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Alternative approaches to the identification and characterization of chemical allergens

I. Kimber[a,*], J.S. Pichowski[a], C.J. Betts[a], M. Cumberbatch[a], D.A. Basketter[b], R.J. Dearman[a]

[a]*Syngenta Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire SK10 4TJ, UK*
[b]*SEAC Toxicology Unit, Colworth House, Unilever Research, Sharnbrook, Beds MK44 1LQ, UK*

Abstract

Chemical allergy can take a variety of forms, those of greatest importance in an occupational setting being skin sensitization resulting in allergic contact dermatitis and sensitization of the respiratory tract associated with asthma and other symptoms. In both cases there is a need for predictive test methods that allow the accurate identification of sensitizing chemicals. Well characterized methods are available for skin sensitization testing, and although to date no tests for respiratory sensitization have been formally validated, progress has been made in defining suitable animal models. In recent years there have been significant advances in our understanding of the cellular and molecular mechanisms through which allergic sensitization to chemicals is induced and regulated. Such progress provides us now with new opportunities to consider alternative approaches to sensitization testing, including the design of in vitro test methods. The greatest investment has been in exploring novel methods for the identification of contact sensitizers and it is upon this aspect of chemical allergy that this article is focused. Described here are some of the general requirements of in vitro test methods for skin sensitization, and progress that has been made in developing suitable approaches with particular emphasis on the utility of dendritic cell culture systems.

Keywords: Skin sensitization; In vitro methods; Predictive tests; Dendritic cells; Interleukin 1β

1. Introduction

Allergic disease resulting from exposure of susceptible individuals to sensitizing chemicals is an important health issue. There is clearly a need to identify accurately chemicals that have the potential to induce allergic sensitization and to assess the risks they pose to human health. In the context of occupational disease two forms of allergic sensitization are of greatest importance; skin sensitization resulting in allergic contact dermatitis and sensitization of the respiratory tract associated with rhinitis, asthma and other symptoms. In both instances methods for hazard identification focused originally on the use of guinea pig tests in which activity is measured usually as a function of challenge-induced allergic reactions (dermal or pulmonary) in previously sensitized animals (for reviews see Maurer, 1996; Sarlo and Ritz, 1997). However, during the last two decades there have been significant advances in allergy research and in the definition of the important immune mechanisms that influence the acquisition and regulation of allergic sensitization to chemicals. Associated with this progress there have been opportunities to design alternative approaches to hazard identification based on analysis of immune responses induced in mice (and rats) by chemical allergens. Among these new methods is one which is already well established, the local lymph node assay, a predictive test for skin sensitizing potential (Kimber et al., 1994; Dearman et al., 1999; Gerberick et al., 2000), and others that show promise but which have not yet been formally validated. Among the latter are included the mouse (and rat) IgE test and cytokine fingerprinting, both of which methods are designed to identify chemicals that have the ability to cause sensitization of the respiratory tract (Kimber and Dearman, 1999a).

Abbreviations: DC, dendritic cells; DMSO, dimethylsulfoxide; DNFB, 2,4-dinitrofluorobenzene; IL, interleukin; LC, Langerhans cells; SLS, sodium lauryl sulfate.

* Corresponding author. Tel.: +44-1625-515408; fax: +44-1625-590249.

E-mail address: ian.kimber@syngenta.com (I. Kimber).

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All of the approaches summarized above have a requirement for animals, and it is legitimate to consider whether there are emerging opportunities to develop alternative strategies for predictive testing that are based solely on in vitro methods. It is timely to address this now since a more sophisticated appreciation of the relevant molecular mechanisms facilitates, in principle at least, the design of cell or tissue culture systems that reflect what is known of the induction phase of sensitization to chemicals. Although some of these opportunities are explored below, it is important to recognize that the development of realistic in vitro methods is not a trivial exercise and poses experimental toxicologists with some substantial challenges.

2. In vitro methods for sensitization: some general considerations

2.1. Contact and respiratory allergens

Chemicals can cause various forms of allergic disease. There are many chemicals that are known to cause skin sensitization and allergic contact dermatitis, but which are believed not to induce sensitization of the respiratory tract. In contrast, there are other chemicals (fewer in number) that have been shown to induce respiratory allergy among exposed subjects, but which only rarely, if ever, induce contact sensitization in humans. There is now a substantial body of evidence that the preferential induction of different forms of allergy is determined by the quality of immune response induced by the chemical sensitizer (Kimber and Dearman, 1997, 1998). Specifically, it has been found that contact and respiratory chemical allergens stimulate the selective development of different functional subpopulations of T lymphocytes which in turn display discrete patterns of cytokine secretion. These subsets of T lymphocytes, acting in concert with their cytokine products, shape the quality of developing immune responses such as to favour either contact or respiratory sensitization (Kimber and Dearman, 1997, 1998). The molecular basis for the stimulation by chemical allergens of qualitatively divergent immune responses is not known, but it is likely that the ways in which the relevant antigen is processed and presented to the immune system by dendritic cells (DC) play significant roles (Kapsenberg et al., 1998). Until the key molecular events have been defined it will not be possible to reproduce in vitro those processes that determine whether a potential chemical allergen is likely to behave as a contact or a respiratory sensitizer. This aspect of hazard characterization with respect to in vitro approaches therefore remains an aspiration for the future. In fact, in the absence of widely accepted or fully validated animal models, the development of in vitro methods for the identification of chemical respiratory allergens is extremely problematic and, other than suggestions that computer-assisted considerations of structure-activity relationships may be useful (Karol et al., 1999), there has been little investment or progress in this area. For this reason, attention here will focus on opportunities that may exist for the development of alternative approaches to skin sensitization testing.

2.2. Hazard identification versus hazard characterization

Examined below are some of the in vitro approaches that have been proposed for the identification of skin sensitizing chemicals. However, the identification of potential hazards is only one part of an holistic safety evaluation, and the development of accurate risk assessments depends also on the availability of information on potency. This may be of particular importance for contact allergy since it has been estimated, on the basis of clinical and experimental studies, that the relative skin sensitizing potency of chemicals may vary by many orders of magnitude (Basketter et al., 2000). Therefore the ideal in vitro test method is one that will allow consideration of relative potency. This may be difficult to achieve in practice and will require the identification of read outs that correlate quantitatively with sensitizing activity.

2.3. Sensitivity and selectivity

If it is going to provide a realistic and complete alternative to current testing strategies, an in vitro method for skin sensitization must be able to identify contact allergens of differing potency and to distinguish these from other chemicals, such as non-sensitizing skin irritants. It is true to say that to date most studies exploring the characteristics of proposed in vitro methods have frequently employed only a limited number of very active skin sensitizing chemicals, and have interrogated test selectivity with only one or more of a few skin irritants. Panels of test chemicals suitable for evaluating the operational characteristics of new test methods for chemical sensitization have been proposed recently (ECETOC, 1999) and these provide one basis for initial assessment of in vitro assay performance.

2.4. Metabolism

An issue relevant to all in vitro test methods is that of metabolism. Low molecular weight chemical allergens are haptens and as such are unable in free form to stimulate an immune response. For immune activation it is necessary that the chemical allergen associates (usually covalently) with protein. Although many skin sensitizing chemicals are inherently protein-reactive, but this is by no means always the case and there are many examples of chemicals that must be metabolized to a protein reactive species in the skin for sensitization to

proceed. It is important therefore that either there is provision for metabolic activation in the cell or tissue culture system chosen for in vitro studies, or that there is an appreciation that sensitizers requiring metabolic conversion may not be recognized.

3. Approaches to skin sensitization testing in vitro

Proposed in vitro test methods are in the main based on an understanding of the cell and molecular processes that characterize the induction phase of contact sensitization. Briefly, the key events are as follows. Epidermal Langerhans cells (LC) recognize, internalize and process chemical allergen in the form of a hapten–protein conjugate. Antigen is transported from the skin to draining lymph nodes by LC. While in transit from the epidermis, LC are subject to a functional maturation such that by the time they arrive in regional lymph nodes they have acquired the characteristics of immunostimulatory DC. It is now well established that the mobilization, migration and maturation of LC are dependent on the availability of relevant skin cytokines (Kimber et al., 2000), a point that will be returned to later. The DC that accumulate in lymph nodes present antigen to responsive T lymphocytes which, as a consequence, are stimulated to divide and differentiate. If the T lymphocyte response is of sufficient vigour, and of an appropriate quality, then skin sensitization will be acquired (Kimber and Dearman, 1999b). Most approaches to skin sensitization testing in vitro have focused on either the induction of T lymphocyte responses or the interaction of chemicals with LC or LC-like cells. In the following sections we focus on these cell types and in particular the use of LC and DC. However, it must be emphasized that there are other immunocompetent cells in the skin and elsewhere which are known to play important roles in contact sensitization and which could form the basis of alternative strategies for in vitro testing.

3.1. T lymphocyte responses

It is relatively straightforward to provoke antigen-induced secondary responses in vitro using T lymphocytes prepared from sensitized animals or humans. What is far more of a challenge is to stimulate antigen-specific primary activation using as responder cells T lymphocytes derived from naive donors. Nevertheless, some progress has been made and it has been found that proliferative responses by naive T lymphocytes can be induced in culture with some, but not all, chemical allergens tested (Hauser and Katz, 1988; Krasteva et al., 1996; Rougier et al., 1998). It remains to be seen whether this approach is sufficiently robust to allow the detection of a wide range of contact allergens and their discrimination from skin irritants.

3.2. Dendritic cell responses

Interest in the use of DC populations for in vitro assessment of skin sensitizing activity probably derived initially from the investigations of Enk and Katz (1992), who analyzed patterns of epidermal cytokine expression following topical exposure of mice to chemical allergens. They found that both skin sensitizers and skin irritants were able to induce changes in cytokine mRNA expression, but that some cytokines appeared to be upregulated selectively, or exclusively, in response to contact allergens. Of particular interest were changes in interleukin (IL) 1β, a cytokine that in mouse epidermis is a product exclusively of LC and which has been shown to be essential for both the induction of LC migration and the acquisition of skin sensitization (Enk et al., 1993; Shornick et al., 1996; Cumberbatch et al., 1997). This suggested that it might be possible to identify skin sensitizers as a function of their ability to induce increased expression of IL-1β. Initially this observation did not provide a realistic basis for the development of an in vitro method due to the difficulties associated with experiments requiring LC. Not only are these cells present in normal skin in very low numbers, but they are also difficult to isolate and subject to rapid phenotypic and functional changes in culture. An attractive alternative to using native LC derived from demonstrations that human DC progenitors could be expanded in culture using an appropriate cocktail of cytokines and that cellular differentiation could be manipulated in such a way as to generate and maintain cells with an LC-like/immature DC-like phenotype (Lenz et al., 1993; Romani et al., 1994; Sallusto and Lanzavecchia, 1994).

Making use of this approach, Reutter et al. (1997) reported that DC derived from human peripheral blood displayed increased IL-1β mRNA expression following treatment with some contact allergens, but not in response to a skin irritant (sodium lauryl sulfate; SLS). More recently we have performed similar investigations using human peripheral blood-derived DC from a variety of donors. DC from all donors constitutively expressed IL-1β, as well as IL-6 and IL-18. The main findings were that several contact allergens, including 2,4-dinitrofluorobenzene (DNFB), *p*-phenylene diamine and methylchloroisothiazolinone/methylisothiazolinone, were able to induce increases in the expression of mRNA for IL-1β (but not for IL-6 or IL-18) in a proportion (approximately 50%) of donors. Fig. 1 summarizes changes in IL-1β mRNA induced by treatment of DC from eight independent donors with DNFB. As the results illustrate, DC derived from only half of these donors displayed discernible increases in IL-1β. In such responsive donors, and under conditions of exposure where contact allergens induced elevated levels of IL-1β mRNA, treatment with the non-sensitizing skin irritants

SLS or benzalkonium chloride failed to influence the expression of this cytokine (Fig. 2). Importantly, the variations between donors with respect to allergen-induced changes in IL-1β mRNA appear to reflect stable differences, insofar as on repeat testing individual donors were found to have consistent responder or non-responder phenotypes (Pichowski et al., 2000; J.S. Pichowski et al., unpublished data).

Collectively these data suggest that some chemical allergens do have the potential to cause an increase in IL-1β expression under conditions where some non-sensitizing skin irritants do not. This is of course encouraging, but it is necessary to temper optimism with caution. First, our evidence indicates that even with very strong contact allergens DC from responsive donors display only a modest elevation in IL-1β mRNA levels (usually approximately twofold) (Fig. 1). The implication is that this approach may not have the sensitivity required to detect sensitizers of lesser potency. Second, although SLS and benzalkonium chloride failed to cause increases in IL-1β in DC from responsive donors, it will be necessary to undertake additional studies with other non-sensitizing chemicals before it can be concluded that the method is truly selective for chemical allergens. Finally, the observation that blood-derived DC from only a proportion of donors have a responder phenotype suggest practical difficulties in ensuring the availability of consistently responsive cells. For these reasons the ability of contact allergens to regulate the expression by cultured DC of IL-1β, at least using this assay configuration, does not appear to represent a viable stand-alone method for the identification of skin sensitizing chemicals. This does not necessarily imply, however, that such responses are without value in safety assessment. It could be argued, for instance, that increased expression of IL-1β by DC may provide a method for selecting out the most potent contact allergens as part of a tiered approach to hazard assessment.

Notwithstanding considerations of the practical utility or otherwise of this approach, the most arresting observation is that some contact allergens appear to interact, probably directly, with cultured DC to induce changes in gene expression under conditions where non-sensitizing skin irritants apparently do not. If this is the case, then it is relevant to consider whether there may be other changes that are provoked under the same or similar conditions of exposure that would be of greater utility as the basis for an in vitro assessment of skin sensitizing activity. Of particular interest of course would be the identification of genes that are regulated selectively by contact allergens and which display a greater dynamic range of expression than does IL-1β.

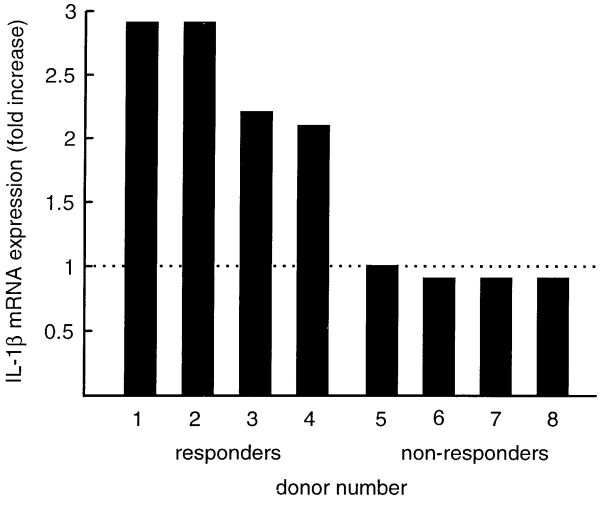

Fig. 1. The influence of DNFB on IL-1β mRNA expression by blood-derived DC: summary of data derived from eight different donors. After isolation and 5 days of culture as described previously (Pichowski et al., 2000), blood-derived DC were exposed to 10.7 pM DNFB in 0.01% dimethyl sulfoxide (DMSO) or to vehicle (0.01% DMSO) alone, for 30 min at 37°C. IL-1β and the housekeeping gene β-actin mRNA expression was analyzed by semi-quantitative reverse transcriptase polymerase chain reaction. Signal strength was assessed by densitometric scanning of the digital pictures and the IL-1β data were standardized to their respective β-actin controls. The data illustrated are expressed as the fold increase in IL-1β mRNA expression after exposure to DNFB compared with concurrent vehicle (DMSO)-treated controls. Dotted line represents zero change in IL-1β expression compared with vehicle (DMSO)-treated controls. Data from four individuals with a responder phenotype and four with a non-responder phenotype are shown.

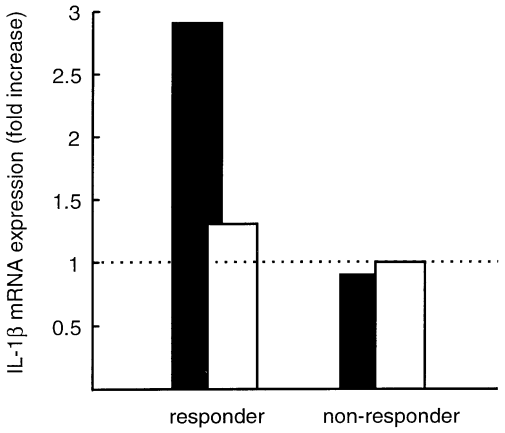

Fig. 2. The influence of DNFB and SLS on IL-1β mRNA production by blood-derived DC from two individual donors. After isolation and 5 days of culture as described previously (Pichowski et al., 2000), blood-derived DC were exposed to 10.7 pM DNFB in 0.01% DMSO (■), 347 μM SLS in 0.01% DMSO (□) or to vehicle (0.01% DMSO) alone, for 30 min at 37°C. IL-1β and the housekeeping gene β-actin mRNA expression was analyzed by semi-quantitative reverse transcriptase polymerase chain reaction. Signal strength was assessed by densitometric scanning of the digital pictures and the IL-1β data were standardized to their respective β-actin controls. The data illustrated are expressed as the fold increase in IL-1β mRNA expression after exposure to DNFB compared with concurrent vehicle (DMSO)-treated controls. Dotted line represents zero change in IL-1β expression compared with vehicle (DMSO)-treated controls. Data from representative individuals with responder and non-responder phenotypes are shown.

There is reason to suppose that if skin sensitizing chemicals are able to interact directly with DC then, in addition to altered IL-1β gene expression, they will stimulate other changes in phenotype and function. Certainly it is the case that topical exposure of mice to contact allergens is associated with a number of important changes in LC that are required for their migration from the skin to draining lymph nodes and their maturation into immunostimulatory DC (Kimber et al., 2000). There is evidence also that in vitro chemical allergens can cause changes in LC or DC other than in IL-1β expression. For example, it has been shown that treatment of human blood-derived DC with strong skin sensitizers, but not with skin irritants, causes a distinct increase in phosphotyrosine (as well as IL-1β mRNA), suggestive of an important role for tyrosine phosphorylation in the activation of DC (Kuhn et al., 1998). In other investigations, flow cytometry was used to measure allergen induced changes in cultured human blood-derived DC. Exposure to some skin-sensitizing chemicals was characterized by a significant increase in the expression by DC of CD86 (a co-stimulatory molecule required for antigen presentation, and which is known to be upregulated during the maturation of LC), in parallel with a decrease in the expression of c-Fms [a membrane receptor for macrophage-colony stimulating factor (M-CSF)/CSF-1] (Manome et al., 1999). Finally, a recent investigation has examined changes induced in human blood-derived DC by treatment with two allergens, nickel chloride or 2,4-dinitrochlorobenzene. In agreement with previous studies, it was found that both sensitizing chemicals caused the increased expression by DC of CD86. In addition, in both cases there was a down-regulation of membrane E-cadherin and an elevated expression of mRNA for the chemokine receptor CCR7 (Aiba et al., 2000). These data are of some interest because the altered expression of these molecules is in accord with what is known of the changes to which LC are subject in vivo, and which facilitate their migration from the epidermis and subsequent localization with draining lymph nodes. As reviewed elsewhere, it is believed that reduced expression by LC of E-cadherin allows these cells to disassociate from surrounding keratinocytes as a first step in their mobilization and that the induced or increased expression of CCR7 confers on activated LC responsiveness to chemokine ligands which direct their localization within the paracortical regions of draining lymph nodes (Kimber et al., 2000).

Taken together, these and other data suggest that many of the changes to which LC are subject following activation in vivo can be modelled in vitro using cultured DC. Such changes include altered expression of certain cytokines, adhesion molecules and membrane receptors for chemokines and cytokines. It seems reasonable to propose therefore that it will be possible to identify genes, or patterns of genes, that are regulated selectively and sensitively by contact allergens and which could form the basis of a robust in vitro method for hazard identification.

4. The way forward

Currently the approach we (and others) are taking is to identify candidate genes that are up- or down-regulated selectively by contact allergens using microarray transcript profiling. For this purpose we are using custom-designed microarrays. The first of these, *ToxBlot I*, comprises approximately 600 genes selected on the basis of their relevance for immune and inflammatory reactions and other biological processes, including the regulation of cell division and differentiation, apoptosis, extracellular matrix interactions and stress reponses. The second custom microarray (*ToxBlot II*) contains cDNA sequences representing over 12,500 genes which embrace more holistically many diverse cellular pathways. These microarrays are being used to characterize changes in gene expression induced following treatment of human blood-derived DC with both sensitizing and non-sensitizing chemicals. Preliminary data suggest that, of the responses observed, and in common with some other recent investigations, regulation of chemokine receptor levels may provide a useful, and biologically relevant, correlate of skin sensitizing potential. These investigations are continuing, with particular emphasis on the search for novel genes that are implicated in allergen-induced DC activation but which have not previously been associated with skin sensitization.

5. Concluding comments

In recent years there has been very substantial progress in our understanding of the cellular and molecular mechanisms that serve to initiate and regulate skin sensitization. Such advances, combined with the application of new technology platforms, is now providing realistic opportunities to design novel approaches to hazard identification. The goal of a robust in vitro method that can serve as a realistic alternative to current predictive tests has yet to be achieved. In practice, realising this goal represents a substantial challenge; the need being for a method that is able to identify selectively a wide range of sensitizing chemicals, including those that require metabolic activation, and which ideally provides information of value in evaluating relative potency. However, although the challenges are great, if our investment in exploring basic molecular mechanisms of chemical sensitization is maintained, and is matched by a willingness to embrace new opportunities, then there is no reason why — for the purposes of hazard identification at least — this objective should not be realized.

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