

# Selective Expression of Immune-Associated Surface Antigens by Keratinocytes in Irritant Contact Dermatitis

Carolyn M. Willis, Catherine J.M. Stephens, and John D. Wilkinson

Department of Dermatology, Wycombe General Hospital, High Wycombe, Buckinghamshire, U.K.

The expression of three immunoregulatory surface antigens by epidermal keratinocytes was studied in irritant contact dermatitis (ICD), in order to assess whether keratinocytes have a modulatory role in the pathogenesis of this disorder. Biopsies were taken from 48-h patch test reactions to six structurally unrelated irritants, and frozen sections immunolabeled with monoclonal antibodies to the major histocompatibility complex class II antigen, HLA-DR, intercellular adhesion molecule-1 (ICAM-1), and the 88-Kd glycoprotein CD36 (OKM5), as well as to the CD3 (T cells) and CD11a (lymphocyte function associated antigen-1, LFA-1) antigens. We found that there was very limited expression of HLA-DR by keratinocytes, with no correlation between the extent of HLA-DR positivity and the degree of T cell infiltration into the epidermis and dermis, suggesting that interferon gamma may not be a significant mediator of ICD at 48 h. In contrast, keratinocytes showed extensive upregulation of ICAM-1, with an excellent spatial association between

ICAM-1 expression and LFA-1 positive leucocytes in the epidermis. This indicates that keratinocyte ICAM-1 induction is not restricted to diseases in which antigen presentation is pivotal, but that it has a generalized role in cutaneous inflammatory reactions, promoting the infiltration of leucocytes into the epidermis. Immunolabeling with OKM5 revealed that CD36 is present to a variable degree on keratinocytes in normal skin. Differential changes in the pattern of keratinocyte expression occurred between irritants, in a manner that suggested that the CD36 antigen does not act as an adhesion molecule in ICD, but rather that its expression is related to the proliferative state of the epidermis. The results of this study demonstrate that immune-associated antigens are selectively expressed on the surface of keratinocytes in 48-h ICD biopsies, implying that these cells play an important regulatory role in the development of the inflammatory response to irritant chemicals. *J Invest Dermatol* 96:505-511, 1991

**D**uring the last few years it has become increasingly apparent that human epidermal keratinocytes play a far more active role in the initiation and perpetuation of cutaneous inflammatory disorders than was first thought. They have been shown to be capable of antigen presentation, albeit in a limited fashion [1] and, under suitable conditions, may be induced to synthesize and secrete a number of important immunomodulating cytokines [2-5].

In diseases characterized by a significant dermal lymphocytic infiltrate, epidermal keratinocytes may also be induced to express several membrane glycoprotein antigens known to be involved in immunoregulation. One of the first of these to be reported was the class II major histocompatibility complex (MHC) molecule, HLA-DR, which, in the epidermis of normal skin, is present only on Langerhans cells and acrosyringeal epithelium [6]. It is expressed by keratinocytes in a wide range of dermatoses including mycosis fungoides, psoriasis, lichen planus, and graft-versus-host disease [7-11]. A number of in vitro and in vivo studies have shown that this upregulation may be due, in part, to the production of interferon gamma (IFN  $\gamma$ ) by activated T lymphocytes at the site of inflammation [12-15]. The significance and function of the aberrant MHC class II expression, however, remains unclear, because there is contradictory evidence as to whether HLA-DR positive keratinocytes enhance or attenuate inflammatory responses [16-21]. It has also been suggested that HLA-DR positive keratinocytes may be the target for activated cytotoxic T lymphocytes [1,21].

More recently, a molecule known as intercellular adhesion molecule-1 (ICAM-1) has also been identified on the surface of keratinocytes in inflammatory dermatoses typified by T-cell infiltration [22,23]. This molecule is known to play a critical role in leucocyte adhesion by acting as the ligand for the lymphocyte function-associated antigen-1 (LFA-1), constitutively expressed by leucocytes [24]. Keratinocytes in normal skin do not express ICAM-1 and its upregulation is believed to relate to the secretion of cytokines, including IFN  $\gamma$  by activated T lymphocytes and tumor necrosis factor alpha (TNF  $\alpha$ ) by macrophages [5,25,26]. Induction of ICAM-1 by non-cytokines such as urushiol and phorbol ester has also recently been demonstrated [27,28].

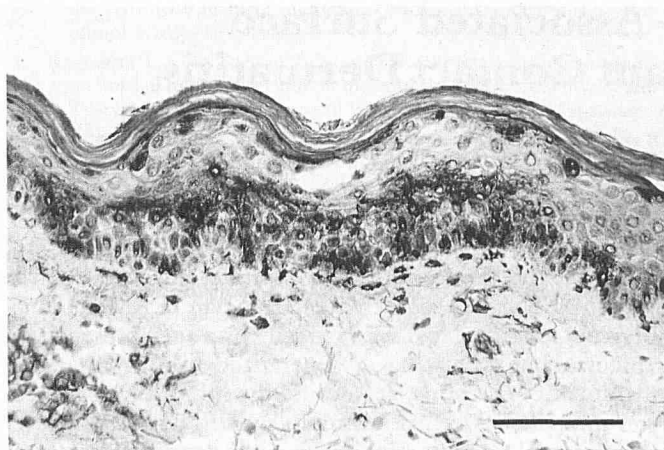
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Reprint requests to: Dr. Carolyn M. Willis, Department of Dermatology, Wycombe General Hospital, High Wycombe, Bucks, HP11 2TT, U.K.

#### Abbreviations:

- ACD: allergic contact dermatitis
- BC: benzalkonium chloride
- CO: croton oil
- HLA-DR: human leukocyte antigen-DR
- ICAM-1: intercellular adhesion molecule-1
- ICD: irritant contact dermatitis
- IFN  $\gamma$ : interferon gamma
- IL-1: interleukin-1
- LFA-1: lymphocyte function associated antigen-1
- NAA: nonanoic acid
- PG: propylene glycol
- SLS: sodium lauryl sulphate
- TNF  $\alpha$ : tumour necrosis factor alpha



**Figure 1.** Immunolabeling of a frozen section from a dithranol reaction, showing one of the few areas in which keratinocytes have been induced to express HLA-DR (bar, 100  $\mu$ m).

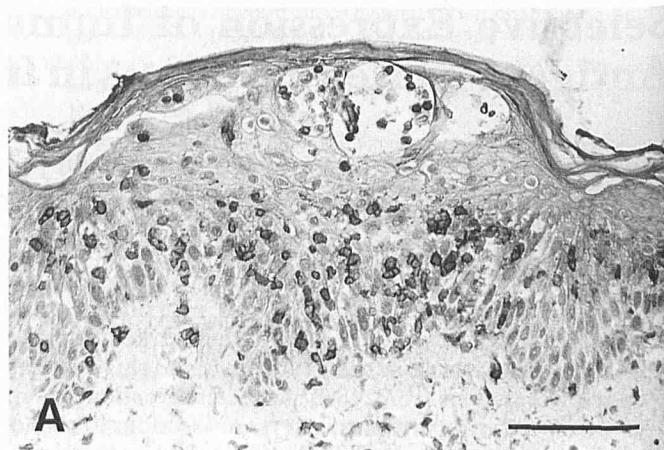
Keratinocytes may also be induced to express another molecule thought to be involved in cell adhesion, an 88-kD glycoprotein known as CD36 (OKM5), which functions as the thrombospondin receptor and is present on the surface of monocytes and platelets [29,30]. Unlike the other two antigens, however, there is some debate as to whether this is expressed by normal keratinocytes [31] but, again, its upregulation is thought to be due, at least in part, to the production of IFN  $\gamma$  by activated T lymphocytes at the site of inflammation [32].

Two diseases that are strongly characterized by the influx of both lymphocytes and macrophages into the epidermis and dermis are allergic (ACD) and irritant contact dermatitis (ICD), and there is reasonable evidence that, in the former delayed-type hypersensitivity reaction, keratinocytes are indeed induced to express HLA-DR, ICAM-1, and OKM5 [11,33–35]. The literature concerning ICD, on the other hand, is somewhat contradictory but appears to suggest that there may be significant differences in keratinocyte expression of antigens between these two etiologically distinct forms of contact dermatitis, particularly with regard to ICAM-1 [33,36–39]. In view of the fact that the upregulation of these membrane antigens can provide valuable information relating to the pathogenesis of a disease, we decided to study, using immunocytochemical methodology, the epidermal expression of HLA-DR, ICAM-1, and OKM5 in 48-h patch test reactions to six structurally unrelated chemical irritants. Immunolabeling with monoclonal antibodies against LFA-1 and the CD3 antigen on T cells (anti-Leu-4) was also performed, in order to allow an assessment of the spatial relationships between lymphocyte exocytosis and keratinocyte ICAM-1 and HLA-DR expression to be made.

#### MATERIALS AND METHODS

**Patch Tests** Full details of the design of this study are given in a previous report [40]. Briefly, each of ten healthy non-atopic male volunteers were patch tested on the volar aspect of the forearm with the following six irritant chemicals, the concentrations having been optimized in a previous study [41]: 5% (w/v) aqueous sodium lauryl sulphate (SLS), 0.8% (w/w) croton oil (CO) in yellow soft paraffin, 0.5% (w/v) aqueous benzalkonium chloride (BC), 80% (v/v) nonanoic acid (NAA) in propan-1-ol, 100% propylene glycol (PG), 0.02% (w/w) dithranol in yellow soft paraffin. In addition, half of the volunteers received patch test controls of yellow soft paraffin and propan-1-ol, the remaining half receiving distilled water and occlusion-alone controls.

After 48 h, the patch tests were removed and the intensity of inflammatory response visually scored as negative, mild (erythema



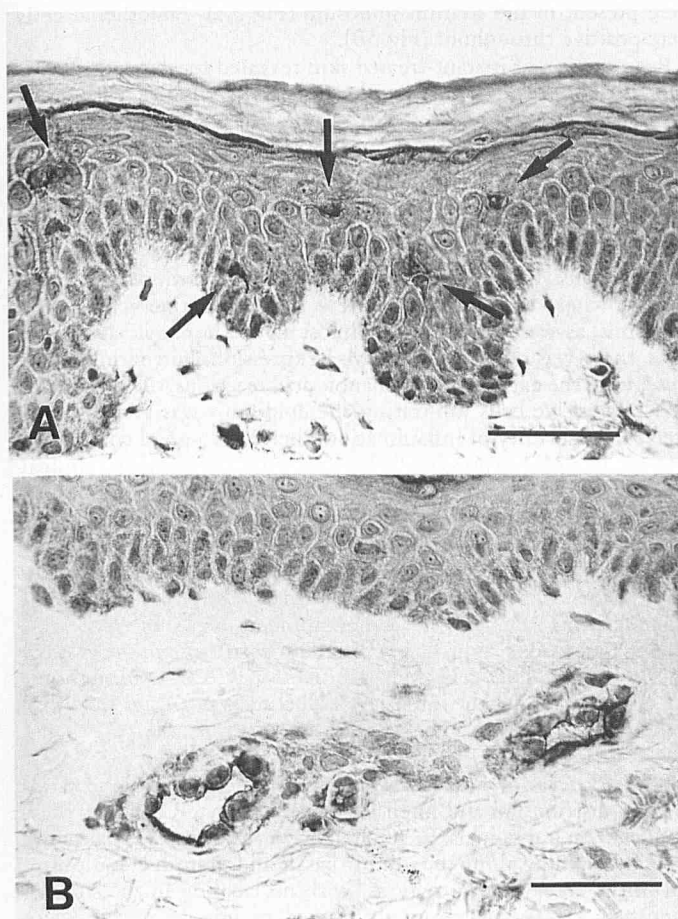
**Figure 2.** Serial sections of a patch test reaction to croton oil showing the infiltration of anti-Leu-4 positive T cells (A: bar, 100  $\mu$ m) and HLA-DR positive mononuclear and dendritic cells (B: bar, 100  $\mu$ m) into the epidermis, but with no associated induction of HLA-DR expression by keratinocytes. This was a common feature of many of the irritant reactions.

alone), moderate (erythema with edema), or severe (erythema, edema, and vesiculation). Punch biopsies (4 mm) were then taken from each site, with an additional sample of normal skin being removed from an adjacent area. The samples were then processed immediately for microscopy.

Approval for the study was obtained from the Wycombe Ethical Research Committee and all volunteers gave informed, written consent.

**Immunocytochemistry** Skin samples were embedded in OCT compound (Miles Scientific, Naperville, IL) and snap frozen in liquid nitrogen. Vertical cryostat sections (4  $\mu$ m) were cut from three different areas (approximately 150  $\mu$ m apart) of each sample and air dried overnight at room temperature on 0.01% poly-L-lysine (Sigma Chemical Co. Ltd., Poole, Dorset, UK)-coated slides. Following fixation in acetone for 10 min at room temperature, the sections were incubated for 20 min at room temperature in the following primary antibodies, all of which were used at a dilution of 1 in 20: anti-ICAM-1 (CD54; Serotec Ltd., Oxford, UK), anti-LFA-1 (CD11a; Dako Ltd., High Wycombe, Bucks, UK), anti-HLA-DR (Becton Dickinson UK Ltd., Oxford, UK), anti-Leu-4 (CD3; Becton Dickinson); and OKM5 (CD36; Ortho Diagnostic Systems Ltd., High Wycombe, Bucks, UK).

Anti-ICAM-1 and anti-LFA-1 were applied on serial sections throughout, as were anti-HLA-DR and anti-Leu-4, so as to enable the spatial relationships between these antigens to be examined.



**Figure 3.** Normal skin immunolabeled with anti-ICAM-1, showing scattered positively stained dendritic cells (arrows) (A: bar, 50  $\mu$ m) and positive endothelial cells (B: bar, 50  $\mu$ m).

An avidin-biotin immunoperoxidase technique (Vectastain ABC kit, Vector Laboratories, Peterborough, UK) with 3'3 diaminobenzidine as chromogen, was used to visualize the antibody/antigen reaction.

**Assessment of Immunolabeling** A qualitative assessment of the distribution of immunolabeling of each antibody was performed by light microscopy. In addition, for anti-HLA-DR and anti-ICAM-1 a semi-quantitative assessment of the degree of keratinocyte positivity in the basal, mid, and upper regions of the epidermis was made, according to the following scale: - (negative),  $\pm$  (very pale or only occasional small patch), + (several small patches), ++ (moderately sized areas), +++ (extensive or large areas). The overall score for each irritant and control group was taken as the mean of the results obtained from all of the individuals tested. Careful examination of the serial sections was also made, to determine the spatial relationships between keratinocyte HLA-DR expression and T-cell exocytosis, and keratinocyte ICAM-1 expression and LFA-1 positive leucocytes. In the interpretation of OKM5 expression, direct comparisons were made between the immunolabeling of each individual's normal skin and that of their patch test sites. For each irritant and control group, a semi-quantitative score for the basal, mid, and upper regions of the epidermis was then obtained, using the following scale: - (no staining in either normal skin or treated sites),  $\leftrightarrow$  (no change from normal skin),  $\uparrow$  or  $\downarrow$  (increase or decrease in 3-5 individuals),  $\uparrow\uparrow$  or  $\downarrow\downarrow$  (increase or decrease in 6-7 individuals),  $\uparrow\uparrow\uparrow$  or  $\downarrow\downarrow\downarrow$  (increase or decrease in >7 individuals).

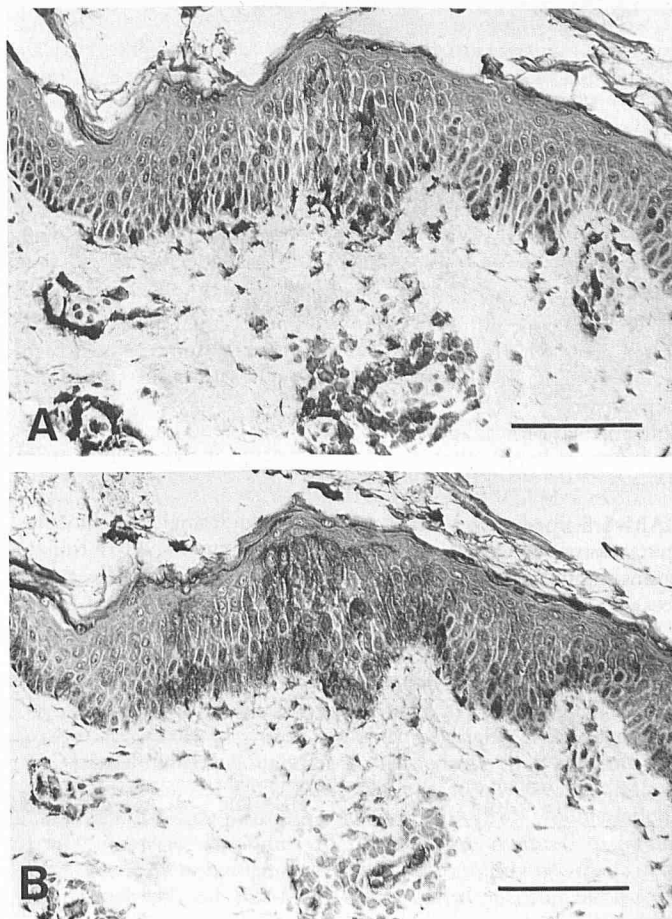
In all patch test reactions, the correlation between the expression of HLA-DR, ICAM-1, and OKM5 by keratinocytes and the intensity of the inflammatory response, as assessed visually, was also examined.

## RESULTS

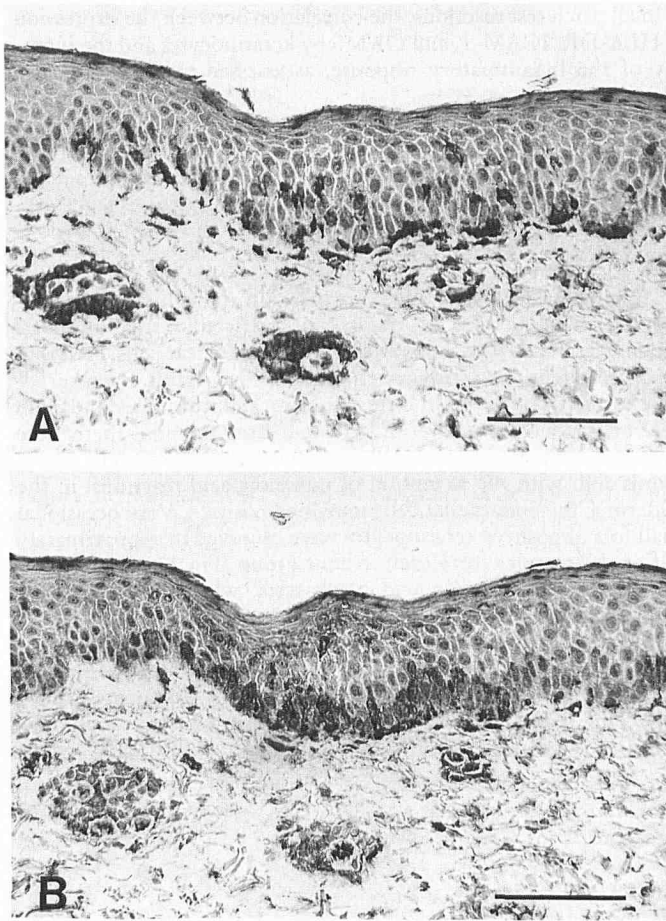
**Clinical Assessment** The majority of irritant patch test reactions were visually graded as mild to moderate, with three severe responses. Vehicle and occlusion controls were macroscopically negative.

**HLA-DR Expression** With normal skin and vehicle and occlusion controls, HLA-DR expression was restricted to small numbers of epidermal and dermal dendritic and mononuclear cells, endothelial cells, and the acrosyringeal regions of the epidermis. Basal keratinocytes and those of the stratum spinosum and stratum granulosum were consistently negative. In irritant-treated samples, there were increased numbers of HLA-DR positive mononuclear cells in the dermis and, with the exception of nonanoic acid reactions, in the epidermis; the endothelial cells remained positive. Very occasional small foci of positive keratinocytes were observed in approximately half of the samples from each irritant group (Fig 1), with the exception of the nonanoic acid patch tests, which were negative throughout.

On examination of the serial sections immunolabeled with anti-HLA-DR and anti-Leu-4, it was found that HLA-DR expression by keratinocytes was unrelated to the degree of T lymphocyte exocytosis. This was particularly striking in many of the reactions to croton oil (Fig 2A,B), sodium lauryl sulphate, and dithranol, where



**Figure 4.** Serial sections of a patch test reaction to propylene glycol demonstrating LFA-1 positive cells infiltrating the epidermis (A: bar, 100  $\mu$ m) with concomitant ICAM-1 expression by keratinocytes (B: bar, 100  $\mu$ m).



**Figure 5.** Serial sections from a benzalkonium chloride reaction demonstrating the close spatial relationship between LFA-1 positive cells in the epidermis (A: bar, 100  $\mu$ m) and ICAM-1 positive keratinocytes (B: bar, 100  $\mu$ m).

large numbers of anti-Leu 4 positive cells had infiltrated the epidermis, with no concomitant keratinocyte positivity. However, in the limited areas where HLA-DR labeling of keratinocytes was seen, there were generally T lymphocytes present within the same region of epidermis.

No correlation was apparent between the induction of HLA-DR expression in keratinocytes and the severity of the inflammatory response.

**ICAM-1 Expression** In normal skin, epidermal keratinocytes were consistently negative, including those in the acrosyringeal regions. However, occasional positively stained mononuclear cells

were present in the stratum spinosum (Fig 3A). Endothelial cells were positive throughout (Fig 3B).

Examination of irritant-treated skin revealed focal areas of keratinocyte ICAM-1 expression in the majority of samples, with staining being invariably heaviest in the basal epidermis. From the serial sections immunolabeled with anti-ICAM-1 and anti-LFA-1, it could be seen that these positively stained areas correlated extremely well with the presence of LFA-1 positive leucocytes in the epidermis (Figs 4A,B and 5A,B). Hence, in those samples with a high degree of exocytosis of LFA-1 positive cells, there was a correspondingly extensive induction of ICAM-1 in the keratinocytes. Conversely, where there were very few LFA-1 positive cells in the epidermis, as was the case with almost all the nonanoic acid reactions, there was little or no ICAM-1 expression. Furthermore, because, with the exception of nonanoic acid reactions, the number of LFA-1 positive cells infiltrating the epidermis was generally related to the severity of inflammation, there was a good correlation between ICAM-1 expression by keratinocytes and the visual clinical score. Interestingly, the nonanoic acid patch tests, although clinically assessed as moderate, showed a remarkable paucity of inflammatory cells in the epidermis.

In common with normal skin, there were scattered positively stained mononuclear and dendritic cells in the stratum corneum of irritant-treated patch test sites. Their numbers, on a subjective basis, did not appear to be appreciably different from those in the control biopsies. Endothelial cells were positive for ICAM-1 throughout, and in some biopsies the intensity of labeling was greater than that of the corresponding control patch tests.

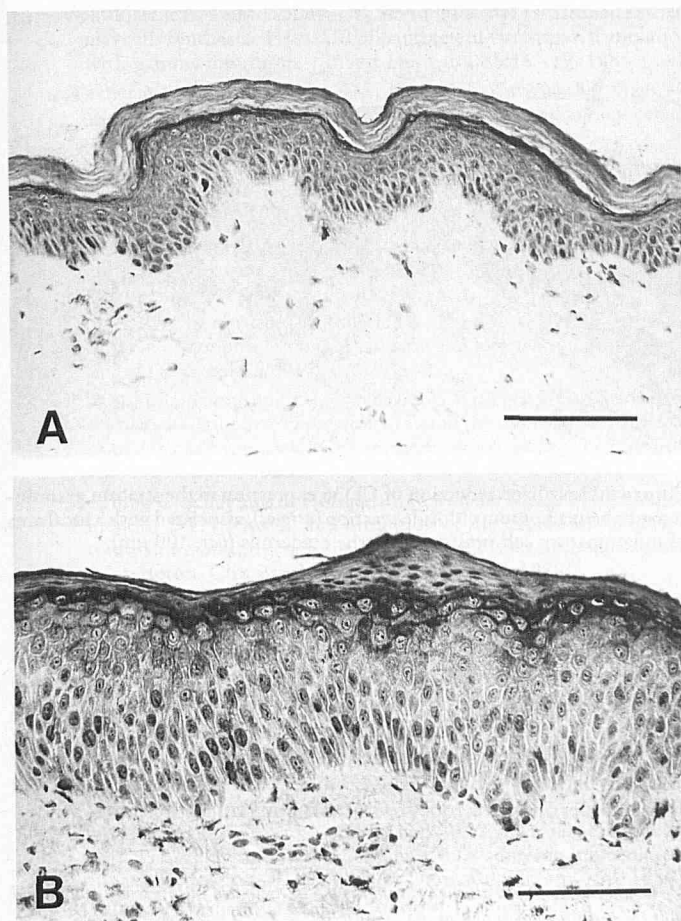
**OKM5 Expression** Samples of normal skin showed some variability in distribution and intensity of keratinocyte OKM5 expression between individuals. In general, however, there was pale and/or patchy staining along the stratum basale and stratum granulosum, and in the acrosyringeal regions, with no labeling in the stratum spinosum. Occasional positively stained mononuclear cells were present in the epidermis, with dermal endothelial cells negative throughout.

Comparison between each individual's normal skin and their irritant-treated sites revealed a degree of differential upregulation and downregulation of keratinocyte OKM5 expression between the different irritant groups, as summarized in Table I (Figs 6A,B and 7A,B). These changes in OKM5 expression were unrelated to the severity of the inflammatory response. Interestingly, in a number of samples where foci of exocytotic cells were present in the epidermis, there was an associated loss of CD36 expression by keratinocytes in the area of stratum spinosum immediately adjacent to the infiltrate (Fig 8). Varying numbers of OKM5-positive mononuclear cells were present in the epidermis, croton oil reactions showing the greatest number, nonanoic acid the least. Endothelial cells remained negative in all samples.

The combined results for the expression of ICAM-1, HLA-DR, and OKM5 by keratinocytes in each irritant and control group are given in Table I.

**Table I.** Changes in the Expression of Immune-Associated Membrane Antigens by Keratinocytes in ICD

Irritant (n = 10)	Keratinocyte Immunolabeling								
	Stratum Basale			Stratum Spinosum			Stratum Granulosum		
	HLA-DR	ICAM-1	OKM5	HLA-DR	ICAM-1	OKM5	HLA-DR	ICAM-1	OKM5
BC	±	+	↓	±	+	-	±	±	↑
SLS	±	++	↓	±	+	-	±	±	↑
CO	±	+++	↓↓	±	++	-	±	+	↓
NAA	-	±	↓↓	-	-	-	-	-	↓
PG	±	+	↓	±	+	-	±	+	↓
Dithranol	±	+++	↓↓	±	++	-	±	++	↓
Vehicle and occlusion controls (n = 5)	-	-	↔	-	-	-	-	-	↔
Normal skin	-	-	Variable	-	-	-	-	-	Variable



**Figure 6.** Immunolabeling of a normal skin biopsy with OKM5 (A: bar, 100  $\mu$ m). Variations between individuals in the expression of CD36 were evident, but in this sample keratinocytes in the stratum granulosum are slightly positive. Patch testing of the same individual with SLS led to an increase in the expression of CD36 in the stratum granulosum (B: bar, 100  $\mu$ m).

## DISCUSSION

We have shown that in ICD, there is selective upregulation of immune-associated antigens on the surface of epidermal keratinocytes, a finding that has important implications regarding the pathogenesis of this disorder.

Expression of the MHC class II antigen HLA-DR by keratinocytes was absent from many reactions and only limited in others, confirming the results of several previous studies conducted in humans and mice [36,37], but contradicting that of Gawkrödger et al [33]. The classic "chickenwire" appearance of HLA-DR positive keratinocytes described in a number of diseases [42,43] was never observed in our 48-h patch test reactions and, although the examination of serial sections revealed a reasonably close spatial association between T-cell exocytosis and the areas of keratinocyte HLA-DR expression, it was readily apparent that the upregulation of HLA-DR in keratinocytes was unrelated to the degree of T-cell infiltration in both the epidermis and dermis. A number of *in vivo* and *in vitro* studies have clearly demonstrated that the production and secretion of IFN  $\gamma$  by activated T lymphocytes induces HLA-DR expression on keratinocytes [25,44,45]. Our findings suggest, therefore, that this immunoregulatory lymphokine may not play a major role in the pathophysiology of ICD at the time period studied,

despite the fact that activated T lymphocytes expressing the interleukin-2 receptor are present in the epidermis and dermis of almost all the irritant reactions examined.\* The majority of T lymphocytes infiltrating the skin in these biopsies are of helper phenotype,\* and there is the possibility that, analogous to the Th 1 and Th 2 subsets of murine helper T cells [46], there are as-yet undefined subsets of human helper T lymphocytes that differentially release IFN  $\gamma$ . The limited upregulation of HLA-DR on keratinocytes in ICD contrasts with the results for ACD [11,33,42], and implies that different regulatory mechanisms may be operating at this time. If the hypothesis that keratinocyte HLA-DR expression perpetuates inflammatory responses [17,18] is correct, the paucity of HLA-DR on keratinocytes in our ICD samples may account, in part, for the rapid recovery of the skin in the majority of primary irritant reactions induced by patch testing [47].

In sharp contrast to our results for HLA-DR, we found that there was extensive expression of the membrane adhesion molecule, ICAM-1, by keratinocytes in irritant reactions, with an excellent spatial correlation existing between LFA-1 positive leukocytes in the epidermis and keratinocyte ICAM-1 positivity. These findings directly contradict a recent publication by Lange Vejlsgaard et al [39], in which it was reported that ICAM-1 expression by keratinocytes does not occur in ICD, but is a feature of ACD reactions [39,48]. We believe that upregulation of ICAM-1 is not, in fact, restricted to those diseases in which antigen presentation is pivotal, as is suggested by these authors, but rather that it has a more generalized and fundamental role, promoting and facilitating lymphocyte traffic into the epidermis in response to direct cellular damage as well as antigenic challenge. From our histopathologic findings [40], the direct association between ICAM-1 positive keratinocytes and LFA-1 positive leukocytes does not, in itself, appear to lead to keratinocyte damage in ICD, as is apparently the case with some other skin diseases [49,50], because there were many instances where exocytotic leukocytes were present in the epidermis adjacent to keratinocytes of normal morphology.

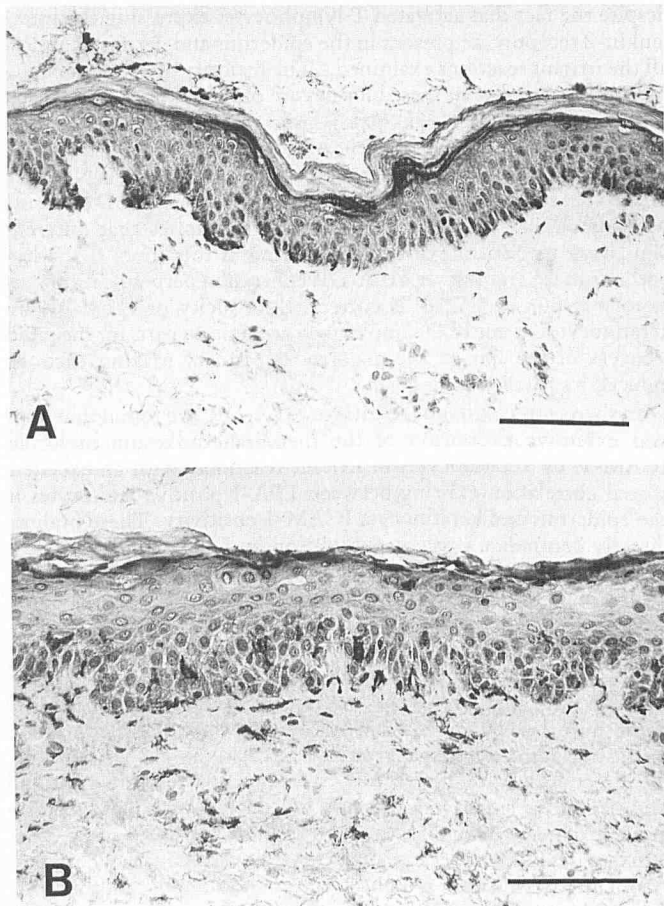
It is known that, of the cytokines that are synthesized and released during the evolution of inflammatory dermatoses, IFN  $\gamma$  and TNF  $\alpha$  are capable of inducing ICAM-1 expression in keratinocytes [25,26,51,52]. In addition, it is possible that endogenously produced IL-1 may similarly upregulate keratinocyte ICAM-1 expression [5], although this is still a matter of debate. Our results for HLA-DR, when taken into consideration with those for ICAM-1, imply that TNF  $\alpha$  and perhaps IL-1, rather than IFN  $\gamma$ , are among the more significant immune-associated inflammatory mediators in 48-h irritant patch test reactions. This view is strengthened by the fact that macrophages, presumably capable of producing TNF  $\alpha$ , are present in abundance in both the epidermis and dermis of ICD reactions.\*

The possibility that the irritant chemicals applied to the skin may themselves have directly induced keratinocyte ICAM-1 expression must also be considered, because it has recently been shown that chemicals such as the allergen urushiol and 12-O-tetradecanoylphorbol-13-acetate have the capacity to do this by altering the keratinocyte signal transduction events involving protein kinase c [27,28].

As well as the observed ICAM-1 expression by keratinocytes, there were also scattered ICAM-1 positive mononuclear and dendritic cells within the epidermis of both irritant-treated and normal skin, the densities of which remained relatively low throughout. These may well be Langerhans cells because they are known to constitutively express ICAM-1, albeit relatively weakly [53], but may also represent infiltrating macrophages [53]. Double labeling or ultrastructural immunocytochemistry are required to identify more precisely the nature of these cells. No attempt was made in this study to quantify this ICAM-1 positive cell population, but it would be interesting to determine whether their density paralleled that of the OKT6 (CD1a) positive dendritic cells immunolabeled in adjacent sections of these same biopsies [54].

Interpretation of the immunolabeling patterns with OKM5 proved to be difficult, particularly as we observed variable basal and

\* Willis CM, Stephens CJM, Wilkinson JD: Characterization of the inflammatory cell infiltrates in irritant reactions to six structurally unrelated chemicals (manuscript in preparation).

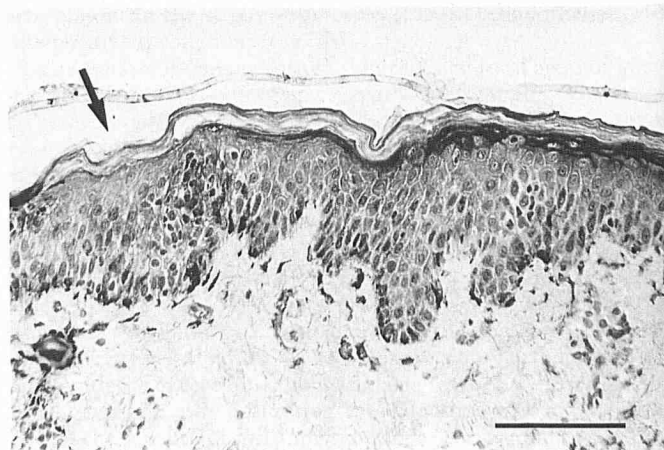


**Figure 7.** Expression of CD36 in a sample of normal skin (A: bar, 100  $\mu\text{m}$ ). Keratinocytes in the stratum granulosum and stratum basale show slight positivity. Patch testing of this individual with dithranol resulted in a loss of keratinocyte CD36 expression (B: bar, 100  $\mu\text{m}$ ).

upper epidermal staining in normal skin biopsies. Our results did, however, confirm the observations by Soyer et al [55] that OKM5 is expressed by keratinocytes in clinically normal skin. The observations that basal staining was lost in the majority of irritant-treated sites and that there was a localized loss of staining in the stratum spinosum associated with foci of exocytotic inflammatory cells suggest that CD36 is not a significant adhesion molecule in the T-cell response to irritant chemicals, as may possibly be the case in the response to allergens [35]. If, as suggested by *in vitro* studies, IFN  $\gamma$  regulates CD36 expression by keratinocytes [32], our findings support our hypothesis that IFN  $\gamma$  does not play a major role in ICD at 48 h.

With regard to the immunolabeling of upper epidermal keratinocytes with OKM5, there appeared to be differential changes in the intensity and extent of staining, related to the chemical nature of the irritant applied. Interestingly, the results suggest that there may possibly be a connection between CD36 expression in the stratum granulosum and the proliferative state of the epidermis, as postulated by Juhlin [56]. In a quantitative study in which sections of these biopsies were immunolabeled with the monoclonal antibody Ki-67,† we found that two of the three irritants that caused slight increases in OKM5 staining, namely sodium lauryl sulphate and propylene glycol, also have increased numbers of proliferating cells in the basal layer. Conversely, dithranol, which caused a distinct decrease in expression of CD36 in the upper epidermis, has significantly reduced numbers of proliferating basal keratinocytes.

† Willis CM, Stephenson CJM, Wilkinson JD: Differential effects of structurally unrelated irritants on the density of proliferating keratinocytes (manuscript in preparation).



**Figure 8.** Localized reduction of CD36 expression in the stratum granulosum of a benzalkonium chloride reaction (arrows), associated with a focal area of inflammatory cell infiltration in the epidermis (bar, 100  $\mu\text{m}$ ).

The results of our investigation demonstrate that there is selective upregulation of surface membrane antigens by keratinocytes in 48-h irritant patch test reactions. At time periods other than this, it is likely that different patterns of expression will be present, reflecting the changing nature of the cytokines released during the inflammatory response. However, our results give further insight into the complex pathophysiology of ICD and confirm the important role of the epidermal keratinocyte in cutaneous inflammation.

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