Dermal Toxicity: Alternative Methods for Risk Assessment

Alan M. Goldberg¹ and Howard I. Maibach²

¹Johns Hopkins University, School of Hygiene and Public Health, Baltimore, Maryland; ²University of California Medical School, San Francisco, California

Conceptually, irritant contact dermatitis (irritation) and allergic contact dermatitis (ACD) in man should provide the ideal platforms to launch in vitro toxicology into the pantheon of in vitro testing assays. In theory, irritant dermatitis has been considered by most a simple area of cutaneous biology, whereas ACD is a complex area of biology. However, both result in responses that are reasonably stereotypical and well characterized. The biology of the underlying mechanisms is becoming characterized and will thus allow development of mechanistically based in vitro assays that will be scientifically validated and thus acceptable to regulatory agencies. — Environ Health Perspect 106(Suppl 2):493-496 (1998). http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-2/493-496goldberg/abstract.html

Key words: skin, dermal, in vitro, irritation, sensitisation, allergic contact dermatitis (ACD)

Irritant Dermatitis Syndrome: Contemporary (Tentative) Definition

Dermatopathologists and toxicologists generally consider cutaneous irritation a homogeneous and monomorphic biologic event, having been lulled by its mundane morphology. However, current knowledge suggests the contrary—a relatively homogeneous appearance but a complex, variegated sequence of mechanisms. Our current clinical and mechanistic classification (Table 1) undoubtedly represents a vast undersimplification, as we are only beginning to understand this common and heterogeneous syndrome.

Irritant Dermatitis Syndrome: Localized or Systemic?

Conventional dogma suggests that irritant dermatitis is a localized (site of contact) phenomenon; surely the reasoning appears impeccable. Yet, current knowledge suggests that, although the point of contact phenomena must be primal, other systemic factors may be decisive. Some possible systemic factors influencing irritant dermatitis are a) age, b) race, c) preexisting and/or previous skin diseases, and d) atopic dermatitis.

Irritation in vitro

Methods to evaluate potential irritation have been well described in In Vitro Skin Toxicology (1). For irritancy testing, physical–chemical measurements, quantitative structure–activity relationship (QSAR), and historical data can provide significant data. In vitro methods that measure cytotoxic interleukins (ILs)1α, arachidonic acid, and the prostaglandins (1,2) should provide adequate information on acute mild irritants through and including corrosivity. Additionally, reconstituted tissue equivalents (RTE) and skin explants may be useful in other situations.

All these systems are in development or in use in research laboratories. They have not gone through adequate optimization yet to be ready for validation, but one can expect that this will begin to happen in the near future. Table 2 is not meant to be inclusive, but to identify current, best-guess approaches to specific end points. There is a substantial need to a) more clearly define relationships between interleukins (both time relationship and biologic interactions); b) understand the biology of adhesion molecules; c) improve and define the conditions of the biologic systems; and d) establish relationships between these biochemical systems, molecules, and exogenous chemicals.

In evaluating acute toxicants (including dermal), it has been suggested that once data from in vitro testing are evaluated, including what is known about the chemicals and evaluation of these chemicals [e.g., QSAR (3,4), literature, physical–chemical measures], it may then be appropriate to establish safety of these materials directly in the human (Figure 1).

Corrosivity, a physical destruction of the skin, is the extreme case of irritancy. It is likely that in the near future we will be able to predict corrosive ability using QSAR, physical and chemical assays, and historical information to fully assess the hazard (3–6). It is inappropriate for us to assess the degree of severe corrosives using either whole animal or human clinical studies.

Phototoxicity

Dermal phototoxicity results from photo-activation of chemicals that cause either a photoirritant or a photoallergic response. A method to examine phototoxicity has recently been described (7,8). The developers of this assay suggest that it is validated, but it has not yet been submitted for fully independent, anonymous peer review.
Table 2. In vitro systems: an approach to dermal toxicity.

| Biologic system       | End point | Irritant                        | Corrosion | ACD |
|-----------------------|-----------|--------------------------------|-----------|-----|
| Cell culture          |           | MTT, arachidonic acid, prostaglandins, IL-1α | Arachidonic acid | B7  | IL-8 |
| RTE and skin explants |           | All of the above plus histochemistry and bioengineering measures e.g., transepithelial water loss | Histochemistry | Arachidonic acid | Prostaglandins | IL-8 |
| Dendritic cells and models | NA       | NA                             | T-lymphocyte activation |

Abbreviations: NA, not applicable; MTT, 3[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide.

Figure 1. System for using in vitro assays as part of a tiered testing structure. The diagram illustrates a sequential process for making the safety assessment of a hypothetical chemical or product. It can be seen that in vitro methods (as well as traditional animal tests) supply only a portion of the information needed to make the safety assessment and that this information is integrated with other data so that a weight-of-evidence decision is finally made. From Curren et al. (36).

...Once Langerhans cells have presented the hapten carrier complex to T cells, the T cells proliferate and differentiate and return to the site of hapten application in the skin where they are responsible for creating an inflammatory response that is recognized clinically as allergic contact dermatitis. Recent studies have shown that not all T cells can recirculate back to the site of antigen application. Only T cells that express specific adhesion molecules do so. Of particular importance in this regard are the C4B1 and C4B7 integrins (25).

...Keratinocytes, the predominant epidermal cell type, contribute to the immunopathogenesis of allergic contact dermatitis in two ways – first, through the induced expression of adhesion molecules that facilitate interactions with T cells and second by the synthesis and secretion of a variety of soluble polypeptide cytokines.
Interleukin-8 (IL-8) is an 8-kDa heparin-binding basic polypeptide that is chemoattractant for T cells (26). There is evidence that it acts in that capacity to bring in T cells into cutaneous sites in urushiol allergic contact dermatitis (27). IL-8 mRNA can be induced in cultured keratinocytes in response to IL-1α (26).

The complexity of the biology, presented alone, provides many opportunities to develop a battery of in vitro tests based on mechanistic understanding (28-33).

The potential systems and end points are summarized in Table 2.

If one uses the schematic in Figure 1 then QSAR, historical data, and literature may provide adequate data to classify a compound or will identify which specific in vitro tests will be appropriate. The next sequential step will be to use cell culture and RTE and measure appropriate cytokines, adhesion molecules, and/or histochemistry.

**Needs and Future Direction**

Many methods have been evaluated by different laboratories. There is a clear need for additional studies to more completely define and identify the underlying biology of the cytokines, adhesion molecules, and other inflammatory molecules. This knowledge will provide the rationale for specific batteries of in vitro tests to provide measures of irritancy, corrosivity, and allergic potential.

What remains to be done is not only validating the assays for man. This is a needed step, but only after appropriate methods are fully developed to generally accepted standards of scientific rigor using in principle the criteria described by the Interagency Coordinating Committee for the Validation of Alternative Methods (34) and the OECD (35) for validation and regulatory acceptance. Then an understanding of how to use the information appropriately for risk assessment will be the next challenge.

**REFERENCES AND NOTES**

1. Rougière A, Goldberg AM, Maibach HI, eds. Alternative Methods in Toxicology. Vol 10: In Vitro Skin Toxicology. Irritation, Phototoxicity, Sensitization. New York:Mary Ann Liebert, 1994.

2. Muller-Decker K, Furstenger G, Marks F. Keratinocyte-derived proinflammatory key mediators and cell viability as in vitro parameters of irritancy: a possible alternative to the Draize skin irritation test. Toxicol Appl Pharmacol 127:99–109 (1994).

3. Baskette DA, Scholes EU, Chamberlain M, Barratt MD. An alternative strategy to the use of guinea pigs for the identification of skin sensitization hazard. Food Chem Toxicol 33(12):1051–1056, (1995).

4. Barratt MD. Quantitative structure–activity relationships (QSAR) for skin corrosivity of organic acids, bases and phenols: principal components and neural network analysis of extended datasets. Toxicol In Vitro 10:85–94 (1996).

5. Barratt MD. Quantitative structure–activity relationships for skin irritation and corrosivity of neutral and electrophilic organic chemicals. Toxicol In Vitro 10:247–256 (1996).

6. Magee PS, Hostynk JT, Maibach HI. Methods in Toxicology. Vol 10: In Vitro Skin Toxicology (Rougier A, Goldberg AM, Maibach HI, eds). New York:Mary Ann Liebert, 1994:281–291.

7. Liebsch M, Spielmann H, Balls M, Brand M, Doring B, Dupuis J, Holtzburger HG, Klenak G, Eplerenier H, Lowell WW et al. First results of the EC/Colipa Validation Project "In Vitro Phototoxicity Testing." In: Alternative Methods in Toxicology. Vol 10: In Vitro Skin Toxicology (Rougier A, Goldberg AM, Maibach HI, eds). New York:Mary Ann Liebert, 1994:243–251.

8. Spielmann H, Balls M, Pechtovich J, Dupuis J, De Silva O, Pape WJW, Holtzburger HG. A validation study of in vitro photoirritation tests in a joint EU/Colipa project: preliminary report. In: Proceedings of the 2nd World Congress on Alternatives to Animal Use in the Life Sciences, Utrecht, The Netherlands, October 1996. Amsterdam:Elsevier Science Publishers, 1997.

9. Elmets C. Progress in the development of an in vitro assay for contact allergens: results of the Aven Project Program experience. In Vitro Toxicol 9:223 (1996).

10. Shelley WB, Juñilin L. Langerhans cells form a reticulopithelial trap for external contact antigens. Nature (London) 261:46–47 (1976).

11. Silberberg-Sinaik I, Thorbecke GJ, Baer RL, Rosenthal SA, Berezowsky V. Antigen-bearing Langerhans cells in skin, dermal lymphatics and in lymph nodes. Cell Immunol 25:137–151 (1976).

12. Sullivan S, Bergstresser PR, Tigelaar RE, Streilein JW. Induction and regulation of contact hypersensitivity by resident, bond marrow-derived, dendritic epidermal cells: Langerhans cells and Thy-1+ epidermal cells. J Immunol 137:2460–2467 (1986).

13. Hauser C, Sting, G. The skin immune response. In: Photoimmunology (Krutmann J, Elmets CA, eds). Oxford: Blackwell Science, 1995:3–10.

14. Macatonia SE, Knight SC, Edwards AJ, Griffiths S, Fryer P. Localization of antigen on lymph node dendritic cells after exposure to the contact sensitizer fluorescein isothiocyanate. Functional and morphological studies. J Exp Med 166:1654–1667 (1987).

15. Dai R, Grammer SF, Streilein JW. Fresh and cultured Langerhans cells display differential capacities to activate hapten-specific T cells. J Immunol 150:59–66 (1993).

16. Schuler G, Steinman RM. Murine epidermal Langerhans cells mature into potent immunostimulatory dendritic cells in vitro. J Exp Med 161:526–546 (1985).

17. Aiba S, Katz SI. Phenotypic and functional characteristics of in vivo-activated Langerhans cells. J Immunol 145:2791–2796 (1990).

18. Cumberbatch M, Peters SW, Gould SJ, Kimber I. Intracellular adhesion molecule-1 (ICAM-1) expression by lymph node dendritic cells: comparison with epidermal Langerhans cells. Immunol Lett 32:105–110 (1992).

19. Teunissen MB, Rongen HA, Bos JD. Function of adhesion molecules lymphocyte function-associated antigen-3 and intercellular adhesion molecule-1 on human epidermal Langerhans cells in antigen-specific T cell activation. J Immunol 152:3400–3409 (1994).

20. Larsen CP, Ritchie SC, Hendrix R, Linsley PS, Hathcock KS, Hodes RJ, Lowry RP, Pearson TC. Regulation of immunostimulatory function and costimulatory molecule (B7-1 and B7-2) expression on murine dendritic cells. J Immunol 152:5208–5219 (1994).

21. Lee MG, Borkowski TA, Udey MC. Regulation of expression of B7 by murine Langerhans cells; a direct relationship between B7 mRNA levels and the level of surface expression of B7 by Langerhans cells. J Invest Dermatol 101:883–886 (1993).

22. Kampgen E, Koch N, Koch F, Stoger P, Heufler C, Schuller G, Romani N. Class II major histocompatibility complex molecules of murine dendritic cells: synthesis, sialylation of invariant chain, and antigen processing capacity are downregulated upon culture. Proc Natl Acad Sci USA 88:3014–3018 (1991).
23. Tang A, Amagai M, Granger LG, Stanley JR, Udey MC. Adhesion of epidermal Langerhans cells to keratinocytes mediated by E-cadherin. Nature (London) 361:82-85 (1993).
24. Heufler C, Koch F, Schuler G. Granulocyte/macrophage colony-stimulating factor and interleukin-1 mediate the maturation of murine epidermal Langerhans cells into potent immunostimulator dendritic cells. J Exp Med 167:700–705 (1988).
25. Ferguson TA, Mizutani H, Kupper TS. Two integrin-binding peptides abrogate T cell-mediated immune responses in vivo. Proc Natl Acad Sci USA 88:8072–8076 (1991).
26. Larsen CG, Anderson, AO, Oppenheim JJ, Matsushima K. Production of interleukin-8 by human dermal fibroblasts and keratinocytes in response to interleukin-1 or tumour necrosis factor. Immunology 68:31–36 (1989).
27. Nickoloff BJ, Griffiths CE, Barker JN. The role of adhesion molecules, chemotactic factors, and cytokines in inflammatory and neoplastic skin disease—1990 uptake. J Invest Dermatol 94:1515–1575 (1990).
28. Bergstresser PR. Cytokine expression by epidermal cell subpopulations in allergic contact dermatitis. In: Alternative Methods in Toxicology. Vol 10: In Vitro Skin Toxicology (Rougier A, Goldberg AM, Maibach HI, eds). New York:Mary Ann Liebert, 1994:303–311.
29. Chamberlain M, Earl L. Use of cell cultures in irritancy testing. In: Alternative Methods in Toxicology. Vol 10: In Vitro Skin Toxicology (Rougier A, Goldberg AM, Maibach HI, eds). New York:Mary Ann Liebert, 1994:59–67.
30. Goodwin BF, Roberts DW. Structure-activity relationships and allergic contact dermatitis. Food Chem Toxicol 24(7):795–798 (1986).
31. Hauser C, Saurat JH. Immunological principles of sensitization. In: Alternative Methods in Toxicology. Vol 10: In Vitro Skin Toxicology (Rougier A, Goldberg AM, Maibach HI, eds). New York:Mary Ann Liebert, 1994:255–261.
32. Perkins MA, Osborne R, Johnson GR. Development of an in vitro method for skin corrosion testing. Fundam Appl Toxicol 31:9–18 (1996).
33. Shivji GM, Gupta AK, Sauder DN. Role of Cytokines in Irritant Contact Dermatitis. In: Alternative Methods in Toxicology. Vol 10: In Vitro Skin Toxicology (Rougier A, Goldberg AM, Maibach HI, eds). New York:Mary Ann Liebert, 1993:13–22.
34. NIEHS. Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the Ad Hoc Interagency Coordinating Committee on the Validation of Alternative Methods. NIH Publ 97–3981. Research Triangle Park, NC:National Institute of Environmental Health Sciences, 1997.
35. OECD. Final Report of OECD Workshop on Harmonization of Validation and Acceptance Criteria for Alternative Toxicological Test Methods, 22–24 January 1996, Solna Sweden. Paris: Organisation for Economic Co-operation and Development, 1996.
36. Curren R, Bruner L, Goldberg A, Walum E. 13th Meeting of the Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC): Validation and Acute Toxicity Testing. Environ Health Perspect 106(Suppl 2):419–425 (1998).