

The Local Lymph Node Assay in 2014

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Toxicology endeavors to predict the potential of materials to cause adverse health (and environmental) effects and to assess the risk(s) associated with exposure. For skin sensitizers, the local lymph node assay was the first method to be fully and independently validated, as well as the first to offer an objective end point with a quantitative measure of sensitizing potency (in addition to hazard identification). Fifteen years later, it serves as the primary standard for the development of in vitro/in chemico/in silico alternatives.

Many substances have the potential to cause skin sensitization and thus give rise to allergic contact dermatitis. For decades, a key aim in toxicology has been to identify such substances accurately and to determine their potency so that likely risks to human health can be assessed and managed.¹ During the final decade of the 20th century, an important step forward was made with the introduction of the mouse local lymph node assay (LLNA), whose key elements are shown in Figure 1. The assay is predicated on quantitation of the selective clonal expansion of lymphocytes in response to chemical allergen exposure (Fig. 1A); the protocol elements of the assay are shown in Figure 1B; the outcome of the assay is displayed in Figure 1C, where a skin sensitizer is defined by a 3-fold or greater stimulation of proliferation in the auricular draining lymph node. A full assay protocol and background details can be found elsewhere.²

With the advent of this assay, there was an evolution of the predictive test end point from the subjective assessment of dermal inflammation in the guinea pig to the quantitative measurement of lymphocyte proliferation in lymph nodes draining the site of exposure to chemical.³ This assay was the first to pass the formal, rigorous, and independent review known in toxicology as “validation.”⁴ As a consequence, it has become a benchmark and is the preferred method for most regulators.

However, the validation process addressed only whether the LLNA accurately identified skin sensitizers. Arguably, what was of greater importance is that the dose response generated in the

LLNA can be interpolated to provide an index of the relative potency of an identified skin sensitizer. This is achieved because the induced proliferation of draining lymph node cells is both causally and quantitatively associated with the extent to which skin sensitization is acquired. The metric used to determine relative potency based upon dose-response relationships in the LLNA became known as the EC3 value; in effect, the concentration of chemical required to cause a threshold (3-fold) level of activation (lymph node cell proliferation) in the lymph node. It proved to be a robust measure of potency⁵ and to correlate well with what was understood concerning the relative potency of skin sensitizers in humans (eg, Gerberick et al⁶). In addition, the fact that the measure used could be expressed in dose per unit area facilitated the emergence of a wholly new and quantitative approach to risk assessment.^{7–9}

So, now that we are well into the second decade of the 21st century, where the great focus in toxicology is the replacement of in vivo testing, what is the legacy of the LLNA? First, the validation process has learned by its experience with the LLNA, such that questions of the applicability domain of a new assay, and its propensity for yielding false-positive and false-negative results, is now addressed with greater intensity in new validation activities. Second, which is critical, the LLNA has generated a database of hundreds of chemicals, the sensitizing activities of which, including potency, have been characterized and fully reported in 2 highly cited publications.^{10,11} These references form an essential resource for those endeavoring to design the next generation of predictive test methods that finally will obviate the use of animals. It has also prompted initiatives to extend our knowledge of the relative potency of skin sensitizing chemicals in humans so that the new methods have the opportunity to reduce interspecies differences in the prediction of sensitizing potency.¹² Ultimately of course, the success of predictive toxicology, risk assessment, and risk management will be determined by what appears in this (and related) journals.

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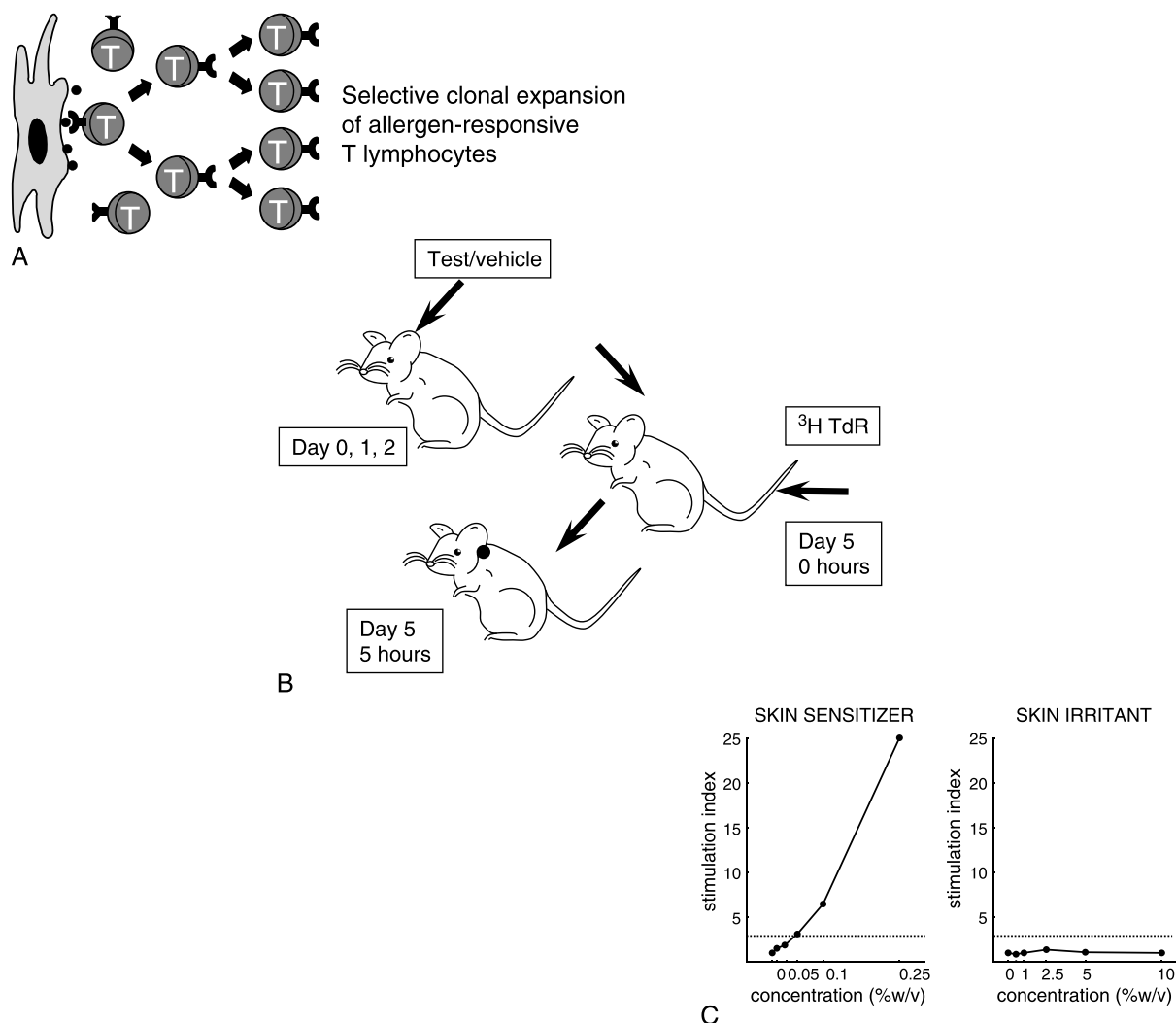


Figure 1. The LLNA in outline. A, The LLNA is based on quantitation of the selective clonal expansion of lymphocytes in response to chemical allergen exposure. B, Groups of mice are exposed to test substance or vehicle, once per day for 3 days on the dorsum of the ear. Two days later, tritiated thymidine is delivered via the tail vein. Five hours later, the draining lymph nodes are removed and thymidine incorporation is quantified. C, Sensitizers are identified via a 3-fold or greater increase in thymidine incorporation compared with concurrent vehicle controls.

REFERENCES

1. Basketter DA. Skin sensitisation: strategies for risk assessment and risk management. *Br J Dermatol* 2008;159:267–273.
2. Kimber I, Basketter DA. The murine local lymph node assay; collaborative studies and new directions: a commentary. *Food Chem Toxicol* 1992;30:165–169.
3. Gerberick GF, Ryan CA, Kimber I, et al. Local lymph node assay validation assessment for regulatory purposes. *Am J Contact Dermat* 2000;11:3–18.
4. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Report. The murine local lymph node assay: a test method for assessing the allergic contact dermatitis potential of chemicals/compounds. NIH Publication No. 99-4494. 1999.
5. Basketter DA, Gerberick GF, Kimber I. The local lymph node assay EC3 value: status of validation. *Contact Dermatitis* 2007;57:70–75.
6. Gerberick GF, Robinson MK, Ryan CA, et al. Contact allergenic potency: correlation of human and local lymph node assay data. *Am J Contact Dermat* 2001;12:156–161.
7. Kimber I, Dearman RJ, Basketter DA, et al. Dose metrics in the acquisition of skin sensitization: thresholds and importance of dose per unit area. *Regul Toxicol Pharmacol* 2008;52:39–45.
8. Robinson MK, Gerberick GF, Ryan CA, et al. The importance of exposure estimation in the assessment of skin sensitization risk. *Contact Dermatitis* 2000;42:251–259.
9. Api AM, Basketter DA, Cadby PA, et al. Dermal sensitization quantitative risk assessment (QRA) for fragrance ingredients. *Regul Toxicol Pharmacol* 2008;52:3–23.
10. Gerberick GF, Ryan CA, Kern PS, et al. Compilation of historical local lymph node assay data for the evaluation of skin sensitization alternatives. *Dermatitis* 2005;16:157–202.
11. Kern PS, Gerberick GF, Ryan CA, et al. Historical local lymph node data for the evaluation of skin sensitization alternatives: a second compilation. *Dermatitis* 2010;21:8–32.
12. Basketter DA, Alepee N, Ashikaga T, et al. Categorization of chemicals according to their relative human skin sensitizing potency. *Dermatitis* 2014;25:11–21.