Contact Dermatitis 2010: 63: 237–247
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CONTACT DERMATITIS

Review Article

Quantifying human susceptibility to contact sensitization; risk assessments now and in the future

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Assessment and quantification of the risk that a chemical will induce allergic contact sensitization presently depend heavily on background data from animal tests. Following the banning of animal testing of chemicals used in cosmetics and personal products in Europe after 2013, alternative approaches will be required. The chemical properties likely to make a given compound a sensitizer can be determined *in vitro* with reasonable certainty, but confirmation that it is a sensitizer comes only from *in vivo* exposure to it. Assessment of the sensitization risks involves consideration of how much of the compound will be applied to skin, for how long, and at which sites. However, the *in vivo* interactions of the chemical with the skin, with regard to its permeability, and biochemical and immune defences, cannot be predicted from a theoretical position. The xenobiotic-metabolizing enzymes and antioxidant defences may degrade chemicals or may generate potentially immunogenic haptens. Many factors can modify the skin and the immune response, including sex, race, age, genetic programming of epidermal permeability, and/or antioxidant and drug-metabolizing pathways. The only certain way to evaluate whether a chemical will sensitize is *in vivo* exposure, and the nature of the hazard is revealed by determination of the dose–response relationship. This review shows there is still a serious gap in our understanding of the biological factors and variables involved in conferring resistance or susceptibility to the development of allergic sensitization by chemicals. We are not yet in a position to predict sensitization by chemicals from a theoretical starting point.

Key words: contact sensitization; DNCB; epidermal barrier; quantitative risk assessment; stratum corneum; review. © John Wiley & Sons A/S, 2010.

Conflicts of interest: An educational grant was received from Unilever plc to support some of the work described herein.

Accepted for publication 26 June 2010

Humans are exposed to a great number of chemicals in the environment, and many people develop allergic sensitization to one or more of these. Understanding the risks and quantifying the likelihood that new chemicals entering the environment will cause trouble by inducing allergic sensitization – so-called quantitative risk assessment – is important. We now understand many of the general principles of why some chemicals are likely to induce immune responses and of how the immune system works to generate such immune responses. Much of our present knowledge has been acquired from research using animals. The challenge that we will soon face is that, after 2013, when animal testing of cosmetic ingredients is banned in Europe, the risks will be assessed by extrapolation of theoretical knowledge linked to general principles.

In order to become visible to the immune system, chemicals must be able to react with proteins to form 'haptens'. This can be predicted both from a theoretical 'structural' point of view and from the results of *in vitro* or *in vivo* experimentation. However, as new chemicals are introduced into the environment, particularly in the domestic and personal product arena, it is important that the risks of possible allergic sensitization resulting from exposure to these substances can be calculated or estimated with some accuracy. In comparison with the robustness of the assessments of the properties of chemicals, knowledge of the human 'susceptibility factors' that

may contribute to how chemicals interact with skin, determining whether a chemical is converted to a skin-sensitizing hapten, or that determine the dose of chemical that reaches the immune system, is decidedly not robust. Although our understanding of these 'host qualities' is steadily increasing, there are very few aspects that we are able to quantify properly. Therefore, a review of to what extent we can be quantitative in our approach to host susceptibility is essential to allow the definition of areas for which an improved quantitative basis is needed.

The process of risk assessment for skin sensitization involves four main steps:

(1) Hazard identification – determination of the physico-chemical properties of a compound that make it likely to be a sensitizer, and subsequent confirmation in animal tests [e.g. local lymph node assay (LLNA)].
(2) Hazard quantification – assessment of how potent a sensitizer it might be, that is, the sensitizing threshold dose.
(3) Assessment of exposure – how much of the substance is applied to the skin (dose) and how the substance will be encountered – whether by long-term or short-term contact, with what sort of frequency, and on which skin areas.
(4) Risk characterization – combining the assessments of exposure and the potential sensitizing potency of the compound to predict the probability that an adverse event will occur.

In recent years, a more quantitative approach has been developed: quantitative risk assessment (QRA) (1). This method endeavours to take into account human variability, product matrix factors and consumer exposure and use by applying assessment factors to adjust the sensitization threshold derived from the hazard quantification described above. The different steps are described in more detail below.

(1) *Hazard identification*: In order to be able to activate an immune response, chemicals have to become bound to proteins. The protein-bound chemical – called a hapten – can then be recognized by T-lymphocytes if it is 'presented' by antigen-presenting cells (APCs) in the correct way, that is, associated with major histocompatibility molecules. In order for an APC to activate a naïve T-cell during the presentation of the 'antigen', the APC itself must have become activated through a series of processes induced because the immunogen is somehow sensed as 'dangerous'. Exactly how danger signals are given by haptens is not clear, but the most potent sensitizers are clearly capable of giving danger signals ensuring that the APCs initiate the specific T-cell response against the hapten.

Chemicals may be spontaneously reactive and capable of binding to nucleophiles such as cysteine or lysine in proteins. This is amenable to relatively certain laboratory assessment. However, the matter is complicated by the possibility of the native chemical undergoing metabolic processing, for example, by enzymes of the cytochrome P450 superfamily, which may result in the formation of reactive intermediate metabolites capable of binding to proteins. Anticipation of this could be very difficult without experimental *in vivo* studies. One source of uncertainty is the possibility that individuals may differ in the effectiveness of the xenobiotic-metabolizing pathways and some individuals may be more likely to generate protein-reactive intermediate metabolites. After the assessments of the physico-chemical properties of a substance suggested that it might be a sensitizer, it was traditional to test this *in vivo* in animals by use of assays such as the Buehler test, guinea pig maximization test, or the murine local lymph node assay (LLNA). In the past, many chemicals were also tested for their sensitizing capacities in humans by use of the human repeated insult provocation test (HRIPT). It is hard to see how, in the future, the judges of ethical science will allow the testing of completely new chemicals in humans – but if they cannot be tested in animals, this may be the only way in which they can be assessed.

(2) *Hazard quantification*: Currently, skin sensitization hazard can only be quantified, that is, the potency/dose response can only be defined, in *in vivo* tests. At present, there is a reasonable body of data on the relative sensitizing potencies of chemicals. These have been systematically established with use of the LLNA (2, 3). Overall, the LLNA has been shown to have very good reproducibility, both between tests and between centres (4, 5). By use of a range of concentrations of the chemical of interest, the dose response can be established for inducing proliferation of murine lymph node cells. Extrapolation from this allows prediction of the EC3 value, which is the concentration that would elicit a three-fold stimulation of lymph node cell proliferation – expressed as percentage or dose per unit area. This has allowed the classification of chemicals into five categories: (i) extreme or potent; (ii)

Table 1. Classification of sensitising potency for chemicals

Sensitizing potency	LLNA EC3 (μg/cm^2)
Extreme/potent	≤10
Strong	10–100
Moderate	100–1000
Weak	1000–10 000
Extremely weak	>10 000

LLNA, local lymph node assay.

strong; (iii) moderate; (iv) weak; and (v) non-sensitizers (6). The quantitative basis for this classification is derived from the EC3 values in the LLNA (Table 1) (7). For the 26 chemicals that have been assessed most rigorously, there has also been a comparison with the best data from human testing (HRIPT), in which the threshold concentration for inducing sensitization was defined. In general, a very good correlation was found (6), the range of EC3 and HRIPT threshold concentrations spanning several orders of magnitude, from as low as 1 μg/cm^2 to nearly 10 000 μg/cm^2.

(3) *Assessment of exposure*: This involves assessments of the type of product, how much is applied at each use, whether it is a leave-on or rinse-off product, how frequently the product is used, and to which body sites it is applied. From this, a quantitative estimate of exposure is derived, and expressed as the dose per unit area.

(4) *Risk characterization*: The threshold for sensitization derived from the hazard characterization is adjusted by taking into account the human variability (factors that relate to the human host, such as race, sex, and age), product matrix (vehicle and presence of irritants or other components), and consumer use (skin sites and areas, and whether there is occlusion), to derive an acceptable and safe level of exposure. The ratio between this figure and that from the assessment of exposure (step 3 above) must be >1; in other words, the consumer exposure must be less than the calculated safe exposure level.

The factors mentioned above will now be reviewed in more detail.

Product-related Considerations

The product-related factors, including type of product, and consumer habits, including site, area and frequency of application, will not be considered here, as they have reviewed in detail by authors experienced in the details of QRA (1, 6, 8).

Vehicle or product matrix

Irritant properties. Research into irritant responses is characterized not only by a lack of understanding but also by a lack of critical thinking. First, most workers talk about irritants as though they are the same, that is, that the mechanisms involved in generating inflammation are the same. However, sodium hydroxide, hydrochloric acid and acetic acid will be irritants at low concentrations, probably through pH disturbances that are likely to be cytotoxic; dithranol and croton oil have tumour-promoting effects that stimulate the transcription factors AP1 and nuclear factor kappaB to induce cytokine expression; and organic compounds such as decanol, nonanoic acid and octanoic acid are likely to have a variety of membrane-disrupting effects. Sodium lauryl sulfate (SLS) is a detergent that is capable of solubilizing intercellular lipids and also cell membranes, disrupting the stratum corneum permeability barrier. It is now known that, following stratum corneum barrier disruption, whether by SLS, by organic solvents, or by tape stripping, there is activation of repair of the stratum corneum (see above), which also activates serine proteases and an inflammatory (irritant) response (9, 10). Many of the more potent contact sensitizers are also irritants. It is assumed that the irritant effects reflect activation of the innate immune response, which in turn promotes the maturation of dendritic cells, potentiating their T-cell-activating properties. However, the mechanisms by which innate immune responses are activated are generally not clear. One of the properties shared by many irritant contact sensitizers is the ability to induce oxidative stress with the generation of reactive oxygen species, and much research is now focusing on the role of antioxidant defences in determining susceptibility both to irritants and to contact sensitizers. The literature on the interaction between irritants and contact sensitizers is filled with the mixing of two different aspects: (i) the barrier-disrupting effects of surfactants/solvent irritants, which may augment the penetration of sensitizers; and (ii) the possible adjuvant effects through concomitant 'irritation' inflammation. The latter was shown by Grabbe, who found that addition of an irritant sensitizer to a non-irritant one at subclinical levels greatly augmented sensitization by the non-irritant compound (11). However, they did not consider the possibility that the irritant may be augmenting penetration.

The overlap of the processes involved in the generation of irritant responses, which are likely to involve activation of innate immune responses by non-sensitizing irritants, and the additional activation of the adaptive immune response by contact-sensitizing chemicals has led to the use of irritant responses as predictors of potential

susceptibility to contact sensitization. Thus, one study investigated 23 individuals with a pre-existing contact allergy to colophonium (12). When susceptibility to irritation by SLS was determined by challenging them with a concentration series of SLS ranging from 0.1% to 20%, 78% of the people with allergies reacted to SLS at or below 2.5%. By contrast, only 44% of non-allergic controls reacted to these concentrations. Another study by the same authors investigated trainee hairdressers, following them over the first 6 months of training (13). The thresholds for irritation by SLS and the presence of any contact sensitivities were determined at the start, and after the 6 months the development of hand dermatitis and new allergic sensitization was determined. Overall, 9/24 developed hand dermatitis, and these had a significantly lower threshold for irritation by SLS. In the whole study, there was also an association between the development of allergic contact sensitivity and a low threshold for irritation by SLS. Thus, tissue defences that might confer resistance to irritation responses do appear to be reduced in people who are more susceptible to becoming contact sensitized.

Penetration enhancement and vehicle effects. It is taken as a given that the vehicle in which a compound is delivered to the skin may be important in determining the amount that penetrates (14). Kligman studied this in humans who had become sensitized to various substances that were either water-soluble or lipid-soluble (15). He made a range of dilutions (2%, 1%, and 0.5%) of each compound in six bases: two hydrophobic oily bases (petrolatum and anhydrous lanolin), two emulsions with amphipathic properties (hydrophilic ointment and Aquaphor®), and two hydrophilic bases (polyethylene glycol and water). He patch tested the allergic individuals and, for each vehicle, scored the numbers who reacted at each dilution to obtain a composite score. Differences only emerged at and below 1%, and interestingly, pet. was the best vehicle for both water-soluble and lipid-soluble substances, with lanolin being second best for water-soluble substances and third best for lipid-soluble substances. The water-based vehicles were worst, and elicited the fewest positive responses. The general pattern was that the lipid-based vehicles would still elicit positive reactions when allergens were diluted below 1%, whereas the water-based vehicles only elicited positive responses with the compounds at 2–10%. Marzulli and Maibach compared pet. and ethanol as vehicles for delivery of sensitizing doses of a range of chemicals to human volunteers (16). The differences were not great, and they concluded there was no compelling reason to choose one vehicle in preference to the other. A more quantitative assessment of the effects of some vehicles was obtained by use of the EC3 value from the LLNA (14, 17). Seven skin-sensitizing chemicals were tested at a range of concentrations in five solvents differing in their hydrophobicity: acetone/olive oil, methylethylketone, dimethylformamide, propylene glycol, and dimethyl sulfoxide. For some chemicals (1,4-dihydroquinone and methylchloroisothiazolinone), there was a >10-fold difference in the EC3 value obtained with different solvents, whereas for other chemicals (cinnamal and isoeugenol), there was an approximately two-fold difference. Surprisingly, the effects of vehicle appear, overall, to be rather smaller than might have been imagined.

Interactions between putative sensitizers and other constituents. In the assessments of whether a potentially immunogenic chemical actually becomes a sensitizer *in vivo*, the metabolic and biochemical processing of the compound is likely to be critical. Xenobiotic-metabolizing enzymes such as glutathione-*S*-transferase (GST) or the cytochrome P450 enzymes inflict oxidative stress on the cells/tissues. The constitutive levels of antioxidant defences such as glutathione may be crucial in allowing the complete detoxification of a chemical. If, within the product, there are other substances that also compete for the antioxidants, there may be incomplete detoxification of the compound of interest and the generation of the sensitizing form of it. In the field of drug allergy, it is well known that drug detoxification pathways that appear just to be coping with a given drug can be overloaded by the addition of a second drug, so that detoxification of the first drug is incomplete, and sensitization and allergy ensue. One of the best-known examples is the addition of sodium valproate to the anti-epileptic regimen of carbamazepine, which results in allergy to the carbamazepine. McLelland and Shuster showed that when two allergens were applied as a mixture at concentrations that were individually below the level capable of eliciting contact allergy in sensitized individuals, in combination they could synergize and elicit reactivity (18). One interpretation of this was that each allergen contributed an increment of activation of the innate immune response, and that these then combined to recruit sufficient numbers of T-cells to trigger the inflammatory response. However, an alternative interpretation is that the two chemicals had interacted to alter the metabolic processing, allowing higher concentrations of one or both to be present. Clearly, this possibility could also operate with regard to the induction of new sensitivities.

Although anti-oxidants are usually added to personal products, this is mainly done to prevent the oxidation and deterioration of various ingredients in the product. A preventative strategy that

might be useful for the manufacturers of personal products would be to increase the quantities of biologically useful antioxidants such as reduced glutathione or *N*-acetylcysteine, so that even as the tissues are encountering the oxidative stress from the chemical, they are being supplemented with the required antioxidants to prevent generation of the sensitizing moiety. The relevance of this was shown by Naisbitt et al., who demonstrated that T-lymphocytes from people allergic to p-phenylenediamine (PPD) could not react to PPD in the presence of excess glutathione (19).

Host-related Factors

The key point that dominates all considerations of how host-related factors may alter susceptibility to sensitization by environmental xenobiotics is that the immune response exhibits clear dose-related responses. Most of the host-related factors considered below cause their effects by modifying the effective dose of a putative sensitizer that reaches the immune system.

Quantitative aspects of the human contact sensitivity response

Experimental studies with the extremely potent experimental sensitizer 2,4-dinitrochlorobenzene (DNCB) have shown that sensitization is directly proportional to the log of the sensitizing dose. Over a nearly 20-fold dose range from 8.8 to 142 μg/cm^2, there are log-linear increases in the proportion of people sensitized and their strength of reactivity (20, 21). There is clear evidence that the variation of responsiveness in healthy humans is normally distributed, so that in a group exposed to any given sensitizing dose of DNCB, a range of strengths of reactivity is induced. It has been shown that towards the 'high responder' end of the distribution, as susceptibility increases, so individuals develop more contact sensitivities. By use of quantitative sensitization with DNCB, it was shown that, in comparison with individuals with no contact sensitivity, individuals with allergy to a single sensitizer were more reactive to DNCB, and individuals with contact allergies to three or more unrelated sensitizers were much more reactive to DNCB (22). The corollary is that towards the 'low-responder' end of the spectrum, there are individuals with low susceptibility (high resistance) to contact sensitization. These individuals would be indicated by the lowest reactivity even at high sensitizing doses of DNCB.

As discussed above, these dose–response relationships were determined by use of single exposures to the sensitizer. However, there is a variety of evidence of different quality showing that repeated exposure to low doses of sensitizers can induce allergic contact sensitization (15, 23), and some of the evidence even suggests that repeated low-dose exposure may be more potent than single higher-dose exposures (24). Thus, Kligman observed the effect of increasing numbers of applications of several potential sensitizers including penicillin V, neomycin sulfate, and ammoniated mercury (15). He used rather high concentrations (10%), but showed, for these and others, that as the number of exposures increased to 15, so sensitization could be induced in healthy humans. Thus, 76% were sensitized to penicillin V, 70% to ammoniated mercury, and 48% to neomycin sulfate (Table 2). In a large prospective study, sensitization by PPD was assessed in groups of healthy human volunteers who used either a low-concentration hair colorant applied frequently for short durations, or a higher-concentration hair dye applied for a longer period but at less frequent intervals (23). Of the group that applied hair colorant (PPD final concentration of 0.48%) for 5 min once a week for 6 months, 7.2% were sensitized. In the comparator group, which applied hair dye (PPD 3%) for 30–40 min once a month for 6 months, only 1.3% were sensitized. The present authors recently investigated the effect of repeated exposures to low doses of an experimental sensitizer (24). DNCB was applied to one group of healthy human volunteers at 60 μg/cm^2, a dose that induced moderate sensitivity in 100% of volunteers when quantitative elicitation doses were applied 4 weeks later (25). The comparison group received three separate doses of 10 μg/cm^2, applied to the same site at weekly intervals, before being challenged. All volunteers became sensitized, and their degree of reactivity to the challenges was identical to that of the group who received the single dose of 60 μg/cm^2. Thus, when applied as small increments, half the total dose (30 versus 60 μg/cm^2) induced equal sensitization. Taken together, the above results suggest that when individuals become strongly sensitized

Table 2. Effects of increasing numbers of exposures on frequency of sensitization

	Number of exposures			
Agent	3	5	10	15
Benzocaine (5%)	0/23	1/22	3/25	6/25
	0[a]	**4.5**	**12**	**24**
Tetramethylthiuramdisulfide (10%)	0/25	0/25	2/22	6/18
	0	**0**	**9**	**33.3**
Neomycin sulpfate (10%)	0/24	1/25	4/23	10/21
	0	**4**	**17.4**	**48**
Penicillin G (10%)	1/25	5/25	10/21	16/21
	4	**20**	**48**	**76**

[a]% positive.
Data modified from Kligman (15).

to some of the ingredients in personal products that are present at very low concentrations, this is likely to be the result of repeated low-dose exposure (26).

In the assessment of sensitizing potency for QRA, the main *in vivo* tests used actually involve repeated applications of the chemicals under investigation. The LLNA involves applications of the chemical under investigation three times at daily intervals; the HRIPT involves applications repeated usually 9 or 10 times over 15 days. The discussion of the 'sensitizing dose' normally focuses on the dose applied singly rather than the cumulative dose. For consideration of relative potency, this is not critical, as it still allows the ranking of chemicals in order. However, for more precise comparisons, the numerical relationships can change. For example, for methylchloroisothiazolinone (MCI)/methylisothiazolinone (MI), the EC3 value is given as 2.25 μg/cm^2 (6), but the cumulative dose would be 6.75 μg/cm^2 in the LLNA. The threshold value for the HRIPT is given as 0.83 μg/cm^2, but the cumulative dose is really 8.3 μg/cm^2. A complicating factor is that it is probably too simplistic to assume that, over the 15 days of the induction applications, the entire dose applied penetrates to reach the immune system. Repeated exposures, either in formal tests such as HRIPTs or through repeated use of personal products, are effectively an accumulation of the administered doses, so it is certainly erring on the side of safety to use the dose applied as single applications.

How do sensitizing dose and eliciting dose correlate? Can eliciting doses be used to predict sensitizing doses?

The general principle that emerges from both the empirical observations in patch test clinics and the formal quantitative studies described above is that when individuals are strongly sensitized, they are highly reactive. In other words, they will react to low concentrations of the relevant allergen. This is clearly seen in the populations of volunteers who were sensitized with DNCB, in that those who received the highest priming doses were most strongly sensitized, and their reactivity could be elicited with less than 1 μg of DNCB (Fig. 1) (26). It might be quite straightforward to use these relationships in a QRA to predict what might be a 'safe' exposure level for DNCB. However, the matter becomes far from straightforward when some real-life situations are examined. Thus, one of the phenomena encountered in patch test clinics is that some individuals can become extremely reactive to chemicals that are normally encountered only in very low concentrations (27, 28). In these studies, sensitized/allergic individuals received 28 or 56 applications, respectively, of chloroatranol or MCI/MI in repeated open application tests. Positive responses were elicited by cumulative doses of 0.84 and 0.7 μg/cm^2, respectively (26). Both of these chemicals are normally found in perfumes and other personal products at about 0.0008 μg/ml (8 ppm) (27, 28). These concentrations appear to be well below the threshold levels at which they might be able to induce allergic sensitization. Therefore,

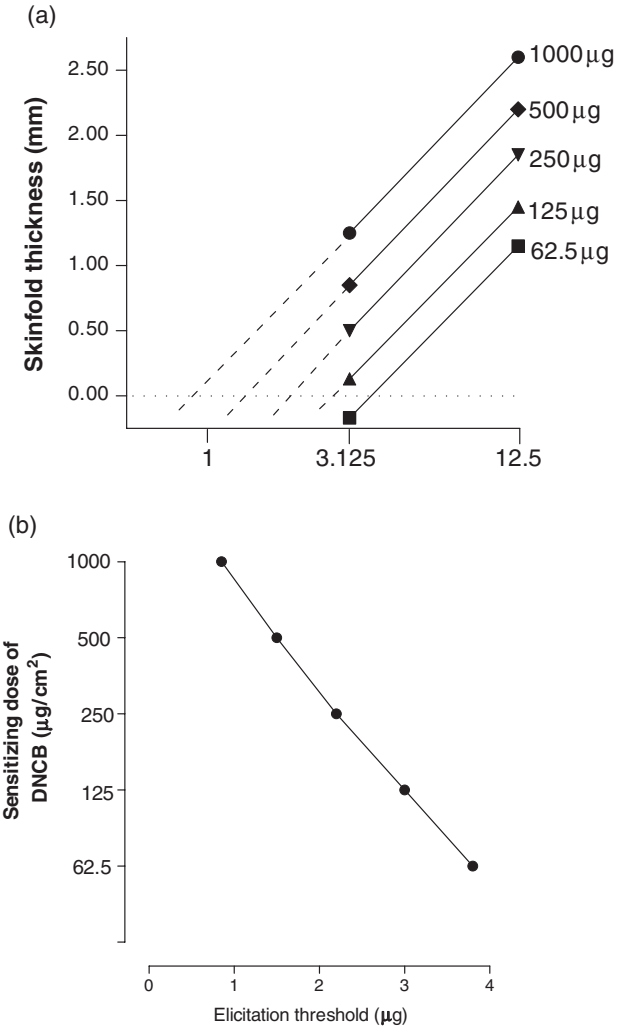

Fig. 1. (a) Dose–response relationships for elicitation challenge in normal volunteers. Five groups of healthy volunteers received initial sensitizing doses of 2,4-dinitrochlorobenzene (DNCB) of 1000 (●), 500 (◆), 250 (▲), 125 (▼) or 62.5 μg (■) of DNCB. Four weeks later, they were challenged with four doses of DNCB (from 3.125 to 25 μg), and responses were measured as thickness with skinfold callipers. The regression lines of the linear portion of the dose–response curve are plotted as solid lines. The calculated extrapolation to $X = 0$ (theoretical threshold elicitation dose) is plotted as dotted lines. Figure reproduced with permission from Friedmann (26). (b) Calculated elicitation thresholds in relation to sensitizing doses of DNCB. The data from (a) are re-expressed as the minimal doses required to elicit reactivity (threshold) against sensitizing doses.

it can only be concluded that it is the repeated exposures to low concentrations that have induced allergic sensitization, and at a very high level of sensitivity. Hence, the working principle is that even though sensitized individuals may be highly reactive to the relevant allergen, in real life it may be impossible to relate this to the dose that would be a risk for inducing sensitization. As shown above, the sensitizing dose may be experienced as multiple repeated exposures to what was thought to be a dose well below the dose that would/could sensitize.

Individual Susceptibility

Individual susceptibility to contact sensitization can be determined at several levels between the skin and the immune system. These include:

(1) The physical integrity of the epidermal barrier (filaggrin gene, lipid types).
(2) The integrity and quality of biochemical defences, including antioxidant defences and drug-detoxifying/metabolizing enzyme systems.
(3) The innate immune responses of the skin.
(4) The adaptive immune response:
    (a) immune response reflected in MHC-linked ability to present immunogens;
    (b) T-cell receptor recognition of immunogens;
    (c) Immune response controls balancing between immunological tolerance and active expression of hypersensitivity (regulatory T-cells and effector T-cells).

In considering the human host factor(s), two approaches will be taken here. The first is to examine how discrete modifying factors such as genes, age and sex can affect overall susceptibility to the development of contact sensitivities. Second, although it has been traditional to focus on the adaptive immune response that generates the specific T-cell-mediated responses to immunogenic chemicals/haptens, there is now a growing realization that the skin is, in fact, the major determinant of whether the adaptive immune system becomes activated. It does this partly by determining the dose of chemical that reaches the adaptive immune system, and partly by contributing activating signals to dendritic cells that augment the chance that they will initiate T-cell activation. Many elements of skin function are variable, as a result of genetic, environmental, hormonal, nutritional and even age-related factors. Many of these functional elements were previously regarded as separate or independent, but are now recognized as being closely interdependent.

The key functions include:

- Stratum corneum integrity/impermeability
- Innate immune defences
    - Tissue defences against microbial invasion
    - Tissue defences against chemical perturbation
    - Responses to physical, microbial or chemical perturbation

Discrete Factors Modifying Susceptibility

Race

The evidence on whether race is a significant factor in susceptibility to contact sensitization is weak. Some reports of susceptibility to irritants have claimed that Asians may be more susceptible than Caucasians. However, the main report collected data from nine studies performed at three different centres in the USA (29). A total of 384 individuals aged 18–74 years had been exposed to a number of irritants, including SLS (all) acetic acid, 1-decanol, and octanoic acid. Patches were applied to the upper outer arm for various times up to 4 hr, and responses were assessed by clinical scoring at 24 and 48 hr. The Asians developed irritant responses to SLS after shorter durations of patch application. A problem with the results from this report is the degree of human variability in responses to SLS. All subjects were tested with SLS as a 'positive control' in each study, and across the nine studies the percentage of people giving positive responses to a 4-hr challenge with 20% SLS varied from 60% to 100%.

Some evidence on racial differences in susceptibility to allergic contact sensitization was obtained by Kligman. He sensitized groups of white and black volunteers (25 per group) with a number of sensitizers (15). Lower frequencies of sensitization were observed in black subjects with four of five allergens tested: monobenzyl ether of hydroquinone, nickel sulfate, penicillin G, and neomycin sulfate (15). There is no evidence on possible reasons, but differences in stratum corneum thickness and possibly increased cutaneous antioxidant defences may contribute.

Age

Age has little overall effect during childhood and adult life. However, infants are clearly less sensitizable. Cassimos used a high dose (1%) of DNCB to sensitize groups of neonates at 1, 3 and 9 months of age (30). It was shown that over the first 9 months of age, the proportions sensitized (as reflected by a positive response to challenge with 0.1%) rose from 6.7% in the first 15 days of life, to 26% at 1 month,

to 63% at 3 months, and to 91% at 9 months. Even though the methodology used was not well quantified, and the results are only expressed as proportion sensitized, it is a clear result. There is clinical evidence that immune function declines in old age, as reflected by the increase in viral infections and tumours, but the evidence on cutaneous immune reactivity shows some interesting divergence. Thus, T-cell-mediated responses, including recall responses to intradermal challenge with tuberculin, streptokinase/streptodornase, or *Candida albicans*, are reduced after the age of 65 years (31). That study also used rather heavy-handed methodology to assess induction of contact sensitivity with DNCB (10%), and found that significantly fewer individuals older than 65 years could be sensitized. The present author (P.S.F.) has used quantitative methods to measure sensitization by DNCB in a wide range of adults, including the very old (up to the late eighties), and found that responsiveness does not diminish until after about 80 years of age (personal observation). As clear evidence that susceptibility to irritants may be different from susceptibility to contact sensitization, there is significant evidence of a decline in skin irritation responses with age. Thus, in the studies described in relation to race, Robinson also looked at the effects of age. A significant fall-off of responses to SLS and octanoic acid was observed after 56 years of age (32). This may be related to the recent demonstration that in older people, there is a failure of production of tumour necrosis factor-α by dermal macrophages, which results in impaired cellular recruitment through interaction with dermal microvascular endothelium (33).

Sex

There is still uncertainty about how important sex is in determining susceptibility to contact sensitization. Some aspects of the immune and inflammatory responses are clearly influenced by sex. Thus, most autoimmune diseases are commoner in females, and certain diseases, such as urticarias and atopic dermatitis, are often exacerbated in relation to the menstrual cycle. Vascular responses following degranulation of mast cells show a distinct variation with the menstrual cycle (34). The magnitudes of weal and flare reactions following prick test challenge with morphine or histamine were measured at different phases of the menstrual cycle, and it was shown that greatest responses occurred at days 12–16 of the cycle, corresponding to maximal oestrogen levels. However, T-cell-mediated responses, such as contact sensitization and delayed-type hypersensitivity, have not been well studied in relation to the sex differences or menstrual cycle. One study used DNCB to quantify contact sensitization, and found significantly greater responses in females (35). In that study, no attention was given to the phase of the menstrual cycle of the volunteers; however, a more recent study, which also measured sensitization by DNCB, compared males and females but carefully avoided the start of the menstrual cycle $\pm$5 days (36). Small but significantly greater reactivity was detected in males. Also, Robinson reported that males gave significantly greater responses to irritants such as SLS and octanoic acid (29).

This is clearly an area where careful investigation of sex differences and the effects of the menstrual cycle is required.

Genetic factors

There will be genetic contributions to all the steps in the pathway between the intact epidermal barrier, the epidermal defences, including biochemical (metabolic and redox-sensitive pathways), innate immunity, and the adaptive immune response, which generates hapten-specific T-cells, including effector and regulatory cells. This could be the subject of a large review by itself. Therefore, only selected and pertinent genetic components will be touched on here. ‘Epidermal’ integrity is complex and clearly important. Mutations in the filaggrin gene have been identified as causing the inherited dry skin of ichthyosis vulgaris. Similar mutations resulting in filaggrin deficiency are closely involved in the pathogenesis of atopic eczema, and up to 10% of the normal population carry at least one copy of these mutations (37). The filaggrin mutations lead to a more water-permeable stratum corneum, but it is not known whether the permeability for lipophilic chemicals is altered. Atopic eczema sufferers are more susceptible to skin irritation by surfactants, which may be a reflection of greater penetration of the surfactant molecules; in turn, this is associated with further barrier disruption and activation of the inflammatory response. However, interestingly, there is no clear evidence that the increased water permeability also has an effect on the permeability of lipid-soluble agents. We have evidence that DNCB penetrates into atopic epidermis and normal epidermis equally (M. Ardern-Jones, personal communication) and, paradoxically, atopic eczema sufferers are sensitized less strongly by DNCB than are non-atopic controls (38, 39).

A number of diseases characterized by ichthyosis (dry skin) have been shown to have genetic mutations affecting the formation of the intraepidermal lipids. There are many examples, but one of the best known is recessive X-linked ichthyosis, in which the enzyme steroid sulfatase is deficient (40). It is

presumed that there are disturbances of the epidermal barrier function in these conditions, but whether it is the water-soluble or lipid-soluble constituents of the environment that are affected has yet to be defined. Similarly, the susceptibility to irritation or contact sensitization of individuals with these rare conditions has yet to be determined.

Integrated Skin Responses to Physical or Chemical Perturbation

Inter-relationships between epidermal barrier integrity and innate defences

The impermeability and integrity of the stratum corneum barrier are the major determinants of the penetration not only of microbes, but also of water-soluble molecules. Susceptibility to irritation by SLS, a water-soluble surfactant, is proportional to the amount that penetrates (41), but how and why it generates an inflammatory reaction is not clear. However, there is now evidence that, following disruption of the stratum corneum barrier either with SLS or by tape stripping, a set of protective/restorative responses is activated. Thus, rapid repair of the stratum corneum is initiated, involving a wave of pseudo-apoptosis of upper epidermal keratinocytes to generate new corneocytes (9, 10, 42). Also, there is a burst of synthesis and secretion of lamellar bodies to restore the intercellular lipid layers (43). Although it has not yet been shown, it is likely that elements of the innate immune response will also be activated in preparedness for defence against microbial invasion through the disrupted physical defences. This will involve expression of antimicrobial peptides and pro-inflammatory cytokines, and activation of dendritic cells.

Many factors, including genetics and environmental agents, may result in impairment of the water permeability barrier of the stratum corneum. This impairment is reflected by increased transepidermal water loss, and clearly has implications for the susceptibility to penetration of water-soluble chemicals, as outlined above. However, regarding the penetration of lipid-soluble molecules, the general view is that it is a passive diffusion process, the rate of which is concentration-dependent, in accordance with Fick's laws, but that is also determined by the relative solubility (partitioning) of the compound between the vehicle and the epidermis. This is reflected by the log P, where P is the octanol–water partition coefficient. If there is passive and easy entry for lipid-soluble agents, the question that arises is 'what form of innate defence is present to prevent chemical perturbation by many lipid-soluble compounds – such as chemical sensitizers?' Within the stratum corneum is a layer of what was previously described as 'sulfur-rich' proteins, thought to be breakdown products of filaggrin or other structural proteins. We have now shown that this layer is, in fact, extremely rich in sulfydryl groups, which can bind thiol-reactive chemicals, thereby impeding their penetration into the viable layers of the epidermis (44). This discovery provides the answer to why there is a large difference between the skin-sensitizing potency of DNCB and that of 2,4-dinitrothiocyanobenzene (DNTB). DNCB is a potent sensitizer in humans, whereas, at comparable doses, DNTB is almost a non-sensitizer. However, DNTB is much more reactive with thiols, as in glutathione, and it binds to the thiol-rich layer in the stratum corneum. It requires a 10-fold greater concentration of DNTB to saturate the thiol-rich layer and to penetrate into the epidermis in similar quantities as DNCB (44). This clearly adds an additional element to the innate barrier defences. Whereas the reactive thiol groups may be components such as cysteines in molecules such as filaggrin, overall, the finding points to the high likelihood of a more substantial redox-based defence system that will be important in defending against tissue damage by reactive chemicals. Other contributors to the redox-based defences include xenobiotic-metabolizing enzymes such as GST and members of the cytochrome P450 superfamily.

Conclusions

The prediction of the risks of skin sensitization by chemicals involves a number of steps. Analysis of the chemical properties that make a substance a potential sensitizer can be performed *in vitro*, and the predictive methods are relatively robust. The assessment of human susceptibility is still highly imprecise, because so many factors can contribute and, for most, there is no good quantitative handle. Although it is very clear that the dose per unit area is the major determinant of sensitization, many factors in the skin are involved in determining the proportion of an applied dose that may actually penetrate and reach the immune system. Thus, the stratum corneum proteins, lipids and biochemical redox barriers are critical. The complement of xenobiotic-metabolizing enzymes may be crucial in determining not only how much of a chemical penetrates, but also whether it is converted to a skin-sensitizing hapten. Also, these defence systems interact with the innate immune response, which generates irritant responses. Hence, they may contribute to potentiating the immunogenicity of potential contact sensitizers and hence to individual susceptibility both to skin irritation and to sensitization. Although some of this is amenable to robust quantification when single doses are applied, a much more complex

situation arises when repeated exposures are experienced. Whereas the quantitative effect of these factors is not known accurately, the human variability in responsiveness, from polysensitized, highly susceptible individuals to those at the low-responding, unsusceptible end of the spectrum, spans about a 20-fold difference in the dose (of DNCB) required to induce sensitization. However, the relative sensitizing potency of a single dose of a chemical may be augmented enormously by delivery of the same total cumulative dose as a series of very low doses – this is one of the areas that most requires clarification. The possible increase in susceptibility to sensitization that may accompany alterations in the stratum corneum barrier have yet to be investigated, and of the constitutive factors, including race, age, and sex, the effects of sex seem to be potentially the most important; however, they require proper quantitation. Racial differences seem to be minor, and only extremes of age appear to be significant. Finally, the hope of having robust numerical factors that will allow accurate prediction of the risks of sensitization by chemicals introduced into the environment does not appear to be a realistic one, even in the intermediate future. It will be important to see what responses the political, regulatory and societal organizations make regarding whether or how chemicals for which there is no background data from *in vivo* tests in animals regarding their potency as skin sensitizers can be introduced. If these bodies remain committed to the view that new *in vitro* methods will be found that will allow the sensitizing potencies of chemicals to be determined without any form of *in vivo* testing, then humankind will find itself back in the dark ages of empiricism accompanied by a growing incidence of outbreaks of allergies to future consumer products. In that case, the signals that any product is accompanied by a significant risk of sensitizing users will emerge through post-marketing surveillance in dermatology contact clinics – as has happened for methyldibromoglutaronitrile (45). The alternative will be a self-imposed moratorium on the introduction of any new products for as many years as it takes to develop the extensive panel of surrogates and substitutes that will be required to mimic the complexities of live test subjects – be they animal or human.

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