

Free radicals and the antioxidant hypothesis of prohaptten activation

Dear Sir,

Lepoittevin (1) has proposed that a new term “prehaptten” be introduced for “non-reactive sensitizing molecules transformed into ‘haptens’ by simple chemical transformation and without requirement of a specific enzymatic system”. He implied that the term “prohaptten” should be applied only to those molecules that require enzymic activation to a protein-reactive species. Aptula et al. (2) then argued “that the term ‘haptten,’ and derived terms such as ‘pro-haptten’ and ‘pre-haptten’ are ambiguous and unnecessary... [and proposed] that their use be abandoned. Instead, [they continued], when considering the chemical basis of skin sensitization, it is preferable to classify compounds according to the chemical reaction mechanisms by which they can modify proteins”. Their conclusion is that all haptens, prohaptens, and prehaptens are or become electrophiles and, accordingly, it makes more sense to categorize them on the basis of the mechanism of electrophilic reaction by which covalent bonding to protein occurs.

While the proposal by Lepoittevin (1) to introduce the term prehaptten is logical, it is based on the premise that we do know how to distinguish a prohaptten from a prehaptten *in vitro* and that we do know how to confirm this *in vivo*. Yes, *in vitro* studies can already give a good indication of pre- or prohapttenic status, but these methods are far

from definitive. And, *in vivo* or *ex vivo* methods lag some way behind, there being no validated methodology available to address this issue. The study published by Basketter et al. (3) demonstrated this admirably in trying, but failing, to reconcile earlier *in vitro* and *ex vivo* data with their new *in vivo* data for dinitrohalobenzenes. Their murine local lymph node assay involved applications of xenobiotics to mouse pinna; the earlier biochemical studies carried out by Schmidt and Chung (4) involved applications of xenobiotics to mouse dorsal skin. In their studies, Schmidt and Chung (4) observed that both prohapttenic and hapttenic processes may be occurring. The murine local lymph node assay, in contrast, showed itself incapable of disentangling these processes because it measures only a single outcome, namely a stimulation index. Indeed, this work may have demonstrated the relative insensitivity of the local lymph node assay as a means of detecting prohapttenic processes, and greater sensitivity in detecting hapttenic processes, ... perhaps because mouse pinna does not express nitroreductase activity, or indeed other putative prohaptten activating enzymes to the extent observed in mouse dorsal skin? Mouse dorsal skin is likely to differ from mouse pinna as regards skin penetration of xenobiotics, presence of nitroreductase and/or other xenobiotic-metabolizing enzymes, and antioxidant status. It is already documented (5) that

the immunological activity of human skin can vary from site to site. There is no reason to believe that a similar situation does not pertain in mouse skin.

In their studies, Schmidt and Chung (4) provided evidence in skin from mouse dorsum for the occurrence of redox cycling of the aromatic nitro groups of the allergenic dinitrohalobenzenes driven by an NADPH-dependent one-electron reductase [most probably a nitroreductase (6)] and leading to oxidative stress, which was quantified in terms of glutathione/glutathione disulfide status. One-electron reductases, by definition, generate free radical species. Although Lepoittevin (1) does allude to the possibility that neutral (i.e. uncharged) free radical species may behave as haptens, Aptula et al. (2), in concluding that all low-molecular-weight contact allergens are electrophiles or proelectrophiles, have overlooked this possibility. Free radicals are almost certainly the ultimate haptens in the case of sensitizing hydroperoxides such as 15-hydroperoxyabietic acid, linalyl hydroperoxide, and limonene hydroperoxide (7). There is also experimental evidence from studies in mice (8) that allergenic alkyl catechols, phenols, and resorcinols found in the plant families Anacardiaceae and Proteaceae are activated in the skin by non-enzymic one-electron oxidation to potentially haptenic free radical species.

Yes, a neutral free radical can have electrophilic character in having an affinity for electron-rich sites on the putative 'hapten-receptor protein' in skin, but some neutral free radical species are nucleophilic and others display no preference (9). The term electrophile is normally used to mean a positively charged species seeking to react with a functional group bearing a lone pair of electrons.

Clearly, Aptula et al. (2) have overlooked the possibility of *in situ* non-enzymic one-electron oxidation or of enzymic one-electron reduction to potentially protein-reactive free radical species as mechanisms of pre- or prohaptens activation.

Once it is recognized that dinitrochlorobenzene, for example, may act as a hapten (electrophile), a prehapten, or a prohaptens, the futility of classifying this substance as a hapten, a prohaptens, a prehapten, an electrophile, or a proelectrophile becomes evident. A similar situation probably pertains with sesquiterpene lactones and isothiocyanates (10), and with urushiols and related alkylphenols (8). This is not to say that the terms hapten, prohaptens, and prehapten should be abandoned. They do, after all, describe discrete characteristics of low-molecular-weight contact allergens. However, the terms should not be used to categorize contact allergens unless it is clear

that only one of these processes is mechanistically possible or dominant *in vivo*.

Schmidt et al. (11) proposed the antioxidant hypothesis of prohaptens activation. Further unpublished work with *para*-phenylenediamine implicated traces of iron as being contributory (12) in that iron present in the stratum corneum (the principal site from which iron is lost from the body other than through blood loss) may produce a so-called Fenton catalyst when it co-ordinates with a penetrating xenobiotic, this iron–xenobiotic complex then catalysing the production of reactive oxygen species that attack the xenobiotic at a hydrogen atom with low bond dissociation energy (i.e. the xenobiotic acts as an antioxidant, forming a potentially protein-reactive free radical species as a result of attack by hydroxyl radical that the iron–xenobiotic complex itself has generated).

Currently available predictive models of contact allergenicity take no account of the redox biochemistry described here. One only has to look at tables of bond dissociation energies (e.g. at <http://www.hbcnetbase.com/>), which are a measure of the ease of homolytic bond cleavage (i.e. free radical formation), to realize that the functional groups on a wide range of prohaptens are candidates for facile hydrogen atom abstraction by endogenous pro-oxidant species, mainly hydroxyl but also the dioxygen radical when co-ordinated with a suitable iron complex. These pro- or pre-haptens bearing hydrogen atoms with low bond dissociation energy (this includes aldehydic, allylic, and benzylic groups as well as methylene groups *alpha* to a carbonyl group) will surely compete with endogenous antioxidants such as vitamin C, vitamin E, and glutathione for a role as an antioxidant, especially under conditions of local endogenous antioxidant depletion caused by oxidative stress. This is the antioxidant hypothesis of prohaptens activation. In turn, the antioxidant hypothesis predicts a role for decompartmentalized iron in the formation of a Fenton catalyst that promotes oxidative stress and a role for endogenous antioxidants in protection against sensitization. Perturbation of glutathione status indicative of oxidative stress [as has been observed with, e.g. *para*-phenylenediamine (13), dinitrohalobenzenes (4), sesquiterpene lactones (9), isothiocyanates (9), and oxazolone and Kathon CG (14)] leads to depletion of endogenous antioxidants and raises the probability that protein-reactive free radical species will be produced from penetrating xenobiotics. Although largely hypothetical at present, the experimental evidence pointing to both non-enzymic one-electron oxidation processes involving iron–xenobiotic complexes and enzymic one-electron reduction processes in the

generation of free radical ultimate haptens needs to be explored further and not simply ignored.

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