



Review

Chemical allergens—What are the issues?

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ABSTRACT

Chemical allergy describes the adverse health effects that may result when exposure to a chemical elicits an immune response. Allergy develops in two phases. In the first phase, exposure of an inherently susceptible subject results in stimulation of an immune response or immunological priming. If the then sensitised subject is exposed on a subsequent occasion to the same chemical then an accelerated and more aggressive secondary immune response will be provoked resulting in inflammation and the signs and symptoms of a clinically discernible allergic reaction. The two forms of chemical allergy of greatest relevance for occupational toxicology are skin sensitisation resulting in allergic contact dermatitis, and sensitisation of the respiratory tract associated with occupational rhinitis and asthma. In this brief survey we identify what we believe currently represent the key issues and key challenges in these areas.

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1. Chemical allergy

Chemical allergy describes the adverse health effects that may result when exposure to a chemical causes allergic sensitisation and allergic disease. By definition allergy, including chemical allergy, is dependent upon specific priming of the adaptive immune system such that subsequent encounter with the same allergen (protein or chemical) will provoke an accelerated and more aggressive secondary immune response resulting in inflammation and the symptoms of allergic disease.

From an industrial/occupational perspective the two forms of chemical allergy that are of greatest relevance are skin sensitisation causing allergic contact dermatitis, and sensitisation of the respiratory tract associated with rhinitis and asthma. For the pur-

pose of this brief commentary it is on these two types of allergic sensitisation on which we will focus.

The purpose here is not to provide a review of chemical allergy, or to even consider in any detail recent progress in this area. Rather the intention is to highlight what arguably may be viewed as being the most important (and in some instances most intriguing) challenges with which we are faced in addressing important immunotoxicological issues.

2. Skin sensitisation

It is probably fair to say that although we know a great deal about the immunological mechanisms that serve to orchestrate the induction of skin sensitisation and the elicitation of allergic contact dermatitis, our understanding remains far from complete.

Nevertheless, an appreciation of some of the key features of immune responses induced by encounter with chemical allergens in the skin has served us well in the development of tools with which to identify and characterise contact allergens (Kimber et

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al., 2002a, 2008; Kimber and Dearman, 2003). There are available guinea pig tests and the mouse local lymph node assay (LLNA) for identification of skin sensitisation hazards (Steiling et al., 2001; Kimber et al., 2002b; Basketter et al., 2007), and the LLNA also provides a method for characterising contact allergens with respect to their relative potency (Dearman et al., 1999; Kimber et al., 2003; McGarry, 2007). Armed with an appreciation of skin sensitisation potency it is then possible to conduct informed quantitative risk assessments with the derivation of valuable metrics such as NESIL (no expected sensitisation induction level) and AEL (acceptable exposure level) (Api et al., 2008).

Much has been achieved, and there are available robust strategies for toxicological evaluation of skin sensitisation, but there remains a reliance on animal-based methods. The LLNA provided important animal welfare benefits compared with the guinea pig test methods that preceded it (Dean et al., 2001), and there have been proposals since then for further reductions in animal numbers used in this test (Kimber et al., 2006; Ryan et al., 2008). However, the aspiration is clearly the development and application of alternative testing paradigms that obviate altogether the requirement for experimental animals.

3. Alternative approaches to assessment of skin sensitisation activity

There has in recent years been a very substantial investment in seeking *in vitro* or *in silico* approaches to skin sensitisation testing. Among the strategies that have found favour are those based on the interaction of chemicals with dendritic cells (DC) or DC-like cells, those that measure the ability of chemicals to interact with proteins or model peptides, or those that seek to identify and exploit structure–activity relationships (SAR) (Kimber et al., 1999, 2001, 2004; Ryan et al., 2001, 2005; Basketter, 2008; Gerberick et al., 2007; Patlewicz et al., 2007; An et al., 2009). Some of these approaches are more advanced than others. Whereas some proposed test methods are now candidates for adoption into a formal validation process, others are best regarded as being at a formative stage. Nevertheless, the investment in this area is yielding some dividends and it is not unreasonable to speculate that there will come a time when a seasoned toxicologist will be able to identify skin sensitising chemicals, with some accuracy, based solely on a consideration of structural characteristics and activity in *in vitro* methods.

What is likely to be much more of a challenge, and the issue on which we focus here, is deriving reliable estimates of relative potency without recourse to animal models. Although dose metrics are required to inform all areas of toxicological evaluation, the importance of potency for effective risk assessment is no better illustrated than in the context of skin sensitisation and allergic contact dermatitis. It is believed that contact allergens vary by up to five orders of magnitude with respect to their relative skin sensitisation potency, and that being the case it is clear that effective risk assessment, and the implementation of appropriate risk management strategies, must necessarily be informed by an appreciation of relative potency (Kimber et al., 2008).

The difficulty is that, from among the many biological and biochemical events that are known to be associated with, and required for, skin sensitisation (and which in some instances form the bases for putative *in vitro* test methods), we have no real appreciation of which (either alone or collectively) drive relative potency.

It is clear that the endpoint used for the standard LLNA (induced lymphocyte proliferative responses in draining lymph nodes of exposed mice) correlates causally and quantitatively with the acquisition of skin sensitisation and is therefore suitable for use as a biomarker for potency. But antigen-driven primary immune

responses by naïve T lymphocytes are difficult to engineer *in vitro*, and even if such responses could be modelled and measured effectively there is no reason to believe that the vigour of T lymphocyte proliferation in culture would necessarily inform sensitising potency *in vivo*. As indicated above, most proposed *in vitro* approaches are focused currently upon either assessment of protein/peptide binding by chemicals, or the ability of chemicals to stimulate changes in the phenotype of DC or DC-like cells. However, while the formation of stable associations with protein and DC activation are both required for the effective acquisition of skin sensitisation (and are therefore, in principle at least, appropriate readouts for hazard identification), there is no evidence that either necessarily reflect potency.

This actually distils down into an intriguing immunotoxicological question—what event that is triggered during the induction of skin sensitisation correlates quantitatively with potency? The answer may turn out to be that there is in fact no single event or process that alone is responsible for driving potency, and that in fact the effectiveness with which sensitisation is acquired depends upon several separate, but interacting, variables.

Such a view is reflected in an analysis published in 2006 in which a paradigm was proposed for determination of the relative potency of contact allergens based upon an integrated, semi-quantitative, assessment of various biological, biochemical and chemical metrics (Jowsey et al., 2006). This paradigm has since been revisited, reviewed and refreshed (Basketter and Kimber, 2009), but the same basic tenets remain unchanged. The argument is that, in the absence of any definitive correlate of potency, a pragmatic way forward is to assign to various endpoints (SAR and *in vitro* assay readouts) a numerical score based, in the case of assay results, on the vigour of induced responses. The overall potency ‘score’ assigned to a chemical is derived from the product of the individual scores assigned to the individual readouts available. The use of the product of individual scores, rather than a cumulative total, has one important implication, insofar as a zero rating for any one readout will necessarily result in an overall zero potency score for the test chemical. The rationale for this is that the failure of a chemical to elicit any signal at all in a readout considered to reflect an essential step in the acquisition of sensitisation should be interpreted as the absence of any significant sensitising activity.

The above approach is crude, and is by way of ‘practicing the art of the possible’ in the absence of any proven paradigm for determining relative potency in the absence of animal models. Crude though it might be, the approach does serve the useful purpose of stimulating discussion about how, in the future, we should be planning to develop robust and integrated approaches to potency assessment, hazard characterisation and risk assessment without the benefit of data derived from studies in animals.

4. Sensitisation of the respiratory tract

While many chemicals are known to have the potential to cause skin sensitisation and allergic contact dermatitis, other materials, fewer in number, are known instead to preferentially result in sensitisation of the respiratory tract and occupational asthma. Here the challenges for immunotoxicologists currently are rather different, but no less demanding, and in some ways rather formidable.

There are available elsewhere reviews, including some recent reviews, of sensitisation of the respiratory tract by chemicals and occupational asthma, and of issues relating to toxicological evaluation (Holsapple et al., 2006; Kimber and Wilks, 1995; Kimber and Dearman, 2005; Kimber et al., 2007; Isola et al., 2008; Roggen et al., 2008; Boverhof et al., 2008). The key issue is that, despite considerable effort, we still do not have available a widely accepted or formally validated method for the identification and character-

isation of chemical respiratory allergens. Without indulging in a detailed survey of the relevant immunological and toxicological landscapes, it is possible to identify at the heart of the problem two areas of uncertainty that have impeded progress. The first of these relates to the immunological mechanisms that result in the induction by chemicals of sensitisation of the respiratory tract. The second is confusion and uncertainty about relevant routes of exposure.

Chemical allergy, including sensitisation by chemicals of the respiratory tract, is by definition dependent upon provocation of an adaptive immune response. About that there is agreement. There is also some agreement, but not complete unanimity, that chemical respiratory allergy is commonly associated with a selective T helper 2 (Th2) cell immune response (Kimber and Dearman, 2005) (this lack of consensus derives both apparently conflicting experimental observations, and differences in interpretation of clinical experience). However, it is now well established that Th2-type immune responses support and sustain IgE antibody responses, the class of antibody associated with allergic sensitisation. Where there remains real controversy is to what extent IgE antibody is associated with, and required for, chemical respiratory allergy. Such uncertainty is founded upon clinical experience which records that not uncommonly symptomatic patients with diagnosed occupational asthma lack detectable IgE antibody (Kimber and Dearman, 2002, 2005). We are of the view that, for various reasons, the extent of correlation between chemical respiratory allergy and IgE antibody has been substantially under-estimated. In fact, it is tempting to suggest that in fact the elaboration of IgE antibody may in most instances represent a mandatory event in the acquisition of respiratory sensitisation. The reasons why IgE antibody is frequently not disclosed in symptomatic subjects has been considered previously in some detail (Kimber and Dearman, 2002), and it is not necessary to rehearse those arguments in full again here. Suffice it so say that the identification of specific IgE antibody to chemical allergens is technically very demanding and challenging. Moreover, it is known that the ability to detect IgE antibody in the serum of sensitised subjects diminishes quickly with time during the weeks following last encounter with the chemical (Kimber and Dearman, 2002).

Therefore, one of the challenges that still requires resolution is greater certainty about the immunological mechanisms through which chemicals cause sensitisation of the respiratory tract, and in particular the role played by IgE antibody. In addressing this question there has been one recent report that may have the potential to shed some new light on this issue. One of the classes of chemical most commonly associated with sensitisation of the respiratory tract and occupational asthma is the acid anhydrides, and a paper published by Helaskoski et al. (2009) has investigated a small group of patients diagnosed with occupational contact urticaria (another manifestation of chemical allergy) to various acid anhydrides, including among others phthalic anhydride. They report sensitisation was invariably associated with a positive skin prick test (SPT) to the causative chemical, a positive skin prick test reflecting the availability of allergen-specific IgE antibody. Based on these observations it would be interesting to determine to what extent SPT reactivity is associated with occupational respiratory allergy (or urticaria) to diisocyanates—a class of chemical respiratory allergen where it has proven particularly difficult to find IgE antibody in symptomatic subjects.

The second issue, that has also been addressed in some detail previously (Kimber and Dearman, 2002), is the relevance of different routes of exposure to the chemical allergen. It has frequently been assumed that sensitisation of the respiratory tract to chemicals demands that exposure is effected via inhalation. This is not necessarily the case, there is mounting evidence, albeit usually indirect evidence, that skin exposure to chemical allergens such as the isocyanates and acid anhydrides, can result in effective

sensitisation of the respiratory tract. This comes as no surprise as adaptive immune responses are not designed to provide only local protection and antibody, including IgE antibody, is distributed systemically. There is no *a priori* reason, therefore, why skin exposure should not result in sensitisation of the respiratory tract. This is important not only in the context of risk assessment and risk management in occupational settings, it is also relevant for consideration of appropriate routes of exposure for animal models.

The argument is, therefore, that a more detailed understanding of the pivotal immunological mechanisms, combined with an appreciation of relevant routes of exposure, would facilitate the more effective development of methods for hazard identification and characterisation, and their more rapid acceptance by the scientific community.

However, despite these barriers, progress has been made in recent years in the development of new testing strategies. Of particular importance have been attempts to move away from traditional inhalation models in rats and guinea pigs that were often technically laborious and costly, and to embrace instead approaches based on the immunobiology of sensitisation (Dearman et al., 2003; Boverhof et al., 2008). There is interest also in establishing SAR relationships, and in exploiting for the purposes of respiratory sensitisation hazard identification progress that is being made in the design of *in vitro* approaches to skin sensitisation testing (Holsapple et al., 2006; Kimber et al., 2007). There is, however, some considerable way still to go.

5. Concluding comments

It will be clear from this brief survey that we now have available robust and reliable approaches for hazard and risk assessment of contact allergens. However, the imperative of developing realistic strategies that do not require the use of animals has thrown up new challenges, perhaps the most taxing of which is assessment of relative potency. In the case of chemical respiratory allergy and occupational asthma, there is still much to play for, but the key challenge is to achieve a really clear understanding of the pivotal immunobiological processes that result in the acquisition of sensitisation.

Although not addressed here, it is worth acknowledging an intriguing question that continues to exercise us and others working in the field of chemical allergy. That is why small molecular weight chemicals that are able to provoke immune responses usually display selectivity with respect to their induction of either contact sensitisation or sensitisation of the respiratory tract. A detailed understanding of the immunobiological factors that underlie such selectivity will undoubtedly play important dividends in enabling us to address more effectively some of the challenges highlighted in this article.

Conflict of interest

None.

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