Toxicology 318 (2014) 32–39

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Review

Chemical respiratory allergy: Reverse engineering an adverse outcome pathway

Ian Kimber[a,*], Rebecca J. Dearman[a], David A. Basketter[b,*], Darrell R. Boverhof[c]

[a] Faculty of Life Sciences, University of Manchester, Manchester, UK
[b] DABMEB Consultancy Ltd, Sharnbrook, Bedfordshire, UK
[c] Toxicology and Environmental Research & Consulting, The Dow Chemical Company, Midland, MI, USA

ARTICLE INFO

Article history:
Received 6 January 2014
Received in revised form 30 January 2014
Accepted 4 February 2014
Available online 15 February 2014

Keywords:
Sensitisation of the respiratory tract
Respiratory allergy
Occupational asthma
Adverse outcome pathway

ABSTRACT

Allergic sensitisation of the respiratory tract by chemicals is associated with rhinitis and asthma and remains an important occupational health issue. Although less than 80 chemicals have been confirmed as respiratory allergens the adverse health effects can be serious, and in rare instances can be fatal, and there are, in addition, related socioeconomic issues. The challenges that chemical respiratory allergy pose for toxicologists are substantial. No validated methods are available for hazard identification and characterisation, and this is due in large part to the fact that there remains considerable uncertainty and debate about the mechanisms through which sensitisation of the respiratory tract is acquired. Despite that uncertainty, there is a need to establish some common understanding of the key events and processes that are involved in respiratory sensitisation to chemicals and that might in turn provide the foundations for novel approaches to safety assessment. In recent years the concept of adverse outcome pathways (AOP) has gained some considerable interest among the toxicology community as a basis for outlining the key steps leading to an adverse health outcome, while also providing a framework for focusing future research, and for developing alternative paradigms for hazard characterisation.

Here we explore application of the same general principles to an examination of the induction by chemicals of respiratory sensitisation. In this instance, however, we have chosen to adopt a reverse engineering approach and to model a possible AOP for chemical respiratory allergy working backwards from the elicitation of adverse health effects to the cellular and molecular mechanisms that are implicated in the acquisition of sensitisation.

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1. Introduction

Chemical respiratory allergy is an important occupational health problem, with serious clinical consequences (Ross and McDonald, 1996; Mapp et al., 2005; Diar Bakerly et al., 2008). Less than 80 chemicals have been shown to cause respiratory allergy in humans

* Corresponding author. Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, Manchester M13 9PT, UK.
E-mail address: ian.kimber@manchester.ac.uk (I. Kimber).

http://dx.doi.org/10.1016/j.tox.2014.02.001
0300-483X/

linked with occupational asthma and rhinitis. Lists are available elsewhere, but among the classes most commonly implicated are the diisocyanates, acid anhydrides, chloroplatinate salts and reactive dyes (Kimber and Dearman, 1997; Baur, 2013).

The challenges for toxicologists in identifying and characterising hazards and assessing health risks for respiratory allergy caused by chemicals are substantial. The situation is very different to that of skin sensitisation for which validated methods for toxicological evaluation are available, together with well established paradigms for risk assessment. No validated methods exist for chemical respiratory allergy. The approaches that have been proposed, or are being explored currently, may have some utility but are far from validation and adoption (Holsapple et al., 2006; Kimber et al., 2007; Boverhof et al., 2008; Isola et al., 2008; Basketter and Kimber, 2011).

The difficulty in developing widely accepted methods for assessment of chemical respiratory allergy have been compounded by two issues.

The first of these is the considerable uncertainty that exists regarding the mechanisms through which chemicals are able to induce allergic sensitisation of the respiratory tract. Respiratory allergy to proteins is associated with, and driven by, specific IgE antibody, and on that basis it would appear reasonable to suppose that the same or a similar mechanism might be relevant for respiratory sensitisation to chemicals. However, although IgE antibody has been associated with many, if not all, chemicals that are known to cause allergic sensitisation of the respiratory tract, the correlation with symptoms is not always strong. In the case of acid anhydrides there has often been found a close association between symptoms and IgE antibody. An example is provided by tetrachlorophthalic anhydride (TCPA) (Howe et al., 1983). However, with some other allergens, and in particular in the case of diisocyanates, only a fraction of symptomatic patients have detectable levels of serum IgE antibody (Cartier et al., 1989; Vandenplas et al., 1993; Cullinan, 1998; Kimber et al., 1998, 2011; Tee et al., 1998; Tarlo, 1999; Kimber and Dearman, 2002a). For instance, it was reported in one study that less than 30% of patients with occupational asthma, and with a positive provocation test, displayed detectable IgE antibody levels (Tee et al., 1998). It can be argued that, for various reasons, the level of association between symptoms of occupational asthma and IgE antibody is much closer than is presently appreciated. For instance, there are significant technical challenges in measuring IgE antibodies specific for a chemical allergen, not least because there is a need to engineer appropriate hapten–protein conjugates for use as substrates in analytical systems (Kimber and Dearman, 2002a). For other reasons as well a case can be made for IgE antibody having a more important role in the acquisition of respiratory sensitisation to chemicals than is generally accepted. However, even if that is the case, the fact remains that an inability to confirm an association with IgE antibody means that there is still doubt as to whether IgE plays a mandatory or universal role in respiratory allergy and occupational asthma (Kimber and Dearman, 2002a, 2005). The inference is, of course, that there may therefore exist immunological mechanisms, other than those that rely on the elaboration of specific IgE antibody, that are able to cause the effective acquisition of respiratory sensitisation. However, there is no consensus on what those other mechanisms are, although by definition the acquisition of allergic sensitisation requires the elicitation of an adaptive immune response of some sort. This is a point that will be returned to later and discussed in greater detail.

The second issue that has contributed to the difficulty of developing methods for assessment of chemical respiratory allergens involves consideration of relevant routes of exposure. It has commonly been assumed that sensitisation of the respiratory tract to chemicals will be acquired solely by inhalation exposure. However, there is reason to be believe, from studies in experimental animals and from clinical experience, that effective sensitisation of the respiratory tract can also be achieved via skin exposure to the relevant chemical allergen (Karol et al., 1988; Botham et al., 1989; Rattray et al., 1994; Kimber and Dearman, 2002a; Tarlo and Malo, 2006; Bello et al., 2007; Redich and Herrick, 2008; Redlich, 2010). This makes sound immunological sense; there is no reason why the skin should not support the quality of immune response required to result in sensitisation of the respiratory tract. In this context it is important to appreciate that the skin is known to be a very effective route for the induction of immune responses to a range of antigens and allergens, and in fact in many instances exposure via the skin may result in more vigorous responses than those from inhalation exposure (Rattray et al., 1994). Acknowledgement that sensitisation of the respiratory tract to chemicals can be achieved via skin exposure has important practical and theoretical implications.

From a practical perspective the ability of skin exposure to support respiratory sensitisation indicates that this is a legitimate route for delivery of test chemicals in assays designed to assess the ability of materials to cause sensitisation of the respiratory tract.

The theoretical implication of the skin being an effective route for sensitisation of the respiratory tract is that whatever mechanisms are involved in the acquisition of sensitisation they must be available both in the skin and in the airways. That is, it is not possible to invoke as potential mechanisms for respiratory sensitisation those that rely on cells, molecules, or tissue microenvironments that are only available in the airways.

Against that background the aim of this article is to describe briefly one potential pathway that may explain the basis for the development of chemical respiratory allergy. This is not an exhaustive or systematic survey of the available literature, but rather an attempt to provide one perspective on the events and processes that may cause sensitisation of the respiratory tract by chemical allergens. For this purpose we have worked backwards from a consideration of the elicitation of adverse reactions in the respiratory tract to the events that cause immunological priming, and from there to speculate how chemical respiratory allergens may be recognised and then processed and presented to the immune system. Working backwards in this way from the adverse health outcome this exercise is, in effect, a reverse engineered adverse outcome pathway (AOP). In 2012 the Organisation for Economic Cooperation and Development (OECD) launched a programme to develop AOPs as analytical constructs that describe series of linked events that are causally related and that lead to an adverse health/environmental effect (Ankley et al., 2010; Vinken, 2013). The general trajectory of an AOP is from a molecular initiating event (MIE), through increasing levels of biological organisation and complexity, to the development of an adverse health effect (outcome) at the tissue and/or organismal level. Using this approach an AOP for skin sensitisation has been developed recently (Organisation for Economic Cooperation and Development (OECD), 2012). Adopting the approach of attempting to define one such pathway, but starting from the adverse outcome, then the first issue that has to be addressed is the nature of the mechanism(s) that cause the symptoms of respiratory allergy and occupational asthma.

2. The elicitation of chemical respiratory allergy/occupational asthma

The task here is to explore how occupational asthma associated with chemical sensitisation might be elicited. There is probably a general consensus that in the sensitised subject respiratory allergic reactions will be provoked following inhalation exposure to a sufficient amount of the inducing chemical allergen in an appropriate form. Where there is considerable uncertainty is regarding the nature of the immunological effector mechanism(s) that trigger such reactions.

It is appropriate here to make the point that, in general, sensitising chemicals differ with respect to the form that allergic reactions will take in affected subjects. There are many hundreds, and perhaps thousands, of chemicals that are known to cause skin sensitisation and allergic contact dermatitis (ACD) (De Groot, 2008). The majority of those chemicals have never been implicated as causes of respiratory allergy or asthma. It is known that skin sensitisation is associated with the development of specific T cell responses, and that ACD is elicited by effector T cells, primarily $CD4^+$ T helper 1 (Th1) cells and $CD8^+$ T cytotoxic (Tc) cells (Kimber and Dearman, 2002b). In contrast, far fewer sensitising chemicals have been shown to cause respiratory allergy, and many of these chemicals are not, or are only rarely, associated with ACD (Baur, 2013). As argued above, those differences are not simply the result of the route of exposure to the inducing chemical through which sensitisation is acquired as it is now believed that effective sensitisation of the respiratory tract can be achieved via skin contact with a relevant chemical allergen (Karol et al., 1988; Botham et al., 1989; Rattray et al., 1994; Kimber and Dearman, 2002a; Tarlo and Malo, 2006; Bello et al., 2007; Redich and Herrick, 2008; Redlich, 2010). It has to be acknowledged, therefore, that contact allergens and chemical respiratory allergens are, to a large extent, discrete classes. Nevertheless, it also has to be acknowledged that in certain circumstances, and maybe in different subjects, some chemicals may be able to elicit either form of allergic sensitisation. In this context it is relevant that in a recent investigation it was found that the quality of immune response elicited in humans by 2,4-dinitrochlorobenzene (DNCB; a potent contact allergen) is influenced by the immunological phenotype of the subject (Newell et al., 2013). However, it is clear that sensitisation induced by exposure to chemical respiratory allergens must take a form that is different from that caused by contact allergens, and an immunological mechanism other than one driven by Th1/Tc effector T lymphocytes specific for the inducing allergen must be sought.

Respiratory allergy to proteins and atopic asthma are dependent upon specific IgE antibodies. During the process of sensitisation a susceptible subject will mount an IgE antibody response against the inducing protein allergen. These antibodies distribute systemically and associate with tissue mast cells, including mast cells in the respiratory tract. Following subsequent inhalation exposure of the sensitised subject to the same allergen the tissue-bound mast cells will be cross-linked causing cellular activation and degranulation resulting in the release of preformed and newly-synthesised inflammatory mediators (including vasoactive amines and leukotrienes). These mediators then act in concert to drive the inflammatory reactions that can result in asthma. Thus, it is not unreasonable to question whether IgE antibodies play a similar role in chemical respiratory allergy.

It is a legitimate question to ask because chemical respiratory allergens are able to induce IgE antibody responses in exposed individuals. In fact, as indicated above, it is probably the case that with all chemical respiratory allergens at least some (and sometimes many) symptomatic subjects have been shown to have detectable IgE antibody specific for the inducing chemical. The difficulty is that for some chemical respiratory allergens, and in particular with the diisocyanates, only a fraction of patients have detectable levels of IgE (Cartier et al., 1989; Vandenplas et al., 1993; Cullinan, 1998; Kimber et al., 1998, 2011; Tee et al., 1998; Tarlo, 1999; Kimber and Dearman, 2002a; Sastre et al., 2003; Jones et al., 2006).

A case can be made that the relationship between chemical respiratory allergy/occupational asthma and IgE antibody is much closer than is generally acknowledged (Kimber and Dearman, 2002a). This is built largely on the fact that it is very difficult technically to identify chemical-specific plasma IgE antibodies using conventional methods. There is a need to prepare protein–hapten conjugates for use as substrates in analytical systems and it is widely appreciated that such conjugates can be very variable and problematic (Campo et al., 2007). However, in view of the fact that it is very commonly reported that in some symptomatic subjects IgE antibody cannot be found (Sastre et al., 2003), it is necessary to accept the possibility that another immunological effector mechanism is at play, at least in those patients that lack detectable IgE antibody (Jones et al., 2006).

What that IgE-independent effector mechanism may be is not clear. The supposition must be that disease progression is based on T lymphocyte function, and although of course there is evidence that occupational asthma is associated with T cell responses this does not necessarily pinpoint the relevant effector mechanism (Bentley et al., 1992; Mamessier et al., 2007).

For the purposes of this exercise it is proposed here that, in absence of definitive characterisation of relevant effector populations, the development of chemical respiratory allergy and the elicitation of occupational asthma is associated with, and probably dependent upon, the induction of selective Th2 cell responses, and the elaboration of type 2 cytokines such as interleukins (IL) 4, 5 and 13. The argument is that a selective Th2 response is required for the elaboration of IgE antibody responses, but even in those instances where (for whatever reason) IgE may not be induced Th2 responses still support the development of allergic reactions in the respiratory tract. The attraction of this proposal is that a mechanism based on preferential Th2-type responses is consistent with both IgE-dependent and IgE-independent sensitisation.

There are several lines of evidence that support a role for selective Th2 responses in chemical respiratory allergy. These can be summarised as follows:

- Although it is argued that there is not a mandatory universal role for IgE antibody in sensitisation of the respiratory tract to chemicals, the fact remains that in many symptomatic patients there is detectable IgE antibody; an observation that suggests chemical respiratory allergens are able in humans to provoke preferential Th2 responses (Sastre et al., 2003).
- It has been reported recently that in patients with occupational asthma to diisocyanates, the interferon γ (IFN-γ) gene promoter is hypermethylated. A reduced potential to express IFN-γ is consistent with the development of preferential Th2 responses (Ouyang et al., 2013).
- In some rodent models it has been found that exposure to chemical respiratory allergens can elicit selective Th2-type responses, whereas in contrast, contact allergens are associated with preferential Th1/Tc1 responses (Kimber and Dearman, 1995; Vento et al., 1996; Vandebriel et al., 2000; Dearman and Kimber, 2001; Van Och et al., 2002; Holsapple et al., 2006; Kimber et al., 2007; Boverhof et al., 2008).

In conclusion, therefore, it is clear that there remains uncertainty about the immune effector mechanisms that drive respiratory allergic reactions to chemicals. This uncertainty derives from the failure to demonstrate a consistent association between IgE antibody and occupational asthma. Although, this 'failure' might actually be due to technical difficulties rather than a true absence of specific IgE antibody, it is necessary to entertain the possibility of effector mechanisms that do not rely on IgE production. It is argued here that a strong candidate for this role would be Th2 lymphocytes. This is because not only is there evidence from studies in both rodents and humans that chemical respiratory allergens elicit selective Th2-type responses, but also since such a mechanism would also accommodate the fact that in many instances symptomatic patients do display detectable IgE antibody.

It is, now necessary therefore to explore what mechanism will support the development of selective Th2 responses to chemical respiratory allergens.

3. The cellular basis for selective Th2 responses to chemical respiratory allergens

It is now very well established that there is a variety of important functional subsets of $CD4^+$ T lymphocytes, including Th1, Th2 and Th17 cells and Tregs (Romagnani et al., 2004; O'Shea and Paul, 2010; Geginat et al., 2013). Collectively these cells, together with subpopulations of $CD8^+$ T lymphocytes, have responsibility for regulating immune responses; determining the quality of response and preventing unwanted, or unnecessarily aggressive, responses that might result in adverse effects. The role of Th subsets in shaping the quality of adaptive immune responses is of considerable importance, allowing the immune system to tailor responses such that particular antigenic challenges can be addressed effectively. The nature of the immune response that is required for effective host resistance to a viral challenge is very different from that needed to combat a multicellular parasitic infection. To accommodate this diversity of challenges the immune system is able to craft variable Th cell responses. With regard to chemical allergy it has been argued that contact allergens and chemical respiratory allergens are being 'read' in different ways by the immune system such that the former provoke selective Th1 responses, and the latter preferential Th2-type responses (Kimber et al., 2011). The question that flows from this is why these classes of chemical allergens induce different qualities of T lymphocyte response resulting, ultimately, in different forms of allergic reaction.

Despite the recognition that selective T lymphocyte responses are commonly induced in response to antigen, there is no clear consensus view on what the master switch is that determines which functional subset(s) of T lymphocytes will be favoured.

Nevertheless, it is safe to assume that dendritic cells (DC) will be influential in guiding the development of qualitatively discrete immune responses. These cells were characterised originally in terms of their potent ability to trigger the antigen-driven activation of naïve T lymphocytes (Banchereau and Steinman, 1998). It has since been established that, in addition to antigen presentation, DC are also important orchestrators of immune function exerting both promotional and regulatory influences. Moreover, it is now clear that there exists a variety of phenotypic subsets of DC, and that these cells also display functional plasticity (Liu et al., 2001; Murphy, 2013).

It is known that DC are able to initiate Th2 responses, and there is evidence also that discrete specialised subsets of DC are able to preferentially drive the development of Th2-type responses (Stumbles et al., 1998; Pulendran et al., 1999; Maldonado-Lopez et al., 1999; Liu et al., 2001; Klechesvsky et al., 2008). Although there may be discrete subpopulations that naturally favour the evolution of Th2 responses, the wider family of DC show considerable plasticity and there are a number of factors that can entrain DC to elicit Th2 responses. Among such are Toll-like receptor (TLR) ligands and other pathogen-associated molecular patterns (PAMPs), allergic mediators such as histamine and thymic stromal lymphopoietin (TSLP), and reactive oxygen species (ROS) (D'Ostiani et al., 2000; Whelan et al., 2000; Caron et al., 2001; De Jong et al., 2002; Edwards et al., 2002; Soumelis et al., 2002; Tang et al., 2010; Bell et al., 2013). Moreover, it has been shown that some protein antigens themselves, or materials with which they are associated, may have intrinsic properties that favour Th2 responses (Ghaemmaghami et al., 2002; Traidl-Hoffmann et al., 2005).

The conclusion that can be drawn is that both nature and nurture are at play. Certain specialised phenotypes of DC may have an inherent bias towards the stimulation of preferential Th2 responses, whereas other DC may acquire a similar Th2 bias due to local microenvironmental conditioning (Eisenbarth et al., 2003).

One important element of Th2 bias among DC appears to be a reduced capacity to produce interleukin 12 (IL-12). This cytokine, a product of DC, is a driver of Th1 development and if production of IL-12 is compromised then this strongly favours the stimulation of selective Th2 responses (Ghaemmaghami et al., 2002; Traidl-Hoffmann et al., 2005; Pulendran et al., 2010).

Taken together the implication is that, for whatever reason, chemical respiratory allergens are preferentially processed and presented by DC that favour the development of Th2 immune responses, and/or that such allergens in some way are able to cause DC to acquire a phenotype that encourages selective Th2 cell development. Although there is no direct evidence to support the hypothesis, it could be argued (for instance) that a common property of chemical respiratory allergens may be to inhibit the local expression of IL-12, either directly or indirectly. Alternatively, chemical respiratory allergens and contact allergens may be associated with the differential availability of ligands for receptors that influence the immunological bias of DC.

There are other considerations also. Thus, it can be argued that the dynamics, location and positioning of DC may also have important influences. For instance, it has been suggested that the ratio of DC to T lymphocytes in regional lymph nodes may impact on Th1/Th2 selectivity (Eisenbarth et al., 2003). Another possibility is that the kinetics of migration of DC to lymph nodes draining the site of exposure to antigen can also influence the balance between Th1 and Th2 responses. Investigations in mice compared the migration of Langerhans cells (LC; epidermal DC) from the skin to regional lymph nodes following topical exposure to either the contact allergen 2,4-dinitrochlorobenzene (DNCB), or to a reference respiratory allergen, trimellitic anhydride (TMA). The migration of LC was significantly more rapid in response to DNCB than to TMA, and this was associated with the elaboration by DNCB of interleukin 1β (IL-1β) in the skin. The reduced kinetics of LC migration in mice exposed to TMA was found to be associated with the anti-inflammatory cytokine interleukin 10 (IL-10) that impaired the production of the cytokines (including IL-1β) that are known to drive LC mobilisation (Cumberbatch et al., 2005). The differential kinetics of LC migration in response to contact and respiratory allergens is intriguing, not least because it is consistent with the view that a journey of longer duration from the time of encounter with antigen to arrival in draining lymph nodes may encourage acquisition of a selective Th2 bias by DC, possibly associated with a reduced potential to elaborate IL-12 (Langenkamp et al., 2000).

It is clear, therefore, that DC play important, and possibly decisive, roles in determining the quality of T lymphocyte responses that will develop, and the assumption is that the activity of DC with a natural or acquired bias towards Th2 development will drive responses to chemical respiratory allergens. But again, the question is why exposure to such allergens is associated with a DC-based pattern of immune activation that develops into a preferential Th2-type response.

A consideration of the evolutionary importance and physiological role of selective Th2 responses is relevant. As argued above, the value of such differentiated responses is to deliver effective host resistance against certain types of infectious agents (such as, for instance, multicellular parasites). It is important, therefore, that the innate and/or adaptive immune systems are able to recognise the signatures of classes of antigen that warrant deployment of Th2 immune responses, or if not the antigens themselves then signals (PAMPs and others) associated with them. What the differential signatures are that separate contact allergens from chemical respiratory allergens is not known, but there are certain aspects of the early molecular interactions

of sensitising chemicals following exposure that deserve attention.

4. Molecular interactions and the acquisition of sensitisation to chemical respiratory allergens

It is widely acknowledged that 'danger signals' are an important element in the initiation of adaptive immune responses. The term danger signal was first coined by Matzinger (1994, 1998) and describes the fact that for full and effective deployment of an adaptive immune response there is required, in addition to antigen, a second signal. That second signal alerts the immune system that encounter with an antigen has been accompanied by signs that denote tissue disruption or trauma, or that identify the antigen as being derived from an infectious microorganism.

The latter signals are characterised as PAMPs; the former as damage-associated molecular patterns (DAMPs) (Oppenheim et al., 2007). The role of such danger signals in the acquisition of skin sensitisation is acknowledged (McFadden and Basketter, 2000; Kimber et al., 2002; Martin and Jakob, 2008; Martin, 2010; Ainscough et al., 2013). The most thoroughly characterised pathogen recognition receptors are TLR, and it has been shown that ligation of these receptors can play an important role in the development of skin sensitisation (Martin, 2010). It is tempting to suggest that chemical allergens of different types may be associated with discrete patterns of receptor activation that in turn impact on DC function and the form that adaptive immune responses will take. Certainly, as would be expected, selective Th1 and Th2 responses have been associated with different microbial products and constituents (De Jong et al., 2002; Eisenbarth et al., 2003), and as chemical allergens are being 'misread' as representing an infectious threat it would appear possible that the mis-recognition of contact and chemical respiratory allergens is effected via different pathogen recognition receptors. This is likely to be a fruitful area of investigation.

Independent of, or in addition to, possible elicitation of different patterns of danger signals, it is important to consider whether the antigenic complex to which sensitisation is acquired may also be influential in driving discrete immune responses. There is reason to believe that the form of the antigen itself, the nature of its interaction with antigen presenting cells, the way it is displayed to responsive T cells, and other related variables, can potentially influence the balance between Th1 and Th2 responses (reviewed in Constant and Bottomly, 1997). However, similar considerations as they relate to chemical allergens need be rather different. Chemicals themselves are not immunogenic, and will not induce immune responses. Sensitisation requires that the chemical forms a stable (covalent) association with proteins, and it is these hapten–protein complexes that are the relevant immunogens. Chemical allergens must, therefore, be inherently protein-reactive, or be converted at (or close to) the site of encounter into a protein-reactive species.

It is legitimate to question whether contact allergens and chemical respiratory allergens display different selectivity with regard to interaction with proteins. In this respect one very interesting observation has been that some chemicals, including respiratory allergens, selectively bind to lysine residues (Banks and Paquette, 1995; Jonsson et al., 1995).

This has been confirmed more recently in investigations of the direct peptide reactivity assay (DPRA). This assay was developed as an alternative (non-animal) method for the prospective identification of skin sensitising chemicals. The DPRA is predicated on the understanding that skin sensitising chemicals will have to form stable associations with protein to assume immunogenic potential, and the assay seeks to reflect this by measurement of the binding of test chemicals to model peptides (Gerberick et al., 2004, 2007; Natsch and Gfeller, 2008). There has been interest in the possibility that this method could be used to reveal differences between contact and respiratory chemical allergens with respect to amino acid selectivity during protein binding (Lalko et al., 2011). In a comparative study, confirmed skin and respiratory sensitisers were reacted with either lysine (Lys) or cysteine (Cys) peptides. The results revealed that, as expected, chemical respiratory allergens were active in the DPRA, but that (in contrast to the majority of contact allergens) they reacted selectively with the Lys peptide (Lalko et al., 2012).

How strong the association is between selectivity for Lys residues and the potential to cause allergic sensitisation is not clear. It might be that the differential peptide binding selectivities of contact allergens and chemical respiratory allergens are disclosed only under defined experimental conditions. It is also not clear whether such relationships will be as clearly discernible when peptide reactivity is measured under competitive conditions, that is where both Cys and Lys peptides are both freely available (Lalko et al., 2013). Such covalent binding, although required, is insufficient in isolation for the induction of sensitisation. Nevertheless, the observations are of considerable potential importance because amino acid selectivity might represent the earliest mechanistic divergence between contact and chemical respiratory allergens.

On the basis that these classes of chemical allergens do display preferences (under some conditions at least) for different amino acid residues, there are two associated phenomena that warrant consideration.

The first is the significance of the nuclear factor erythroid 2-related factor 2 (Nrf2)-Kelch-like ECH-associated protein-1 (Keap1)-antioxidant response element (ARE) pathway for the development of skin sensitisation (Natsch and Emter, 2008; Natsch, 2010). The Keap1 protein is a sensor that possesses Cys residues. In the steady state Keap1 is associated with the transcription factor Nrf2. However, when there is covalent interaction between Cys residues on Keap1 and electrophiles, including skin sensitising chemicals, the protein dissociates from Nrf2. This allows for the accumulation of Nrf2 in the nucleus triggering the transcriptional activation of genes that have an ARE in their promoter region (Natsch, 2010). The activation of Nrf2 is now used as the mechanistic basis for several cell-based assays for the identification of skin sensitising chemicals. It has been proposed that the differential selectivity of chemical allergens for amino acid residues may, in turn, drive discrete activation of signalling pathways and gene expression patterns in DC and other cells (Natsch, 2010). The argument is that the covalent modification of Cys residues on Keap1 by Cys-selective contact allergens will provoke patterns transcriptional activation that favour the development of Th1 responses. The corollary is that chemical respiratory allergens that associate exclusively or selectively with Lys residues will stimulate an as yet unidentified pathway, other than Keap1/Nrf2, that will initiate patterns of gene expression required for the development of Th2 responses and sensitisation of the respiratory tract (Natsch, 2010). Although this remains speculative, it does serve to provide a potential link between the very earliest events during which chemical allergens must form stable associations with proteins and the biological events that result in allergic sensitisation of different types.

A related phenomenon derives from investigations of the interactions of chemical allergens with native proteins. In a series of comparative studies the association of contact allergens and chemical respiratory allergens with cell-associated or soluble (serum) proteins was measured. Both types of chemical allergen were able to bind covalently with cell associated or soluble proteins when incubated with either cells or serum alone. However, when incubated with cells and serum together contact allergens associated selectively with cellular proteins, whereas chemical respiratory allergens bound selectively to serum proteins (Hopkins et al., 2005).

These data prompted speculation that the preferential association of chemical respiratory allergens with serum proteins, rather than cell-associated proteins, was attributable to the fact that albumin is an abundant serum protein and that lysine is a major component of this protein (approximately 10% of the total amino acid content) (Hopkins et al., 2005).

Collectively these data make an interesting case that chemical respiratory allergens are characterised by a selective for binding with Lys rather than Cys residues, and that this distinguishes them from contact allergens. This view is supported by report of Johannesson et al. (2001) that serum albumin is a major target for hapten–protein conjugate formation in humans and guinea pigs exposed to the chemical respiratory allergen hexahydrophthalic anhydride. Up to 60% of exposed workers were found to have antibodies reactive with haptenated serum albumin (Johannesson et al., 2001)

Attractive as this hypothesis is, there are a number of important considerations that must be borne in mind. The first of these is that the difference between contact allergens and chemical respiratory allergens in this context is not absolute and may be dependent on the experimental conditions under which protein/peptide binding is measured (Lalko et al., 2012, 2013). In addition, a number of contact allergens, including 2,4-dinitrofluorobenzene (DNFB), a homologue of DNCB, is able to bind to both Cys and Lys residues (Hopkins et al., 2005). Finally, it has been demonstrated that the association of chemical allergens with proteins is characterised by a high level of selectivity over and above a preference for particular amino acid residues. Thus, for instance, hexahydrophthalic anhydride has been shown to haptenate only seven of the many lysine residues that available in albumin (Kristiansson et al., 2003).

Despite those reservations it does appear that chemical respiratory allergens are characterised by a selectivity for Lys residues, and although the differences between contact and respiratory allergens in terms of amino acid residue preferences may not be absolute, they may be sufficient to drive cellular responses down discrete paths that ultimately result in the evolution of differentiated immune responses. It must be acknowledged that there may be other differences between contact and chemical respiratory allergens with respect to the nature of their interactions with proteins (Enoch et al., 2012), and if this is the case then those might also contribute to the variable triggering of cellular responses.

5. Synthesis of a pathway

In working backwards from the development of occupational asthma, through relevant cellular and molecular events that are required to support allergic sensitisation of the respiratory tract to chemicals, it is possible to propose a potential pathway. This is based on the evidence reviewed. However, the evidence is not complete and a number of uncertainties remain. Clearly more research is required to provide the information necessary where our understanding of events and mechanism is incomplete. However, in advance of the fruits of such research being available it is possible to propose a pathway leading to the development of allergic sensitisation of the respiratory tract as follows:

- Exposure to chemical respiratory allergen, at an appropriate dose, and via a relevant route, will drive the acquisition of sensitisation of the respiratory tract.
- The acquisition of sensitisation of the respiratory tract will be favoured by the preferential association of chemical allergen with Lys residues to configure an appropriate hapten–protein conjugate. The selectivity of chemical respiratory allergen for Lys will also induce in DC and other cell types, signalling pathways and patterns of gene expression that differ from those provoked by contact allergens.
- The patterns of cellular activation, gene expression and protein production induced by chemical respiratory allergens will favour the bias of DC towards initiation of Th2-type responses, possibly via reduced or down-regulated expression of IL-12.
- DC biased in this way will drive preferential Th2 responses to the chemical allergen
- Such Th2 responses will favour the elaboration of specific IgE antibody that will cause sensitisation of the respiratory tract. However, even if the development of selective Th2 responses is not (for whatever reason) accompanied by IgE antibody production then cellular sensitisation of the respiratory tract will still be achieved by mechanisms that have yet to be elucidated.

In the absence of any other information this pathway is plausible and accommodates much of the data that are available and reviewed in this article.

6. Implications and applications

Constructing a plausible pathway in this way certainly serves to identify important gaps in our current understanding of the development of chemical respiratory allergy, and may therefore serve to guide future research.

In addition, it provides a stimulus for reconsideration of the options available for hazard identification and characterisation. On the basis of the information reviewed here, and the pathway proposed, it is clear that the acquisition of respiratory sensitisation to chemicals will normally be associated with Th2 immune responses, and this therefore provides one important correlate of likely hazard. It could be argued that as the development of Th2 responses will usually result in IgE antibody production that measurement of such antibody responses (even thought they are not necessarily always associated with occupational asthma) may also provide a useful and relevant biomarker. In seeking to explore non-animal approaches for toxicological assessment, the two events that suggest themselves as likely candidates for *in vitro* test method development are the acquisition of a Th2 bias among DC, and the selective binding of chemical respiratory allergens with Lys residues. Naturally such opportunities can be supplemented by the development and further refinement, of appropriate Quantitative Structure Activity Relationship (QSAR) models.

There is still some considerable way to go until we will be able, with some certainty, to identify and characterise chemicals that have the potential to cause allergic sensitisation of the respiratory tract. However, with a continued investment in well directed research aimed at providing an increased understanding of the relevant biochemical and immunological mechanisms, real progress should be possible.

Conflict of interest

The authors declare that there are no conflicts of interest.

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