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Induction of ICAM-1 expression by epidermal keratinocytes via a paracrine pathway possibly involving dermal dendritic cells

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Recent studies have attributed a central role to epidermal keratinocytes (KC) in the initiation of cutaneous inflammation [1]. This hypothesis was based on the observation that contact with various environmental stimuli causing skin inflammation clinically, such as contact allergens (e.g. poison ivy, nickel sulphate), toxic chemicals (e.g. dithranol, croton oil) and physical stimuli (ultraviolet radiation), can directly induce epidermal KC to produce specific cytokines (e.g. IL-1, IL-6, IL-8, TNF-α) and to express adhesion molecules (e.g. ICAM-1). These molecules may play an important role in: (1) activation of dermal endothelial cells with subsequent recruitment of inflammatory cells from the circulation (TNF-α, IL-1); (2) migration of inflammatory cells to the epidermis (IL-1, IL-8); and (3) binding of T lymphocytes to epidermal KC through the ICAM-1/LFA-1 pathway (for review see references 1 and 8).

Recently, in our laboratory, the capacity of a large number of cytokines and other inflammatory mediators to induce ICAM-1 expression in epidermal KC was investigated, both in monolayers of cultured KC and in skin biopsy specimens kept in short-term organ culture. Whereas some of the stimuli led to KC ICAM-1 expression in both assays, lipopolysaccharide (LPS), an important constituent of gram-negative bacterial cell walls, was found to induce KC ICAM-1 expression in the skin biopsy specimens, but not in the KC monolayers. The latter result is consistent with recent studies in which it was shown by Northern blot analysis that ICAM-1 mRNA was absent in KC monolayers following incubation with LPS [6]. The results of these and further studies, suggesting that this apparent discrepancy reflects different pathways of KC activation, prompted us to report these observations.

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Monolayers of multi-passaged human KC, cultured in serum-free medium (KGM, Clonetics, San Diego Calif., USA), were incubated for 24 and 48 h (37 °C, 5% CO_2) with IL-1 (10 U/ml), IL-2 (200 U/ml), IL-4 (5 U/ml), IL-6 (1000 U/ml) (Janssen Biochimica, Beerse, Belgium), IL-8 (10 ng/ml) (a kind gift from AO Anderson, Frederick, M., USA), IFN-α (1000 U/ml, Hoffman-La Roche, Basel Switzerland), IFN-γ (1000 U/ml), TNF-α (2000 U/ml) (Genzyme, Cambridge, Mass., USA), TGF-α (10 ng/ml) (Biomedical Technology Inc., Oxford, UK), GM-CSF (1000 U/ml) (Genzyme), PMA (5 ng/ml) (Sigma, St. Louis, Mo., USA) and LPS (12.5, 25, 50, 100, 250 μg/ml) (Difco Laboratories, Detroit, Mich., USA). KC ICAM-1 expression was studied immunohistochemically, using the monoclonal antibody (moab) RR1/1 (CD54, IgG1, provided by TA Springer, Boston, Mass., USA) and a biotin–avidin–peroxidase technique (Vectastain ABC Kits, Vector Laboratories, Inc., Burlingame, Calif., USA) with 3,3′-diaminobenzidene tetrahydrochloride as the chromogen. In addition, induction of ICAM-1 expression on KC was investigated in skin biopsy specimens kept in organ culture, as described previously [5]. Briefly, 2 mm punch biopsies of normal human skin, obtained from abdominal and breast reconstructive surgery, were cultured by complete submersion in Dulbecco's Modified Eagles Medium, supplemented with 5% heat-inactivated fetal calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml) and glutamine (2 mM) (ICN, Biomedicals, Costa Mesa, Calif., USA), and incubated for 24 and 48 h with the above-mentioned immune mediators. Skin biopsies were snap-frozen in liquid nitrogen and cryostat sections were examined for KC ICAM-1 expression.

KC monolayers expressed ICAM-1 following incubation with IFN-γ, TNF-α and PMA, but not with LPS or any of the other cytokines tested. The ICAM-1 expression was strongly positive on more than 90% of the KC after IFN-γ and TNF-α incubation and was strongest after 48 h. PMA led to ICAM-1 expression on 5–10% of the KC after 24 and 48 h. In the short-term organ culture, ICAM-1 was induced on epidermal KC not only by IFN-γ, TNF-α and PMA, but also by LPS. LPS and PMA induced focal ICAM-1 expression on

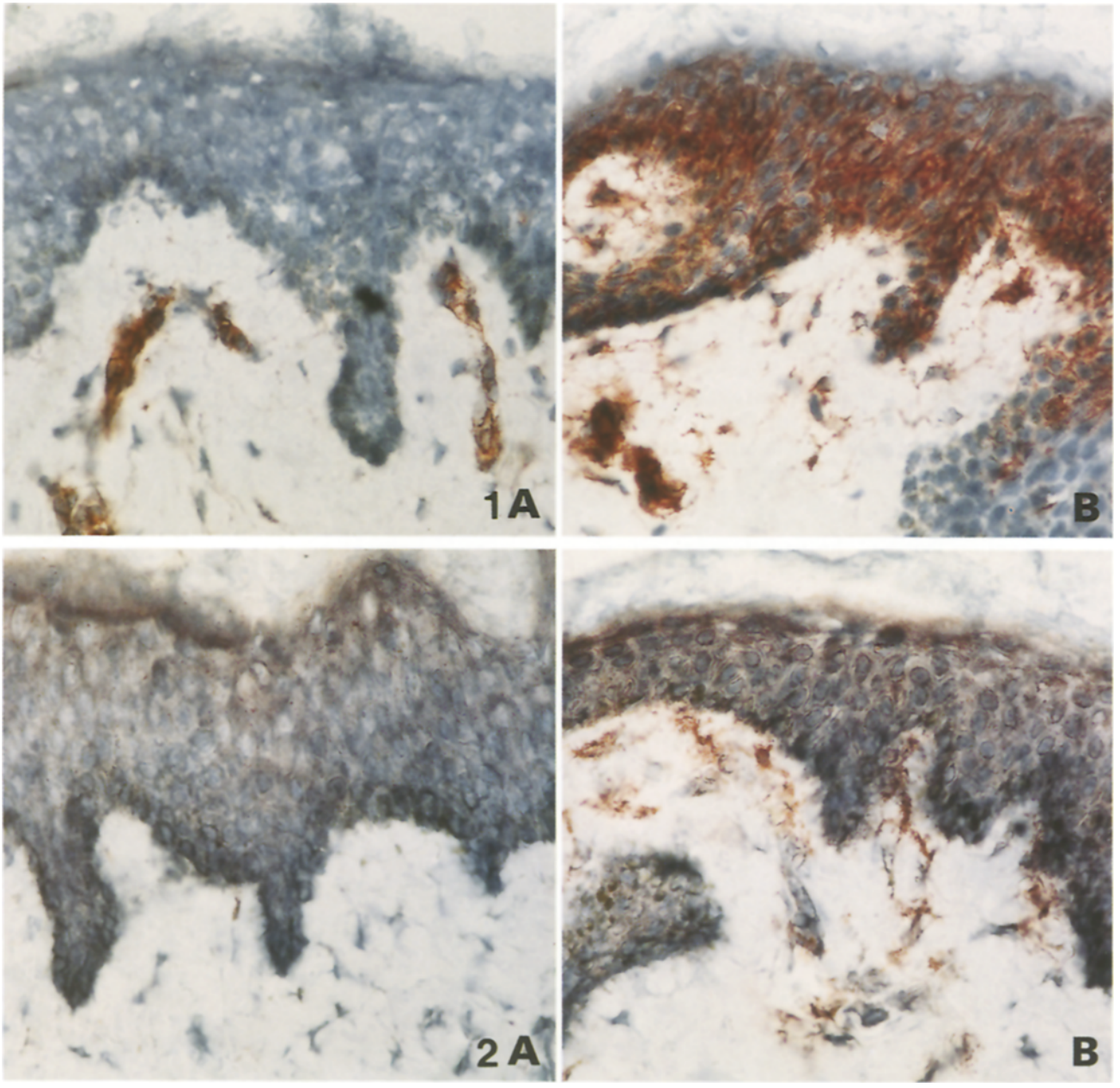

Fig. 1A, B. ICAM-1 expression in normal skin after short-term organ culture without LPS (**A**) and in the presence of LPS (**B**)

Fig. 2A, B. TNF-α expression in normal skin after short-term organ culture without LPS (**A**) and in the presence of LPS (**B**)

basal and suprabasal KC, whereas IFN-γ and TNF-α induced ICAM-1 expression throughout the entire epidermis.

Because of the apparent discrepancy in KC ICAM-1 induction by LPS in the two assays used, the suggestion was raised that the ICAM-1 induction observed in skin biopsy specimens kept in short-term organ culture was an indirect rather than a direct effect of LPS on KC, probably mediated by cytokines produced by other epidermal or dermal cells. It was anticipated that this effect was mediated by TNF-α, since: (1) IFN-γ and TNF-α are the only known cytokines able to induce KC ICAM-1 expression; (2) IFN-γ, but not TNF-α, induces HLA-DR on KC [2–4]; and (3) KC in the skin biopsies stimulated by LPS in short-term organ culture expressed ICAM-1, but no HLA-DR. To test this hypothesis, additional immunohistochemical studies were performed on cryostat sections of skin biopsy specimens cultured with or without LPS (50 μg/ml, 24 h), using polyclonal antibodies against TNF-α and IL-8 (both provided by S Kunkel, Ann Arbor, Mich., USA), and moab against VCAM-1 (BBA 5, IgG1), ELAM-1 (BBA 1, IgG1) (ITK Diagnostics, De Kwakel, The Netherlands), CD1a (OTK6, IgG1) (Ortho Diagnostics, Raritan, NJ, USA) and HLA-DR (CLB.H2.5.10, IgG1) Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands).

In cryostat sections from skin biopsies incubated with LPS, the epidermis showed a strong focal staining of ICAM-1 in the basal and suprabasal layers (Fig. 1B) and

a weak diffuse staining of TNF-α in the suprabasal layer (Fig. 2B). This staining pattern did not correspond with the presence of epidermal CD1+, HLA-DR+ Langerhans cells. There was no epidermal staining for IL-8. In the papillary dermis dendritic cells strongly expressed TNF-α (Fig. 2B) and IL-8. Endothelial cells strongly expressed ICAM-1 (Fig. 1B) and ELAM-1, and weakly VCAM-1. In cryostat sections from skin biopsies not stimulated by LPS, staining for ICAM-1, TNF-α, IL-8, ELAM-1 and VCAM-1 was completely negative, except for ICAM-1 expression on dermal endothelial cells (Fig. 1A) and a faint staining of the suprabasal KC for TNF-α (Fig. 2A). These findings support our hypothesis that LPS induces ICAM-1 expression by epidermal KC indirectly via induction of TNF-α production by dermal dendritic cells. It is possible that TNF-α released by these dendritic cells is also responsible for the induction of IL-8 in these cells (autocrine stimulation) and for the expression of ELAM-1 and VCAM-1 by dermal endothelial cells. However, a direct effect of LPS on these cells can not be excluded.

The observation that KC can be induced to express ICAM-1, not only by direct stimulation by environmental factors, but also indirectly via cytokines produced by dermal cells, further demonstrates that not only epidermal cells, such as KC and Langerhans cells, but also dermal dendritic cells may act as initiators of cutaneous inflammation [7].

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