Differential Regulation of Epidermal Cell Tumor-Antigen Presentation by IL-1α and IL-1β

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IL-1 exists in two forms, termed IL-1α and IL-1β, which exert similar effects in a number of biologic models. Recently, there have been reports of some differences in the activities of these two species in some systems. To address this issue with regard to Langerhans cells, Langerhans cell-enriched preparations of epidermal cells were treated with either IL-1α or IL-1β before pulsing with S1509a tumor-associated antigens and subsequent use for immunization of naïve mice to S1509a. While epidermal cells treated with 100 U IL-1β per ml were able to induce protective tumor immunity (as indicated by the rejection of a subsequent tumor challenge with viable S1509a tumor cells), epidermal cells treated with 100 U IL-1α per ml failed to confer protective immunity. At 1000 U per ml, IL-1β also inhibited the ability of epidermal cells to induce tumor immunity. To investigate the effects of the two IL-1 forms on elicitation of tumor immunity, naïve mice were immunized against the S1509a tumor by s.c. injection of dead S1509a cells. Epidermal cells enriched for Langerhans cells were treated with either 100 U IL-1α or IL-1β per ml before tumor-associated antigens-pulsing. Epidermal cells were then washed and injected into a hind footpad of tumor immune mice and 24 h footpad swelling was assessed as a measure of delayed-type hypersensitivity. Exposure to IL-1α led to suppressed elicitation of delayed-type hypersensitivity, whereas IL-1β treated epidermal cells elicited a normal (100 U per ml) or enhanced (1000 U per ml) level of delayed-type hypersensitivity. Previous experiments indicated that the suppressive effects of IL-1α on induction of immunity may be mediated by TNFα. Therefore, the ability of IL-1α or IL-1β to induce epidermal cell production of TNFα was assessed. IL-1α induced epidermal cells to secrete significantly higher amounts of TNFα protein compared with stimulation with IL-1β. IL-1α and IL-1β appear to differentially regulate epidermal cell antigen presenting capability. ***Key words: Langerhans cells/TNFα/tumor immunology. J Invest Dermatol 111:609–615, 1998***

IL-1 plays an important role in immune and inflammatory reactions. There are two different forms of IL-1, IL-1α and IL-1β, which have similar activities in many systems (Dinarello, 1988, 1991). Within the murine skin, keratinocytes are able to produce large amounts of IL-1α, whereas IL-1β is expressed primarily by macrophages and as an early event of Langerhans cell activation (Toshiyuki *et al*, 1995). IL-1α production can be significantly enhanced by chemical or mechanical stimuli and by ultraviolet radiation (UVR) (Gahring *et al*, 1981), and is thought to be important in the regulation of inflammatory, proliferative, and immunologic events (Dinarello and Wolff, 1993). Interestingly, IL-1α is induced by various tumor promoters applied to the skin, suggesting a possible role of IL-1α in the early development of skin cancer (Lee *et al*, 1993). IL-1β, however, appears to be upregulated in Langerhans cells rapidly after contact allergen exposure. Furthermore, after intradermal injection of murine IL-1β into mouse ears, the gene expression of several biologic response modifiers was upregulated in a way that mimicked the changes caused by allergen exposure (Enk *et al*, 1993). Epidermal cells from IL-1β injected sites demonstrated increased alloantigen-presenting ability, whereas epidermal cells from IL-1α injected sites had decreased alloantigen-presenting function. Also, after systemic administration, IL-1β could enhance the number of specific antibody producing cells in the spleen of mice immunized to specific antigen, whereas IL-1α inhibited this antibody response (Boraschi *et al*, 1990). Taken together, these findings are highly suggestive for a dichotomy in the activity of the two interleukins.

Langerhans cells, the principal antigen-presenting cells within the epidermis, are able to induce and elicit CD4-dependent immune responses *in vitro* as well as *in vivo* (Inaba *et al*, 1986). Recently, extensive research are centered around the role of Langerhans cells in the development of tumor immunity. Langerhans cells are capable of presenting tumor-associated antigens (TAA) for the induction of both primary and the elicitation of secondary immune responses (Grabbe *et al*, 1991). Interestingly, exposure of Langerhans cells to granulocyte macrophage-colony stimulating factor (GM-CSF) seems to be a requirement for successful priming of naïve animals for substantial tumor immunity. Several carcinogens, including UVR, are known to alter the number and morphology of Langerhans cells (Aberer *et al*, 1981; Stingl *et al*, 1983; Greene *et al*, 1984). UVR has been of particular interest because it is the most relevant agent with regard to human nonmelanoma skin cancer. At skin sites irradiated *in vivo* the induction

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Abbreviations: CM, complete medium; TAA, tumor-associated antigens.

of immunity against epicutaneously applied haptens is suppressed (Shimizu and Streilein, 1994). At higher doses of UVR the ability to induce contact or delayed-type hypersensitivity (DTH) is systemically impaired (Ullrich, 1986; Molendijk *et al*, 1987). The role of cytokines in these effects is suggested by the ability of UVR to induce the release of cytokines from keratinocytes (Ullrich, 1995a, b). Several of those factors, including interleukin-10 (IL-10), tumor necrosis factor-α (TNFα), and interferon-γ (IFNγ), were able to inhibit or, in the case of GM-CSF, to enhance antigen-presenting function of epidermal Langerhans cells. In this context previous work from our laboratory demonstrated that IL-1α is able to suppress the ability of epidermal antigen-presenting cells to present TAA for protective tumor immunity and that this inhibition can be abrogated by anti-TNFα (Grabbe *et al*, 1994).

In this study, we have investigated the issue of functional distinction between IL-1α and IL-1β with regard to presentation of tumor antigens. To address this question, we have examined the ability of either IL-1α or IL-1β to regulate Langerhans cell presentation of TAA for *in vivo* anti-tumor immunity. Moreover, by using epidermal cell preparations pulsed with TAA to elicit DTH activity in tumor immune mice, we used this system to examine separately the ability of the two IL-1 forms to regulate Langerhans cell presentation for induction of primary and elicitation of secondary immune responses.

MATERIALS AND METHODS

Mice Five to 8 wk old female (BALB/C × A/J)F_1[CAF_1](H-$2^{d/a}$) mice were obtained from The Jackson Laboratory (Bar Harbor, ME).

Tumors The S1509a methylcholanthrene-induced spindle cell tumor line, originally derived from A/J mice, was kindly provided by Dr. Mark I. Greene (University of Pennsylvania, Philadelphia, PA). It was maintained in tissue culture at 37°C and 5% CO_2 in RPMI-1640 supplemented with 10% heat inactivated fetal calf serum (Gibco Laboratories, Grand Island, NY), 100 U penicillin per ml, 100 mg streptomycin per ml, 0.1 mM essential and nonessential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate, and 0.01 M HEPES buffer ["complete medium" (CM)]. S1509a usually grows progressively in normal syngeneic recipients and has been demonstrated to induce a variety of immunologic responses in the host (Grabbe *et al*, 1991).

Reagents Cytokines and growth factors used in this study included recombinant murine GM-CSF, recombinant murine IL-1β (Genzyme, Cambridge, MA; specific activity, $>1 \times 10^7$ U per mg), recombinant murine IL-1α (Genzyme; specific activity, $>1 \times 10^7$ U per mg), and EGF (Collaborative Biomedical Products, Bedford, MA). The two IL-1 forms were tested for endotoxin contamination with an E-TOXATE assay (Sigma, St. Louis, MO) and were found to be endotoxin negative. Monoclonal rat anti-mouse IL-1 receptor type 1 and anti-TNFα antibodies were purchased from Genzyme and used according to the manufacturer's instructions for inhibition of IL-1α bioactivity. Anti-IL-1β was purchased from R&D Systems (Abington, U.K.). Anti-Thy 1.2 monoclonal antibody (clone NEI-001 TS) was obtained from Sigma and used at 1:2000. Low toxicity rabbit complement was obtained from Cedarlane Laboratories (Hornby, Ontario, Canada) and used at 1:40 in phosphate-buffered saline. Enzymes used during preparation and dissociation of epidermal cells were dispase (Collaborative Research, Bedford, MA), DNase (Sigma), and trypsin (Gibco, Grand Island, NY). A commercially available enzyme-linked immunosorbent assay for murine TNFα/β (Genzyme) had a sensitivity of 10 pg per ml and was utilized according to the manufacturer's instructions. For culturing primary keratinocytes, 10 cm tissue culture dishes were coated with 1 mg bovine serum albumin per ml (Boehringer, Indianapolis, IN), 10 mM HEPES buffer (Gibco), and 60 mg Vitrogen 100 per ml (Celtrix, Palo Alto, CA) in Hank's balanced salt solution (Gibco).

Soluble TAA were prepared from freeze-thaw lysates of S1509a tumor cells as described elsewhere (Grabbe *et al*, 1991). Briefly, S1509a cells (10^7 per ml in complete medium) were disrupted by three freeze-thaw cycles and centrifuged at 600 × *g* for 20 min. The supernatant was collected and spun again at 13,000 × *g* for 1 h. The remaining supernatant was used as a source of soluble TAA.

Preparation of epidermal cells and immunization protocol Epidermal cells were prepared using a standard protocol as described (Grabbe *et al*, 1991). Briefly, truncal skins of shaved and chemically depilated (Neet, Whitehall Laboratories, New York, NY) mice were removed and depleted of s.c. fat and panniculus carnosus. The skins were floated dermis side down on 0.5 U dispase per ml and 0.4% trypsin in Ca^{++}/Mg^{++}-free phosphate-buffered saline for 40 min at 37°C; epidermal sheets were then collected and dissociated by gentle strirring for 20 min. The resulting epidermal cells were filtered through nylon gauze (Nitex, Tetco, Elmsford, NY) and washed. Thy 1^+ cells were deleted by incubation in anti-Thy 1.2 monoclonal antibody for 30 min on ice, followed by washing and subsequent incubation in low-toxicity rabbit complement for 30 min at 37°C. Dead cells were removed by treatment with 0.05% trypsin and 80 mg DNase per ml in Ca^{++}/Mg^{++}-free phosphate-buffered saline for 5 min at room temperature. Typically, the epidermal cell population contained between 5% and 15% I-A^+ cells. Viability and percentage of I-A^+ cells were assessed by flow cytometry immediately before injection into mice, and differences in I-A^+ cell percentage between groups within experiments were negligible.

In order to rule out effects of IL-1α or IL-1β on epidermal cell viability, epidermal cells were cultured for 16 h in 100 U per ml and 1000 U per ml of either IL-1α or IL-1β. Controls were incubated in CM. There were no differences between the viability of IL-1 treated epidermal cells compared with controls. Also, the proportion of Ia^+ cells among the groups was not altered by IL-1 treatment as investigated by flow cytometry.

Epidermal cells were then treated with either IL-1α or IL-1β in CM containing 50 U GM-CSF per ml for 16 h at 37°C. In another experiment an additional group of epidermal cells was cultured for 1 h at 37°C with monoclonal rat anti-mouse IL-1 receptor type 1 antibodies before washing and incubation in 100 U IL-1α per ml plus 50 U GM-CSF per ml in CM for 16 h. Another group of epidermal cells was incubated with anti-IL-1 for 1 h, washed, and cultured in 50 U GM-CSF per ml in CM for 16 h. Treatment of epidermal cells with neutralizing anti-TNFα antibodies was performed at a concentration of 1:1000 during all incubation periods until injection of epidermal cells into mice. Control cells were cultured only in CM containing GM-CSF. After the incubation periods dead cells were depleted, epidermal cells were washed three times, and incubated in CM containing TAA or in CM alone (negative control) for 2 h at 37°C. After TAA-pulsing, the epidermal cells were washed extensively to remove unbound TAA. Two hundred thousand epidermal cells were then injected s.c. into each naïve recipient on the lower back. This immunization was repeated three times at weekly intervals. One week after the last immunization, mice were challenged with 2×10^6 live S1509a tumor cells s.c. on the lower lateral abdomen and tumor growth was assessed every 48 h by measurement with a Vernier caliper.

Earlier studies showed that epidermal cells treated with GM-CSF overnight and pulsed with TAA generate tumor immunity in immunized mice, leading to immunologic rejection of the tumor over a period of 7–14 d (Grabbe *et al*, 1991). The specificity of tumor immunity in this system was previously demonstrated by showing that immunization with tumor cell lysates from an unrelated tumor line (UV-5496–1) does not lead to immunity against S1509a (Grabbe *et al*, 1991).

Elicitation of S1509a tumor immunity and measurement of DTH Mice were immunized against S1509a by three injections of $0.5–1.0 \times 10^6$ dead S1509a (killed by repetitive freeze-thawing) s.c. at 5–7 d intervals. Generation of protective immunity in these mice was confirmed by rejection of a subsequent tumor challenge and induction of DTH against this tumor (data not shown).

Epidermal cells from naïve donor mice were generated and Thy-1^+ cells depleted as described above. Epidermal cells were then pulsed with S1509a TAA for 2 h, washed extensively, and 5×10^5 cells were then injected into a hind footpad of tumor-immune mice. Some groups of epidermal cells were treated with 100 U IL-1α or IL-1β per ml for 3 h before TAA pulsing. Specific footpad swelling was measured as the mean difference between the footpad thickness of the injected *versus* the uninjected side 24 h after challenge.

For heat-inactivation, 1000 U IL-1β per ml was heated at 60°C for 30 min.

Thymocyte proliferation assay Thymocytes were aseptically harvested from 3 wk old C3H/HeJ mice. Samples of 1×10^5 thymocytes were cultured in 200 μl of CM containing 4 mg PHA per ml and either IL-1α or IL-1β at concentrations 1, 10, 100, and 1000 U per ml. After 72 h of incubation at 37°C in 5% CO_2, cells were pulsed with 1 μCi of [^{3}H]TdR and incubated for an additional 6 h. At that time cells were harvested with a semiautomated cell harvester (Harvester 96, Model II, Tomtech, Orange, CT). Incorporation of [^{3}H]TdR was examined by liquid szintillation counting.

Culture of epidermal cells Mouse primary keratinocytes were prepared from 4 to 8 wk old CAF_1 mice as above and maintained in low-calcium (0.05 mM) minimal essential medium supplemented with 4% Chelex-treated fetal calf serum and epidermal growth factor (10 ng per ml; Collaborative Research, Cambridge, MA) in collagen type I coated tissue culture dishes. IL-1α or IL-1β (10, 100, 1000 U per ml) were added to the medium from the beginning of the culturing to the medium. Supernatants were harvested after 24 h and TNFα protein content evaluated with a TNFα/β enzyme-linked immunosorbent assay (Genzyme, Cambridge, MA).

Data generation and statistical evaluation Tumor volumes were calculated as the product of the maximal tumor diameter in three perpendicular directions, measured with a Vernier caliper. This method was previously confirmed to correlate well with the tumor weight (Grabbe *et al*, 1991). To avoid unnecessary pain to the experimental animals, mice were sacrificed after the tumor volume exceeded 1000 mm^3. To evaluate statistical differences between the mean tumor volume in the various experimental groups, the "best-fit" slope of the tumor growth in each animal was determined using Cricket Graph software (version 1.3.2, Cricket Software, Malvern PA) on a Macintosh computer and the significance of differences between the means of the slopes for the groups of interest tested by the two-tailed Student's t test for unpaired data. The significance of differences between the mean values obtained for DTH experiments was assessed by the two-tailed Student's t test for unpaired data.

RESULTS

Dichotomy in the effects of IL-1α and IL-1β on the induction of tumor immunity To examine the effects of IL-1α and IL-1β on the ability of epidermal antigen-presenting cells to present TAA for the induction of protective immunity in naïve recipient mice, epidermal cells were prepared and enriched for Langerhans cells as described. One group of cells was incubated in 100 U IL-1α per ml and 50 U GM-CSF per ml, whereas the other group was cultured in 100 U IL-1β and GM-CSF per ml for 16 h. Those IL-1α and IL-1β concentrations induce identical stimulation in a thymocyte *in vitro* assay (data not shown). Epidermal cells were then washed, pulsed with TAA, washed again, and injected s.c. into naïve mice for immunizations. Control groups were incubated in GM-CSF for 16 h, washed, and either pulsed (positive control) or not pulsed (negative control) with TAA before injection. These procedures were repeated with each group at weekly intervals for 3 wk. One week after the last immunization all mice were challenged with viable S1509a tumor cells at a site different from that of immunization and tumor growth was scored over time. **Figure 1**(***A***) shows that GM-CSF-treated epidermal cells can effectively present TAA for induction of substantial immunity (group A), as reported previously. GM-CSF is required for epidermal cells to present TAA efficiently for induction of tumor immunity in this system; thus exposure to GM-CSF was performed in all experimental groups. Exposure of epidermal cells to IL-1α inhibited their ability to induce protective tumor immunity, whereas culture in IL-1β did not suppress epidermal antigen-presenting cell function. The inhibition of the induction of tumor immunity by IL-1α was abrogated by neutralizing anti-TNFα antibodies (**Fig 1*B***, group D). These results were observed in three of three experiments.

Epidermal cells were prepared, enriched for Langerhans cells, and divided into three groups (**Fig 2**). One group of cells was incubated in IL-1α and GM-CSF as above (**Fig 1**, group C), another group of cells was incubated in rat anti-mouse IL-1 receptor type 1 antibody for 30 min at 37°C followed by addition of IL-1α and GM-CSF for an additional 16 h of incubation. The third group of epidermal cells was pretreated with anti-IL-1 as above and then cultured only in GM-CSF. All groups were washed, pulsed with TAA, washed again, and used for immunization. Positive and negative control groups were treated as described before. **Figure 2**, group D, demonstrates that preincubation of epidermal cells in anti-IL1 receptor type 1 antibody abrogated the inhibitory effects of IL-1α on the induction of tumor immunity by epidermal cells. Pretreatment of epidermal cells with anti-IL-1R1 alone did not result in a significant suppression (**Fig 2**, group E).

IL-1α induces a greater production of TNFα by epidermal cells than IL-1β According to previous studies in our laboratory, the suppressive effects of IL-1α on induction of immunity could be inhibited by anti-TNFα (Grabbe *et al*, 1994). We therefore investigated the modulatory effects of IL-1α and IL-1β on the production of TNFα by cultured murine keratinocytes. Keratinocytes were cultured at a concentration of 0.8×10^6 per ml for 24 h in low-Ca^{2+} medium in the presence of 10, 100, and 1000 U per ml of either IL-1α or IL-1β in collagen type I coated tissue-culture dishes. Control epidermal cell cultures were incubated in medium alone for the same amount of time. Afterwards, supernatants were collected and assayed for the presence of TNFα protein with a TNFα enzyme-linked immunosorbent assay. **Table I** shows that IL-1α induced dose dependently more TNFα compared with IL-1β. These data are from three independent experiments.

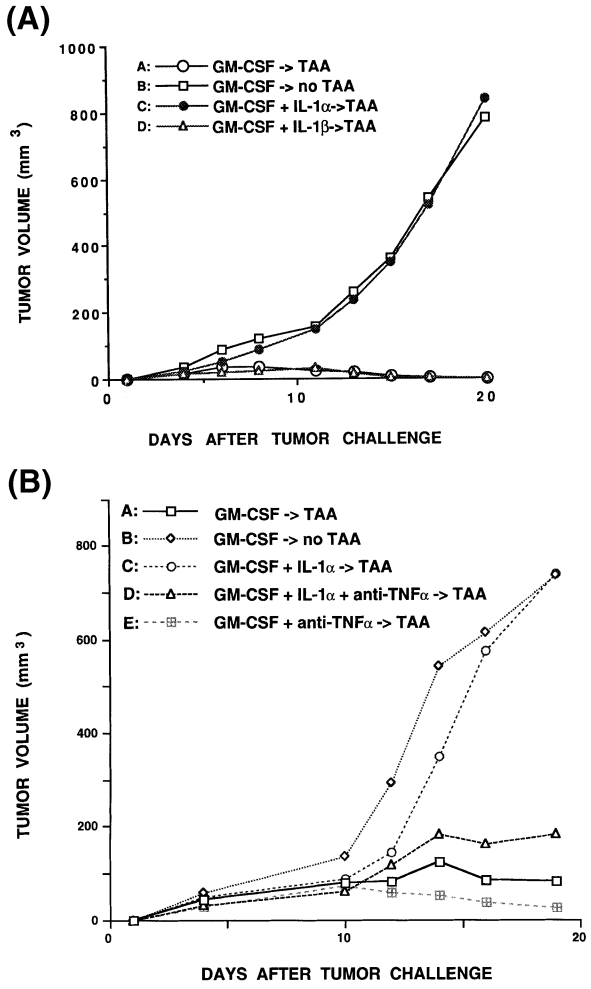


Figure 1. Differential effects of IL-1α and IL-1β on epidermal cell presentation of TAA for induction of immunity. (*A*) Epidermal cells were incubated in 50 U GM-CSF per ml plus either 100 U IL-1α per ml (group C) or 100 U IL-1β per ml (group D) for 16 h. Epidermal cells were then pulsed with TAA as described in *Materials and Methods*. Control groups were cultured in GM-CSF alone and either pulsed with TAA (group A) or not (group B). Groups of mice were immunized with 2×10^5 of these differentially treated epidermal cells at weekly intervals for a total of three immunizations. All mice were challenged with 2×10^6 viable S1509a tumor cells 1 wk after the last immunization and tumor growth was scored over time. The graph shows the mean tumor volume in mice immunized with differentially treated epidermal cells. $p < 0.001$ for A *versus* B, C; not significant for A *versus* D. (*B*) Epidermal cells were incubated in 50 U GM-CSF per ml plus either 100 U IL-1α per ml (group C) or 100 U IL-1α per ml plus anti-TNFα (group D) for 16 h. Epidermal cells were then pulsed with TAA as described. Control groups were cultured in GM-CSF alone and either pulsed with TAA (group A) or not pulsed (group B). Immunization was performed as above. $p < 0.001$ for A *versus* B, C; not significant for A *versus* D, E.

A high concentration of IL-1β can inhibit induction of tumor immunity Taking the experimental data from **Table I** into consideration, we hypothesized that epidermal antigen-presenting cell function might be suppressible by IL-1β if a much higher concentration was used in order to stimulate sufficient TNFα production. To test this hypothesis, epidermal cells were prepared, enriched for Langerhans cells, and incubated in 1000 U per ml or 100 U per ml of IL-1β without GM-CSF for 16 h. Control groups of epidermal cells were treated as above. After washing and pulsing with TAA, epidermal cells were used to immunize naïve mice three times at weekly intervals. In separate groups, Langerhans cell-enriched epidermal cells were prepared

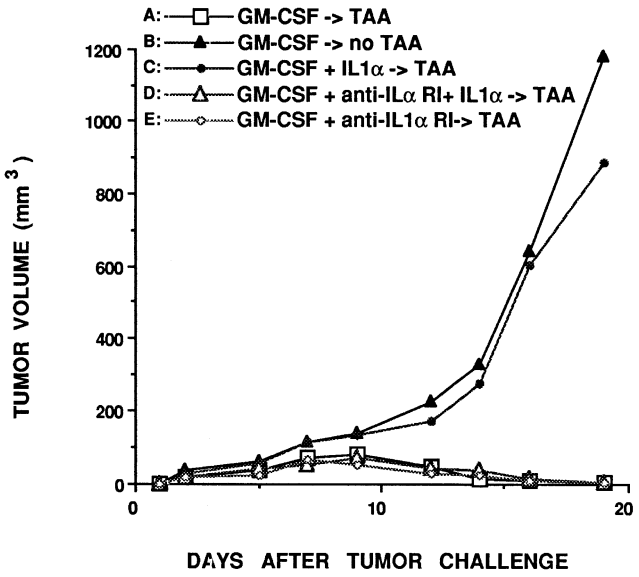


Figure 2. The suppressive effects of IL-1α could be abrogated with anti-IL-1α type 1 receptor antibody. In this experiment epidermal cells were incubated with anti-IL-1α type 1 receptor antibody for 30 min before 100 U IL-1α per ml and 50 U GM-CSF per ml were added (group D). Other groups were treated either with IL-1α plus GM-CSF (group C) or with anti-IL-1α plus GM-CSF (group E). Subsequently, these differentially treated epidermal cells were pulsed with TAA and used for immunization. Control groups of mice were treated and immunized and challenged as was described in the legend to **Fig 1**. $p < 0.001$ for A *versus* B, C; not significant for A *versus* D, E.

Table I. IL-1α is a stronger inducer of TNFα from epidermal cells than IL-1β[a]

Cytokine	Concentration (U per ml)	TNF content of supernatants (pg per ml)	p value[b]
None	–	38.8 ± 35.5	
IL-1α	10	94.1 ± 29.3	–
IL-1β	10	85.6 ± 34.3	0.171
IL-1α	100	222.4 ± 10.1	–
IL-1β	100	97.3 ± 31.0	0.009
IL-1α	1000	669.0 ± 115.2	–
IL-1β	1000	181.5 ± 88.1	0.015

[a]800,000 CAF_1 epidermal cells per ml were cultured in medium alone or cytokines as indicated for 24 h. Supernatants were then collected and assayed by enzyme-linked immunosorbent assay for TNF content.
[b]IL-1α *versus* IL-1β at each concentration.

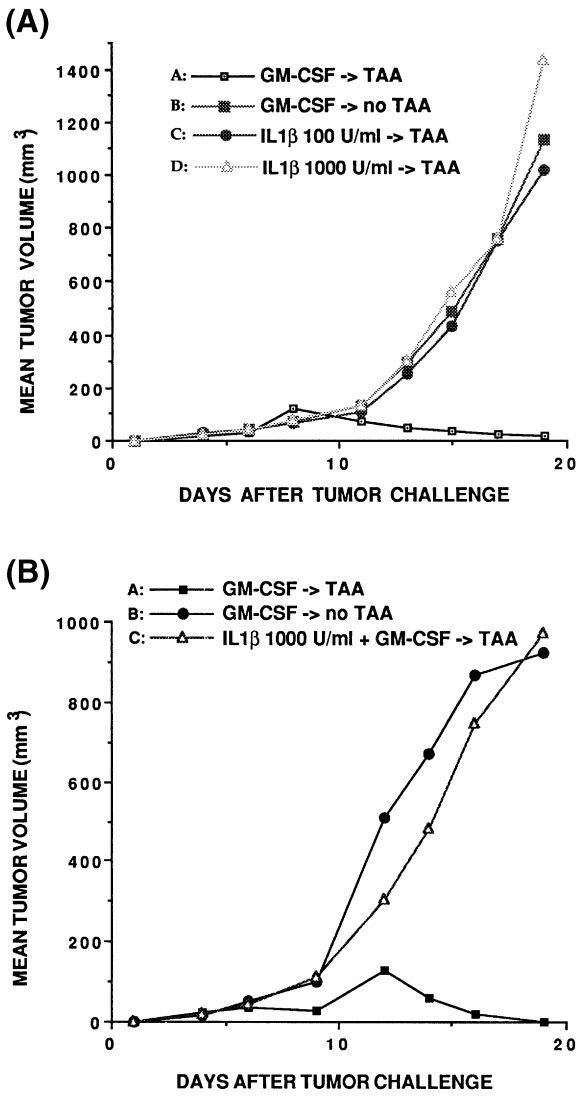


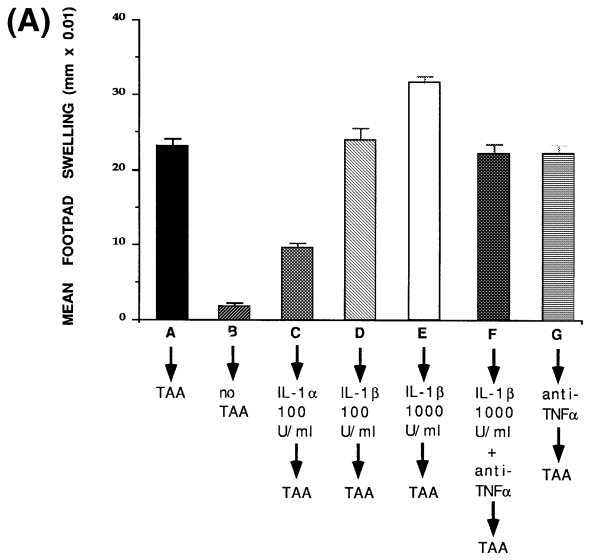
Figure 3. Very high concentrations of IL-1β suppress epidermal cell antigen-presenting function. (*A*) Epidermal cells were cultured in either 100 U per ml or 1000 U per ml of IL-1β alone before TAA pulsing and use for immunizations. Control groups were treated as above. $p < 0.001$ for A *versus* B, C, D. (*B*) In another experiment epidermal cells were coincubated with 1000 U IL-1β per ml and 50 U GM-CSF per ml, washed and pulsed with TAA, and washed again before immunization. Control groups as above. $p < 0.001$ for A *versus* B, C.

and treated with 1000 U IL-1β per ml in the presence of 50 U GM-CSF per ml. Control groups were treated as described and all groups of differentially cultured epidermal cells were, after washing and TAA pulsing, used for immunization as above. **Figure 3**(***A***, groups C and D) demonstrates that culture of Langerhans cell-enriched epidermal cells in either 100 U per ml or 1000 U per ml of IL-1β without the addition of GM-CSF fails to give epidermal cells the capability of inducing tumor immunity in this system. The data in **Fig 3**(***B***), however, shows that at the concentration of 1000 U per ml, IL-1β does inhibit the ability of GM-CSF-treated epidermal cells to induce protective tumor immunity. These results were observed in three of three experiments.

IL-1α but not IL-1β suppresses the elicitation of DTH to TAA

As the regulation of primary immune responses may differ significantly from that of secondary immune responses, experiments were performed to investigate the effects of IL-1α and IL-1β on TAA presentation by epidermal cells in a primed system. For this purpose, we employed the mouse footpad model of DTH to the S1509a tumor. Mice were immunized three times at 5 d intervals by s.c. injection of tumor fragments generated by repetitive freeze/thaw cycles of S1509a tumor cells. Those mice were immune to a subsequent tumor challenge. Groups of mice were challenged 5 d after the last immunization by injection of TAA-coupled epidermal cells into one hind footpad, and 24 h DTH was assessed as the difference in footpad thickness between the injected *versus* noninjected site. To study the effects of IL-1α and IL-1β on this system, epidermal cells that had been incubated in either 100 U IL-1α or IL-1β per ml, 1000 U IL-1β per ml or anti-TNFα for 3 h prior to exposure to TAA were examined. Another group of epidermal cells was pretreated with anti-TNFα for 30 min at 37°C, before 1000 U IL-1β per ml were added. All differentially treated epidermal cells were washed and pulsed with TAA for 2 h. Groups of cells were washed again and then injected into a hind footpad of tumor immune mice. After 24 h the DTH reaction was measured. For control groups, epidermal cells were incubated in medium alone prior to pulsing with TAA (positive control) or were not pulsed with TAA (negative control) before injection.

Figure 4(***A***) demonstrates that IL-1α significantly and substantially suppressed the elicitation phase of tumor immunity; however, incuba-



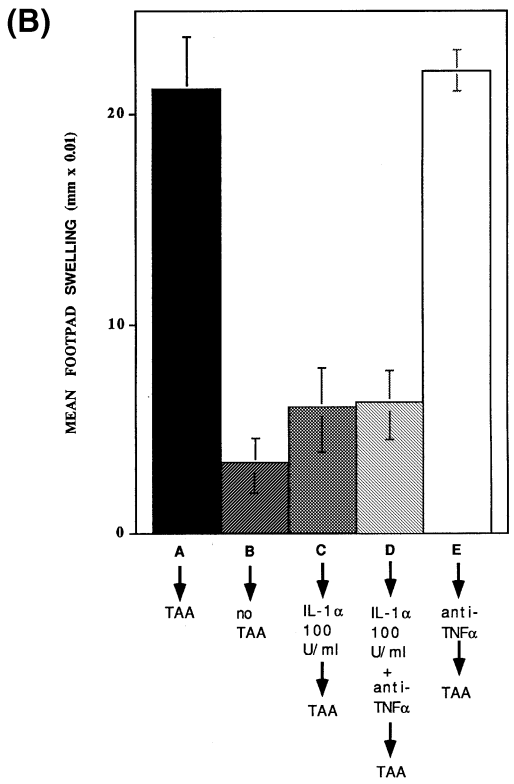


Figure 4. Incubation of epidermal cells in IL-1α but not IL-1β inhibits their ability to elicit DTH in tumor immune mice. (*A*) Mice were immunized against S1509a by s.c. injection of 1×10^6 S1509a tumor cell fragments (freeze/thaw S1509a lysates) three times at 5 d intervals. One week after the last immunization, mice were challenged for a DTH response by injection of 5×10^5 epidermal cells treated as below and footpad swelling assessed as a measure of DTH response 24 h later. Epidermal cells were incubated in either CM plus 100 U IL-1α per ml, 100 U IL-1β per ml, 1000 U IL-1β per ml, or anti-TNFα for 3 h. Another group was preincubated in anti-TNFα for 30 min at 37°C before 1000 U IL-1β per ml were added. Control groups were cultured for the same amount of time in CM only. Groups of differentially treated cells were then washed and pulsed with TAA for an additional 2 h. The control groups were not exposed to cytokines but were pulsed with TAA (positive control) or not pulsed (negative control). Error bars ± SEM. $p < 0.001$ for A *versus* B, C, E; not significant for A *versus* D, F, G. (*B*) Mice were immunized as above. Challenge was performed with epidermal cells treated with 100 U IL-1α per ml (group C), IL-1α and anti-TNFα (group D), or anti-TNFα (group E). Controls were performed as described above. $p < 0.001$ for A *versus* B, C, D; not significant for A *versus* E.

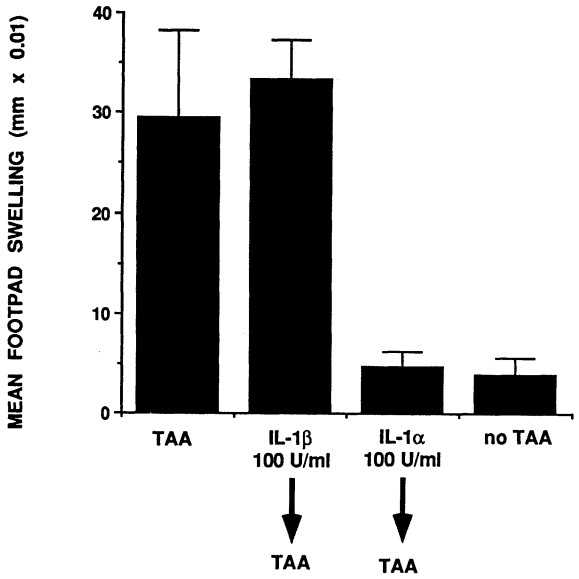


Figure 5. Incubation of epidermal cells in neutralizing anti-IL-1β antisera abrogates the increased DTH response induced by very high concentrations of IL-1β. Several groups of mice were immunized against the S1509a tumor as described. Langerhans cell-enriched epidermal cells were either treated with 1000 U IL-1β per ml (group C), pretreated with anti-IL-1β for 30 min before 1000 U IL-1β per ml were added (group D), or cultured in anti-IL-1β (group E) alone. Another group was exposed to heat-inactivated (30 min at 60°C) IL-1β (group F). Controls were performed as above (groups A and B). Subsequently, all groups were TAA pulsed and injected after extensive washing into one hind footpad as described. Error bars + SEM. $p < 0.001$ for A *versus* B, C; not significant for A *versus* D, E, F.

tion in IL-1β resulted in a slight, but not statistically significant, increase of DTH reaction against S1509a TAA. These findings were observed in three of three experiments. Interestingly, incubation with IL-1β at doses large enough to induce significant TNFα protein production (1000 U per ml) yielded a significantly increased DTH response to TAA when compared with the positive control group. This augmenting effect could be abrogated with neutralizing anti-TNFα; anti-TNFα treatment alone had no effect. This result is in agreement with our earlier report indicating that TNFα treatment of EC significantly augments DTH responses. The suppressive effects of 100 U IL-1α per ml, however, could not be abrogated by exposure to neutralizing anti-TNFα antibodies (**Fig 4*B***, group D). This suggests that at least for the S1509a-DTH assay IL-1α treatment has strong inhibitory effects, which dominate over the effects of the IL-1α-induced TNFα production.

In order to rule out the possibility that endotoxin contamination could account for our findings, an additional experiment was performed in which IL-1β was heat-inactivated by incubation at 60°C for 30 min. Tumor immune mice were injected with Langerhans cell-enriched epidermal cells that were treated with 1000 U IL-1β, anti-IL-1β per ml and 1000 U IL-1β, anti-IL-1β per ml alone or heat-inactivated IL-1β. The data in **Fig 5** (one of three independent experiments) shows that anti-IL-1β treatment abrogated the augmentation of DTH to TAA induced by treatment of epidermal cells with a high concentration of IL-1β. Neither antibody exposure alone nor exposure to heat-inactivated IL-1β had a significant effect on elicitation of DTH. These data suggest that endotoxin contamination is unlikely to be responsible for the effects demonstrated by the data in **Fig 4**.

DISCUSSION

The data presented herein indicate a dichotomy in the effects of IL-1α and IL-1β on tumor antigen presentation by epidermal antigen-presenting cells. In contrast to IL-1α, IL-1β at a concentration of 100 U per ml failed to inhibit either the induction or the elicitation of tumor immunity in mice. By blocking the IL-1 type I receptor

(80 kDa) with a monoclonal antibody prior to IL-1α incubation, IL-1α-induced suppression of the induction of immunity could be abrogated. Interestingly, freshly prepared epidermal cells produced significantly more TNFα when stimulated with IL-1α than after IL-1β stimulation. Earlier experiments performed by *Grabbe et al* showed that the inhibition of the induction of tumor immunity by IL-1α could be abrogated by anti-TNFα (Grabbe *et al*, 1994). Very high concentrations of IL-1β (1000 U per ml) were able to inhibit the induction of tumor immunity. These findings suggest that the difference seen in the effects of IL-1α and IL-1β on induction of tumor immunity may relate to the relative ability of the two cytokines to induce TNFα production. Additionally, IL-1α inhibits presentation of TAA by epidermal cells for elicitation of DTH, whereas IL-1β does not. Indeed, at 1000 U IL-1β per ml, augmentation of the DTH response was seen. This is similar to the augmentation observed in this primed system with TNFα. These observations support the concept of a differential regulation of primary *versus* secondary cellular immune responses. They also suggest that the IL-1α effect in primed systems is not mediated through TNFα and that inhibition by IL-1α dominates over the TNFα effect.

The finding that IL-1α and IL-1β have very different effects on antigen presentation by Langerhans cells has a number of important implications. First, IL-1α in the epidermis is produced primarily by keratinocytes, whereas IL-1β is produced by Langerhans cells. Thus, keratinocytes may tend to downregulate antigen presentation by Langerhans cells, and production of IL-1β by Langerhans cells may promote antigen presentation. This idea is supported by the fact that very early in contact hypersensitivity responses, IL-1β expression rises rapidly (Enk *et al*, 1993). Production of IL-1α by keratinocytes may play a role in limiting antigen presentation within the epidermis. For example, IL-1α induction is known to occur after exposure to ultraviolet radiation, a mediator of suppressed cellular immune responses. The concept that IL-1α may play a role in UVR-induced immunosuppression is supported by the knowledge that IL-1α induces TNFα expression, a cytokine shown to play an important role in UVR-mediated suppression of the induction of contact hypersensitivity (reviewed by Kurimoto and Streilein, 1992; Streilein, 1993).

Observations of differential activities of the two IL-1 forms have been seen in other experimental systems. IL-1β enhanced the number of specific antibody producing spleen cells in mice after specific antigen stimulation, whereas IL-1α was inhibitory (Boraschi *et al*, 1990). Furthermore, after injection of IL-1β into the skin, harvested Langerhans cells were more potent stimulators in alloantigen presentation assays than Langerhans cells derived from noninjected skin (Enk *et al*, 1993); however, Langerhans cells from IL-1α injected sites were suppressed in alloantigen presenting ability. Additionally, intradermally administered IL-1β led to an enhancement of major histocompatibility complex class II expression.

IL-1α is present within the murine epidermis in substantial concentrations. After midrange wavelength ultraviolet irradiation (280–320 nm) of a murine keratinocyte cell line, PAM 212, *Gahring et al* demonstrated convincingly that IL-1 release was upregulated (Gahring *et al*, 1984). Increased levels of IL-1 were also detectable in the serum of UVB treated mice. Serum concentrations peaked 48 h after irradiation and returned to normal after 96 h. In another study, IL-1α mRNA expression was found to be upregulated after application of several tumor promoters on mouse skin (Lee *et al*, 1993). No significant upregulation was seen after nontumor promoting phorbol esters or analogs of tumor promoters were used. These findings in concert with our observations, may suggest a role for IL-1α in inhibiting immune recognition of incipient tumors within the epidermis.

IL-1β was originally found to be produced primarily by macrophages; however, it was reported that IL-1β gene expression was upregulated as an early event of Langerhans cell activation with antigen. The physiologic concentration of IL-1β in the epidermis may be below that which is necessary to inhibit antigen presentation. Indeed, the work of Enk *et al* suggests it augments the induction of primary immunity *in situ* (Enk *et al*, 1993).

IL-1 binds to two cell-surface receptors. The IL-1 type I receptor is an 80 kDa protein with a larger cytoplasmatic domain than the smaller 60 kDa IL-1 type II receptor (Dower *et al*, 1986, 1990). The type I receptor mediates IL-1 effects, whereas the type II receptor acts as a "decoy" and does not signal (Colotta *et al*, 1993). *In vivo* many IL-1-responsive cells express relatively low concentrations of the type I receptor. Within the skin, high levels of type II receptors are expressed on basal epithelial cells. In our experiments the suppressive effects of IL-1α on induction of tumor immunity could be successfully blocked by preincubation of epidermal cells in anti-IL-1 receptor type I antibodies or with anti-TNFα. Interestingly in this context, is the report by Probert *et al* showing that after systemic treatment with a neutralizing monoclonal murine IL-1 receptor type I antibody, the development of TNF-induced arthritis in an murine experimental model could be abrogated (Probert *et al*, 1995). Moreover, serum TNF levels were significantly lower in IL-1 receptor type I antibody treated mice than in isotype control antibody treated mice.

Taken together these data suggest that, at least for the suppression of the induction phase of tumor immunity, IL-1α signal transduction is mediated through the type I receptor that results in the increased release of TNFα. At very high concentrations, IL-1β might be suppressive by itself or might induce enough TNFα to suppress the induction phase of tumor immunity as well.

These data demonstrate a novel aspect in the differential regulation of epidermal immunity by the two IL-1 forms.

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