

Allergic contact dermatitis: is the reactive chemistry of skin sensitizers the whole story? A response

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To the Editor,

In their commentary (1), Basketter and Kimber address two themes. The first may be summarized as 'What are we to make of clinical reports of allergic contact dermatitis caused by chemicals that do not seem to be reactive in any way?' and the second as 'the relationship between protein reactivity and relative skin potency – why do we need kinetics?' My response is structured similarly.

Clinical Reports of Allergic Contact Dermatitis Caused by Chemicals Lacking Reactivity

As Basketter and Kimber correctly point out, the literature is replete with clinical reports of contact allergy to

chemicals that show no evidence of reactivity or potential to be converted to reactive derivatives. Such chemicals are typically weakly sensitizing. Basketter and Kimber suggest that such cases may be interpreted in terms of the possibility that there are alternative mechanisms, so far unidentified, by which some chemicals produce contact allergy, with all of the elements of induction, elicitation, and specificity, without requiring reactive chemistry.

Basketter and Kimber suggest that 'in seeking to insist that all allergic contact dermatitis is dependent on reactive chemistry, it may be that we are sometimes in danger of force fitting the facts to accommodate the dogma, ignoring those instances that point to the existence of complementary mechanisms.' It is not impossible that alternative mechanisms sometimes apply in allergic contact dermatitis, although Basketter and Kimber do not suggest how this possibility should be investigated. However, unless and until such mechanisms are shown to

exist and are characterized, I see no value in assuming that they do exist, when simpler explanations are available.

A simple explanation for apparent cases of non-reactive chemicals being weak sensitizers is that some patients may be hypersensitive to minute levels of reactive impurities that, not having been specifically looked for, have not been identified but are commonly present in these chemicals. People who have been sensitized by exposure to a batch of material that contains a higher than usual level of a sensitizing impurity may well react subsequently to material that contains the impurity at much lower levels.

This interpretation of weak sensitization potency has proved itself to be useful on several occasions, as it suggests a strategy of investigating the composition of the material and its manufacturing chemistry in sufficient detail to identify the chemically reactive sensitizing impurities and set quality control specifications. There are many examples where this strategy has been successful. From personal experience, I can cite: the identification of halogenated sultones and unsaturated sultones as sensitizing impurities in a sodium lauryl ether sulfate (SLES) surfactant (2), leading to manufacturing specifications to completely avoid formation of these by-products (3); identification of saturated γ -sultones and δ -sultones as sensitizing impurities in olefin sulfonate surfactants, leading to quality control specifications and modified manufacturing technology to meet these specifications (3, 4); and identification of dimethyl esters of α -sulfocarboxylic acids as sensitizing impurities in ester sulfonate surfactants (5), again leading to quality control specifications and modified manufacturing technology to meet these specifications (6).

One might ask why, if chemically reactive impurities are the main reason for allergy to apparently non-reactive chemicals, they have not been identified for all the many cases that can be cited – such as those mentioned by Basketter and Kimber. The simple answer is probably that there has not been sufficient incentive to invest the quite large amount of research effort required. To put this in some sort of perspective, when the SLES sensitization issue arose in 1968, it took about 5 years of extensive research, including the development of a special animal model, to identify the reactive impurities (2). This expenditure could be justified because of the global high-tonnage use of SLES in consumer products. In contrast, with most of the non-reactive 'weak sensitizers', a cheaper and adequate solution has been for clinicians simply to advise affected patients to avoid the substance, and the substance has not been considered to be enough of a problem for its continued use to be threatened on the grounds of its allergenicity. No doubt if allergy to any of the 'non-reactive clinical allergens' cited by Basketter

and Kimber was a bigger problem, and was a major commercial issue, intensive research would be performed to try to identify the cause of the problem and to overcome it. It is worth mentioning that, because of the advances that have been made in determining structure–activity relationships and analytical technology, investigations to identify allergenic impurities can often be performed more easily and efficiently than in the past. A good example is the identification of the impurity 4-isoamyl-benzene-1,2-diol as the main cause of the unexpectedly high local lymph node assay (LLNA) potency of azurone (7). Another example is the demonstration by clinical patch testing that clinical allergy to 'coumarin' can be attributed to impurities in the commercial materials, pure coumarin having no significant allergenicity (8).

Another explanation that may apply in some cases is that an apparent case of sensitization is a false positive. Some chemicals are accepted as consistent false positives in animals, so why not in humans? A good example of a false positive in animals is sodium lauryl sulfate (SLS), which consistently gives positive results, near the borderline of the moderate/weak classification (EC3, 10%). Rather contrary to what they argue in their editorial, Basketter and Kimber have cited lack of reactivity alerts as one of the reasons for classifying SLS as a false positive (9).

To conclude this section, the conjecture that there are unidentified alternative mechanisms, not based on reactive chemistry, by which some chemicals produce contact allergy cannot currently be proved or disproved. However, the most practically useful interpretation is that sensitization to nominally non-reactive (including not pro-reactive) substances is usually caused by reactive allergenic impurities.

The Relationship between Protein Reactivity and Relative Skin Potency – Why Do We Need Kinetics?

The second section of the Basketter and Kimber commentary begins with the statement 'there has been the view that inherent or acquired electrophilicity is a universal mandatory requirement for the successful acquisition of skin sensitization, it has been proposed that this property will therefore provide a sound correlate of sensitizing potency' (10) (this is ref(8) in their commentary). As the article continues, this concept, attributed to reference (10) (of this letter), is considered as though it were a proposal made on the basis of abstract reasoning. There is no recognition of the fact that the concept of sensitizing potency being correlated quantitatively with reactivity was proposed over 40 years

ago (11), was demonstrated in the early 1980s (12, 13), and is supported by a large body of published evidence that has been summarized up to 2008 in ref (10), since when further correlations have been reported (14, 15).

The commentary goes on to pose three questions:

1. 'Even if it is accepted, for the purposes of this discussion, that protein reactivity is indeed the parameter that determines relative skin-sensitizing potency, then it is legitimate to question what aspect of the property would be the most relevant.'

It would have been legitimate about 75 years ago to raise this question, but this question has been addressed and answered by numerous experimental studies. The aspects of reactivity that are relevant are the reaction mechanistic domain (i.e. the nature of the reaction) (16, 17) and the degree of reactivity (12) – which is conventionally represented by a rate constant.

2. 'It has been argued that the kinetics of peptide or protein reactivity will correlate best with potency, but why should this be the case?'

The question may be rhetorical, but the simple answer is factual – it is the case because it has been found to be so; see the many quantitative correlations between rate constants, or their surrogates, and sensitization potency that have been published. It would be the case irrespective of whether or not the reason was understood. A simple mechanistic explanation of why this is so, which can be found in the literature, is that the rate constant models the proportion of the applied dose that reacts with the protein as opposed to being eliminated (12).

3. 'Why should the speed of conjugate formation with proteins be influential in driving the magnitude of immune priming and the ease with which sensitization is acquired?'

Here, the commentary gives the impression of some confusion about kinetics and chemical reactivity. The 'speed' of a reaction is more commonly referred to as the reaction rate, and it is not usually constant throughout the reaction. For present purposes, kinetics is the study of how the reaction rate is related to concentrations of the reactants. The reaction rate usually decreases over the course of the reaction, as the concentrations of reactants decrease. The rate constant relates the reaction rate to the concentrations of reactants, and is the 'gold standard' chemical reactivity parameter preferred by chemists.

The commentary continues 'There are a number of other variables that could be considered relevant also. Among these are: the nature and location of the proteins with which a chemical associates, the number of different proteins that are haptenated, and the molar substitution ratio of conjugated proteins. It is entirely possible that

these factors may also impact on the induction of immune responses, so there is no reason to suppose that kinetics is the only, or even the most important, metric'.

Yes, these variables could be considered, but all of the evidence shows that they do not need to be considered. (Although variables such as the nature and location of the proteins with which a chemical associates, the number of different proteins that are haptenated, and the molar substitution ratio of conjugated proteins do not need to be considered explicitly, this does not necessarily mean that they play no part. It is quite plausible that differences in these variables can explain why it is necessary to group chemicals into reaction mechanistic domains or categories, in order to apply read across and to develop models to predict sensitization potency.) Good quantitative correlations [quantitative mechanistic models (QMMs)] between allergenic potency and rate constants or their surrogates, in some cases supplemented by a hydrophobicity parameter, have been developed without any knowledge of these variables (10, 12–15). The statistical quality of these QMMs leaves no scope for further variables to improve them. Therefore, the statement that 'there is no reason to suppose that kinetics is the only, or even the most important, metric' is refuted by the findings. The skin sensitization process is, like many other toxicological processes, complex and still not fully understood. Much remains unknown concerning the nature of the protein-binding step, but, for the purpose of developing predictive non-animal models, it does not need to be known. Failure to distinguish between what needs to be known and what does not is probably the main reason for the continued failure, despite a decade of intensive and heavily funded collaborative international research projects, to deliver fully satisfactory non-animal methods for sensitization potency that can be used by industry and regulators for risk assessment purposes.

The commentary continues: 'there may be many events that are essential for the acquisition of sensitization, but that do not correlate with potency. These, like the previous minimum height requirement for UK policemen, are threshold effects without a direct quantitative relationship with the effectiveness of the process. It is perfectly possible that the electrophilic properties of chemicals fall into this category. That is, there is a requirement for threshold levels or greater of conjugate formation with protein, but, after that threshold level has been achieved, any further conjugate production is without impact on skin sensitization.'

Here, there seems to be some confusion about the consequences of thresholds. If this conjecture were true, that after the threshold level of conjugate formation has been achieved then any further conjugate production is

without impact on skin sensitization, then dose–response relationships above the sensitization threshold would not be observed. This conjecture implies that sensitization is a binary phenomenon – subjects cannot be sensitized to varying degrees; they are simply either sensitized or not. It would not be possible to derive LLNA EC3 values by interpolation between stimulation index (SI) values above and below 3, because SI values significantly above 3 would not be observed. This, of course, is in complete contradiction to the findings of numerous publications by Basketter and Kimber showing dose–response trends in the LLNA and describing how to derive EC3 values from dose–response plots (e.g. 18). In guinea pigs, it has also been long established that there are varying degrees of sensitization, depending on the induction dose of allergen, for example the extensive induction/elicitation dose–response studies for unsaturated sultones (19, 20) and sulfonate esters (20) in guinea pigs. Thus, this conjecture is completely counter-factual.

But even if this conjecture, that any further conjugate production above the threshold is without impact on skin sensitization, were true, it would still not imply a reactivity threshold above which an increase in reactivity would be without effect. The reason is simple: the more reactive the chemical, the less of it is needed to reach the conjugate formation threshold.

The following example illustrates these points. Dichloronitrobenzene (DCNB) is reactive enough to be a sensitizer in the LLNA, and, at a dose concentration of 20%, it produces enough protein conjugation to lead, via a cascade of *in cutaneo* processes, to the three-fold

increase in lymph node T cell proliferation (SI = 3) that is considered to be the threshold for sensitization to be induced (21). Therefore, the level of protein conjugation produced by 20% DCNB is the threshold for protein conjugation to give sensitization. DNCB is much more reactive, and a dose concentration of only about 0.05% is required to reach the protein conjugation threshold for producing the three-fold increase in lymph node T cell proliferation (SI = 3) that is the threshold for sensitization. Now consider what happens if a 0.25% concentration of DNCB is applied in the LLNA. The protein conjugation threshold for sensitization is already reached with 0.05%, so the extra 0.2% should, according to the Basketter and Kimber conjecture, have no further impact on sensitization, and the resulting SI value should still be 3. In fact, the reported SI value is ~ 25 (22).

To conclude, the second part of the commentary, in which Basketter and Kimber address the relationship between protein reactivity and relative skin-sensitizing potency, seems to be an exercise in abstract reasoning with no reference to, or consideration of, the large body of published evidence on the subject. This abstract reasoning leads to a series of unsubstantiated conjectures, all of which run contrary to what is already known.

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