

# Diisocyanates, occupational asthma and IgE antibody: implications for hazard characterization

Ian Kimber<sup>a\*</sup>, Rebecca J. Dearman<sup>a</sup> and David A. Basketter<sup>b</sup>

**ABSTRACT:** Sensitization of the respiratory tract by chemicals resulting in rhinitis and asthma is an important occupational health issue. Occupational asthma is associated with significant morbidity and can be fatal. Tests for the identification and characterization of chemicals with the potential to cause sensitization of the respiratory tract are lacking. In spite of sustained interest there are no validated or widely accepted methods available, and this presents toxicologists with a considerable challenge. One important constraint on the development of appropriate testing strategies has been uncertainty and controversy about the immunological mechanisms through which chemicals may induce sensitization of the respiratory tract. By analogy with protein respiratory allergy it is legitimate to consider that IgE antibody-dependent mechanisms may play a pivotal role. However, although many aspects of chemical respiratory allergy are consistent with reactions caused by IgE antibody, uncertainty remains because among patients with occupational asthma caused by chemical respiratory allergens there are commonly a proportion, and sometimes a significant proportion, of subjects that lack detectable IgE antibody. Here we consider the relevance of IgE antibody responses for the development of a chemical respiratory allergy to diisocyanates. A case is made that IgE antibody responses are, either directly or indirectly, closely associated with occupational asthma to the diisocyanates (and to other chemical respiratory allergens). As such the argument is advanced here that IgE antibody represents an appropriate readout for the characterization of chemical respiratory allergens, and that uncertainty about mode of action should no longer represent a hurdle in the development of suitable test methods. Copyright © 2014 John Wiley & Sons, Ltd.

**Keywords:** occupational asthma; chemical respiratory allergy; IgE antibody; hazard characterization; diisocyanate

## Introduction

Chemical respiratory allergy associated with occupational asthma is an important health issue commonly characterized by considerable morbidity (Ross and McDonald, 1996; Mapp *et al.*, 2005a; Bakerly *et al.*, 2008; Kenyon *et al.*, 2012). Compared with known contact allergens far fewer chemicals have been implicated as being respiratory allergens, among that number being diisocyanates, acid anhydrides, certain chloroplatinate salts, and some reactive dyes (Baur, 2013; Baur and Bakehe, 2014).

From a toxicological perspective chemical respiratory allergy presents a number of significant challenges, and these have been the subject of previous commentaries and reviews (Kimber and Dearman, 2002; Holsapple *et al.*, 2006; Isola *et al.*, 2008; Boverhof *et al.*, 2008; Basketter and Kimber, 2011). Perhaps the most significant issue is uncertainty regarding the mechanisms through which sensitization of the respiratory tract to chemicals is achieved, and the impact that this uncertainty has had on the development of methods for the identification and characterization of chemical respiratory allergens.

It is still the case that, in spite of a prolonged research investment, together with the use of a variety of *in vivo* models for research purposes, there are still not available validated methods, or even widely accepted methods, for hazard identification (Kimber *et al.*, 2014). The absence of appropriate strategies for evaluation of respiratory sensitization potential is a major constraint on the development of effective risk assessments and risk management measures, and on addressing satisfactorily the

requirements of regulations such as the Regulation, Evaluation, Authorisation and restriction of CHemicals (REACH) (REACH, 2006). There is an increasingly important need, therefore, to seek new approaches to toxicity testing. However, that is likely to be achieved only when there is a clearer view on the critical events and immunological pathways that are required for the acquisition of sensitization.

A significant hurdle, and arguably the most important hurdle, to achieving this remains uncertainty concerning the role played by IgE antibody. It is, of course, entirely legitimate to consider IgE antibody as being a relevant effector mechanism. The pivotal role played by IgE in allergic responses to proteins, and in allergic asthma, is well established. Moreover, there is available evidence that exposure to chemicals that are known to cause respiratory allergy can induce detectable levels of serum IgE antibody. Thus, with all, or certainly almost all, in chemicals which have been shown to cause respiratory sensitization there are at least some symptomatic patients that have specific IgE

\*Correspondence to: Ian Kimber, Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, Manchester M13 9PT, UK.  
Email: ian.kimber@manchester.ac.uk

<sup>a</sup>Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, Manchester M13 9PT, UK

<sup>b</sup>DABMEB Consultancy Ltd, Normans Road, Sharnbrook, Bedfordshire, UK

antibody. However, although this confirms that, in principle, exposure to chemical respiratory allergens is able to provoke in humans the class of adaptive immune response required for the elaboration of IgE antibodies, the fact remains that there have been many patients with occupational asthma in whom IgE antibody has not been found. The correlation of IgE with clinical disease varies between chemical allergens, or classes of chemical allergens. In some instances, for example with the acid anhydrides, there has often been reported a fairly close association with IgE antibody. Examples are provided by tetrachlorophthalic anhydride (Howe *et al.*, 1983) and hexahydrophthalic anhydride (Nielsen *et al.*, 1994). The same is true for sensitization to platinum salts (Murdoch *et al.*, 1986). The picture is, however, very different with the diisocyanates. Here it is commonly the case that only a fraction, and sometimes only a small fraction, of symptomatic subjects have detectable IgE antibody (Cartier *et al.*, 1989; Vandenplas *et al.*, 1993; Cullinan, 1998; Tee *et al.*, 1998; Tarlo, 1999; Ott *et al.*, 2007; Pronk *et al.*, 2007; Hur *et al.*, 2008). As a result, it has been proposed that occupational asthma to diisocyanates is an IgE-independent disease (Mapp *et al.*, 2005b; Jones *et al.*, 2006).

It is this uncertainty about the role of IgE antibody in chemical respiratory allergy specifically, and about the important pathogenic mechanisms generally, that have made it difficult to reach agreement on relevant readouts for predictive test methods.

The purpose of this article is to examine whether a case can be made for: (a) IgE antibody being more commonly associated with occupational asthma than is sometimes assumed, and (b) irrespective of the strength of that association, whether IgE antibody responses are a relevant endpoint for the purposes of hazard identification.

To address those questions attention will focus here on diisocyanate occupational asthma where there has been greatest doubt about the requirement for IgE antibody.

## Diisocyanates

The diisocyanates have a long history of use in diverse industrial applications. Among those that have been used most commonly are 2,4- and 2,6-toluene diisocyanate (TDI), hexamethylene diisocyanate (HDI), diphenylmethane diisocyanate (MDI), isophorone diisocyanate (IPDI) and naphthalene diisocyanate (NDI). The first cases of occupational asthma to TDI were reported in the 1950s (Fuchs and Valade, 1951; Woodbury, 1956), and it is now well established that TDI and other diisocyanates are important respiratory allergens (Mapp *et al.*, 1988; Cartier *et al.*, 1989; Bakerly *et al.*, 2008).

As indicated above, it is with diisocyanate asthma that it has proven most difficult to discern a relationship between symptoms of respiratory hypersensitivity and the presence of specific IgE antibody (Cartier *et al.*, 1989; Vandenplas *et al.*, 1993; Cullinan, 1998; Tee *et al.*, 1998; Tarlo, 1999; Ott *et al.*, 2007; Pronk *et al.*, 2007; Hur *et al.*, 2008). The issue that needs to be addressed is whether the extent of an association between IgE antibody and diisocyanate asthma has previously been underestimated. The related question is of course: if, in at least a proportion of cases, sensitization of the respiratory tract to chemicals can be acquired in the absence of an IgE antibody response, then what are the relevant immunological mechanisms through which this is achieved?

## Diisocyanate Allergy and IgE Antibody

It has been argued previously that, for a number of reasons, the association between IgE antibody and chemical respiratory allergy is stronger, and possibly considerably stronger, than is often suggested (Kimber and Dearman, 2002). There are an increasing number of lines of evidence to support this position, and these are summarized below.

### (a) IgE antibody has high specificity for diisocyanate asthma

Although IgE antibody has low predictive sensitivity for isocyanate asthma, it is clear that there is a very high level of predictive specificity. Most commentators agree that serum IgE antibody is highly predictive of occupational asthma (Cartier *et al.*, 1989; Tee *et al.*, 1998; Malo *et al.*, 2006; Wisnewski, 2007; Ott *et al.*, 2007; Budnik *et al.*, 2013). It can be concluded, therefore, that the diisocyanates as a class do have the inherent potential to provoke the class of adaptive immune response required to initiate an IgE antibody response, and that when detectable, IgE antibody is very closely associated with chemical respiratory allergy (Sastre *et al.*, 2003).

### (b) Difficulty of detecting hapten-specific IgE antibody

There is a case to be made that the failure to demonstrate a close relationship between occupational asthma and hapten-specific IgE antibody is due, in large part, to the technical and logistical problems associated with detecting the latter (Kimber and Dearman, 2002). One important factor is that it has proven difficult to engineer in a reliable and reproducible way that hapten-protein conjugates can be used successfully as substrates for the detection of specific IgE antibody. In this context it is known that the characteristics of such conjugates are dependent upon the reaction conditions employed, and that there is a need for rigorous standards and controls. There is a similar requirement for optimization and standardization of the configuration of the immunoassays in which such conjugates are used (Kimber *et al.*, 1998; Wisnewski *et al.*, 2004; Wisnewski, 2007; Campo *et al.*, 2007; Budnik *et al.*, 2013). Ott *et al.* (2007) have reviewed these issues in some detail and have identified, and listed in tabular form, a number of important factors relating to both conjugate preparation and assay standardization that need to be addressed for improved detection of diisocyanate-induced antibodies. Among the key considerations for effective conjugate production are: the molar ratio of the hapten-protein reaction mixture, the choice of carrier protein, and characterization of the conjugate (Ott *et al.*, 2007). The conclusion drawn is that the measurement of chemical allergen-specific IgE antibody is problematic, of variable effectiveness and frequently unreliable. The implication is that the presence of plasma IgE antibody may commonly be missed through reliance on inappropriate hapten-protein conjugates and/or their use in incorrectly configured assay systems.

The other factor that influences the detectability of IgE antibody is persistence. Although IgE antibody bound to mast cells has a much longer half-life, the half-life of unbound plasma IgE is approximately 2 days (Hellman, 2007). It is not surprising, therefore, that the detection of IgE antibody becomes less successful as the period from last exposure increases (Tee *et al.*, 1998). The relationship between exposure and identifiable IgE antibody may be complicated further as contact with the chemical allergen is likely to be sporadic and limited to the work place

(Pronk *et al.*, 2007). The view stated by Wisnewski (2007) is that 'without accurate exposure information, negative isocyanate-specific IgE assays may lead to misdiagnosis and false conclusions about pathogenic mechanisms'. Taken together the available evidence suggests that the measurement of IgE antibody to chemical respiratory allergens is uncertain and unreliable, and that the correlation between IgE and disease is probably considerably stronger than the literature suggests. In fact, a case can be made that, given the difficulties outlined above, there may exist a mandatory universal role for the IgE antibody in the development of sensitization of the respiratory tract to chemical allergens.

(c) *Immune responses to chemical respiratory allergens in experimental animals*

It is well established that the production of IgE antibody requires the induction of T helper (Th)2 cells; these cells favouring the induction of IgE responses through the elaboration of interleukin 4 (IL-4) and other cytokines (Kimber and Dearman, 1997). Experimental studies have revealed that diisocyanates, and other chemicals known to cause respiratory allergy in humans, are able to elicit in mice preferential Th2-type immune responses associated with increases in the total serum concentration of IgE (Hilton *et al.*, 1996; Dearman *et al.*, 2003; Kimber *et al.*, 2011). It must be acknowledged that experience with chemical respiratory allergens and Th2-type immune responses in experimental animal systems has been somewhat variable. Nevertheless, the available data suggest that in many circumstances exposure of experimental animals to chemical respiratory allergens is associated with preferential Th2-type responses (Van Och *et al.*, 2002; Herrick *et al.*, 2003; Goutet *et al.*, 2005; Farraj *et al.*, 2006; Ku *et al.*, 2008; De Jong *et al.*, 2009; Kimber *et al.*, 2011; Vandebriel *et al.*, 2011).

(d) *Selective type 2 responses in humans*

There is limited information available on the phenotype of T lymphocyte responses and diisocyanate asthma (and occupational asthma associated with other chemical respiratory allergens). Nevertheless, what data are available commonly point to the involvement of a predominantly CD4<sup>+</sup> T lymphocyte response that is characterized by Th2-type cytokine production (Bentley *et al.*, 1992; Saetta *et al.*, 1992; Redlich *et al.*, 1997; Wills-Karp, 1999; Liu and Wisnewski, 2003). A second independent line of evidence suggestive of the selective elicitation by diisocyanates of selective Th2-type responses in humans has been published recently. Ouyang *et al.* (2013) reported that the expression of interferon- $\gamma$  (IFN- $\gamma$ ) may be down-regulated, via epigenetic mechanisms, in subjects with occupational asthma. This cytokine, a product of Th1 cells, is known to antagonize the development of Th2 responses and IgE antibody production. It was found that in patients with confirmed diisocyanate asthma the promoter region for IFN- $\gamma$  was hypermethylated, consistent with a reduced expression of this cytokine, and a consequential facilitation of Th2-type responses.

However, support for the proposal that chemical respiratory allergens preferentially initiate selective Th2-type responses must be tempered by the report of Jones *et al.* (2006) who found that there was an absence of mRNA for both IL-4 and IgE (transcripts for the epsilon constant region of IgE; C $\epsilon$ ) in bronchial biopsies of patients after positive inhalation challenge with diisocyanates.

The reason for the failure to detect these mRNA species is not clear, but it is likely that kinetics – and the timing of analyses – may have a decisive impact on the success or failure of identification of mRNA for IL-4 and C $\epsilon$  (Jones *et al.*, 2006; Maestrelli *et al.*, 1997; Wisnewski and Jones, 2010).

## Concluding Comments

The evidence summarized above suggests that, in spite of the difficulty in identifying allergen-specific antibody in symptomatic patients, there may be a close and causal relationship between specific IgE antibody and chemical respiratory allergy. Indeed, it is possible that there is a mandatory requirement for IgE antibody in respiratory sensitization to chemical allergens. If that is the case then there is no doubt that the elaboration of IgE antibody responses would be an entirely legitimate endpoint for the identification of chemical respiratory allergens. Moreover, if sensitization to chemical respiratory allergens is IgE antibody-dependent then, in theory at least, a quantitative relationship can be inferred in which case evaluation of induced IgE antibody responses would also inform considerations of relative respiratory sensitizing potency.

If the argument were to be entertained that in at least some instances the development of chemical respiratory allergy can proceed in the absence of IgE antibody then it is necessary to seek an alternative immunological mechanism through which sensitization is acquired. It has been proposed that cellular immune mechanisms may be responsible. However, even if that were the case, then those cellular mechanisms would appear to be Th2-like in nature. As IgE antibody is clearly a consequence of the development of a selective Th2 response then even if sensitization could be acquired in some instances by the activity of Th2 cells alone, then IgE antibody might still provide a relevant read-out for the identification of chemical respiratory allergens in experimental systems.

The proposition advanced here is that IgE antibody provides an appropriate basis for the identification and characterization of chemical respiratory allergens, and that uncertainty about the mechanisms through which sensitization of the respiratory tract to chemicals is acquired should no longer represent a barrier to the development of appropriate predictive test methods.

## Conflict of Interest

The Authors did not report any conflict of interest.

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