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Tumour necrosis factor-α is required for accumulation of dendritic cells in draining lymph nodes and for optimal contact sensitization

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SUMMARY

Following skin sensitization epidermal Langerhans' cells (LC), many of which bear antigen, are stimulated to migrate from the skin and traffic via afferent lymphatics to lymph nodes draining the site of exposure. It has been proposed previously that tumour necrosis factor-α (TNF-α), a keratinocyte-derived epidermal cytokine (the expression of which is augmented following cutaneous sensitization), provides one signal for LC migration. In the experiments described here the influence of systemically administered neutralizing anti-TNF-α antibody on dendritic cell (DC) accumulation in draining lymph nodes has been investigated. Treatment with anti-TNF-α inhibited markedly the frequency of DC in draining nodes measured 18 hr following exposure to the skin allergens oxazolone and fluorescein isothiocyanate or to the non-sensitizing skin irritant sodium lauryl sulphate. Similar treatment with anti-TNF-α 2 hr prior to primary exposure to oxazolone impaired significantly the efficiency of skin sensitization measured 5 days later as a function of challenge-induced increases in ear thickness. The same antibody administered 18 hr following initial exposure to oxazolone was without effect on skin sensitization. These data confirm the importance of TNF-α for the migration of LC from the skin to draining lymph nodes and demonstrate that this cytokine is required for optimal contact sensitization.

INTRODUCTION

Epidermal Langerhans' cells (LC) are considered to play a central role in the induction of cutaneous immune responses.[1] Following skin sensitization a proportion of LC local to the site of exposure are stimulated to migrate from the skin via afferent lymphatics and to accumulate in draining lymph nodes.[2–6] The cells which arrive in draining nodes, many of which bear high levels of antigen, have the characteristics of dendritic cells (DC), are immunocompetent and provoke T-lymphocyte responses both *in vitro* and *in vivo*.[2,4,7,8] The acquisition of immunostimulatory activity reflects the functional and phenotypic maturation of LC while in transit from the epidermis.[9–11] Compared with LC resident in the skin, the DC which arrive in draining lymph nodes exhibit a significant increase in the membrane expression of both major histocompatibility complex (MHC) class II (Ia) antigen[10] and intercellular adhesion molecule-1 (ICAM-1; CD54).[11] By analogy with *in vitro* studies it is probable that the functional maturation of LC is associated also with other phenotypic changes.[12,13] The maturation of LC *in vitro* is effected by epidermal cytokines and, in particular, by granulocyte–macrophage colony-stimulating factor (GM-CSF).[14,15]

In previous studies we have questioned whether, in addition to mediating the maturation of LC, epidermal cytokines provide the signal for LC to migrate from the epidermis. We have reported that the intradermal administration of homologous recombinant tumour necrosis factor-α (TNF-α) causes both an accumulation of DC in draining lymph nodes and a reduction in the frequency of local epidermal LC.[16,17] Although under the same conditions GM-CSF was found to be inactive,[16,17] it was not possible to conclude that other epidermal cytokines or other products of keratinocytes were without effect. To address this issue we have in the present investigation examined the influence of a neutralizing anti-TNF-α antibody on the accumulation of DC in draining lymph nodes induced by the topical exposure of mice to the skin sensitizers oxazolone and fluorescein isothiocyanate (FITC). It has been reported previously that the stimulation of LC migration is not an exclusive property of chemical allergens. It was found that topical treatment of mice with the skin irritant sodium lauryl sulphate (SLS) also resulted in the accumulation of DC in draining lymph nodes.[18] In parallel studies reported here we have investigated the requirement for TNF-α in the accumulation of lymph node DC associated with SLS exposure.

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Abbreviations: ADBP, 1 : 1 acetone : dibutylphthalate; A00, 4 : 1 acetone : olive oil; DC, dendritic cells; DMF, dimethylformamide; FITC, fluorescein isothiocyanate; GM-CSF, granulocyte–macrophage colony-stimulating factor; ICAM-1, intercellular adhesion molecule-1; LC, Langerhans' cells; LNC, lymph node cells; MHC, major histocompatibility complex; NRS, normal rabbit serum; PBS, phosphate-buffered saline; SLS, sodium lauryl sulphate; TNF-α, tumour necrosis factor-α.

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Finally, it is assumed that the migration of LC during skin sensitization and effective transport of the inducing allergen to the lymph nodes plays a pivotal role in the effective induction of contact allergy. To test this assumption the influence of anti-TNF-α on the effectiveness of skin sensitization to oxazolone has been examined also.

MATERIALS AND METHODS

Animals

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

Chemicals and exposure

The skin sensitizing chemicals 4-ethoxymethylene-2-phenyloxazol-5-one (oxazolone; Sigma Chemical Co., St Louis, MO) and FITC (Aldrich Chemical Co, Gillingham, UK) were dissolved, respectively, in 4 : 1 acetone : olive oil (AOO) and 1 : 1 acetone : dibutylphthalate (ADBP). SLS (BDH Ltd, Poole, UK) was dissolved in dimethylformamide (DMF). Groups of mice received 25 μl of the test chemical (1% oxazolone, 1% FITC or 10% SLS), or an equivalent volume of the relevant vehicle alone, on the dorsum of both ears.

Anti-TNF-α antibody

Neutralizing polyclonal rabbit anti-mouse TNF-α was purchased from Genzyme Diagnostics (West Malling, UK) and supplied as a filter-sterilized hyperimmune antiserum with a specific activity of approximately 10^6 neutralizing units/ml. Mice received 100 μl of antibody diluted 1:5 in sterile phosphate-buffered saline (PBS) by intraperitoneal injection. Control mice received intraperitoneal injections of an equal volume of sterile normal rabbit serum (NRS) diluted with PBS to an equivalent extent.

Isolation and enumeration of lymph node dendritic cells

Draining auricular lymph nodes were excised 18 hr following exposure to the test chemical or to vehicle alone. Chemical exposure was preceded by intraperitoneal injection 2 hr previously of either anti-TNF-α or normal rabbit serum. Nodes were pooled for each experimental group and single-cell suspensions of lymph node cells (LNC) prepared by mechanical disaggregation through 200-mesh stainless steel gauze. LNC were washed with and resuspended in RPMI-1640 growth medium (Gibco, Paisley, UK) supplemented with 25 mM HEPES, 400 μg/ml streptomycin, 400 μg/ml ampicillin and 10% heat-inactivated fetal calf serum (RPMI–FCS). Viable cell counts were performed by exclusion of 0·5% trypan blue and the cell concentration adjusted to 5×10^6 cells/ml in RPMI–FCS. DC-enriched populations were prepared as described previously.[19] Briefly, 2 ml of Metrizamide (Nygaard, Oslo, Norway; 14·5% in RPMI–FCS) was layered gently under 8 ml of the cell suspension and tubes centrifuged for 15 min (600 *g*) at room temperature. Cells accumulating at the interface were collected, washed once and resuspended in RPMI–FCS. The frequency of DC in such low buoyant density fractions was assessed routinely by direct morphological examination using phase contrast microscopy. Results are expressed as DC/node.

Sensitization for, and elicitation of, contact sensitivity

Groups of mice received 100 μl of anti-TNF-α antibody or NRS either 2 hr prior to or 18 hr following sensitization. Mice were treated topically with 50 μl of 1% oxazolone in AOO on both shaved flanks. Control mice received an equivalent volume of AOO alone. Five days later the ear thickness of each mouse was measured using an engineers' micrometer (Moore and Wright, Sheffield, UK). The dorsum of both ears was then treated immediately with 25 μl of the challenge concentration (0·25%) of oxazolone. Elicitation reactions were measured 24 hr later as the mean percentage increase in ear thickness relative to prechallenge values. The significance of changes in contact hypersensitivity reactions was evaluated using the paired Student's *t*-test.

RESULTS

In preliminary experiments it was confirmed that intraperitoneal injection of mice with NRS failed to influence the accumulation of DC in draining nodes induced by topical sensitization (data not presented). Subsequently, experiments were performed in which groups of mice ($n = 10$) received

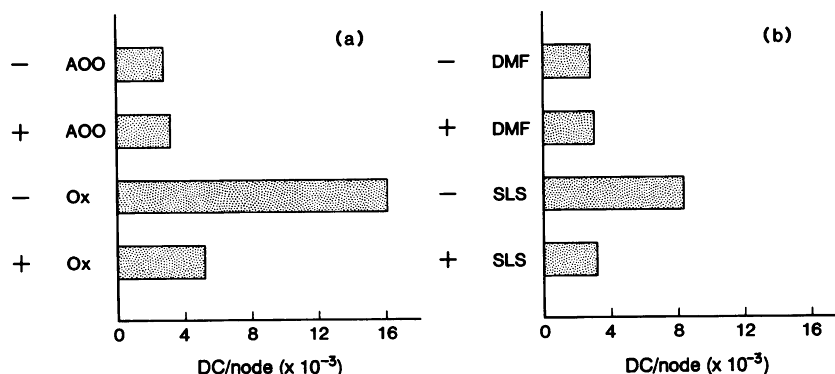

Figure 1. Influence of anti-TNF-α on the accumulation of DC in draining lymph nodes following topical exposure to oxazolone (a) or SLS (b). Groups of mice ($n = 10$) received a single 100 μl injection (i.p.) of either anti-TNF-α (+) or NRS (–). Two hours later mice were treated on the dorsum of both ears with 25 μl of (a) 1% oxazolone (Ox) in AOO or AOO alone or (b) 10% SLS in DMF or DMF alone. Draining auricular nodes were removed 18 hr later and the number of DC/node measured.

Table 1. Influence of anti-TNF-α on the accumulation of DC in draining lymph nodes following topical exposure to FITC

Exp.	Treatment	DC/node ($\times 10^{-3}$) ADBP	FITC
1	NRS	3·56	8·62
	anti-TNF-α	3·11	3·22
2	NRS	3·07	8·57
	anti-TNF-α	3·65	4·09

Groups of mice ($n = 10$) received a single 100 μl injection (i.p.) of either anti-TNF-α or NRS. Two hours later mice were treated on the dorsum of both ears with 25 μl of either 1% FITC in ADBP or ADBP alone. Draining auricular lymph nodes were excised 18 hr following sensitization and the number of DC/node measured.

either anti-TNF-α or NRS by intraperitoneal injection. Two hours later mice were treated on the dorsum of both ears with 25 μl of 1% oxazolone or with an equal volume of vehicle (AOO) alone. After a further 18 hr draining auricular nodes were excised and the frequency of DC measured. A representative experiment is illustrated in Fig. 1a in which it was found that in control mice, administered NRS alone, topical sensitization with oxazolone induced a greater than five-fold increase in the numbers of DC found within draining nodes 18 hr later (from a mean value of $2{\cdot}87 \times 10^3$ DC/node in vehicle-treated mice to $16{\cdot}12 \times 10^3$ DC/node in animals exposed to oxazolone). In animals treated with anti-TNF-α prior to sensitization, oxazolone caused a significantly less marked change in lymph node DC number (from $3{\cdot}16 \times 10^3$ in AOO-treated controls to $5{\cdot}16 \times 10^3$ DC/node in oxazolone-sensitized mice). We have reported previously that exposure of mice to the non-sensitizing skin irritant SLS also induces the accumulation of DC in draining nodes.[18] It was found, as illustrated in Fig. 1b, that prior administration of neutralizing anti-TNF-α also served to inhibit DC accumulation resulting from topical exposure to SLS.

Systemic treatment with anti-TNF-α also inhibited DC accumulation in draining lymph nodes induced by another chemical. The results of two experiments performed with FITC, a contact sensitizing chemical, are described in Table 1.

As the data described above demonstrated clearly that prior administration of anti-TNF-α caused a marked inhibition of allergen-induced DC accumulation in draining nodes, we examined whether such treatment also resulted in a reduced effectiveness of contact sensitization. In the experiments described below contact sensitization was measured as a function of challenge-induced increases in ear thickness. The results of three independent experiments are illustrated in Fig. 2. In each of these experiments sensitization and challenge with oxazolone in mice treated with NRS resulted in vigorous contact hypersensitivity responses, with challenge-induced increases in ear thickness of between 65 and 87%. Treatment with vehicle alone caused only very modest changes in ear thickness (Fig. 2). Systemic administration of anti-TNF-α 2 hr prior to sensitization with oxazolone caused a marked inhibition of contact hypersensitivity reactions in response to challenge 5 days later. The inhibition of contact hypersensitivity resulting from pretreatment with anti-TNF-α ranged from 31·4% (experiment 3) to 48·1% (experiment 1). In each case the depression of cutaneous hypersensitivity reactions was statistically significant ($P < 0{\cdot}005$). In contrast, treatment of mice with anti-TNF-α 18 hr following, rather than 2 hr prior to, sensitization with oxazolone failed to influence the vigour of challenge-induced reactions. In no instance did anti-TNF-α significantly influence the ear thickness of mice exposed to vehicle alone.

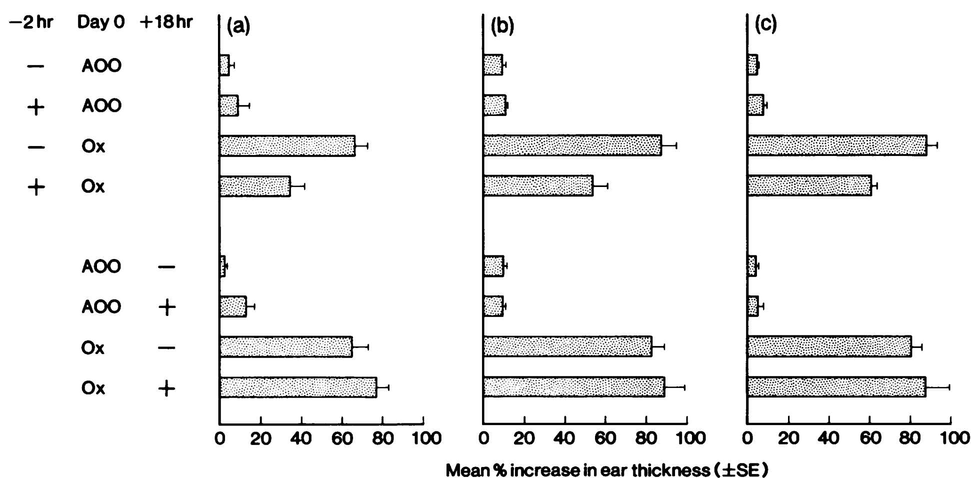

Figure 2. Influence of anti-TNF-α on contact sensitization to oxazolone. Groups of mice ($n = 5$) received a single 100 μl injection (i.p.) of anti-TNF-α (+) or NRS (−) either 2 hr prior to, or 18 hr following, epicutaneous application to both shaved flanks of 50 μl of 1% oxazolone (Ox) or of vehicle (AOO) alone. Five days following topical exposure the ear thickness of each mouse was measured immediately prior to challenge of the dorsum of both ears with 0·25% oxazolone. Elicitation reactions were measured 24 hr later as the mean per cent increase in ear thickness (± SE) relative to prechallenge values. A summary of three independent experiments: (a) Exp. 1; (b) Exp. 2; (c) Exp. 3.

DISCUSSION

We have reported previously that TNF-α provides one signal for the migration of epidermal LC from the skin and their accumulation as DC in draining lymph nodes. Thus, intradermal injection of homologous recombinant TNF-α was found to induce in mice a reduced frequency of LC in epidermis local to the site of injection and an increase in the number of DC within draining lymph nodes.[16,17] The assumption was that TNF-α acts directly upon a proportion of LC resident in the epidermis rather than inducing migration via a secondary signal such as the local induction of other epidermal cytokines. This assumption was based partly upon the rapidity of changes observed following injection of TNF-α, but also upon the demonstration of species selectivity. It was found that, under identical conditions of exposure, recombinant human TNF-α of equivalent specific activity caused in mice neither a reduced frequency of epidermal LC, nor an accumulation of DC in draining nodes.[16,17] Two types of membrane receptor for TNF-α exist in both mouse and man; 55 000 MW form designated TNF-R1 and a 75 000 MW form designated TNF-R2.[20,21] Most conserved in the extracellular domain is TNF-R1 and this receptor exhibits similar affinity for the human and mouse cytokine. In contrast TNF-R2 is most conserved in the intracellular domain and has less homology in the extracellular sequence. Consequently TNF-R2 exhibits species specificity.[20,21] While keratinocytes express TNF-R1[22] and in mice respond to both human and murine TNF-α with respect to induced expression of intercellular adhesion molecule-1 (ICAM-1),[17] there is evidence that LC express only TNF-R2.[23] This is consistent with a direct and species selective effect of exogenous TNF-α on epidermal LC.

Evidence is now available that cutaneous trauma resulting from skin sensitization, skin irritation or ultraviolet B (UVB) irradiation causes the increased synthesis of TNF-α by keratinocytes.[24,25] The experiments described here indicate that, compatible with an important role for this cytokine in providing a stimulus for LC migration following exposure to skin sensitizing or skin irritant chemicals, systemic treatment of mice with neutralizing anti-TNF-α antibody results in a very marked inhibition of DC accumulation in draining nodes induced by contact allergens or SLS. In previous studies the same antibody has been found also to inhibit significantly the accumulation of DC in draining lymph nodes resulting from local UVB irradiation.[26] The conclusion drawn is that, in some circumstances at least, the migration of LC from the skin to draining nodes is mediated largely or wholly by the action of TNF-α. This being the case experiments were designed to examine whether inhibition of allergen-induced LC migration by administration of anti-TNF-α would impair the effectiveness of skin sensitization. Exposure of mice to the neutralizing antibody 2 hr prior to sensitization with oxazolone caused a significant inhibition of contact hypersensitivity measured 5 days later as a function of challenge-induced increases in ear thickness. It has been reported previously that TNF-α is an essential mediator of contact hypersensitivity reactions in previously sensitized mice.[27] In view of these data we investigated whether, when given 18 hr following, rather than 2 hr prior to, skin sensitization, anti-TNF-α would serve to inhibit the elicitation of contact hypersensitivity reactions. Under these circumstances anti-TNF-α was without effect. It is suggested therefore that when given prior to sensitization anti-TNF-α inhibits the migration of LC from the epidermis and as a consequence compromises the effectiveness of skin sensitization. The interpretation is that when given following chemical exposure the same antibody is ineffective in influencing sensitization as there has been sufficient time for the accumulation of antigen-bearing DC in draining nodes. These data are consistent with the report of Bromberg *et al.*[28] in which neutralizing anti-TNF-α was found to impair contact hypersensitivity reactions to picryl chloride when given during the induction, but not elicitation, phase. The failure in the present study to inhibit completely the induction of contact sensitization might be attributable to the use of insufficient antibody. Alternatively other cells, such as the Ia^+ dermal DC described by Tse & Cooper[29] may also play a role in antigen transport and the initiation of skin sensitization.

A question remains regarding the nature of changes induced in responsive LC by TNF-α. It is possible that altered expression of adhesion molecules by LC and adjacent cells may play an important role in this process. Interestingly it has been found that both LC and keratinocytes express the homophilic adhesion molecule E-cadherin, the suggestion being that this determinant serves to maintain LC in the epidermis.[30] An induced down-regulation of E-cadherin expression by LC and/or by keratinocytes would therefore facilitate the disassociation of LC from the surrounding tissue matrix and permit the initiation of migration away from the skin. Of potentially great relevance is expression of the adhesion molecule Pgp-1 (CD44). This molecule has been found to influence markedly the migration and motility of various cell types.[31–33] In a recent study it was reported that treatment with TNF-α of DC, which with respect to surface phenotype resembled LC, resulted in the increased expression of CD44 and induced the appearance of an isoform of this molecule.[34] The latter isoform has been associated previously with the increased metastic potential of carcinoma cells.[32] In this context it is also relevant that mice infected with Rauscher leukaemia virus display impaired migration of LC to draining lymph nodes following skin sensitization with FITC and that this inhibition was associated with a reduced expression by LC of CD44.[35] Finally, a recent study has revealed that protein kinase C may transduce the signal for LC migration.[36]

It is proposed, therefore, that TNF-α provides an important and, in some instances, the sole signal for LC migration from the skin. It is possible that the critical effect of TNF-α on epidermal LC is to cause altered expression of adhesion molecules which facilitate disengagement of responsive cells from surrounding keratinocytes (E-cadherin) and which permit movement away from the skin towards draining lymph nodes (CD44). It is possible also that protein kinase C is necessary for such TNF-α-mediated changes.

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