Toxicology and Applied Pharmacology **169,** 231–237 (2000)
doi:10.1006/taap.2000.9085, available online at http://www.idealibrary.com on IDEAL®

# Induction and Localization of Cutaneous Interleukin-1$\beta$ mRNA during Contact Sensitization

Faiz Kermani,* Melanie S. Flint,† and Sharon A. M. Hotchkiss*

**Molecular Toxicology, Imperial College School of Medicine, Sir Alexander Fleming Building, London SW7 2AZ, United Kingdom; and †Toxicology and Molecular Biology Branch, CDC, NIOSH, 1095 Willowdale Road, Morgantown, West Virginia 26505-2888*

Received May 26, 2000; accepted October 11, 2000

**Induction and Localization of Cutaneous Interleukin-1$\beta$ mRNA during Contact Sensitization. Kermani, F., Flint, M. S., and Hotchkiss, S. A. M. (2000).** *Toxicol. Appl. Pharmacol.* **169, 231–237.**

**Chemical allergens that induce contact sensitivity cause changes in levels of epidermal cytokines. In mice one of the earliest epidermal cytokines to be upregulated following sensitization is interleukin-1$\beta$ (IL-1$\beta$). The present study investigated the kinetics and *in situ* localization of induced IL-1$\beta$ expression in mouse skin following topical exposure to the contact allergen oxazolone. Mice were exposed topically to 1% oxazolone, with control mice exposed to vehicle (acetone:olive oil 4:1) alone, and at various times thereafter skin was excised for IL-1$\beta$ mRNA and protein determination by *in situ* hybridization and enzyme-linked immunosorbant assay (ELISA), respectively. IL-1$\beta$ mRNA was found to be expressed constitutively at low levels in skin from naïve (untreated) and vehicle-treated mice, with mRNA localized in some hair follicles and sebaceous glands; no IL-1$\beta$ mRNA was detected in the epidermis of control animals. Following topical exposure of mice to oxazolone for 5–15 min, upregulation of IL-1$\beta$ mRNA was observed in the epidermis, dermis, hair follicles, and sebaceous glands; at 90 min and beyond the pattern of IL-1$\beta$ mRNA expression declined toward control. Analysis of whole skin homogenates by ELISA demonstrated cutaneous IL-1$\beta$ protein to be present constitutively in both vehicle-treated and naïve mice. Following exposure to oxazolone, cutaneous IL-1$\beta$ protein expression was elevated at 30 min, decreased at 1 h, and fell below the limit of detection of the assay at 2 h before returning to constitutive levels at 4 and 24 h. IL-1$\beta$ protein levels in vehicle-treated mice, naïve mice, and mice treated with the respiratory allergen trimellitic anhydride were unchanged over this time period. The present study demonstrated that IL-1$\beta$ mRNA expression was upregulated rapidly and transiently in well-defined regions of mouse epidermis and dermis during contact sensitization, and was succeeded by an elevation in IL-1$\beta$ protein. This early highly localized upregulation of IL-1$\beta$ lends further support to the hypothesis that this cytokine plays a key role in the initial stages of skin sensitization. Such information will enhance our understanding of the molecular processes involved in allergic contact dermatitis and may provide a mechanistic basis for designing refined animal and *in vitro* alternatives to existing models of skin sensitization.** 

*Key Words:* **IL-1$\beta$ mRNA; IL-1$\beta$ protein; contact sensitization; *in situ* hybridization.**

Chemical allergens that induce contact sensitivity cause changes in levels of epidermal cytokines. In mice one of the earliest epidermal cytokines to be selectively upregulated following sensitization is interleukin-1$\beta$ (IL-1$\beta$):[1] Langerhans cell-derived IL-1$\beta$ mRNA is increased within 15 min following primary topical exposure of mice to contact allergens but not irritants (Enk and Katz, 1992a,b; Enk *et al.*, 1993). It has been postulated that this selective upregulation of IL-1$\beta$ may provide a useful marker for detecting sensitizing chemicals and distinguishing them from skin irritants. Epidermal cells, both Langerhans cells and keratinocytes, are important sources of cellular cytokines (Luger and Schwarz, 1990; Matsue *et al.*, 1992). During the induction phase of contact sensitization, Langerhans cells migrate from the epidermis (Knight *et al.*, 1985; Kripke *et al.*, 1990; Macatonia *et al.*, 1986), undergo maturation (Larsen *et al.*, 1990; Ozawa *et al.*, 1996), and accumulate as dendritic cells in the draining lymph nodes where they present antigen to T cells (Knight *et al.*, 1985; Kripke *et al.*, 1990). Freshly isolated Langerhans cells are poor stimulators of T cells (Schüler *et al.*, 1985) and must undergo a cytokine-mediated maturation process before they can stimulate T cells (Heufler *et al.*, 1992; Witmer-Pack *et al.*, 1987). Certain epidermal cytokines, particularly IL-1$\beta$ (Heufler *et al.*, 1992; Ozawa *et al.*, 1996), granulocyte macrophage colony stimulating factor (GM–CSF) (Witmer-Pack *et al.*, 1987), and tumor necrosis factor-$\alpha$ (TNF-$\alpha$) (Kimber and Cumberbatch, 1992) are believed to play a key role in the induction of contact sensitization. TNF-$\alpha$ and IL-1$\beta$ are major stimuli for Langerhans cell migration (Cumberbatch *et al.*, 1997; Kimber and Cumberbatch, 1992), while Langerhans cell maturation has been linked to the cytokines GM–CSF (Ozawa *et al.*, 1996) and IL-1$\beta$ (Ozawa *et al.*, 1996; Xu *et al.*, 1995). The induction of IL-1$\beta$ in the epidermis enhances the antigen presenting cell function of Langerhans cells by upregu-

[1] Abbreviations used: IL-1$\beta$, interleukin-1$\beta$; ISH, *in situ* hybridization; ELISA, enzyme-linked immunosorbant assay; TNF-$\alpha$, tumor necrosis factor-$\alpha$; DNCB, 2,4-dinitrochlorobenzene; DNFB, 2,4-dinitrofluorobenzene; TNCB, 1,3,5-trinitrochlorobenzene; TMA, trimellitic anhydride; AOO, acetone/olive oil.



0041-008X/00 $35.00

lating the expression of major histocompatibility complex (MHC) class II and co-stimulatory molecules such as intercellular adhesion molecule-1 (ICAM-1), B7-1, B7-2, and CD40 on Langerhans cells (Ozawa *et al.,* 1996), and also stimulates keratinocytes to produce TNF-$\alpha$ which induces Langerhans cell migration (Cumberbatch *et al.,* 1997; Junghans *et al.,* 1998).

Previously, we have demonstrated that the induced expression of TNF-$\alpha$ mRNA is tightly controlled, both temporally and spatially, following topical application of mice to the contact allergen oxazolone, and that a similar pattern of TNF-$\alpha$ mRNA expression is induced by intradermal injection of IL-1$\beta$ protein (Flint *et al.,* 1998). It was suggested that the areas of TNF-$\alpha$ mRNA expression were coincident with sites where Langerhans cells were induced, by interaction with contact allergen, to synthesize and secrete IL-1$\beta$ mRNA and protein (Flint *et al.,* 1998). In the present study, the kinetics of induction and *in situ* localized expression of IL-1$\beta$ mRNA and protein were investigated in mouse skin during sensitization with oxazolone. Information from this study will enhance our understanding of the molecular processes involved in allergic contact dermatitis and may provide a mechanistic basis for designing refined animal and *in vitro* alternatives to existing models of skin sensitization.

## MATERIALS AND METHODS

***Animals.*** Female BALB/c strain mice (6–8 weeks old) were obtained from Harlan Olac (Bicester, UK) and maintained on a diet of Biosure CRM pellets (Manea, Cambs, UK) and water *ad lib.*

***Chemicals.*** Oxazolone (4-ethoxymethylene-2-phenyloxazol-5-one) was obtained from Sigma Chemical Co. (St. Louis, MO). Trimellitic anhydride (TMA) was obtained from Aldrich (Gillingham, UK). These chemicals were dissolved in acetone/olive oil (AOO), 4:1 (v/v) (Sigma, Poole, UK).

***Skin sensitization.*** Four mice per time interval for each treatment group were used. Groups of mice received 100 $\mu$l of 1% oxazolone (contact allergen), 10% TMA (respiratory allergen), or an equal volume of vehicle (AOO) alone, on the dorsum of the shaved flanks. In some experiments, mice received 25 $\mu$l of the same concentration of oxazolone on the dorsum of both ears. Additional control mice ($n = 3$) were left untreated.

***Processing of skin samples.*** At various times (0–24 h) following treatment, mice were killed and the application sites excised. The samples were divided into two halves, each approximately 1 $cm^2$. For subsequent analysis of cytokine mRNA by *in situ* hybridization, one half of the sample was placed in 4% paraformaldehyde (w/v). The remaining sample was used for the measurement of cytokine protein by enzyme-linked immunosorbant assay (ELISA). The samples for ELISA were finely chopped on ice, weighed and homogenized in RPMI 1640 culture medium (Gibco Ltd., Paisley, Renfrewshire, UK) supplemented with 5% fetal calf serum (FCS), 2 mM glutamine, 400 units/ml penicillin, and 400 $\mu$g/ml streptomycin (RPMI–FCS). The homogenates were snap frozen in liquid nitrogen, brought rapidly to room temperature, and sonicated for 15 s (50 Hz). Supernatants were collected by centrifugation (2000*g,* 5 min) and stored at −70°C until analysis.

***In situ hybridization.*** Glass slides were soaked for 1 h in 1% v/v Decon, rinsed in distilled water, and air-dried. Slides were then dipped successively in 70% v/v ethanol, 95% v/v ethanol and 3-aminopropyltriethoxysilane (2% v/v in acetone, Sigma, Poole, UK) and rinsed in 0.1% v/v diethylpyrocarbonate (Sigma) in water. Sections (5 $\mu$m) were cut of paraffin wax-embedded, paraformaldehyde-fixed skin and were mounted onto the slides.

*In situ* hybridization was performed as described by Flint *et al.* (1998), using a method adapted from that of Howie *et al.* (1992). Sections were deparaffinized in xylene (Merck Ltd., Lutterworth, UK), rehydrated in ascending alcohols, and pretreated for 1 h at 37°C with 1 $\mu$g/ml proteinase K (Sigma). The reaction was terminated by washing with phosphate-buffered saline (PBS, pH 7.4) and refixing in 4% paraformaldehyde. Slides were prehybridized for 1 h at 37°C with 60 $\mu$l of hybridization buffer (final concentrations: 0.6 M sodium chloride, 0.1% v/v sodium pyrophosphate, 50 mM Tris–HCl, 0.2% v/v polyvinylpyrrolidone, 5 mM EDTA, 0.2% w/v Ficoll, 10% w/v dextran sulfate, 10% v/v single-stranded salmon DNA, and 50% w/v deionized formamide (Sigma)). The buffer was drained from the slide and was replaced with 10 $\mu$l of the hybridization buffer containing 10 ng of the biotin-labeled oligonucleotide probe (R & D Systems Europe, Abingdon, UK). The probe used was an antisense mouse IL-1$\beta$ cocktail, containing equimolar ratios of probes to exons 5, 6, and 7 of murine IL-1$\beta$. Controls included no probe, RNase prior to hybridization, and antisense $\beta$-actin (R & D Systems), as published previously (Flint *et al.,* 1998).

The slides were covered with Gelbond (Sigma) and incubated overnight at 37°C in a humid atmosphere. The sections were then washed four times under increasingly stringent conditions in sodium saline citrate solution followed by washing twice in Tris–saline buffer (pH 7.5) and blocking for 30 min with 3% normal goat serum (Vector Laboratories, Peterborough, UK). Streptavidin alkaline phosphatase conjugate (Eugene, OR) diluted 1:800 in 3% normal goat serum (containing 0.1% v/v levamizole, Eugene) was added for 5 h at room temperature. The slides were then washed and treated overnight with Sigmafast nitrobluetetrazolium/bromo-4-chloro-indolyl phosphate (NBT/BCIP) containing 0.1% levamizole. The next day, the slides were washed thoroughly in distilled water, counterstained with 1% malachite green in distilled water, washed under a running tap, dried for 1 h at 37°C, and mounted under Aquamount aqueous mountant (Merck Ltd.). Sections were examined under a standard light microscope with camera attachment (Leica, Milton Keynes, UK) and photographs were taken.

The *in situ* hybridized sections were assessed using a visual scoring system to determine NBT/BCIP staining intensity (absence, weak, moderate, or intense) which correlated with levels of expression of mRNA for IL-1$\beta$. Supplementary assessment of the sections was undertaken by observers who were blind to the *in situ* hybridization procedure.

***Measurement of cutaneous IL-1$\beta$ protein by ELISA.*** IL-1$\beta$ protein levels in murine whole skin homogenates were measured using a commercial sandwich ELISA (Genzyme, West Malling, UK). Plastic microtitre plates (Genzyme) were coated with 2 $\mu$g/ml monoclonal hamster anti-mouse IL-1$\beta$ antibody (Genzyme) in 0.1 M carbonate buffer (pH 9.5) (Sigma) by overnight incubation at 4°C. The plates were washed four times with wash buffer (PBS, pH 7.3, containing 0.05% Tween 20) (Sigma) and blocked by treatment with PBS containing 4% bovine serum albumin (Sigma) for 2 h at 37°C. Aliquots (100 $\mu$l) of tissue homogenates and murine IL-1$\beta$ standards were added to the plates for 1 h at 37°C, following which the plates were washed four times and incubated for 1 h at 37°C with 0.8 $\mu$g/ml biotinylated rabbit anti-mouse secondary antibody (Genzyme). Following a further washing step the plates were incubated with 1:1000 dilution of HRP-conjugated streptavidin (Genzyme) for 15 min at 37°C. Enzyme substrate (tetramethyl benzidine/peroxide) (Genzyme) was added and the reaction terminated within 30 min by the addition of 1 M sulfuric acid (Merck Ltd.). The concentration of IL-1$\beta$ in test samples was calculated from a standard curve derived with murine IL-1$\beta$ (range 0–500 pg/ml). The limit of detection of IL-1$\beta$ was 20 pg/ml.

***Statistics.*** IL-1$\beta$ protein levels determined by ELISA were compared using the Dunnett Multiple Comparisons test and where $P < 0.05$ were considered significantly different from controls.

## RESULTS

### *IL-1$\beta$ mRNA Expression Following Exposure to Oxazolone*

*In situ* hybridization analysis revealed that IL-1$\beta$ mRNA was expressed constitutively at low levels in skin from naïve (untreated) and vehicle (AOO)-treated mice, with localization

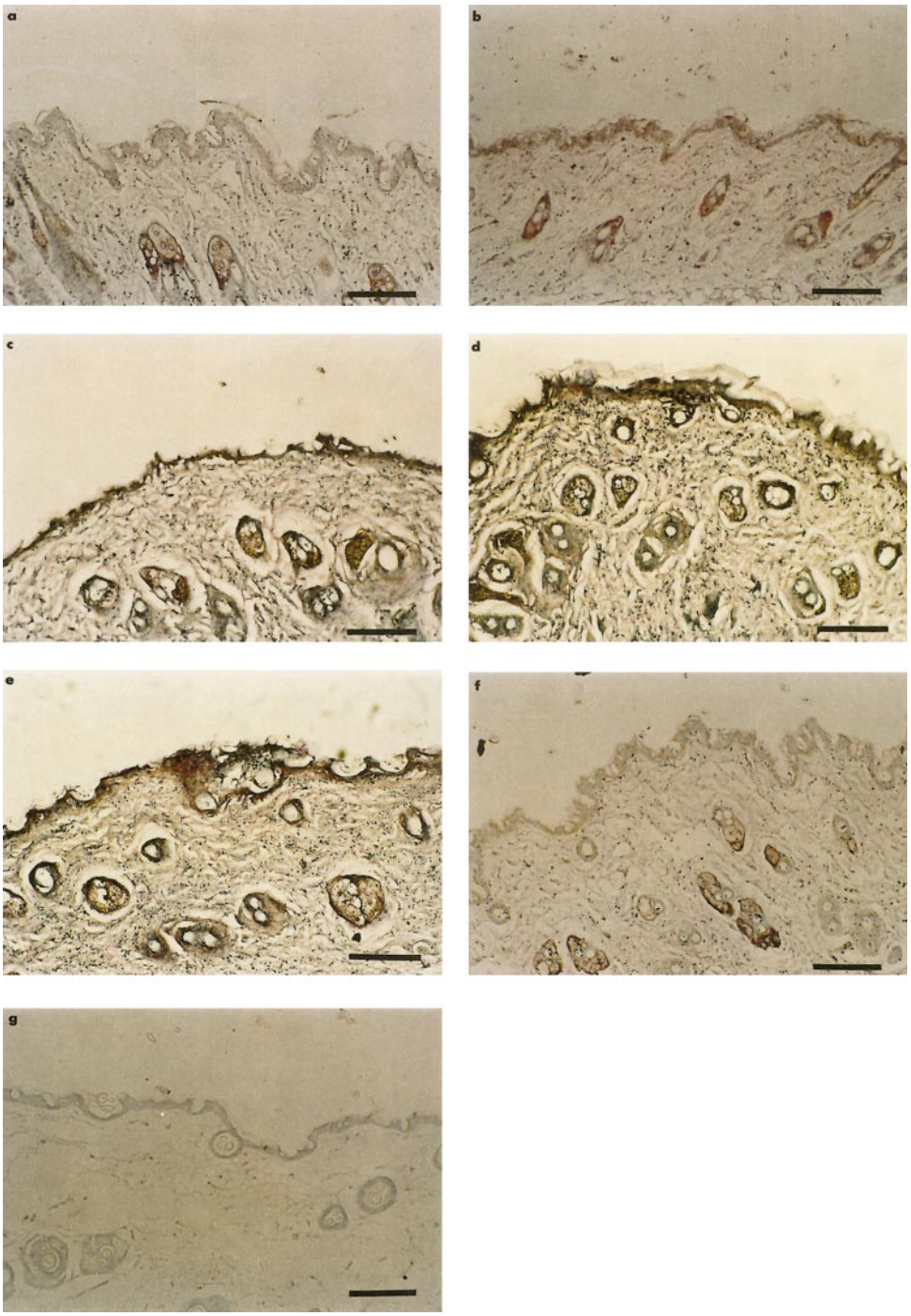


**FIG. 1.** *In situ* hybridization analyses of IL-1β mRNA expression in mouse flank skin. (a) Constitutive expression of IL-1β mRNA in skin excised from naïve (untreated) mice. (b) Constitutive expression of IL-1β mRNA in skin excised from mice treated with acetone:olive oil vehicle. (c) Expression of IL-1β mRNA in skin excised from mice treated with the contact allergen, at 5 min. (d) Expression of IL-1β mRNA 10 min following exposure to oxazolone. (e) Expression of IL-1β mRNA 15 min following exposure to oxazolone. (f) Expression of IL-1β mRNA 90 min following exposure to oxazolone. (g) Lack of signal for IL-1β mRNA in mouse skin in the absence of antisense IL-1β probe. Scale bar, 50 μm.

in some hair follicles and sebaceous glands and in certain dermal cells; no constitutive expression was observed in the epidermis of these animals (Figs. 1a and 1b). Following exposure of mice to oxazolone, cutaneous IL-1β mRNA was upregulated rapidly and transiently in both the epidermis and the dermis (Figs. 1c–1f). Increased expression of IL-1β mRNA was first observed 5 min following exposure to oxazolone (Fig. 1c), was maximal between 10–15 min (Figs. 1d and 1e), and declined to background levels thereafter (Fig. 1f). No signal was observed in tissue sections that had undergone *in situ*

**TABLE 1**
**Visual Scoring of Staining Intensity of IL-1β mRNA Expression in Mouse Skin Determined by *in Situ* Hybridization**

| | Epidermis | Dermis | Hair follicles and sebaceous glands |
|---|---|---|---|
| Naïve (untreated) | − | + | + |
| Vehicle (AOO)-treated | − | + | + |
| 5 Min oxazolone | ++ | ++ | +++ |
| 10 Min oxazolone | ++ | +++ | +++ |
| 15 Min oxazolone | ++ | +++ | +++ |
| 90 Min oxazolone | − | + | ++ |

*Note.* The *in situ* hybridization sections (Figs. 1a–1g) were analyzed using a visual scoring system, whereby the NBT/BCIP staining intensity was scored as follows: −, absence of staining; +, weak staining; ++, moderate staining; +++, intense staining for IL-1β mRNA. Assessment of each section was undertaken on three separate occasions by observers who were blind to the *in situ* hybridization procedure.

hybridization in the absence of the IL-1β antisense probe (Fig. 1g) or pretreatment with RNase prior to hybridization (data not shown). In untreated skin from naive animals, β-actin mRNA (positive control) was ubiquitously distributed throughout the epidermis and dermis (data not shown), as published previously (Flint *et al.,* 1998). The level of *in situ* expression of IL-1β mRNA, assessed using a visual scoring system, is presented in Table 1.

### *IL-1 β Protein Expression Following Exposure to Oxazolone*

ELISA analysis showed that IL-1β protein was present constitutively in whole skin from naïve (untreated) mice (36.3 ± 0.9 pg/ml, mean ± SEM, $n = 3$ mice) and in mice exposed to vehicle (AOO) alone (33.1 ± 1.9 pg/ml, mean ± SEM, $n = 4$ mice).

In oxazolone-treated mice cutaneous IL-1β protein levels were elevated significantly ($P < 0.05$) at 30 min (77.0 ± 2.9 pg/ml), decreased at 1 h (48.1 ± 17.8 pg/ml), and fell below the detection limit (20 pg/ml) of the assay at 2 h, before returning to constitutive levels at 4 h (32.6 ± 9.0 pg/ml) and 24 h (45.3 ± 10.8 pg/ml) (Fig. 2). Oxazolone-induced levels of IL-1β protein at 2 h were statistically significantly different from control, but since they were below the limit of detection of the assay this was not considered to be of biological relevance. In untreated mice and mice treated with the vehicle (AOO) or with the respiratory allergen TMA, IL-1β protein was not elevated above control levels at any time up to 24 h (Fig. 2).

## DISCUSSION

The data presented here demonstrate that there is early and transient upregulation of IL-1β mRNA in the epidermis and dermis of mouse skin in response to topical treatment with the contact allergen oxazolone. IL-1β mRNA was not present constitutively in the epidermis, but was upregulated from 5 to 15 min following exposure to oxazolone and was not apparent in the epidermis at times thereafter. Such a rapid induction is important for a key proinflammatory cytokine such as IL-1β which upregulates MHC class II and co-stimulatory molecules to transform immature Langerhans cells into mature dendritic cells capable of efficient antigen presentation to T cells in the

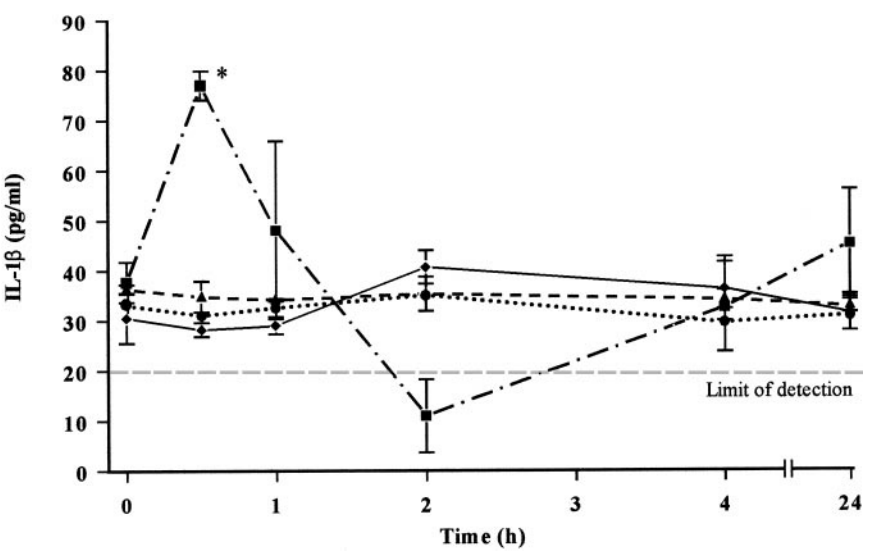


**FIG. 2.** ELISA analysis of IL-1β protein levels in whole mouse skin homogenates. Groups of mice were exposed on the shaved dorsum to 100 μl acetone:olive oil (AOO) vehicle ($n = 4$) (dotted line), respiratory allergen TMA ($n = 4$) (solid line), or the contact allergen oxazolone (dotted and dashed line). One group of mice ($n = 3$) was left untreated (naïve) (dashed line). At various times following exposure, the application site was excised and homogenates were prepared for analysis of IL-1β protein by ELISA. Results are expressed as mean ± SEM and compared by the Dunnett Multiple Comparisons test. Where $P < 0.05$, the value was considered statistically significantly different from control and is marked with an asterisk. (The value for oxazolone treatment at 2 h was statistically significantly different from control, but since it was below the limit of detection of the assay this was not considered to be of biological relevance and is not marked with an asterisk.)

draining lymph nodes (Hopkins *et al.,* 1990; Ozawa *et al.,* 1996) and to stimulate TNF-α production from keratinocytes to induce Langerhans cell migration from the epidermis (Cumberbatch *et al.,* 1997; Enk and Katz, 1992; Junghans *et al.,* 1998). The rapid induction in IL-1β mRNA expression is in agreement with the studies by Enk and Katz (1992a,b) who observed induction of IL-1β mRNA at 15 min in epidermal cell suspensions from the skin of mice treated *in vivo* with the contact allergens TNCB, DNFB, and DNCB using RT–PCR. However, this article elucidates this mechanism further by defining for the first time the precise localization and distribution of the mRNA for this cytokine within the skin at various times during the induction phase of contact sensitization.

The present study has shown that the localized upregulation of IL-1β mRNA is rapid in murine skin and precedes that observed for TNF-α mRNA in previous studies (Flint *et al.,* 1998). *In situ* hybridization analyses revealed IL-1β mRNA was increased as early as 5 min following exposure to oxazolone whereas TNF-α mRNA was not elevated until 10 min under the same exposure conditions (Flint *et al.,* 1998). These data support the findings of Enk and Katz (1992a,b) using RT–PCR: following treatment of mice with contact allergens, cutaneous IL-1β mRNA was increased at 15 min, preceding the increase in TNF-α mRNA at 30 min. The temporal difference between IL-1β and TNF-α was also observed at the protein level: IL-1β protein was elevated at 30 min following exposure to oxazolone in the present study while TNF-α was increased at 1 h under the same exposure conditions (Flint *et al.,* 1998). These observations all support the hypothesis that IL-1β is one of the earliest cytokines to be induced during contact sensitization and that following its release, IL-1β acts to stimulate TNF-α production. Presumably the release of IL-1β from Langerhans cells stimulates neighboring keratinocytes, in a paracrine manner via the IL-1 type I receptor (Kupper *et al.,* 1988), to produce TNF-α which then acts on the TNF type 2 receptor (TNF-R2) on Langerhans cells (Larregina *et al.,* 1996) to induce their migration (Cumberbatch *et al.,* 1997).

The induced expression of IL-1β mRNA was succeeded by an elevation in IL-1β protein. At the protein level IL-1β was present constitutively in whole skin homogenates and was upregulated twofold within 30 min following exposure to oxazolone. The rapid induction of IL-1β protein could be the consequence of transcriptional upregulation of the IL-1β gene followed by rapid translation of IL-1β mRNA into protein and stabilization of the protein product, as reported for lipopolysaccharide-induced IL-1β mRNA and protein in human peripheral blood mononuclear cells (Schindler *et al.,* 1990). The kinetics are in line with other studies: when the murine macrophage cell line $P388D_1$ was grown in a 1:1 mixture of DMEM and Ham's F12 cell culture media, IL-1β protein precursor synthesis could be detected as early as 30 min after completion of the cell culture protocol while between 3 and 19 h the mature 18-kDa form was detected and the 33-kDa precursor decreased (Giri *et al.,* 1985).

According to in-house data from the manufacturers of the ELISA used in the present study (Genzyme), the anti-IL-1β antibody raised against the mature form of IL-1β had 13% cross-reactivity with IL-1β precursor. Therefore, some of the protein detectable by ELISA in the present study was likely to be the precursor form of IL-1β. This is a limitation of currently available commercial ELISA kits as antibodies specific to the precursor or mature forms of IL-1β are not yet available. In support of the approach taken in these studies, Dinarello (1992) stated that it is important to measure the synthesis of both the primary translational IL-1β precursor product as well as the processed mature form of IL-1β as this reflects the translational control of the IL-1β gene under the pathological condition being studied.

The rapid downregulation of IL-1β mRNA in the present study, whereby the expression of the cytokine mRNA declined essentially to control levels within 90 min, is of potential toxicological significance in limiting the local effects of this proinflammatory cytokine. The mechanism of downregulation could involve mRNA destabilization which has been shown to occur for other transiently expressed cytokines such as GM–CSF (Shaw and Kamen, 1986). The 3′ untranslated regions of mRNA coding for a number of proinflammatory cytokines, including IL-1β, contain an adenine- and uridine-rich sequence which targets mRNA for rapid turnover (Kruys *et al.,* 1989; Shaw and Kamen, 1986). The half-life of GM–CSF mRNA in T cells stimulated with lectins was determined to be less than 30 min and this was linked to the presence of these adenine- and uridine-rich sequences within the GM–CSF mRNA (Shaw and Kamen, 1986). It has been demonstrated that when the human monocyte cell line THP-1 is stimulated with lipopolysaccharide there are at least two kinetically distinct populations of IL-1β mRNA produced (Fenton *et al.,* 1988). It is not known if these mRNA populations are due to distinct genes, alternative message processing, or to a subpopulation of mRNA that the cell has made unavailable to degradation (Fenton *et al.,* 1988). Similar mechanisms of selective cytokine mRNA degradation due to conserved adenine- and uridine-rich sequences in the 3′ region have been shown for TNF-α mRNA (Brown and Beutler, 1990) and interferon-β (IFN-β) mRNA (Kruys *et al.,* 1989).

In the present study, the decline in IL-1β mRNA was followed by a decline in IL-1β protein, with protein levels falling from their maximum at 30 min to below constitutive at 2 h. The mechanism by which IL-1β protein is downregulated may involve suppression, either at the transcriptional level as mediated by glucocorticoids (Lew *et al.,* 1988) or at the translational level as mediated by prostaglandins (Dinarello, 1996; Mauviel *et al.,* 1991). Alternatively, levels of IL-1 β protein could be reduced by binding to naturally occurring soluble IL-1 receptors (Giri *et al.,* 1994). Elevated levels of soluble IL-1 type II receptors have been demonstrated in sepsis and are postulated to modulate the effects of IL-1 during such inflammatory conditions (Giri *et al.,* 1994). Effective downregulation of IL-1β at the mRNA and/or protein level is required as IL-1β

is a multifunctional cytokine with diverse biological actions which, once the immune response has been initiated, must be controlled to avoid prolonged stimulation (Dinarello, 1996). IL-1$\beta$ activates T lymphocytes and enhances production of interleukin 2 (IL-2), stimulates the production of chemokines from fibroblasts and endothelial cells, upregulates adhesion molecules such as ICAM-1, and induces fever (Miller *et al.*, 1997; Ozawa *et al.*, 1996; Schröder *et al.*, 1990).

IL-1$\beta$ protein levels in the present study only varied in response to the contact allergen oxazolone, remaining unaffected in skin from naïve (untreated) mice, vehicle-treated mice, and mice treated with the respiratory allergen TMA. The observation that IL-1$\beta$ protein production was elevated with the cutaneous allergen, but not the respiratory allergen, is of interest and may be of biological and toxicological significance. Although only observed under the precise experimental conditions described here using a single chemical for each class, it is possible that other chemicals in the same class could induce the same responses in IL-1$\beta$ production. This is in line with the fact that topical exposure of mice to contact and respiratory sensitizers stimulates divergent responses, presumably due to the selective activation of T helper 1 (Th1) and T helper 2 (Th2) type cells, respectively (Dearman and Kimber, 1991; Dearman *et al.*, 1996). IL-1$\beta$ mRNA has been reported to be enhanced in mice specifically in response to contact allergens, unlike other proinflammatory cytokines such as TNF-$\alpha$, IFN-$\gamma$ (interferon-$\gamma$), and GM–CSF which are upregulated in response to nonspecific stimuli, for example skin irritants (Enk and Katz, 1992a,b). This observation lends mechanistic support to the approach currently under examination in some laboratories of using specific IL-1$\beta$ cytokine upregulation as a marker of skin sensitization. Furthermore, it has been postulated that the selective upregulation of IL-1$\beta$ by allergens, but not irritants, may provide a useful marker for distinguishing skin sensitizing chemicals from skin irritants, although in human skin lymph this would not appear to be the case (Brand *et al.*, 1996).

In addition to describing the *in situ* localization of induced IL-1$\beta$ mRNA in the epidermis, the present study has allowed the examination of *in situ* changes in the dermis over a similar time period. The *in situ* hybridization data indicate constitutive expression of IL-1$\beta$ mRNA in the dermis and certain hair follicles and sebaceous glands, with induction kinetics similar to that for epidermal IL-1$\beta$ mRNA, although to confirm this constitutive expression unequivocally a negative control using an irrelevant biotinylated anti-sense oligo should also be tested. The significance of the constitutive expression and contact allergen-induced upregulation of IL-1$\beta$ mRNA in murine dermis, hair follicles, and sebaceous glands is unclear. Dermal dendritic cells, equivalent to Langerhans cells in that they express MHC class II antigens as well as the dendritic marker DEC-205, have been reported in murine skin (Lenz *et al.*, 1993; Belkaid *et al.*, 1996). These dermal dendritic cells are believed to be a resident population in the dermis and could represent the source of the dermal IL-1$\beta$ detected in the present study. In human studies, the monocyte/macrophage cell population has been suggested as a potential dermal source of IL-1$\beta$ (Hazuda *et al.*, 1988) and it is possible that these cells may also represent a dermal source of IL-1$\beta$ in the mouse. The dermal distribution of constitutive IL-1$\beta$ mRNA in murine skin in the present study accords with that observed in human skin: Boehm *et al.* (1995) reported constitutive expression of IL-1$\beta$ mRNA in human skin hair follicles and sweat glands using *in situ* hybridization, while Ahmed *et al.* (1996) reported constitutive expression of IL-1$\beta$ mRNA in the inner root sheath cell layer of human hair follicles and suggested that the cytokine had a role in hair growth. However, allergen-induced *in situ* changes in the expression of IL-1$\beta$ mRNA in human skin have not been examined.

In conclusion, the studies reported in this article have shown that IL-1$\beta$ mRNA is rapidly and transiently upregulated in mouse skin, in highly localized regions of the epidermis, dermis, hair follicles, and sebaceous glands, following topical exposure to the contact allergen oxazolone. This temporal and spatial profile of induced IL-1$\beta$ mRNA expression lends further support to the hypothesis that this cytokine plays a key role in the initial stages of skin sensitization. Such information will enhance our understanding of the molecular processes involved in allergic contact dermatitis and may provide a mechanistic basis for designing refined animal and *in vitro* alternatives to existing models of skin sensitization.

## ACKNOWLEDGMENTS

We are grateful to Dr. Gary Coulton, Imperial College, London, for helpful discussions regarding the *in situ* hybridization methodology, and to Dr. Peter Clark, Dr. Camilla Smith, and Ms. Connie Cheung for valuable assistance during preparation of the manuscript.

## REFERENCES

Ahmed, A. A., Nordlind, K., Schultzberg, M., Brakenhoff, J., Bristulf, J., Novick, D., Svenson, S. B., Azizi, M., and Lidén, S. (1996). Immunohistochemical studies of proinflammatory cytokines and their receptors in hair follicles of normal human skin. *Acta. Derm. Venereol.* **76,** 348–352.

Belkaid, Y., Jouin, H., and Milon, G. (1996). A method to recover, enumerate and identify lymphomyeloid cells present in an inflammatory dermal site: A study in laboratory mice. *J. Immunol. Methods.* **199,** 5–25.

Boehm, K. D., Yun, J. K., Strohl, K. P., and Elmets, C. A. (1995). Messenger RNAs for the multifunctional cytokines interleukin-1$\alpha$, interleukin-1$\beta$ and tumour necrosis factor-$\alpha$ are present in adnexal tissues and in dermis of normal human skin. *Exp. Dermatol.* **4,** 335–341.

Brand, C. U., Hunziker, T., Yawalkar, N., and Braathen, L. R. (1996). IL-1 beta protein in human skin lymph does not discriminate allergic from irritant contact dermatitis. *Contact Derm.* **35(3),** 152–156.

Brown, J. H. T., and Beutler, B. (1990). Endotoxin-responsive sequences control cachectin/tumor necrosis factor biosynthesis at the translational level. *J. Exp. Med.* **171,** 465–475.

Cumberbatch, M., Dearman, R. J., and Kimber, I. (1997). Langerhans cells require signals from both tumour necrosis factor-$\alpha$ and interleukin-1$\beta$ for migration. *Immunology* **92,** 388–395.

Dearman, R. J., Basketter, D. A., and Kimber, I. (1996). Characterization of

chemical allergens as a function of divergent cytokine secretion profiles induced in mice. *Toxicol. Appl. Pharmacol.* **138,** 308–316.

Dearman, R. J., and Kimber, I. (1991). Differential stimulation of immune function by respiratory and contact chemical allergens. *Immunology* **72,** 563–570.

Dinarello, C. A. (1992). ELISA kits based on monoclonal antibodies do not measure total IL-1β synthesis. *J. Immunol. Methods* **148,** 255–259.

Dinarello, C. A. (1996). Biologic basis for interleukin-1 in disease. *Blood* **87,** 2095–2147.

Enk, A. H., and Katz, S. I. (1992a). Early molecular events in the induction phase of contact sensitivity. *Proc. Natl. Acad. Sci.* **89,** 1398–1402.

Enk, A. H., and Katz, S. I. (1992b). Early events in the induction phase of contact sensitivity. *J. Invest. Dermatol.* **99,** 39S–41S.

Enk, A. H., Angeloni, V. L., Udey, M. C., and Katz, S. I. (1993). An essential role for Langerhans cell-derived IL-1 β in the initiation of primary immune responses in skin. *J. Immunol.* **150(9),** 3698–3704.

Fenton, M. J., Vermeulen, M. W., Clark, B. D., Webb, A. C., and Auron, P. E. (1988). Human pro-IL-1β gene expression in monocytic cells is regulated by two distinct pathways. *J. Immunol.* **140,** 2267–2273.

Flint, M. S., Dearman, R. J., Kimber, I., and Hotchkiss, S. A. M. (1998). Production and in situ localization of cutaneous tumour necrosis factor α (TNF-α) and interleukin 6 (IL-6) following skin sensitization. *Cytokine* **10 (3),** 213–219.

Giri, J. G., Lomedico, P. T., and Mizel, S. B. (1985). Studies on the synthesis of interleukin 1. *J. Immunol.* **134,** 343–349.

Giri, J. G., Wells, J., Dower, S. K., McCall, C. E., Guzman, R. N., Slack, J., Bird, T. A., Shanebeck, K., Grabstein, K. H., Sims, J. E., and Alderson, M. R. (1994). Elevated levels of shed type II IL-1 receptor in sepsis. *J. Immunol.* **153,** 5802–5809.

Hazuda, D. J., Lee, J. C., and Young, P. R. (1988). The kinetics of interleukin 1 secretion from activated monocytes. *J. Immunol.* **263,** 8473–8479.

Heufler, C., Topar, G., Koch, F., Trockenbacher, B., Kämpgen, Romani, N., and Schuler, G. (1992). Cytokine gene expression in murine epidermal cell suspensions: Interleukin 1β and macrophage inflammatory protein 1α are selectively expressed in Langerhans cells but are differentially regulated in culture. *J. Exp. Med.* **176,** 1221–1226.

Hopkins, S. J., Humphreys, M., Kinnaird, A., Jones, D. A., and Kimber, I. (1990). Production of interleukin-1 by draining lymph node cells during the induction phase of contact sensitization in mice. *Immunology* **71,** 493–496.

Howie, S. E. M., Aldridge, R. D., McVittie, E., Thornton, E., Ramage, E., and Hunter, J. A. A. (1992). A non-radiolabelled in situ hybridization method for the detection of epidermal cytokine mRNA. *J. Exp. Dermatol.* **1,** 230–235.

Junghans, V., Gutgesell, C., Jung, T., and Neumann, C. (1998). Epidermal cytokines IL-1β, TNF-α, and IL-12 in patients with atopic dermatitis: Response to application of house dust mite antigens. *J. Invest. Dermatol.* **111,** 1184–1188.

Kimber, I., and Cumberbatch, M. (1992). Stimulation of Langerhans cell migration by tumour necrosis factor α (TNF-α). *J. Invest. Dermatol.* **99,** 48S–50S.

Knight, S. C., Krejci, J., Malkovsky, M., Collizzi, V., Gautam, A., and Asherson, G. L. (1985). The role of dendritic cells in the initiation of immune responses to contact sensitizers. *Cell. Immunol.* **94,** 427–434.

Kripke, M. L., Munn, C. G., Jeevan, A., Tang, J.-M., and Bucana, C. (1990). Evidence that cutaneous antigen-presenting cells migrate to regional lymph nodes during contact sensitization. *J. Immunol.* **145,** 2833–2838.

Kruys, V., Marinx, O., Shaw, G., Deschamps, J., and Huez, G. (1989). Translational blockade imposed by cytokine-derived UA-rich sequences. *Science* **245,** 852–855.

Kupper, T. S., Lee, F., Birchall, N., Clark, S., and Dower, S. (1988). Interleukin 1 binds to specific receptors on human keratinocytes and induces granulocyte macrophage colony-stimulating factor mRNA and protein. *J. Clin. Invest* **82,** 1787–1792.

Larregina, A., Morelli, A., Kolkowski, E., and Fainboim, J. (1996). Flow cytometric analysis of cytokine receptors on human Langerhans cells: Changes observed after short-term culture. *Immunology* **87,** 317–325.

Larsen, C. P., Steinmann, R. M., Witmer-Pack, M., Hankins, D. F., Morris, P. J., and Austyn, J. M. (1990). Migration and maturation of Langerhans cells in skin transplants and explants. *J. Exp. Med.* **172,** 1483–1493.

Lenz, A., Heine, M., Schuler, G., and Romani, N. (1993). Human and murine dermis contain dendritic cells. *J. Clin. Invest.* **92,** 2587–2596.

Lew, W., Oppenheim, J. J., and Matsushima, K. (1988). Analysis of the suppression of IL-1α and IL-1β production in human peripheral blood mononuclear adherent cells by a glucocorticoid hormone. *J. Immunol.* **140,** 1895–1902.

Luger, T. A., and Schwarz, T. (1990). Evidence for an epidermal cytokine network. *J. Invest. Dermatol.* **95,** 100S–104S.

Macatonia, S. E., Edwards, A. J., and Knight, S. C. (1986). Dendritic cells and the initiation of contact sensitivity to fluorescein isothiocyanate. *Immunology* **59,** 509–514.

Matsue, H., Ponciano, D. C., Bergstresser, P. R., and Takashima, A. (1992). Cytokine expression by epidermal cell subpopulations. *J. Invest. Dermatol.* **99,** 42S–45S.

Mauviel, A., Temime, N., Charron, D., Loyau, G., and Pujol, J. P. (1991). Induction of interleukin-1 beta production in human dermal fibroblasts by interleukin-1 alpha and tumor necrosis factor-alpha. Involvement of protein kinase-dependent and adenylate cyclase-dependent regulatory pathways. *J. Cell Biochem.* **47 (2),** 174–183.

Miller, A. J., Hopkins, S. J., and Lluheshi, G. N. (1997). Sites of action of IL-1 in the development of fever and cytokine responses to tissue inflammation in the rat. *Br. J. Pharmacol.* **120,** 1274–1279.

Ozawa, H., Nakagawa, S., Tagami, H., and Aiba, S. (1996). Interleukin-1β and granulocyte-macrophage colony-stimulating factor mediate Langerhans cell maturation differently. *J. Invest. Dermatol.* **106,** 441–445.

Schindler, R., Clark, B. D., and Dinarello, C. A. (1990). Dissociation between interleukin-1β mRNA and protein synthesis in human peripheral blood mononuclear cells. *J. Biol. Chem.* **265(18),** 10232–10237.

Schröder, J. M., Sticherling, M., Henneicke, H. H., Preissner, W. C., and Christophers, E. (1990). IL-1α or tumor necrosis factor-α stimulate release of three NAP-1/IL-8 related neutrophil chemotactic proteins in human dermal fibroblasts. *J. Immunol.* **144,** 2223–2232.

Schüler, G., Romani, N., and Steinman, R. M. (1985). A comparison of murine epidermal Langerhans cells with spleen dendritic cells. *J. Invest. Dermatol.* **85,** 99S–106S.

Shaw, G., and Kamen, R. (1986). A conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediates selective mRNA degradation. *Cell* **46,** 659–667.

Witmer-Pack, M. D., Olivier, W., Valinsky, J., Schuler, G., and 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,** 1484–1498.

Xu, S., Ariizumi, K., Edelbaum, D., Bergstresser, P. R., and Takashima, A. (1995). Cytokine-dependent regulation of growth and maturation in murine epidermal dendritic cell lines. *Eur. J. Immunol.* **25,** 1018–1024.