of atopic asthma—ie, childhood. Further work in this area should focus on the development of early diagnostic markers and clarification of the mechanisms that lead to asthma persistence. Other insights might be expected to emerge as our understanding of asthma continues to evolve.

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