



EPIDERMAL CYTOKINES AND SKIN SENSITIZATION HAZARD

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Abstract—The induction phase of skin sensitization is associated with the passage of antigen-bearing Langerhans cells (LC) from the epidermis to the draining lymph nodes. Recent investigations have revealed that the induction of LC migration following topical sensitization is dependent on tumour necrosis factor α (TNF- α), an epidermal cytokine. While in transit to the lymph nodes LC are subject to both phenotypic and functional maturation which, by analogy with *in vitro* studies, is also effected by epidermal cytokines (granulocyte/macrophage colony-stimulating factor, GM-CSF and interleukin-1, IL-1). It is now apparent that Langerhans cell function, the induction of cutaneous immune responses and effective sensitization are dependent on the availability of such cytokines and that contact allergens are able to provoke their production by keratinocytes and by Langerhans cells themselves. The development of screening strategies for the evaluation of skin sensitization potential as a function of epidermal cytokine production is discussed.

Introduction

The induction phase of contact sensitization following first encounter with a chemical allergen is characterized by various immunobiological events. Of central importance is the accumulation in lymph nodes draining the site of exposure of dendritic cells (DC), many of which carry high levels of allergen (Kimber and Cumberbatch, 1992). These allergen-bearing DC derive from epidermal Langerhans cells (LC) and have the ability to provoke T lymphocyte activation and to initiate immune responses. It is now apparent that associated also with skin sensitization is the production, or increased production, by epidermal cells of cytokines (Enk and Katz, 1992a,b). We have examined the influence of cytokines on LC function during skin sensitization, and in this paper the relevance of epidermal cytokine production for the evaluation *in vitro* of sensitizing activity is considered.

Cytokines and Langerhans cell function

The DC which arrive in draining lymph nodes following skin sensitization of mice are immunologically mature and stimulate T lymphocyte activation efficiently. In contrast, the epidermal LC from which they derive are relatively poor antigen-presenting cells. It has been shown that while in transit to

the lymph nodes, LC are subject to phenotypic and functional maturation. Thus, compared with LC resident in the epidermis, the allergen-bearing DC found in lymph nodes following sensitization express increased levels of intercellular adhesion molecule-1 (ICAM-1) and of MHC class II (Ia) antigens; membrane determinants which facilitate interaction with, and presentation of antigen to, T lymphocytes (Cumberbatch *et al.*, 1991 and 1992). By analogy with *in vitro* studies it appears likely that some of these changes are effected by granulocyte/macrophage colony-stimulating factor (GM-CSF) and interleukin 1 (IL-1), both of which are cytokines known to be produced by keratinocytes (Heufler *et al.*, 1988). There is evidence also that keratinocyte cytokines, in addition, may provide a signal for LC to leave the skin and migrate to the draining lymph nodes. Dermal tumour necrosis factor α (TNF- α) causes the arrival in local lymph nodes of DC (Table 1), coincident with a reduction in the frequency of LC within the epidermis. Moreover, systemic administration of a neutralizing anti-TNF- α antibody has been found to inhibit markedly the accumulation of DC in draining nodes during sensitization (Cumberbatch and Kimber, 1992; and unpublished observations, 1993).

Epidermal cytokines

Keratinocytes produce, or can be stimulated to produce, a variety of cytokines. Some of those which have been identified to date are illustrated in Fig. 1. More recently, evidence has accumulated that LC, and/or the DC into which they mature, are also able to elaborate cytokines. It has been shown that LC are the major or exclusive source of epidermal interleukin

Abbreviations: GM-CSF = granulocyte/macrophage colony-stimulating factor; DC = dendritic cell; ICAM-1 = intercellular adhesion molecule-1; IL = interleukin; IP-10 = interferon-induced protein 10; LC = Langerhans cell; MHC = major histocompatibility complex; MIP = macrophage inflammatory protein; TGF = transforming growth factor; TNF- α = tumour necrosis factor α .

Table 1. The influence of dermal tumour necrosis factor α (TNF- α) on the frequency of dendritic cells (DC) in draining lymph nodes

TNF- α (ng/ear)	DC/node	% increase
0	1834	—
12.5	2172	18.4
25	3092	68.6
50	3888	112.0
100	5011	173.2

Mice received a 25- μ l intradermal injection of various concentrations of recombinant murine TNF- α in the dorsum of each ear pinna. The frequency of DC in the draining (auricular) lymph nodes was measured 18 hr later.

(MIP-1 α). Epidermal LC also produce macrophage inflammatory protein 2 (MIP-2) and transforming growth factor β (TGF- β) and cultured LC, which are in many ways analogous to the immunologically active LC which have migrated to the lymph node, synthesize interleukin 6 (IL-6) (Enk and Katz, 1992a; Gruschwitz and Hornstein, 1992; Heufler *et al.*, 1992; Matsue *et al.*, 1992; Schreiber *et al.*, 1992). Although the local production and availability of certain epidermal cytokines (such as TNF- α and GM-CSF) are necessary for LC migration and maturation and for the initiation of effective sensitization, it is clear that local trauma, in addition to that induced by chemical allergens, will result in the increased production of these mediators. It is relevant, therefore, that the expression of certain epidermal cytokines appears to be modulated only by chemical allergens. Thus, epidermal mRNA for IP-10, MIP-2, IL-1 β and interleukin 10 (IL-10) has been shown to be up-regulated only following topical exposure to skin sensitizers (Enk and Katz, 1992a, b) (Table 2). Of particular

Table 2. Epidermal cytokines which may be regulated selectively by chemical allergens*

Cytokine	Source
Interleukin 1 β (IL-1 β)	Langerhans cells
Interferon-induced protein 10 (IP-10)	Keratinocytes
Macrophage inflammatory protein 2 (MIP-2)	Langerhans cells and keratinocytes
Interleukin 10 (IL-10)	Keratinocytes

*After Enk and Katz, 1992a,b; Heufler *et al.*, 1992.

interest is interleukin 1 β . Not only is IL-1 β produced exclusively by LC in epidermal populations (Matsue *et al.*, 1992), but also production is increased significantly following maturation (Heufler *et al.*, 1992). Moreover, as this is one of the cytokines considered to be up-regulated only by chemical allergens (Enk and Katz, 1992a), and in view of the fact that the presence of IL-1 β appears to be essential for effective skin sensitization (Enk *et al.*, 1993), this protein may provide a relevant and useful marker for allergenic potential.

Opportunities for *in vitro* analysis

A greater understanding of the role of epidermal cytokines in skin sensitization, their cellular sources and requirements for induction yields realistic opportunities to consider *in vitro* approaches for evaluation of sensitizing activity. While it is possible, on the basis of recent data, to conclude that the local availability of TNF- α and GM-CSF will be essential for effective sensitization, it is not possible to identify skin sensitizing chemicals solely on the basis of their ability to induce production of these cytokines. Other forms of cutaneous trauma, including irradiation with UV light and skin irritation, also result in increased epidermal cell production of TNF- α . It is nevertheless true, however, that chemicals which fail to cause the local synthesis of these cytokines are unlikely to induce sensitization. A more attractive approach is provided by the observations that epidermal cytokines such as IL-1 β , IP-10, MIP-2 and IL-10 are apparently up-regulated only by chemical allergens. If this selectivity can be confirmed with a wider range of chemicals of differing sensitizing potential and with non-sensitizing skin irritants then it may prove possible to develop *in vitro* methods using epidermal cells, or (in the case of IL-1 β) purified LC, to assess activity as a function of cytokine production.

REFERENCES

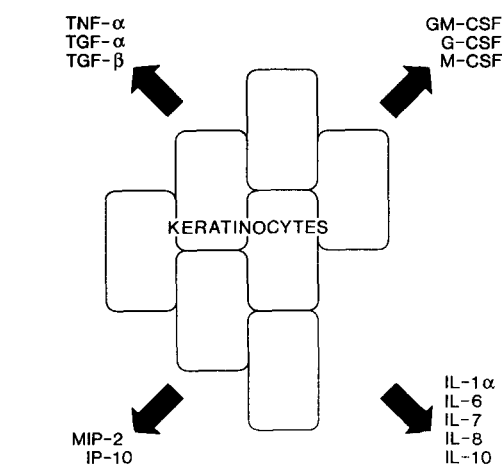


Fig. 1. Keratinocyte-derived cytokines. Keratinocytes produce constitutively, or can be stimulated to produce, a variety of cytokines including tumour necrosis factor α (TNF- α), transforming growth factors α and β (TGF- α and TGF- β), interferon-induced protein 10 (IP-10), macrophage inflammatory protein 2 (MIP-2), granulocyte/macrophage, granulocyte and macrophage colony-stimulating factors (GM-CSF, G-CSF and M-CSF), and interleukins 1 α , 6, 7, 8 and 10 (IL-1 α , IL-6, IL-7, IL-8 and IL-10).

- Cumberbatch M., Gould S. J., Peter S. W. and Kimber I. (1991) MHC class II expression by Langerhans cells and lymph node dendritic cells: possible evidence for maturation of Langerhans cells following contact sensitization. *Immunology* **74**, 414-419.
- Cumberbatch M. and Kimber I. (1992) Dermal tumour necrosis factor- α induces dendritic cell migration to draining lymph nodes and possibly provides one stimulus for Langerhans cell migration. *Immunology* **75**, 257-263.

- Cumberbatch M., Peters S. W., Gould S. J. and Kimber I. (1992) Intercellular adhesion molecule-1 (ICAM-1) expression by lymph node dendritic cells. Comparison with epidermal Langerhans cells. *Immunology Letters* **32**, 105–110.
- Enk A. H., Angeloni V. L., Udey M. C. and Katz S. I. (1993) An essential role for Langerhans cell-derived IL-1 β in the initiation of primary immune responses in the skin. *Journal of Immunology* **150**, 3698–3704.
- Enk A. H. and Katz S. I. (1992a) Early molecular events in the induction phase of contact sensitivity. *Proceedings of the National Academy of Sciences of the U.S.A.* **89**, 1398–1402.
- Enk A. H. and Katz S. I. (1992b) Identification and induction of keratinocyte-derived IL-10. *Journal of Immunology* **149**, 92–95.
- Gruschwitz M. S. and Hornstein O. P. (1992) Expression of transforming growth factor type beta on human epidermal dendritic cells. *Journal of Investigative Dermatology* **99**, 114–116.
- Heufler C., Koch F. and Schuler G. (1988) Granulocyte/macrophage colony-stimulating factor and interleukin 1 mediate the maturation of murine epidermal Langerhans cells into potent immunostimulatory dendritic cells. *Journal of Experimental Medicine* **167**, 700–705.
- Heufler C., Topar G., Koch F., Trockenbacher B., Kampgen E., Romani N. and Schuler G. (1992) Cytokine gene expression in murine epidermal cell suspensions: interleukin 1 β and macrophage inflammatory protein 1 α are selectively expressed in Langerhans cells but are differentially regulated in culture. *Journal of Experimental Medicine* **176**, 1221–1226.
- Kimber I. and Cumberbatch M. (1992) Dendritic cells and cutaneous immune responses to chemical allergens. *Toxicology and Applied Pharmacology* **117**, 137–146.
- Matsue H., Cruz P. D., Jr, Bergstresser P. R. and Takashima A. (1992) Langerhans cells are the major source of mRNA for IL-1 β and MIP-1 α among unstimulated mouse epidermal cells. *Journal of Investigative Dermatology* **99**, 537–541.
- Schreiber S., Kilgus O., Payer E., Kutil R., Elbe A., Mueller C. and Stingl G. (1992) Cytokine pattern of Langerhans cells isolated from murine epidermal cells. *Journal of Immunology* **149**, 3525–3534.