Research News

Measurement of allergenic potency using the local lymph node assay

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Chemicals that can act as contact allergens have been identified successfully using guinea-pig models. However, contact allergy is still common, probably because of, at least in part, failures of risk assessment. A new method, the local lymph node assay, replaces the guinea-pig as a tool for hazard identification and offers the real prospect of accurate prediction of allergen potency, the missing link in skin sensitization risk assessment.

Chemicals that have the potential to cause delayed contact hypersensitivity in the skin have, for many years, been identified using one of several guinea-pig predictive assays[1]. Although these methods worked well for the purposes of hazard identification, their use in risk assessment has been more problematic because such methods do not lend themselves readily to the measurement of the potency of chemical allergens. With an increasingly sophisticated appreciation of the immunobiological mechanisms that initiate and regulate allergic responses to chemicals, opportunities to consider alternative approaches to hazard identification and characterization have arisen. One such novel approach is the murine local lymph node assay (LLNA), which is a method for the identification of skin sensitizing chemicals[2–5].

Hazard identification using the LLNA

The LLNA has been evaluated exhaustively and a standard protocol has been developed (Fig. 1). This method is based on the measurement of proliferative responses induced in draining lymph node cells (LNCs) following topical exposure of mice to a test chemical, and is used to identify chemicals that are skin sensitizers. The LLNA was the first new toxicology test to be considered by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), an organization established by 14 Federal regulatory and research agencies to harmonize the development, validation and acceptance of toxicological test methods. A peer-review panel (PRP) was appointed by ICCVAM and charged with the development of a scientific consensus on the utility of the LLNA. The extensive intra- and inter-laboratory validation data that were reviewed (on ~200 chemicals) have been published elsewhere[5]. The PRP were asked to address two major questions: (1) has the LLNA been evaluated sufficiently and is its performance sufficiently satisfactory to warrant its adoption as a stand-alone alternative to the guinea-pig maximization test and the Buehler assay?; and (2) does the LLNA offer advantages with respect to animal welfare considerations (i.e. refinement, reduction and/or replacement)?

The answer to both of these questions was 'yes' (detailed comments and recommendations have been published elsewhere[6]). Importantly, in relation to animal welfare benefits, the LLNA does indeed provide distinct reduction and refinement opportunities. The ICCVAM conclusions were endorsed more recently by the official validation body in Europe, the European Centre for the Validation of Alternative Methods (ECVAM)[7]. The LLNA is therefore now recognized as a fully validated method and as such represents a stand-alone alternative to guinea-pig methods for the identification of skin sensitizing chemicals. However, as mentioned above, it is not hazard identification, but risk assessment, that poses the most significant challenge, and for accurate risk assessment it is necessary to understand intrinsic potency. It is in this context that the LLNA offers considerable advantages compared with the standard guinea-pig methodologies.

Potency estimation using the LLNA

In its most basic form, the LLNA provides a limited evaluation of dose responses and delivers objective, quantitative data. LNC proliferative activity not only provides a marker for skin sensitization but also correlates quantitatively with the extent to which sensitization is acquired[8]. Therefore, there was an opportunity to use dose–response data to provide information on the relative potencies of skin sensitizers[9]. The approach taken is to derive mathematically the amount of chemical necessary to provoke a threefold increase in the proliferative activity in draining LNCs compared with concurrent vehicle-treated controls. This is termed the EC3 value[10] (Fig. 2). The reliability and stability of this measure has already been demonstrated[11], as has its inter-laboratory reproducibility[12].

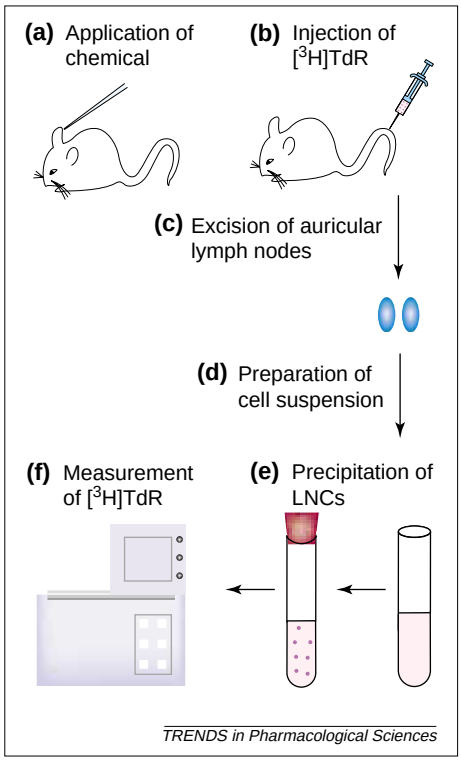

Fig. 1. The local lymph node assay (LLNA). (a) Groups of mice (CBA strain) receive topical applications, once a day for three consecutive days, of the test chemical on the dorsum of both ears. In standard analyses, three concentrations of the test material are evaluated together with the relevant vehicle control. (b) Five days following the initiation of exposure, all mice receive an intravenous injection of [^{3}H]-labelled thymidine {[^{3}H]TdR} into their tail vein. (c) Five hours later, animals are sacrificed and draining (auricular) lymph nodes are excised. (d) A single cell suspension of lymph node cells (LNCs) is prepared by gentle mechanical disaggregation and the cells are washed and resuspended in trichloroacetic acid (TCA) for at least 12 hours at 4 °C. (e) Precipitates are resuspended in TCA and transferred to an appropriate scintillation fluid. (f) The incorporation by draining LNCs of [^{3}H]TdR is measured by β-scintillation counting and recorded as mean disintegrations per minute (dpm). For each concentration of the test material a stimulation index (SI) is derived relative to the concurrent vehicle control. Those chemicals that at one or more test concentrations induce a SI of three or greater are classified as skin sensitizers.

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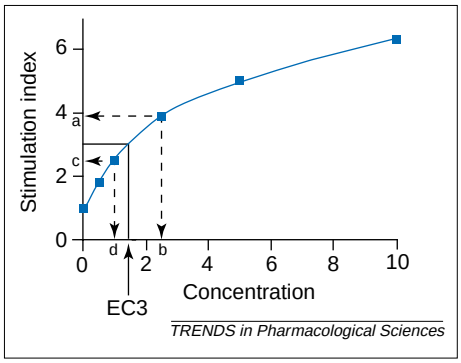

Fig. 2. Determination of the local lymph node assay (LLNA) EC3 value for estimation of allergenic potency. The graph shows a dose–response curve using the LLNA. The concentration of the test chemical required to produce a stimulation index (SI) of 3 (the EC3 value) is calculated using the formula EC3 = 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.

Preliminary investigations have demonstrated that the EC3 value might represent a useful index of potency[13,14]. A question of primary importance, however, is whether and to what extent EC3 values derived from the LLNA represent information of relevance to the potency of contact allergens in humans. The most important demonstrations of the potential utility of EC3 values have derived from more extended comparisons of these values with what is known about the relative potency in humans of a wider range of contact allergens[15–17]. In these investigations, EC3 values for 30 chemicals of widely varying skin sensitizing potency in humans have been 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 under way[18].

Table 1. Correlation of LLNA and human potency classifications[a,b]

Chemical	Human potency class	LLNA potency class
Methyl/chloromethylisothiazolinone	Strong	Strong
p-Phenylenediamine	Strong	Strong
Diphencyclopropenone	Strong	Strong
2,4-Dinitrochlorobenzene	Strong	Strong
Glutaraldehyde	Moderate	Strong
Formaldehyde	Moderate	Moderate
Isoeugenol	Moderate	Moderate
Tetramethylthiuramdisulfide	Moderate	Moderate
Cinnamic aldehyde	Moderate	Moderate
Phenylacetaldehyde	Moderate	Moderate
Citral	Weak	Weak
Eugenol	Weak	Weak
Hydroxycitronellal	Weak	Weak
5-Methyl-2,3-hexanedione	Weak	Weak
p-Methylhydrocinnamic aldehyde	Weak	Weak
Hexylcinnamic aldehyde	Weak	Weak
p-tert-Butyl-α-methyl hydrocinnamal	Weak	Weak
Cyclamen aldehyde	Weak	Weak
p-Methylhydrocinnamic aldehyde	Weak	Weak
Linalool	Extremely weak	Weak
Penicillin G	Extremely weak	Extremely weak
Ethyleneglycoldimethacrylate	Extremely weak	Extremely weak
Propylene glycol	Extremely weak	Non-sensitizer
Vanillin	Extremely weak	Non-sensitizer
Propyl paraben	Extremely weak	Non-sensitizer
Ethyl vanillin	Non-sensitizer	Non-sensitizer
Glycerol	Non-sensitizer	Non-sensitizer
Hexane	Non-sensitizer	Non-sensitizer
Diethylphthalate	Non-sensitizer	Non-sensitizer
Tween 80	Non-sensitizer	Non-sensitizer

[a]Data taken from Refs 15–17.
[b]Abbreviation: LLNA, local lymph node assay.

References

1 Botham, P.A. *et al.* (1991) Skin sensitization: a critical review of predictive test methods in animal and man. *Food Chem. Toxicol.* 29, 275–286
2 Kimber, I. and Basketter, D.A. (1992) The murine local lymph node assay: a commentary on collaborative studies and new directions. *Food Chem. Toxicol.* 30, 165–169
3 Basketter, D.A. *et al.* (1995) An alternative strategy to the use of guinea pigs for the identification of skin sensitization hazard. *Food Chem. Toxicol.* 33, 1051–1056
4 Dearman, R.J. *et al.* (1999) Local lymph node assay: use in hazard and risk assessment. *J. Appl. Toxicol.* 19, 299–306
5 Gerberick, G.F. *et al.* (2000) Local lymph node assay: validation assessment for regulatory purposes. *Am. J. Contact Derm.* 11, 3–18
6 NIH (1999) The murine local lymph node assay: a test method for assessing the allergic contact dermatitis potential of chemicals/compounds. NIH No. 99–4494
7 Balls, M. and Hellsten, E. (2000) Statement on the validity of the local lymph node assay for skin sensitization testing. ECVAM Joint Research Centre, European Commission, Ispra, Italy, 21 March. *ATLA* 28, 366–367
8 Kimber, I. and Dearman, R.J. (1991) Investigation of lymph node cell proliferation as a possible immunological correlate of contact sensitizing potential. *Food Chem. Toxicol.* 29, 125–129
9 Kimber, I. and Basketter, D.A. (1997) Contact sensitization: a new approach to risk assessment. *Hum. Ecol. Risk Assess.* 3, 385–395
10 Basketter, D.A. *et al.* (1999) A comparison of statistical approaches to derivation of EC3 values from local lymph node assay dose responses. *J. Appl. Toxicol.* 19, 261–266
11 Dearman, R.J. *et al.* (1998) Temporal stability of local lymph node assay responses to hexyl cinnamic aldehyde. *J. Appl. Toxicol.* 18, 281–284
12 Warbrick, E.V. *et al.* (1999) Local lymph node assay responses to para-phenylenediamine: intra- and inter-laboratory evaluations. *J. Appl. Toxicol.* 19, 255–260
13 Hilton, J. *et al.* (1998) Estimation of relative skin sensitizing potency using the local lymph node assay: a comparison of formaldehyde and glutaraldehyde. *Am. J. Contact Derm.* 9, 29–33
14 Basketter, D.A. *et al.* (1999) Skin sensitization risk assessment: a comparative evaluation of three isothiazolinone biocides. *Contact Derm.* 40, 150–154
15 Basketter, D.A. *et al.* (2000) Use of the local lymph node assay for the estimation of relative contact allergenic potency. *Contact Derm.* 42, 344–348
16 Gerberick, G.F. *et al.* Contact allergenic potency: correlation of human and local lymph node assay data. *Am. J. Contact Derm.* (in press)
17 Basketter, D.A. *et al.* Human potency predictions for aldehydes using the local lymph node assay. *Contact Derm.* (in press)
18 Robinson, M.K. *et al.* (2000) The importance of exposure estimation in the assessment of skin sensitization risk. *Contact Derm.* 42, 251–259

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