REVIEW ARTICLE

Keratinocytes as initiators of inflammation

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Environmental stimuli responsible for inducing cutaneous inflammation include contact allergens and ultraviolet light. We postulate that these diverse stimuli trigger a cutaneous inflammatory response by directly inducing epidermal keratinocytes to elaborate specific pro-inflammatory cytokines and adhesion molecules. The consequences are activation of dermal microvascular endothelial cells and selective accumulation of specific mononuclear cells in the dermis and epidermis. Thus, keratinocytes may act as "signal transducers", capable of converting exogenous stimuli into the production of cytokines, adhesion molecules, and chemotactic factors (acting in an autocrine and paracrine fashion) responsible for initiation of "antigen-independent" cutaneous inflammation. The initiation phase may facilitate or promote an amplification phase with additional production of tumour-necrosis factor alpha and interferon gamma via an "antigen-dependent" pathway, and keratinocyte/T cell/antigen-presenting dendritic cellular associations. The direct activation of keratinocytes, with their ability to produce the complete repertoire of pro-inflammatory cytokines, can profoundly influence endogenous and recruited immunocompetent cells, thereby providing the critical trigger responsible for the swift and clinically dramatic alterations that occur following contact between the epidermis and a host of "noxious" agents.

Lancet 1991; **337**: 211–14.

Introduction

Keratinocytes make up about 95% of the cell mass of human epidermis, and are responsible for the biochemical and physical integrity of skin via their production of numerous high-molecular-weight molecules such as cytokeratins and mucopolysaccharides. Under normal homoeostatic conditions, the epidermis protects the body against external injury. In certain disease states, it used to be presumed that keratinocytes were passive targets for immunological attack from infiltrating T cells, while epidermal immune responses were actively directed by dendritic, antigen-presenting, major histocompatibility complex (MHC) class II expressing, Langerhans cells.[1] Over the past decade, however, it has become clear that keratinocytes participate actively in such immune responses.[2] In many inflammatory skin diseases characterised by accumulation of T lymphocytes, keratinocytes aberrantly express MHC class II HLA-DR antigens,[3] intercellular adhesion molecule 1 (ICAM-1),[4] and CD36 antigen;[5] in psoriasis, keratinocytes additionally produce interleukin 6 (IL-6) and IL-8.[6,7] In vitro, mononuclear-cell-derived cytokines, such as interferon gamma (IFN-γ) and tumour-necrosis factor alpha (TNF-α), stimulate keratinocytes to produce HLA-DR,[8] ICAM-1,[9] IL-1,[10] IL-6,[11] IL-8,[12] monocyte chemotactic and activating factor (MCAF),[13] and transforming growth factor alpha (TGF-α).[14]

These data demonstrate that keratinocytes possess the capacity to interact with infiltrating mononuclear cells, either by release of pro-inflammatory cytokines or via intercellular adhesion reactions. There is still uncertainty, however, about the mechanism responsible for the genesis of accumulation of mononuclear cells at sites of cutaneous inflammation caused by environmental cues including low-molecular-weight contact allergens (eg, poison ivy, nickel sulphate), toxic chemicals (eg, dithranol, croton oil), and physical stimuli such as ultraviolet light. There is increasing evidence that specific immunologically active products of keratinocytes generated in response to

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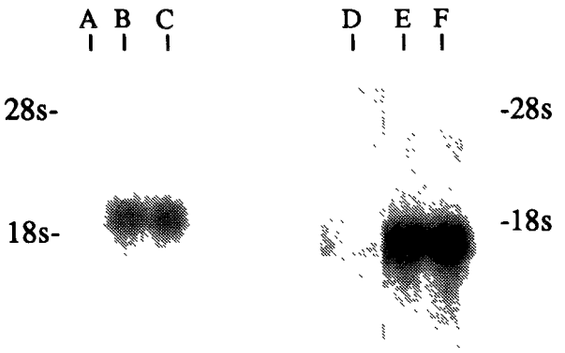

Fig 1—Urushiol induction of genes in cultured keratinocytes.

Lanes A–C hybridised with probes for TNF-α mRNA, lanes D–F hybridised with probes for IL-8 mRNA Keratinocytes harvested at 0 h (lanes A and D), 4 h (lanes B and E), and 8 h (lanes C and F).

environmental cues may orchestrate the initiation of cutaneous inflammation.

Proinflammatory responses of keratinocytes

Urushiol (the pentadecylcatechol responsible for poison ivy dermatitis),[15] all-*trans* retinoic acid (RA), and 12-O-tetradecanoyl-13-phorbol acetate (TPA) are low-molecular-weight compounds, which, when applied topically to normal skin, produce erythema and selective accumulation of CD4 and CD8 positive T lymphocytes at the dermal/epidermal interface. This suggests that specific chemotaxins and adhesion factors for these cells are produced in the epidermis during onset of inflammation.

To elucidate the potential role of keratinocytes in these inflammatory reactions, monolayers of normal multi-passaged human keratinocytes grown in a serum-free medium (Clonetics, San Diego) were cultured with urushiol (5 μmol/l) (courtesy of Dr C. Anderson), RA (1 μg/ml) (Sigma), or TPA (10 nmol/l) (Sigma). Cellular RNA was extracted and hybridised by northern blot to cDNA probes specific for ICAM-1 (an adhesion ligand for lymphocytes), IL-8 (a T-cell chemotaxin), and IL-1 and TNF-α (IL-1 is a T-cell chemotaxin and both are potent activators of endothelial cells[12]).

Urushiol induced TNF-α mRNA and IL-8 mRNA at 4 and 8 h after exposure (fig 1). Culture supernatant TNF-α and IL-8 bioactivity paralleled mRNA production, as measured by cytolysis of WEHI cells (courtesy of Dr S. Kunkel) and IL-8 antibody-inhibitable T cell and neutrophil chemotaxis (courtesy of Dr M. Jones), respectively. Urushiol, TPA, and RA all induced ICAM-1 mRNA (the calcium ionophore ionomycin had no effect by itself) (fig 2), confirming our previous detection of ICAM-1 protein.[15]

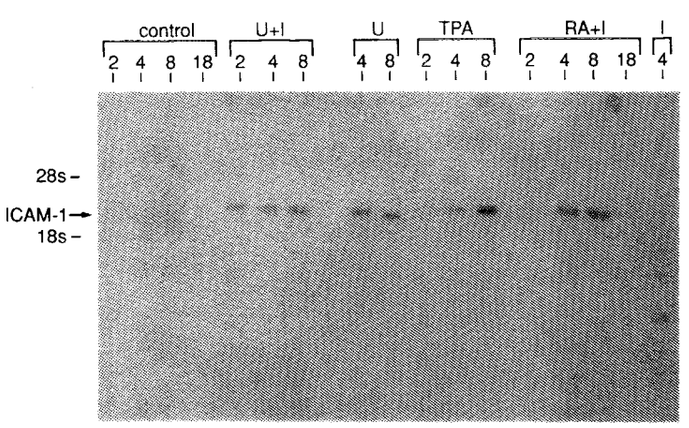

Fig 2—Induction of ICAM-1 mRNA by urushiol, TPA, and RA.

Keratinocytes grown without stimuli (control), or exposed only to ionomycin (I), for 2–18 h did not express detectable ICAM-1 mRNA However, urushiol (U), TPA, and RA plus I induced ICAM-1 mRNA detectable at 4 h

While we observed that TPA could induce IL-8 mRNA and protein, RA could not, even after addition of 1 μmol/l ionomycin to potentiate protein kinase C signal transduction, an important mediator of the biological activity of RA, TPA,[16] and urushiol.[17] Urushiol, TPA, and RA all induced IL-1β mRNA 4 and 8 h after exposure. The ability of RA to induce ICAM-1 and IL-1β but not IL-8 is of interest, since the inflammatory response to RA in vivo is relatively devoid of large accumulations of mononuclear leucocytes.

These studies complement our in-vivo data showing keratinocyte ICAM-1 expression in urushiol dermatitis (preceding the lymphocytic infiltrate),[15] psoriasis[4] and retinoid dermatitis,[18] and epidermal IL-8 expression in urushiol dermatitis[19] and psoriasis.[7] Furthermore, given the close spatial apposition of basal keratinocytes to papillary dermal vascular endothelium (within 20–40 μm),[15] production by keratinocytes of IL-1 and TNF-α may explain endothelial cell activation in urushiol dermatitis[19] and psoriasis, as indicated by endothelial leucocyte adhesion molecule 1 (ELAM-1)[20] and vascular cell adhesion molecule 1 (VCAM-1)[21] expression.

Similar results have been reported with ultraviolet B light as an inducer of cutaneous inflammation. After ultraviolet B irradiation in vitro, keratinocytes express ICAM-1,[22] IL-1,[23,24] IL-6,[11] and TNF-α.[25] Exposure to ultraviolet B in vivo leads to dermal vascular cell expression of ELAM-1 within 6 h (Norris PG et al, unpublished). Furthermore, IL-1 and IL-6 are detectable in serum following whole body ultraviolet irradiation,[26,27] and may account for the fever after sunburn. Nickel sulphate, the most common contact allergen in the UK, also directly upregulates keratinocyte IL-1 in vitro.[28] The activation of keratinocytes is, however, not completely indiscriminate, as indicated by the fact that lipopolysaccharide, an important constituent of gram-negative bacterial cell walls, does not induce cultured keratinocyte to express ICAM-1.[9]

Initiation phase of cutaneous inflammation

Epicutaneous contact with environmental stimuli, including those detailed above, leads directly to keratinocyte activation, with release of IL-1 and TNF-α, and expression of the surface protein ICAM-1 (fig 3). Secretion of IL-1 and TNF-α results in activation of dermal endothelial cells, production of the surface leucocyte adhesion ligands ICAM-1, ELAM-1,[20] and VCAM-1,[21] and consequent recruitment and sequestration of predominantly memory-type mononuclear cells from the circulation. Concomitant secretion by keratinocytes of IL-8 and IL-1 promotes directional T-lymphocyte migration along a chemotactic concentration gradient to the epidermis, where T cells expressing lymphocyte function-associated antigen 1 (LFA-1) adhere to keratinocytes via interactions with the LFA-1 ligand, ICAM-1. T cell adherence to keratinocytes in vitro is LFA-1/ICAM-1-dependent,[2,9] and in-vivo immunohistochemical studies of inflamed skin in numerous dermatoses reveal co-localisation of keratinocytes expressing ICAM-1 with LFA-1-positive T cells.[4,29]

Amplification phase of cutaneous inflammation

Scanning laser densitometry of northern blots of ICAM-1, IL-8, and MCAF mRNA shows that stimulation of these genes in keratinocytes is considerably greater by cytokines than by non-cytokine, low-molecular-weight compounds.[12] It seems likely, therefore, that close apposition of mononuclear cells and keratinocytes in vivo

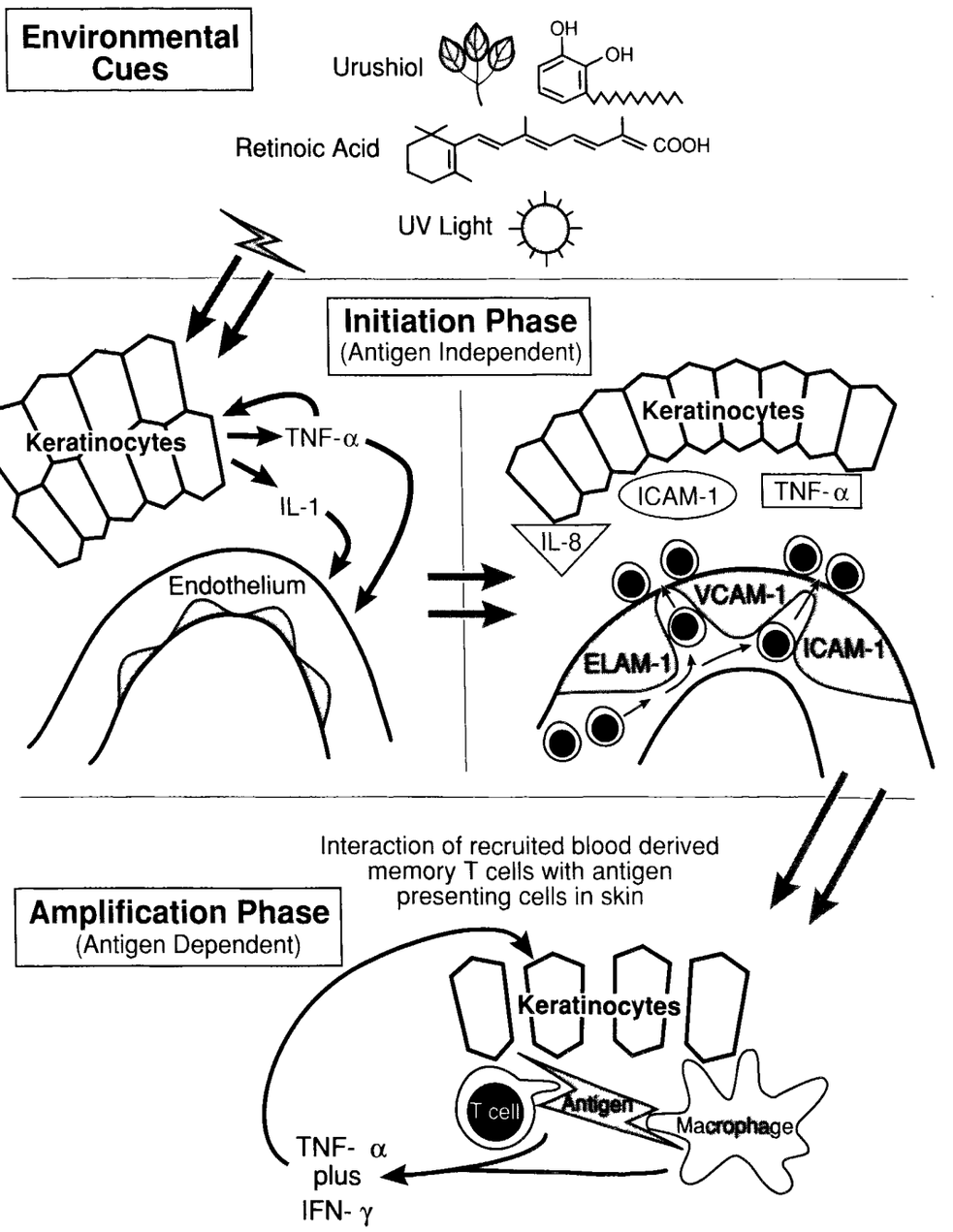

Fig 3—How keratinocytes function as environmental signal transducers converting exogenous stimuli into production of pro-inflammatory cytokines.

leads to amplification, both in intensity and in number, of the factors produced by keratinocytes and mononuclear cells, and proliferation of T cells. In support of this hypothesis, cell cycling T cells in cutaneous T-cell lymphoma and psoriasis are confined primarily to the epidermis, having migrated from the dermis.[29,31] Furthermore, T-cell proliferation can be induced by TPA-stimulated keratinocytes via an ICAM-1-dependent pathway.[32] Evidence from other cell systems shows that cellular adhesion leads directly to cytokine release.[33] Therefore, interactions between mononuclear cells and epidermal keratinocytes lead to an amplification of cutaneous inflammatory events, with increased sequestration of circulating leucocytes and accumulation of these cells in the epidermis.

Autocrine induction of keratinocyte-derived genes may also play a vital role in the amplification of responses—eg, both IL-1 and TNF-α are auto-induced in human keratinocytes. Moreover, the initial and direct "antigen-independent" keratinocyte activation, which could be mediated via altered signal transduction involving protein kinase C,[15,29] may facilitate the recruitment and functional interaction between epidermal cells and dendritic antigen-presenting cells. Such interactions leading to IFN-γ/TNF-α release in the amplification phase may then be driven by more of an "antigen-dependent" component. We suggest that the "antigen" becomes recognisible to the immune system either by chemical alteration of extracellular proteins to form hapten-protein conjugate, or by direct modification of cell surface MHC molecules to function as "superantigens".[34] If the environmental stimulus is ultraviolet light, then only an "initiation-phase" type reaction may occur, since no new antigen would be formed; thus, most sunburns are relatively mild and transient. This compares with the intense and persistent inflammation produced by exposure to poison ivy/oak leaf surfaces, where urushiol could generate both "initiation-phase" and "amplification-phase" reactions mediated via protein kinase C signal transduction and hapten-protein ("antigen") conjugates, respectively.

Besides triggering the start of inflammation, keratinocyte-derived cytokines have other significant roles in the

pathophysiology of cutaneous inflammation. Firstly, a common sequel to inflammation is epidermal hyperproliferation, as manifested by thickened skin and scale formation. TGF-α is a potent autocrine growth factor for keratinocytes that can be induced by IFN-γ and TNF-α,[7,14] and IL-6 and IL-8 are also known growth factors for keratinocytes.[6,35] Thus, abnormal hyperproliferation of keratinocytes may be linked directly to cytokines released by inflamed epidermis. Secondly, glucocorticosteroids and cyclosporin A are highly efficacious in a wide variety of inflammatory dermatoses with multiple aetiologies. We suggest that a logical explanation for their action is that they block production of pro-inflammatory cytokines, adhesion molecules, and chemotoxins by keratinocytes and immunocytes, as outlined above. Finally, calcium channel inhibitors have been shown to block IL-8-induced T-cell chemotaxis,[36] and ICAM-1-dependent T-cell adhesion to keratinocytes,[29] suggesting that such agents may be of therapeutic benefit in the managment of inflammatory skin disease.

While one of the basic immunodermatological dogmas historically has concerned the critical interaction between T cells and epidermal Langerhans cells, the ability of keratinocytes to produce a complete repetoire of immunoregulators suggests they also have an important pathophysiological role in the genesis and evolution of cutaneous inflammation.

Supported in part by the Wellcome Trust, British Association of Dermatologists and the Psoriasis Association of Great Britain (J. N. W. N. B.), and National Institutes of Health grants AR31857, AR40065 and AR08123 (B. J. N.), and HL-39415 (V. M. D.)

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