Immunology 1994 **81** 395–401

Modulation of epidermal Langerhans' cell frequency by tumour necrosis factor-α

M. CUMBERBATCH, I. FIELDING & I. KIMBER *Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire*

SUMMARY

During the induction phase of skin sensitization, dendritic cells (DC), many of which bear high levels of antigen, accumulate in lymph nodes draining the site of exposure. These DC derive from epidermal Langerhans' cells (LC) which are induced to migrate from the skin, via the afferent lymphatics, to lymph nodes. We demonstrated previously that intradermal exposure of mice to homologous, but not human, recombinant tumour necrosis factor-α (TNF-α) also causes an accumulation of DC in draining nodes, the implication being that the local production of this cytokine by epidermal cells provides one stimulus for LC migration. In the present study we have examined the influence of dermal TNF-α on the frequency of LC within the epidermis. Intradermal injection of mice with 25 ng or greater murine recombinant TNF-α caused a significant reduction in LC numbers within 30 min of exposure. The same treatment did not influence the frequency of Thy-1$^+$ epidermal DC. The density of LC was unaffected by the same amount of human TNF-α of comparable specific activity or by murine granulocyte–macrophage colony-stimulating factor (GM-CSF). These data provide additional evidence that TNF-α provides an important signal for the migration of LC from the epidermis.

INTRODUCTION

The induction phase of skin sensitization is accompanied by the accumulation in draining lymph nodes of dendritic cells (DC), many of which bear high levels of antigen.[1–4] Direct and indirect evidence indicate that these DC derive from epidermal Ia$^+$ Langerhans' cells (LC).[2,5,6] While in transit from the skin, LC are subject to both phenotypic and functional maturation, such that by the time they reach the draining lymph nodes they have acquired the characteristics of immunostimulatory DC.[1,7–9] Studies in mice have revealed that such maturation is associated with, and facilitated by, increased membrane expression of several molecules required for effective antigen presentation, including major histocompatibility complex (MHC) class II (Ia) antigens,[10] intercellular adhesion molecule-1 (ICAM-1)[11] and the costimulatory molecule B7/BB1, a ligand for CD28.[12] By analogy with *in vitro* studies, it is likely that some such changes are effected by granulocyte–macrophage colony-stimulating factor (GM-CSF), possibly acting in concert with interleukin-1 (IL-1)[13,14] and it is now known that the production or increased production of these and other cytokines by epidermal cells is stimulated following topical exposure of mice to skin-sensitizing chemicals.[15,16]

Our recent studies have suggested that tumour necrosis factor-α (TNF-α), a keratinocyte-derived cytokine, the expression of which is likewise up-regulated by skin sensitization,[15] may also have an important influence on LC function.[17] Intradermal injection of mice with homologous recombinant TNF-α was shown to cause a time- and dose-dependent accumulation of DC in draining lymph nodes and it was proposed that this epidermal cytokine provides a stimulus for LC migration from the skin.[17]

In the present investigation we have examined the influence of dermal TNF-α on the frequency of LC in the epidermis. There is resident within murine epidermis a second population of bone marrow-derived dendritic cells which lack Ia and which express the membrane glycoprotein Thy-1 and the γδ T-cell receptor.[18–20] Although the function of these Thy-1$^+$ epidermal DC remains unclear, it has been suggested that they exert a regulatory influence on cutaneous immune responses.[21–23] In comparative studies we have also examined the influence of TNF-α on the frequency of Thy-1$^+$ DC within the epidermis.

Received 2 September 1993; revised 6 October 1993; accepted 1 November 1993.

Abbreviations: BSA, bovine serum albumin; DC, dendritic cells; EDTA, ethylenediamine tetra-acetic acid; FITC, fluorescein isothiocyanate; GM-CSF, granulocyte–macrophage colony-stimulating factor; ICAM-1, intercellular adhesion molecule-1; IL-1, interleukin-1; LC, Langerhans' cells; MHC, major histocompatibility complex; PBS, phosphate-buffered saline; TNF-α, tumour necrosis factor-α.

Correspondence: M. Cumberbatch, Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire SK10 4TJ, U.K.

MATERIALS AND METHODS

Animals

Young adult (6- to 8-week-old) BALB/c strain mice were obtained from Harlan Olac Ltd (Bicester, U.K.) and used throughout these studies.

Cytokines

Recombinant murine TNF-α (specific activity 4×10^7 U/mg) was obtained from NBS (Hatfield, U.K.). Recombinant human TNF-α, of comparable specific activity,[17] was a generous gift from Dr S. J. Foster (Zeneca Pharmaceuticals, Alderley Park, Macclesfield, U.K.). In one series of experiments recombinant mouse GM-CSF (NBS; specific activity 10^6 U/mg) was used. All preparations were supplied as sterile solutions in phosphate-buffered saline (PBS) containing 0·1% bovine serum albumin (BSA) as carrier protein.

Exposure

Mice received 30 μl intradermal injections of TNF-α (or in some experiments GM-CSF) or an equivalent volume of carrier protein (BSA) alone into both ear pinnae. Preparations were diluted with sterile PBS containing 0·1% BSA and administered using 1-ml syringes with 30-gauge stainless steel needles. Additional control mice were untreated.

Preparation and analysis of epidermal sheets

To prepare intact epidermal sheets, ears were split with the aid of forceps into dorsal and ventral halves. The dorsal halves were incubated for 2 hr at 37° with 0·02 M ethylenediamine tetra-acetic acid (EDTA; Sigma Chemical Co., St Louis, MO) dissolved in PBS. The epidermis was separated from the dermis using forceps and washed in PBS.

To monitor the expression by epidermal cells of Ia (LC) and Thy-1 (Thy-1$^+$ DC) determinants, epidermal sheets were fixed in acetone for 20 min at −20°. Following fixation, epidermal sheets were washed for a total of 30 min in PBS and then incubated at room temperature for 30 min with either anti-mouse I-A^d (mouse IgG2a; Becton Dickinson, Mountain View, CA) or anti-mouse Thy-1.2 (rat IgG2b; Becton Dickinson) both diluted 1 : 100 in PBS. Sheets were then washed in three changes of PBS prior to incubation for a further 30 min with the appropriate fluorescein isothiocyanate (FITC)-conjugated second antibody, either an F(ab′)$_2$ sheep anti-mouse IgG (Sigma) or an F(ab′)$_2$ rabbit anti-rat IgG (Serotec, Kidlington, U.K.), both diluted 1 : 100 in PBS. Finally, sheets were washed in three changes of PBS and mounted on microscope slides in Citifluor (Citifluor Ltd, London, U.K.) and sealed with nail varnish. Concurrent experiments were performed using appropriate isotype-matched control antibodies in place of the primary antibody, either mouse anti-H-2^k (mouse IgG2a; Becton Dickinson) or rat anti-HLA class I (rat IgG2b; Serotec).

In one series of experiments epidermal sheets were examined, using the same methods, for the expression of intercellular adhesion molecule-1 (ICAM-1) using an anti-mouse ICAM-1 antibody (rat IgG2b, clone YN-11.7.4 from the European Collection of Animal Cell Cultures, Salisbury, U.K.). Control experiments were performed using rat IgG2b anti-HLA class I in place of the primary antibody, as already described.

Samples were examined by fluorescence microscopy and the frequency of stained cells measured using an eyepiece with a calibrated grid (0·265 × 0·175 at ×40 magnification). Four epidermal sheets were prepared from each experimental group and for each sample 10 random fields were examined. In no instance was any fluorescence detected following treatment of epidermal sheets with isotype-matched control antibodies.

The statistical significance of differences between experimental groups was calculated using the Student's *t*-test.

RESULTS

Influence of TNF-α on the frequency of epidermal LC and Thy-1$^+$ DC

During the course of these investigations it was established that the frequency of LC and Thy-1$^+$ DC within epidermal sheets prepared from the dorsum of the ears of untreated BALB/c mice varied between approximately 800 and 1100 LC/mm^2 and between 50 and 150 Thy-1$^+$ DC/mm^2. These values are in broad agreement with results reported previously by other investigators who have examined epidermal dendritic cells in BALB/c mice,[19,22] and confirm that in this strain the frequency of LC within the epidermis is considerably higher than that of Thy-1$^+$ DC. In preliminary experiments the influence of intradermal injection *per se* on the frequency of LC and Thy-1$^+$ DC was examined. Mice received a single intradermal injection of 30 μl of PBS or of PBS containing 0·1% BSA. Epidermal sheets were prepared 2 hr later. In repeated independent experiments neither treatment was found to induce any change in the frequency of LC or Thy-1$^+$ DC. Thus, in a single representative experiment the frequencies of Ia$^+$ LC/mm^2 (mean ± SE) in epidermal sheets prepared from untreated mice, or mice which had received PBS alone or PBS containing 0·1% BSA were, respectively, 830·7 ± 62·7, 867·5 ± 51·1 and 838·3 ± 44·5. In the same experiment the frequencies of Thy-1$^+$ DC in the same groups were 89·1 ± 8·0, 92·1 ± 22·8 and 102·2 ± 21·7, respectively.

Epidermal sheets were then examined in the same way following intradermal administration of 50 ng of murine recombinant TNF-α; an amount of this cytokine shown previously to cause a marked accumulation of DC in draining lymph nodes.[17] As demonstrated by the representative experiment illustrated in Fig. 1, mouse TNF-α resulted in a significant

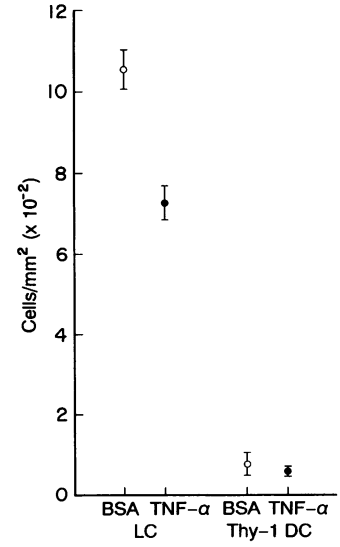

Figure 1. Influence of murine TNF-α on the frequency of Ia$^+$ LC and Thy-1$^+$ DC in the epidermis. Groups of mice ($n = 6$) received 30 μl intradermal injections into both ear pinnae of either 50 ng murine TNF-α in 0·1% BSA (●) or 0·1% BSA alone (○). Ears were removed 2 hr later and epidermal sheets prepared. The frequencies of Ia$^+$ LC and Thy-1$^+$ DC were measured by indirect immunofluorescence. Results are expressed as the mean number of cells/mm^2 (± SE) derived from examination of 10 fields per sample for each of four samples.

($P < 0{\cdot}01$) reduction in the frequency of Ia$^+$ LC. Thus, compared with mice which had received 0·1% BSA alone, there was an approximately 30% reduction in LC numbers. No change in the frequency of Thy-1$^+$ DC was observed (Fig. 1).

Kinetics of TNF-α-induced changes in epidermal LC frequency

Intradermal injection of murine TNF-α was found to cause a rapid reduction in the frequency of Ia$^+$ LC. A representative experiment is shown in Fig. 2. A significant ($P < 0{\cdot}01$) reduction in LC numbers (to approximately 75% of control values in the experiment illustrated in Fig. 2) was observed when epidermal sheets were prepared 30 min following administration of TNF-α. A reduction in LC frequency was found also 1 and 2 hr after treatment (Fig. 2). In no instance was the number of epidermal Thy-1$^+$ DC affected by exposure to TNF-α and treatment with 0·1% BSA alone failed to affect the frequency of either Ia$^+$ LC or Thy-1$^+$ DC (data not presented).

In one series of experiments the frequency of epidermal dendritic cells was examined following intradermal administration of GM-CSF, a cytokine known to promote the functional maturation of LC *in vitro*.[13,14] Mice were injected intradermally with 10 ng GM-CSF/ear, a concentration comparable with that used in *in vitro* studies[13,14] and which in previous investigations was found not to cause an accumulation of DC in draining lymph nodes.[17] Epidermal sheets were prepared at various periods following exposure but in no instance did GM-CSF influence the density of either Ia$^+$ LC or Thy-1$^+$ DC. Some morphological changes were observed, however. Within 1 hr of treatment with GM-CSF epidermal LC displayed a more dendritic appearance compared with BSA-exposed controls (data not presented).

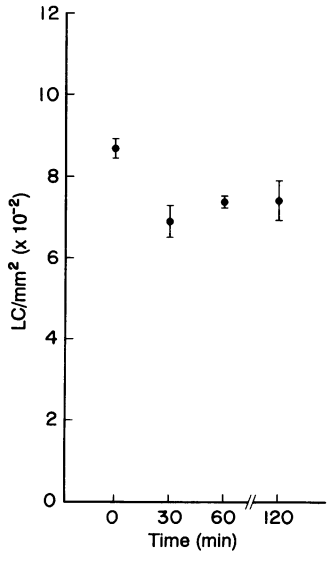

Figure 2. Kinetics of induced changes in epidermal Ia$^+$ LC frequency. Groups of mice ($n = 3$) received 30 μl intradermal injections of 50 ng murine TNF-α. Control mice received 0·1% BSA. Ears were removed immediately after treatment and at various times thereafter and epidermal sheets prepared. The frequency of Ia$^+$ LC was measured by indirect immunofluorescence. Results are expressed as the mean number of cells/mms^2 (±SE) derived from examination of 10 fields per sample for each of four samples.

The rapid loss of LC from the epidermis within 30 min of intradermal exposure to murine TNF-α was confirmed in a separate series of experiments recorded in Table 1. In six independent experiments the frequency of Ia$^+$ LC in epidermal sheets prepared 30 min following treatment with the cytokine was found to vary between 73·4% and 85·2% of that observed in sheets obtained from control mice exposed to BSA alone.

Reduction of Ia$^+$ LC frequency in the epidermis. Influence of TNF-α concentration

We have reported previously that intradermal exposure of mice to TNF-α results in a dose-dependent increase in the number of DC found within draining lymph nodes.[17] In those studies maximal or near maximal responses were observed with 50 ng TNF-α/ear, a concentration of the cytokine shown in the experiments reported here to cause a loss of Ia$^+$ LC from the epidermis. However, lower concentrations of intradermal TNF-α can also induce DC accumulation in draining nodes,[17] and we therefore examined the dose dependency of alterations in epidermal LC density resulting from exposure to this cytokine. As the representative experiment illustrated in Fig. 3 demonstrates, treatment of mice with 25 ng or greater TNF-α caused a significant ($P < 0{\cdot}01$) reduction in the density of LC. Exposure to 12·5 ng TNF-α had a small, but statistically insignificant effect on LC frequency (Fig. 3).

Comparison of murine and human TNF-α

We have shown previously that unlike murine TNF-α, the recombinant human cytokine fails to induce in mice an accumulation of DC in draining lymph nodes.[17] Similar comparisons were therefore performed in the present investigations. Groups of mice were treated with 50 ng of murine TNF-α or with an identical amount of human TNF-α of comparable specific activity (as judged by cytotoxicity *in vitro* for L929 cells[17]). Control mice received 0·1% BSA alone. Murine TNF-α caused a significant ($P < 0{\cdot}01$) reduction in the frequency of Ia$^+$

Table 1. Frequency of epidermal Ia$^+$ LC 30 min after intradermal exposure to murine TNF-α

Experiment	Cells/mm^2 (mean ± SE)		% reduction in LC frequency induced by TNF-α
	BSA	TNF-α	
1	832·6 ± 26·1	669·0 ± 20·3**	19·7
2	777·2 ± 35·6	604·4 ± 21·4**	22·2
3	828·8 ± 34·9	706·5 ± 16·3*	14·8
4	941·3 ± 39·9	691·3 ± 42·7**	26·6
5	989·0 ± 56·7	792·8 ± 43·7*	19·8
6	817·4 ± 23·1	600·0 ± 26·4**	26·6

Groups of mice ($n = 3$) received 30 μl intradermal injections into both ear pinnae of either 50 ng murine TNF-α or 0·1% BSA alone. Ears were removed 30 min later and epidermal sheets prepared. The frequency of Ia$^+$ LC was measured by indirect immunofluorescence. Results derive from examination of 10 fields per sample for each of four samples. * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

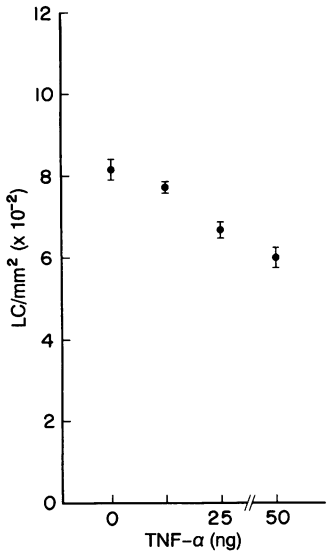

Figure 3. Influence of concentration on murine TNF-α-induced reduction in the density of epidermal Ia$^+$ LC. Groups of mice ($n = 3$) received 30 μl intradermal injections of various concentrations of murine TNF-α into both ear pinnae. Control mice received 0·1% BSA alone. Ears were removed after 30 min and epidermal sheets prepared. The frequency of Ia$^+$ LC was measured by indirect immunofluorescence. Results are expressed as the mean number of cells/mm^2 (±SE) derived from examination of 10 fields per sample for each of four samples.

LC. Human TNF-α did not influence LC number (Fig. 4). Under these conditions of exposure human TNF-α was not without effect in the mouse epidermis, however. The induction of ICAM-1 expression by keratinocytes was examined 24 hr after intradermal exposure of mice to 50 ng of either murine or human TNF-α, or following exposure to an equal volume of 0·1% BSA alone. Keratinocytes from untreated mice fail to express ICAM-1 and expression was not induced by treatment with BSA alone. In contrast, both mouse and human TNF-α induced detectable levels of ICAM-1, the response to the former being more vigorous (Fig. 5).

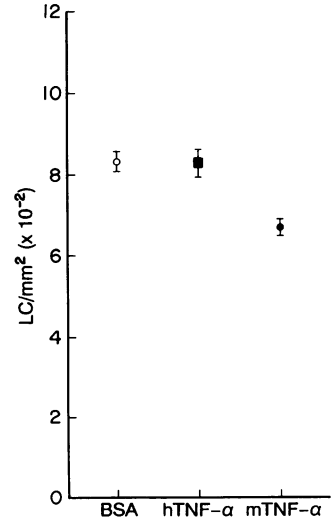

Figure 4. Induced changes in epidermal Ia$^+$ LC frequency. A comparison of murine and human TNF-α. Groups of mice ($n = 3$) received 30 μl intradermal injections of 50 ng of either murine (●) or human (■) TNF-α. Control mice received 0·1% BSA alone (○). Ears were removed after 30 min and epidermal sheets prepared. The frequency of Ia$^+$ LC was measured by indirect immunofluorescence. Results are expressed as the mean number of cells/mm^2 derived from examination of 10 fields per sample for each of four samples.

DISCUSSION

In previous studies we have found that intradermal exposure of mice to homologous TNF-α causes a time- and dose-dependent increase in the frequency of DC in draining lymph nodes.[17] The available evidence indicates that the DC which accumulate in lymph nodes local to the site of TNF-α administration are unlikely to derive from peripheral blood. It has been found that DC introduced into the blood of rats and mice do not migrate to the peripheral nodes.[24,25] Moreover, it is clear from studies in mice that the DC which arrive in draining lymph nodes following skin sensitization derive from epidermal LC which traffic from the skin via the afferent lymphatics.[2,5,6] We proposed, therefore, that the local production, or increased production, of TNF-α by epidermal cells in response to contact sensitization, or other forms of cutaneous trauma, might provide an important signal for LC migration from the skin.[17] The data presented here are consistent with that hypothesis insofar as TNF-α has been shown to cause a rapid and dose-dependent loss of LC from the epidermis. The tempo of changes in the density of epidermal LC is compatible with the accumulation of DC in draining lymph nodes following cutaneous exposure to TNF-α. A significant reduction in the frequency of LC resident within the epidermis was apparent within 30 min of treatment and increased numbers of lymph node DC were, in previous studies, first detectable 2 hr following exposure and maximal by 4 hr.[17] That the two phenomena are related is also suggested by the equivalence of dose–response relationships. Although the accumulation of DC in draining lymph nodes was maximal or near maximal with 50 ng TNF-α, less marked changes were observed following treatment with 25 and 12·5 ng of the cytokine.[17] Similarly, a rapid loss of Ia$^+$ LC from the epidermis was induced with 50 ng of TNF-α and, to a lesser extent, with 25 ng. It is relevant also that GM-CSF, a cytokine known to influence LC function *in vitro* failed to induce changes in the frequency of DC in draining nodes,[17] and, at the same concentration, did not influence the density of Ia$^+$ LC in the epidermis.

An interesting feature of these investigations is the species specificity of changes induced by TNF-α. An equal amount of human TNF-α of comparable activity was without influence on LC density. Similarly, we have shown previously that intradermal administration of human TNF-α failed to induce in mice an accumulation of DC in draining lymph nodes.[17] A precedent for species selectivity in the interaction of TNF-α with LC exists. Koch *et al.*[26] demonstrated that culture with homologous TNF-α maintained the viability of murine LC in the absence of functional maturation. In those experiments it was found that human TNF-α was inactive.[26] We propose that these observations reflect the selective expression by LC of membrane receptors for TNF-α. Two distinct receptors for this cytokine have now been recognized in both mouse and man; a 55,000

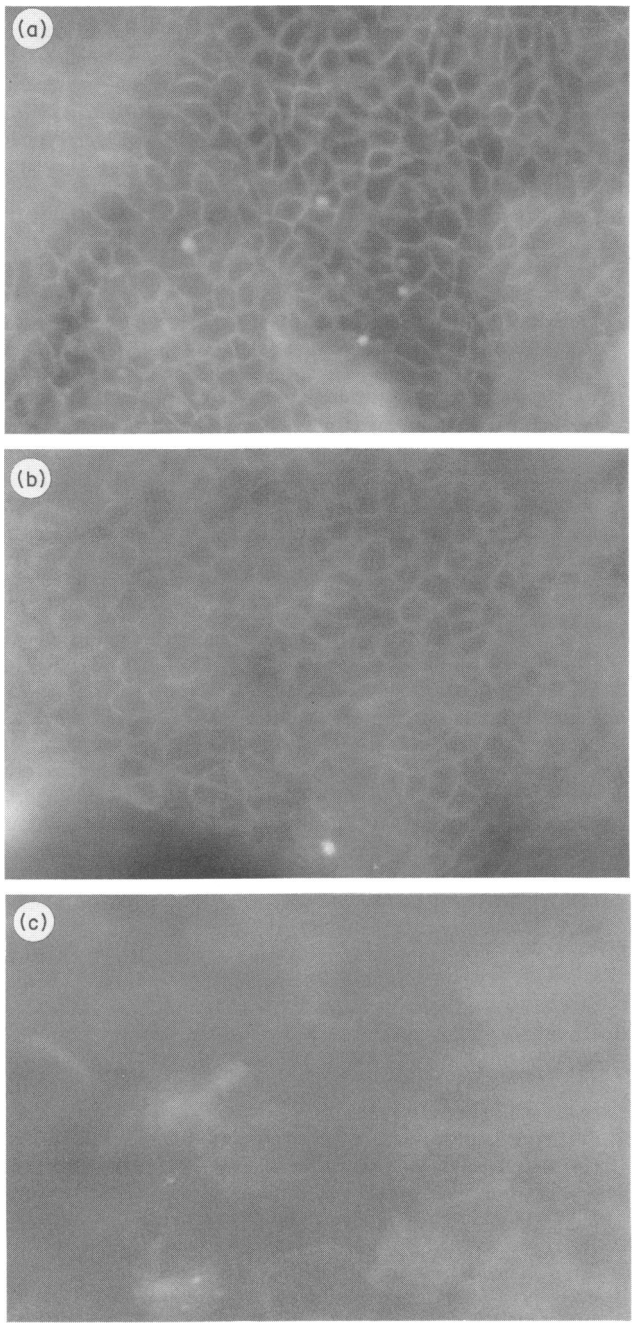

Figure 5. Induced expression of ICAM-1 by keratinocytes following intradermal exposure to murine or human TNF-α. Groups of mice ($n = 3$) received 30 μl intradermal injections of 50 ng of either murine (a) or human (b) TNF-α, or an equal volume of 0·1% BSA alone (c). Ears were removed after 24 hr and epidermal sheets prepared. The expression of ICAM-1 by keratinocytes was assessed by indirect immunofluorescence. Magnification × 56·5.

MW form designated TNF-R1 and a 75,000 MW form, TNF-R2.[27,28] Mouse and human TNF-R1 exhibit greatest homology in the extracellular domain and, as a consequence, mouse TNF-R1 has similar affinity for the mouse and human cytokine.[27] In contrast, TNF-R2 is most conserved in the intracellular domain. There is weaker homology in the extracellular region and as a result this receptor exhibits strong selectivity for the homologous cytokine.[27]

It is suggested, therefore, that LC express only TNF-R2 receptors. This is supported by the results of a recent analysis of TNF receptor expression in human lymphoid tissue.[29] Using monoclonal antibodies specific for the two forms of TNF-α receptor it was found that human LC express only the 75,000 MW TNF-R2. That the divergent effects of murine and human TNF-α in LC migration and DC accumulation do indeed reflect LC receptor expression is supported by the fact that in the experiments described here human TNF-α was available in the epidermis in biologically relevant amounts. Thus, intradermal injection of human TNF-α (and the mouse cytokine) provoked the expression of ICAM-1 by keratinocytes. Keratinocytes are known to express TNF-R1 (but not TNF-R2) and it has been proposed that possession of this receptor plays an important role in the regulation of epidermal ICAM-1 expression during inflammatory responses.[30]

In normal epidermis only LC express Ia antigen.[31] It was for this reason that in the present investigations we chose membrane Ia as the marker for LC. It might be argued, however, that, in theory, the apparent loss of LC from the epidermis is attributable instead to a reduced expression of Ia antigen. The rapidity of TNF-α-induced changes in the density of Ia^+ LC, combined with the observation that only a proportion (never greater than 30%) of LC were affected, argue against a down-regulation of Ia. Moreover, it has been found *in vitro* that culture of purified LC with TNF-α does not down-regulate Ia or inhibit induced increases in the expression of this molecule.[26] For these reasons the data reported here reflect a loss of LC from the epidermis rather than a complete down-regulation of Ia expression on a proportion of cells.

Although we propose that TNF-α provides a signal for the initiation of LC migration, it is clear that the efficient movement of cells from the epidermis to lymph nodes will require a number of other biological interactions. It has been demonstrated recently that LC adhere to keratinocytes in the epidermis through expression of E-cadherin.[32] The fact that the expression of this molecule by LC is down-regulated during functional maturation *in vitro* suggests that loss of E-cadherin may be an important event in the release of LC from keratinocytes prior to migration away from the skin.[32] Furthermore, it has been proposed that the expression by LC of integrins may also play an important role in the interaction of LC with the tissue matrix and movement through the dermis to regional lymph nodes.[33,34] It will be of interest to examine the influence of TNF-α on the expression by LC of E-cadherin and integrins. Such studies are in progress.

The data presented here demonstrate that intradermal TNF-α, at concentrations which reduce LC density, does not influence the number of $Thy\text{-}1^+$ DC found within the epidermis. This is consistent with the results of a previous investigation in which cell-bound antigen in the draining lymph nodes of contact-sensitized mice was shown to be associated only with Ia^+ DC. No antigen-bearing $Thy\text{-}1^+$ DC were observed.[4]

It is of interest to consider the present studies in the context of recent investigations performed by Streilein and colleagues[35,36] who also examined the influence of dermal TNF-α on LC function. On the basis of these studies it was suggested that TNF-α immobilizes LC in the epidermis and thereby prevents

effective migration to the draining nodes.[35,36] It is relevant, however, that a reduction in the density of epidermal Ia$^+$ LC induced by TNF-α was also observed.[35,36]

In conclusion, we propose that in response to skin sensitization keratinocytes are induced to synthesize TNF-α, or increased amounts of TNF-α, and that the local availability of this cytokine serves to provide one signal for LC migration from the skin to the draining lymph nodes. In support of this is the demonstration that topical exposure of mice to skin-sensitizing chemicals up-regulates epidermal cell mRNA for this cytokine.[15] In view of the rapidity of induced changes in the epidermis and the fact that TNF-α has been shown to influence LC behaviour *in vitro*,[26] it is likely that the action of this cytokine is direct. What remains unclear is the nature of the stimulus provided by TNF-α. One possibility, however, is that altered expression of adhesion molecules may play an important role in facilitating LC movement away from the skin.[32-34] It has been suggested recently that protein kinase C transduces the signal for LC migration,[37] and it is possible that this second messenger is necessary for TNF-α-induced changes in LC function.

Finally, cutaneous trauma other than that resulting from skin sensitization will also result in the production or increased production of TNF-α by keratinocytes. There is evidence that both ultraviolet B (UVB) irradiation and topical exposure to non-sensitizing skin irritants will induce keratinocytes to produce this cytokine.[15,38] It is of interest, therefore, that both such treatments also cause the accumulation of DC in draining lymph nodes.[39,40] The synthesis and secretion of TNF-α and the consequent stimulation of LC migration may represent an important early step in cutaneous responses to injury and infection.

REFERENCES

1. Macatonia S.E., Edwards A.J. & Knight S.C. (1986) Dendritic cells and the initiation of contact sensitivity to fluorescein isothiocyanate. *Immunology*, **59,** 509.
2. Macatonia S.E., Knight S.C., Edwards A.J., Griffiths S. & Fryer P. (1987) 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.
3. Kinnaird A., Peters S.W., Foster J.R. & Kimber I. (1989) Dendritic cell accumulation in draining lymph nodes during the induction phase of contact allergy. *Int. Archs Allergy appl. Immunol.* **89,** 202.
4. Cumberbatch M. & Kimber I. (1990) Phenotypic characteristics of antigen-bearing cells in the draining lymph nodes of contact sensitized mice. *Immunology*, **71,** 404.
5. Munn C.G., Bucana C. & Kripke M.L. (1989) Antigen-binding cells from lymph nodes of mice sensitized epicutaneously with hapten have features of epidermal Langerhans cells. *J. invest. Dermatol.* **92,** 487.
6. Kripke M.L., Munn C.G., Jeevan A., Tang J.-M. & Bucana C. (1990) Evidence that cutaneous antigen-presenting cells migrate to regional lymph nodes during contact sensitization. *J. Immunol.* **145,** 2833.
7. Knight S.C., Krejci J., Malkovsky M., Colizzi V., Gautam A. & Asherson G.L. (1985) The role of dendritic cells in the initiation of immune responses to contact sensitizers. 1. *In vivo* exposure to antigen. *Cell. Immunol.* **94,** 427.
8. Jones D.A., Morris A.G. & Kimber I. (1989) Assessment of the functional activity of antigen-bearing dendritic cells isolated from the lymph nodes of contact-sensitized mice. *Int. Archs Allergy appl. Immunol.* **90,** 230.
9. Cumberbatch M., Illingworth I. & Kimber I. (1991) Antigen-bearing dendritic cells in the draining lymph nodes of contact sensitized mice: cluster formation with lymphocytes. *Immunology*, **74,** 139.
10. Cumberbatch M., Gould S.J., Peters S.W. & Kimber I. (1991) MHC class II expression by Langerhans cells and lymph node dendritic cells: possible evidence for the maturation of Langerhans cells following contact sensitization. *Immunology*, **74,** 414.
11. Cumberbatch M., Peters S.W., Gould S.J. & Kimber I. (1992) Intercellular adhesion molecule-1 (ICAM-1) expression by lymph node dendritic cells: comparison with epidermal Langerhans cells. *Immun. Lett.* **32,** 105.
12. Larsen C.P., Ritchie S.C., Pearson T.C., Linsley P.S. & Lowry R.P. (1992) Functional expression of the costimulatory molecule, B7/BB1, on murine dendritic cell populations. *J. exp. Med.* **176,** 1215.
13. Witmer-Pack M.D., Olivier W., Valinsky J., Schuler G. & Steinman R.M. (1987) Granulocyte/macrophage colony-stimulating factor is essential for the viability and function of cultured murine epidermal Langerhans cells. *J. exp. Med.* **166,** 1484.
14. Heufler C., Koch F. & Schuler G. (1988) Granulocyte/macrophage colony-stimulating factor and interleukin 1 mediate the maturation of murine epidermal Langerhans cells into potent immunostimulatory dendritic cells. *J. exp. Med.* **167,** 700.
15. Enk A.H. & Katz S.I. (1992) Early molecular events in the induction phase of contact sensitivity. *Proc. natl. Acad. Sci. U.S.A.* **89,** 1398.
16. Enk A.H. & Katz S.I. (1992) Identification and induction of keratinocyte-derived IL-10. *J. Immunol.* **149,** 92.
17. Cumberbatch M. & 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.
18. Bergstresser P.R., Tigelaar R.E., Dees J.H. & Streilein J.W. (1983) Thy-1 antigen bearing dendritic cells populate murine epidermis. *J. invest. Dermatol.* **81,** 286.
19. Tschachler E., Schuler G., Hutterer J., Leibl H., Wolff K. & Stingl G. (1983) Expression of Thy-1 antigen by murine epidermal cells. *J. invest. Dermatol.* **81,** 282.
20. Kuziel W.A., Takashima A., Bonyhadi M., Bergstresser P.R., Allison J.P., Tigelaar R.E. & Tucker P.W. (1987) Regulation of T-cell receptor γ-chain RNA expression in murine Thy-1$^+$ dendritic epidermal cells. *Nature*, **328,** 263.
21. Sullivan S., Bergstresser P.R., Tigelaar R.E. & Streilein J.W. (1986) Induction and regulation of contact hypersensitivity by resident, bone marrow-derived, dendritic epidermal cells: Langerhans cells and Thy-1$^+$ epidermal cells. *J. Immunol.* **137,** 2460.
22. Bigby M., Kwan T. & Sy M-S. (1987) Ratio of Langerhans cells to Thy-1$^+$ dendritic epidermal cells in murine epidermis influences the intensity of contact hypersensitivity. *J. invest. Dermatol.* **89,** 495.
23. Welsh E.A. & Kripke M.L. (1990) Murine Thy-1$^+$ dendritic epidermal cells induce immunological tolerance *in vivo*. *J. Immunol.* **144,** 883.
24. Fossum S. (1998) Lymph-borne dendritic leukocytes do not recirculate but enter the lymph node paracortex to become interdigitating cells. *Scand. J. Immunol.* **27,** 97.
25. Kupiec-Weglinski J.W., Austyn J.M. & Morris P.J. (1988) Migration patterns of dendritic cells in the mouse. Traffic from the blood and T cell-dependent and -independent entry to lymphoid tissues. *J. exp. Med.* **167,** 632.
26. Koch F., Heufler C., Kampgen E., Schneeweiss D., Bock G. & Schuler G. (1990) Tumor necrosis factor α maintains the viability of murine epidermal Langerhans cells in culture, but in contrast to granulocyte/macrophage colony-stimulating factor, without inducing their functional maturation. *J. exp. Med.* **171,** 159.
27. Lewis M., Tartaglia L.A., Lee A., Bennett G.L., Rice G.C., Wong G.H.W., Chen E.Y. & Goeddel D.V. (1991) Cloning and

expression of cDNAs for two distinct murine tumor necrosis factor receptors demonstrate one receptor is species specific. *Proc. natl. Acad. Sci. U.S.A.* **88,** 2830.

28. TARTAGLIA L.A. & GOEDDEL D.V. (1992) Two TNF receptors. *Immunol. Today*, **13,** 151.
29. RYFFEL B., BROCKHAUS M., GREINER B., MIHATSCH M.J. & GUDAT F. (1991) Tumour necrosis factor receptor distribution in human lymphoid tissue. *Immunology*, **74,** 446.
30. TREFZER U., BROCKHAUS M., LOETSCHER H., PARLOW F., KAPP A., SCHOPF E. & KRUTMANN J. (1991) 55-kD tumor necrosis factor receptor is expressed by human keratinocytes and plays a pivotal role in regulation of human keratinocyte ICAM-1 expression. *J. invest. Dermatol.* **97,** 911.
31. TAMAKI K., STINGL G., GULLINO M., SACHS D.H. & KATZ S.I. (1979) Ia antigens in mouse skin are predominantly expressed on Langerhans cells. *J. Immunol.* **123,** 784.
32. TANG A., AMAGAI M., GRANGER L.G., STANLEY J.R. & UDEY M.C. (1993) Adhesion of epidermal Langerhans cells to keratinocytes mediated by E-cadherin. *Nature*, **361,** 82.
33. LE VARLET B., STAQUET M.J., DEZUTTER-DAMBUYANT C., DELORME P. & SCHMITT D. (1992) *In vitro* adhesion of human epidermal Langerhans cells to laminin and fibronectin occurs through β1 integrin receptors. *J. Leukoc. Biol.* **51,** 415.
34. AIBA S., NAKAGAWA S., OZAWA H., MIYAKE K., YAGITA H. & TAGAMI H. (1993) Up-regulation of α4 integrin on activated Langerhans cells: analysis of adhesion molecules on Langerhans cells relating to their migration from skin to draining lymph nodes. *J. invest. Dermatol.* **100,** 143.
35. STREILEIN J.W., GRAMMER S.F., YOSHIKAWA T., DEMIDEM A. & VERMEER M. (1990) Functional dichotomy between Langerhans cells that present antigen to naive and to memory/effector T lymphocytes. *Immunol. Rev.* **117,** 158.
36. VERMEER M. & STREILEIN J.W. (1990) Ultraviolet B light-induced alterations in epidermal Langerhans cells mediated in part by tumor necrosis factor-alpha. *Photodermatol. Photoimmunol. Photomed.* **7,** 258.
37. HALLIDAY G.M. & LUCAS A.D. (1993) Protein kinase C transduces the signal for Langerhans' cell migration from the epidermis. *Immunology*, **79,** 621.
38. KOCK A., SCHWARZ T., KIRNBAUER R., URBANSKI A., PERRY P., ANSEL J.C. & LUGER T.A. (1990) Human keratinocytes are a source for tumor necrosis factor α: evidence for synthesis and release upon stimulation with endotoxin or ultraviolet light. *J. exp. Med.* **172,** 1609.
39. MOODYCLIFFE A.M., KIMBER I. & NORVAL M. (1992) The effect of ultraviolet B irradiation and urocanic acid isomers on dendritic cell migration. *Immunology*, **77,** 394.
40. CUMBERBATCH M., SCOTT R.C., BASKETTER D.A., SCHOLES E.W., HILTON J., DEARMAN R.J. & KIMBER I. (1993) Influence of sodium lauryl sulphate on 2,4-dinitrochlorobenzene-induced lymph node activation. *Toxicology*, **77,** 181.