

Review article

Predictive testing in contact allergy: facts and future

D. A. Basketter¹, I. Kimber²

¹SEAC Toxicology Unit, Unilever Research, Sharnbrook;

²Syngenta Central Toxicology Laboratory,
Macclesfield, UK

D. A. Basketter
SEAC Toxicology Unit
Unilever Research
Colworth House
Sharnbrook
Bedfordshire MK44 1LQ
UK

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Allergic contact dermatitis results from skin sensitization. Topical exposure of susceptible subjects to a contact allergen induces a cutaneous immune response that will, if of sufficient magnitude, result in the elicitation of a cutaneous inflammatory reaction if the same individual is exposed to the inducing chemical allergen on a subsequent occasion. Thus, a key question for toxicologists is how to predict which chemicals possess this property. In fact, several questions are associated with toxicologic evaluation of contact allergy. If the protocols used in the last half of the 20th century were as good as some believe, notwithstanding special cases such as nickel or plant dermatitis, why is there currently such a clinical burden related to this condition? How can toxicologists identify potential contact allergens and estimate their potency? Why do some individuals appear to be relatively readily sensitized, while others may undergo substantial exposure to the same contact allergens without any apparent ill effect? These questions may not seem at once related, but there is in fact an important correlation. Existing predictive tests may do a good job of identifying chemicals with the intrinsic ability to behave as a contact allergen, but they are not perfect. It is our thesis that while the process of hazard identification is amenable to modest improvement, proper risk assessment and consequent risk management can be enhanced significantly by taking advantage of recent improvements in predictive methods and by understanding better the immunobiology of contact allergy.

In particular, knowledge of the latter will pave the way in the future for the development of appropriate non-animal models of contact allergy.

Contact allergy predictive tests: the facts

What are the “facts” surrounding predictive testing in contact allergy? For the second half of the 20th century, several guinea pig models were used as a surrogate for the human immune system. Chemicals under investigation for their potential to behave as contact allergens were applied to groups of guinea pigs, by one or more routes of exposure with or without adjuvant, in an effort to induce contact allergy; the extent to which sensitization had been acquired was then judged by elicitation of allergic inflammation, normally after epicutaneous challenge. The potential confusion with cutaneous irritation was minimized by the use of sham-treated controls and the use of an appropriate challenge dose. These methods have been reviewed extensively elsewhere (1–3). Of particular importance, however, was the development of the guinea pig maximization test (GPMT) during the 1960s by Magnusson & Kligman (4). For the first time, a protocol was developed after an examination of the many parameters that would affect the sensitivity of the test procedure. The work was described in detail in a monograph published in 1970 (5). The aim was to produce a test method that, unlike the other procedures available at the time, would permit the identification of weak

contact allergens. Of particular relevance in this respect was the use of Freund's complete adjuvant, the intradermal injection of substances, and the use of moderately irritating test concentrations. Broadly speaking, it was judged that this aim was achieved, and subsequent assessment of the GPMT supported this position (1, 2, 6, 7). Nevertheless, it is true to say that at the time no rigorous assessment of the sensitivity and specificity of the GPMT was conducted; this only occurred much later, as an alternative method went through formal validation (see below). Perhaps not surprisingly, given this effort to optimize the sensitivity of guinea pig assays as predictive animal models, difficulties with the GPMT were noted; false positive results were identified (8, 9), and problems with the interpretation of apparent weakly positive reactions were described (10, 11).

The other commonly used guinea pig predictive method has been the Buehler test (12, 13). This protocol is regarded widely as being less sensitive than the GPMT, not least because it uses neither Freund's complete adjuvant nor the intradermal injections of test chemical employed in the GPMT. In addition, it is only relatively recently that extensive published data on the performance of the method compared with other procedures have become available (14, 15). Nevertheless, when properly conducted, the Buehler test appears to perform as well as the GPMT as a screen for potential contact allergens (15). Furthermore, it may well be less prone to false positives and interpretative difficulties that can confound results from the GPMT (9). A wide variety of other test protocols involving the use of guinea pigs have been described, but all follow a similar pattern of induction and challenge, with subjective assessment of challenge-induced erythema as the endpoint (reviewed in Ref. 1).

What are the facts about the ability of these guinea pig predictive tests to identify chemicals which represent a significant contact allergenic hazard? The most rigorous assessment was published only relatively recently (16, 17). Although the primary focus of these publications was the formal validation of a new predictive method, the local lymph node assay (LLNA), as part of that process, data were collated which served to substantiate the sensitivity and specificity of guinea pig-based predictions compared with what is known of human contact allergy. Analysis of 30 chemicals indicated that the overall accuracy of the guinea pig methods was 91%. A recent update to that data set, in which a total of 74 chemicals was examined, has provided a more robust assessment. This indicates that the overall accuracy of the guinea pig methods as predictors of human sensitization is 88% (18).

Various problems arose with the conduct of guinea pig tests, particularly with the GPMT (6). Technical aspects of the protocol as described originally (4, 5) were open to quite wide interpretations, leaving

important details to the choice of individual investigators. These, however, could have a major impact on the outcome of the test (19). Furthermore, little clear guidance was available on the selection of vehicles for use in these tests, despite the fact that it was well understood that the choice of vehicle might alter substantially the test result (20, 21).

Before moving to current and future trends in predictive skin sensitization testing, it is worth noting in passing that human studies have been conducted in the past. Of special relevance are those published during the 1960s by Kligman which describe the human maximization test (20, 22, 23). Also of significance were the studies of Marzulli & Maibach (24, 25). This work involving human volunteers achieved a number of objectives at the time, but, importantly, it has given us a valuable legacy in the 21st century of human predictive test data which can be used as a benchmark for new test methods. This data set has been used already for this purpose (16, 18, 26). No doubt, it will prove of considerable value for the evaluation of novel *in vitro* and/or *in silico* approaches.

One of the failures of the standard guinea pig test methods is their inability to provide the type of data which is required for further characterization of a skin sensitization hazard once it has been identified. In particular, it is difficult to interpret guinea pig results so as to provide information on the relative potency of a contact allergen (27, 28). (Note that here the term "potency" defines the intrinsic strength of the allergen and is separate from any considerations of exposure.) There are several reasons why this should be the case, including the use of adjuvant, occluded application, or injection of test substances and the subjective nature of the endpoint. In reality, it is not practicable in guinea pig assays to examine in detail multiple induction concentrations of the test chemical, and even if this were to be done, an endpoint that relies on a subjective assessment of the frequency of responses, rather than the vigour of responses, is not well suited to determination of the inherent potency of a sensitizing chemical. Attempts have been made to overcome some of these important limitations of guinea pig assays (29, 30), but for various reasons they have not met with much success, not least because the problem of the subjective endpoint has always remained. This topic has been reviewed recently (31).

Contact allergy predictive tests: the future

In reality, the future, or at least part of it, is already upon us. The guinea pig tests that have for decades dominated the field of predictive testing for skin sensitization have begun to give way to a new mouse model, the LLNA, which offers a number of advantages. In terms of hazard identification, the LLNA has

passed the necessary formal validation steps within the USA (17) and Europe (32). Thus, it can act as a complete alternative to guinea pig tests, and a draft test guideline has been circulated by the OECD (33). Details of the validation processes have been published extensively elsewhere and so will not be revisited here (16, 17). What is important to mention, however, is that the LLNA has an accuracy for hazard identification (in relation to human skin sensitization) of 86% (18), closely comparable with the figure for the guinea pig tests of 88% mentioned above, but obtained with a considerably larger data set of some 100 chemicals. This is achieved with a simpler methodology, fewer animals, and considerable animal welfare benefits (30, 34). Importantly, the LLNA results have been shown to be highly reproducible between laboratories (16, 35), a success not easily achieved with guinea pig results (2, 13, 36). However, in our view, the future prospects for predictive testing in contact allergy can be considered to fall into two main areas: methods for the *in vitro* hazard identification of allergens and methods for the estimation of relative allergenic potency.

***In vitro* methods for identification of contact allergenic hazard**

In this section, both *in vitro* and computer-based approaches to the prospective identification of chemicals which possess the ability to cause skin sensitization will be considered. Typically, *in vitro* predictive tests in toxicology examine cells which are thought to be relevant to the biological response and measure some relatively simple endpoint, such as cytotoxicity or release of mediators of the reaction, such as cytokines. For contact allergy, the state of the art has been considered quite recently (37, 38). In brief, there are three cell types that are of key importance in the development of contact allergy, Langerhans cells (LC), T cells, and keratinocytes. With the present level of understanding of the mechanisms of contact allergy (reviewed in Ref. 38), the process may be summarized as follows. After topical exposure of a susceptible individual to sufficient amounts of a contact allergen, a cutaneous immune response is initiated. Essential for this are the cellular-molecular interactions between epidermal cells and their cytokine and chemokine products. LC play pivotal roles; it is these cells that recognize, internalize, and process chemical allergen – in the form of a hapten-protein complex. Under the regulation of epidermal cytokines and various chemokines, LC are induced to migrate from the epidermis and to accumulate in the lymph nodes draining the site of exposure. During this migration, and again under the control of epidermal cytokines, LC are subject to a phenotypic change, losing the properties of antigen-processing cells and acquiring instead the features of mature, immunostimulatory dendritic cells (DC). It is

these antigen-bearing DC that present antigens to responsive T cells in the draining nodes. These T cells become activated and are stimulated to divide and differentiate. The cytokines that are of particular importance in regulating LC migration and maturation are interleukin-1 β (IL-1 β), tumour necrosis factor- α (TNF- α), and granulocyte macrophage-colony stimulating factor (GM-CSF).

Each stage of the induction of contact allergy has been examined as a potential route to the provision of an *in vitro* predictive test method. A number of groups have examined whether the interaction of hapten with keratinocytes might provide a practical approach (40–43). Some investigators have suggested that there are differences in the responses to allergens compared with skin irritants. Frequently, such studies have been performed with only a small number of compounds in which one or two very potent contact allergens (which typically are also strongly irritant) are compared with a classic moderate irritant, such as sodium lauryl sulphate. Not surprisingly, differences have been observed; the same would be true if different families of irritants were compared, or, perhaps, if varying doses of the same irritant were examined. However, the recurring overall problem is that the response of this cell type appears to be relatively nonspecific – it is the immune system which provides exquisite sensitivity and selectivity. In reality, the function of the keratinocyte appears to be to signal “danger” and so alert and activate elements of the immune system (44, 45).

A more promising candidate for the identification of a specific response to contact allergens *in vitro* is the LC or its *in vitro* cultured equivalent (see below). One pivotal response of the LC is to migrate from the epidermis to the draining lymph node, and it has been suggested that monitoring of this migration in skin explants might provide one approach to an *in vitro* test (46). However, a broader consideration of this matter confirms that in fact LC migrate from the epidermis in response to a wide range of stimuli (47). In addition, there is at present no evidence that there is a consistent pattern of phenotypic changes in the LC that is specific for contact allergens (48). Interestingly, a recent study using blood-derived, cultured, LC-like DC found a more consistent upregulation of certain markers, including CD54 (ICAM-1) and CD86 (B7-2) in the presence of one of a small number of allergens, but without significant augmentation by an irritant (49). However, the limited response to the very strong contact allergen, 2,4-dinitrochlorobenzene, is a cause for concern and indicates the need for further work, as does the observation of the stimulation of CD86 expression by a range of metal salts, including some that are not recognized as being human contact allergens (50).

The ability to derive LC-like human DC by selective culture of blood-derived monocytes has offered new

Table 1. Correlation of LLNA and human potency classifications^{a,b}

Chemical	Human potency class ^c	LLNA EC3 value (%)	LLNA potency class
2,4-Dinitrochlorobenzene	Strong	0.08	Strong
Diphenylcyclopropenone	Strong	0.05	Strong
Methyl/chloromethylisothiazolinone	Strong	0.05	Strong
<i>p</i> -Phenylenediamine	Strong	0.06	Strong
Cinnamic aldehyde	Moderate	2.0	Moderate
Formaldehyde	Moderate	0.4	Moderate
Glutaraldehyde	Moderate	0.2	Strong
Isoeugenol	Moderate	1.3	Moderate
Methyldibromoglutaronitrile	Moderate	5.2	Moderate
Tetramethylthiuramdisulphide	Moderate	6.0	Moderate
Phenylacetaldehyde	Moderate	4.7	Moderate
<i>p</i> - <i>t</i> -Butyl- α -methyl hydrocinnamal	Weak	19	Weak
Citral	Weak	13	Weak
Cyclamen aldehyde	Weak	21	Weak
Eugenol	Weak	13	Weak
Hexylcinnamic aldehyde	Weak	8.0	Weak
Hydroxycitronellal	Weak	20	Weak
5-Methyl-2,3-hexanedione	Weak	26	Weak
<i>p</i> -Methylhydrocinnamic aldehyde	Weak	14	Weak
Ethylene glycoldimethacrylate	Extremely weak	35	Extremely weak
Isopropyl myristate	Extremely weak	44	Extremely weak
Linalool	Extremely weak	30	Weak
Propylene glycol	Extremely weak	Nonsensitizing	Nonsensitizer
Propyl paraben	Extremely weak	> 50	Nonsensitizer
Vanillin	Extremely weak	> 50	Nonsensitizer
Acetanilole	Nonsensitizer	Nonsensitizing	Nonsensitizer
Aniline	Nonsensitizer	> 50	Nonsensitizer
Dextran	Nonsensitizer	Nonsensitizing	Nonsensitizer
Diethylphthalate	Nonsensitizer	Nonsensitizing	Nonsensitizer
Ethyl vanillin	Nonsensitizer	> 50	Nonsensitizer
Glycerol	Nonsensitizer	Nonsensitizing	Nonsensitizer
Hexane	Nonsensitizer	Nonsensitizing	Nonsensitizer
Isopropanol	Nonsensitizer	Nonsensitizing	Nonsensitizer
Octanoic acid	Nonsensitizer	Nonsensitizing	Nonsensitizer
Tween 80	Nonsensitizer	Nonsensitizing	Nonsensitizer

^a Data taken from references and combined with additional unpublished information.^b LLNA: local lymph node assay.^c Classes strong, moderate, and weak would classify as skin sensitizers (R43) in EU legislation (83).

opportunities for the investigation of novel *in vitro* predictive methods. The most widely investigated approach has been the expression by LC of mRNA for IL-1 β . This cytokine has attracted particular interest, not least because IL-1 β appears to be produced exclusively by LC in the mouse epidermis (51, 52). IL-1 β is known to be required for the induction of LC migration by chemical allergens (53, 54); if it is absent or neutralized, the acquisition of contact sensitization is impaired or inhibited (55, 56). A seminal observation was made in 1992 by Enk & Katz, who found that topical treatment of mice with contact allergens, but not with a skin irritant, resulted in a rapid increase in the expression by LC of mRNA for IL-1 β (57).

Collectively, these data suggested that it might be possible to identify contact allergens as a function of their ability to stimulate the elevated expression of IL-1 β by cultured LC (58). Exploration of this approach has been facilitated by the availability of methods for

the maintenance in culture of DC, derived from precursors in human peripheral blood or other sources, with phenotypic characteristics resembling those of LC (59, 60). Study of cultured human LC-like cells has shown that contact allergens do indeed have the ability to stimulate increased mRNA expression by cultured DC under conditions where nonsensitizing skin irritants do not do so (61, 62). However, these investigations also have shown that there may be stable differences between blood donors with respect to the responsiveness of DC to allergen-induced changes in IL-1 β expression, and that this, coupled with the relatively low level of response seen even with very strong allergens, seems likely to compromise the ability of this system to be a reliable predictive tool (62, 63).

If biological methods are still in the developmental phase, it is worth remembering that much can be learned from the chemistry of contact allergy. This has been the subject of two recent publications (64, 65)

which detail how our understanding of hapten chemistry has advanced sufficiently to allow us to predict protein reactivity and metabolic activation/inactivation. Perhaps the most fruitful approach has been to capture existing knowledge of the chemical structures which can give rise to contact allergenic hazards in an expert computer system, DEREK (66–68). The advantage of this type of system is that it is built on rules which can evolve and thus improve as our understanding of the chemistry of contact allergy increases (69). Thus, for example, the newly reported understanding of the differing reaction mechanisms for aldehydes can be incorporated, so that chemical structural alerts for this type of allergen are improved (70). Such an approach is more likely to prove of general value than the construction of quantitative structure-activity relationships (QSAR), which tend to describe only very restricted sets of chemicals (reviewed in Ref. 64).

Methods for the estimation of contact allergenic potency

The LLNA provides a basic evaluation of contact allergen dose responses, delivered as objective, quantitative data (16). However, it is known also that LNC proliferative activity not only provides a marker for skin sensitization, but also correlates quantitatively with the extent to which sensitization is acquired (71). Consequently, it was recognized that there was a real possibility to make better use of the dose-response data and so provide information on the relative potencies of skin sensitizers (72). The approach taken is to derive mathematically the amount of chemical necessary to provoke a threefold increase in the proliferative activity in draining lymph-node cells compared with concurrent vehicle-treated controls. This is termed the EC3 value

(73). The reliability and stability of this measure have already been demonstrated (74), as has its interlaboratory reproducibility (75). The EC3 value is calculated by the following equation:

$$EC3 = c + [(3 - d)/(b - d)](a - c)$$

where the data points lying immediately above and below the SI value of 3 on the LLNA dose-response plot have the coordinates (a,b) and (c,d), respectively.

A question of primary importance, however, is whether and to what extent EC3 values derived from the LLNA provide information of relevance to the potency of contact allergens in man. Preliminary investigations have demonstrated that this is a useful measure (76, 77). However, the most important demonstrations of the potential utility of EC3 values have derived from more extended comparisons with what is known of the relative potency in man of a wider range of contact allergens (78–80). EC3 values for 35 chemicals of widely varying skin sensitizing potency in man can be shown to be extremely well correlated (Table 1). Thus, the challenge for the future is to determine the best way to incorporate EC3 data into quantitative risk assessments, an effort that is already underway (81, 82).

Conclusions

The way in which all aspects of the toxicologic evaluation of skin sensitization have developed is a compelling example of how investment in defining basic molecular and cellular mechanisms has paid great dividends in terms of our ability to identify hazards and risks to human health.

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