THE JOURNAL OF IMMUNOLOGY

# By IL-1 Signaling, Monocyte-Derived Cells Dramatically Enhance the Epidermal Antimicrobial Response to Lipopolysaccharide



Lide Liu, Alice A. Roberts and Tomas Ganz

*J Immunol* 2003; 170:575-580; ;
http://www.jimmunol.org/content/170/1/575



*The Journal of Immunology* is published twice each month by
The American Association of Immunologists, Inc.,
9650 Rockville Pike, Bethesda, MD 20814-3994.

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

# By IL-1 Signaling, Monocyte-Derived Cells Dramatically Enhance the Epidermal Antimicrobial Response to Lipopolysaccharide[1]

**Lide Liu,* Alice A. Roberts,† and Tomas Ganz[2]***

**Epithelia react to microbial pathogens by mounting a defensive response that includes the production of antimicrobial peptides. In this study, we show that, in human epidermal cultures, *Escherichia coli* LPS was a very weak direct inducer of human β-defensin (HBD)-2 mRNA and peptide, but the induction was greatly amplified when monocyte-derived cells (MoDeC) acted as intermediaries between LPS and the epidermis. IL-1R antagonist largely reversed the effect of MoDeC on epidermal HBD-2, indicating that, from among the many products of MoDeC, IL-1 was the dominant inducer of HBD-2 synthesis. In normal fresh human skin, which contains Langerhans cells and other myeloid cell types, in addition to keratinocytes, LPS also induced HBD-2 in an IL-1-dependent manner. In DNA microarray expression studies, HBD-2 was one of the most abundant mRNAs induced in epidermis by LPS-treated MoDeC, and its induction was reversed by IL-1Ra. Thus, epidermal response to LPS is potently amplified by MoDeC through IL-1-mediated signaling, leading to a selective increase in the synthesis of the antimicrobial peptide HBD-2. This pattern of responses establishes a key role for both IL-1 and HBD-2 in the host defense reaction of the epidermis. *The Journal of Immunology*, 2003, 170: 575–580.**

Epithelia serve not only as a physical barrier against infections but also secrete substances that inhibit or neutralize invading microbes. Antimicrobial peptides and proteins are prominent components of epithelial secretions, and are either produced constitutively or induced by infection (1). In the epidermis, the antimicrobial peptide human β-defensin (HBD)[3]-2 was first isolated from psoriatic scales (2) and was later shown to be expressed in other epithelia as well (3). Our studies have shown that cell differentiation as well as a bacterial or cytokine signal is required for the induction of HBD-2 expression in keratinocytes (4, 5).

LPS (or endotoxin), an abundant glycolipid of the outer membrane of Gram-negative bacteria, is a well-studied and potent activator of inflammatory and host defense responses (6, 7). In early inflammatory response to LPS, macrophages are the major cellular targets for LPS action. Experiments with mice that lack individual Toll-like receptors (TLR) provide strong evidence that LPS-induced signal transmission depends on TLR4 (8). In a complex with LPS-binding protein and CD-14, LPS binds to TLR4 on macrophages, inducing the production of cytokines and other inflammatory mediators that have widespread effects on the host. Functional TLRs have been detected not only in macrophages but also in epithelia (9–15). Thus, two kinds of responses can be envisioned. The direct response depends on the detection of pathogen-associated molecules by epithelial TLRs or other receptor proteins and intracellular epithelial signaling. The indirect epithelial response is mediated by macrophages or other myeloid cells that detect pathogen-associated molecules and then signal to the epithelium. The relative roles of direct and indirect epithelial responses to pathogen-associated molecules have not been explored.

IL-1, present in two main agonist forms IL-1α and IL-1β, is a multifunctional cytokine produced by activated macrophages and other cell types, including keratinocytes. IL-1 mediates a wide spectrum of inflammatory, metabolic, physiologic, and immunological reactions, both locally and systemically (16, 17). IL-1R type I, the principal signaling receptor for IL-1α and IL-1β, also belongs to the Toll-like family of mammalian receptors. In the skin, and especially in the epidermis, IL-1 induces the transcription of genes involved in skin inflammation via activation of NF-κB and mitogen-activated protein kinase pathways (18). There are several physiological IL-1 inhibitors, among which IL-1R antagonist (IL-1Ra) is a central regulatory molecule for IL-1 activity (19, 20). It competes with IL-1 for receptor binding, but does not activate the receptor, and has no other molecular target as determined by epistatic genetic analysis (21). IL-1Ra has been shown to block pathological inflammatory responses induced by IL-1 (22).

We show here that the ordinarily weak effect of LPS on the epidermis is greatly enhanced by MoDeC. The resulting massive and selective induction of antimicrobial peptide HBD-2 is almost entirely mediated by IL-1.

## Materials and Methods

### *Organotypic culture and stimulation*

Primary epidermal cultures were obtained from MatTek (EPI-200-3S; Ashland, MA). The cultures, grown from normal human epidermal keratinocytes on collagen-coated Millicell CM membranes (0.45-μm pores, 24-well size; Millipore, Bedford, MA), were placed in 12-well plates with the medium supplied by the manufacturer and incubated at 37°C in a 5% $CO_2$-humidified air atmosphere. On day 4, the epidermal cultures were lifted to the air-liquid interface and then cultured in air-liquid interface for another 4 days according to the supplier's instruction. IL-1α (50 ng/ml; R&D Systems, Minneapolis, MN), *Escherichia coli* LPS (100 ng/ml; *E.*

*Will Rogers Pulmonary Research Laboratory, Departments of Medicine and Pathology, and †Department of Pathology, Geffen School of Medicine, University of California, Los Angeles, CA 90095

Received for publication July 3, 2002. Accepted for publication October 25, 2002.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by Grant HL 46809 from the National Institutes of Health.

[2] Address correspondence and reprint requests to Dr. Tomas Ganz, CHS 37-055, Department of Medicine, Geffen School of Medicine, University of California, Los Angeles, CA 90095-1690. E-mail address: tganz@mednet.ucla.edu

[3] Abbreviations used in this paper: HBD, human β-defensin; MoDeC, monocyte-derived cell; IL-1Ra, IL-1R antagonist; TLR, Toll-like receptor; CM, conditioned medium; AP, alkaline phosphatase; SPRR-2B, small proline-rich protein 2B.



0022-1767/03/$02.00

*coli* 055:B5), or diluted conditioned medium (CM; produced by incubating monocytes with LPS as detailed below) were used to stimulate the cultured epidermis with or without IL-1Ra (200 ng/ml; R&D Systems) for 2–48 h.

### *Isolation of monocytes and cell culture*

Human monocytes were isolated from three healthy volunteer donors under a protocol approved by the University of California, Los Angeles, Institutional Review Board. Freshly obtained heparinized whole blood was diluted with 0.2 volume of 1× PBS and centrifuged through Ficoll-Paque Plus (Amersham Pharmacia Biotech, Piscataway, NJ). The buffy coat cells were collected and washed with 1× PBS to remove platelets. The monocytes were enriched by centrifugation on a step 46% iso-osmotic Percoll (Sigma-Aldrich, St. Louis, MO) gradient overlaid with RPMI 1640 with 10% FCS, recovered from the interface between the Percoll and the medium, washed with 1× PBS, and counted and cultured at a concentration of $1.2 \times 10^6$/ml in RPMI 1640 with 20% autologous serum. The monocytes were 80–85% pure as judged by blood stain morphology. To prepare CM, LPS (100 ng/ml; *E. coli* 055:B5) was added into the culture medium and incubated with monocytes for 4 days. The cell-free supernatant is referred to as CM.

### *Normal human skin culture and stimulation*

The use of human skin samples in this study was approved by UCLA Institutional Review Board. Normal human skin specimens (from reduction mammoplasties) were obtained immediately after surgery. After removing the fat tissues and part of the dermis, the skin was then cut into small strips ~1 mm wide and 5 mm long. The samples were incubated in keratinocyte basal medium (Invitrogen, San Diego, CA) containing 2.5% human serum, and stimulated with LPS (100 ng/ml) with or without IL-1Ra (200 ng/ml) for 24 h.

### *Northern blot analysis*

Total RNA was isolated by using TRIzol (Invitrogen). The RNA was further purified by using RNeasy minipreps (Qiagen, Valencia, CA), according to the manufacturer's instructions. Ten micrograms of the purified total RNA from each sample was loaded onto 1% agarose formaldehyde denaturing gel, and electrophoresis was performed at 5 V/cm for 2~3 h. After rinsing the gel in distilled water and 10× SSC, the gel was transferred to GeneScreen Plus (NEN, Boston, MA) overnight. The resulting blots were rinsed with 2× SSC, and then stained with 0.04% methylene blue in 0.3 M sodium acetate for 1 min and destained with distilled water for 2 min. After photography, the blots were baked for 2 h at 80°C before hybridization. The full-length HBD2 cDNA was labeled and hybridized to the blots at 42°C in a solution containing 50% formamide, 5× SSC, 5× Denhardt solution, 1% SDS, and 100 $\mu$g/ml denatured salmon sperm DNA. Membranes were washed twice with 0.2× SSC/0.1% SDS at 42~55°C and exposed at −80°C.

### *Affymetrix GeneChip arrays and analysis*

The Affymetrix GeneChip HuGene U133 set (Affymetrix, Santa Clara, CA) was used to define gene expression profiles of the epidermis stimulated by IL-1$\alpha$, LPS, or CM from LPS-exposed monocytes, with or without IL-1Ra. Total RNA was prepared as for Northern blots, from triplicate or quadruplicate epidermal samples to average differences between individual wells. RNA labeling and microarray hybridization was performed by University of California, Los Angeles, Microarray Core Facility. Briefly, 15 $\mu$g of total RNA was used to synthesize biotinylated cRNAs. The GeneChip sets were hybridized with 15 $\mu$g of fragmented cRNA probes and then washed. The staining was performed with streptavidin-PE. Images were scanned at 3-$\mu$m resolution by using a GeneArray Scanner made for Affymetrix by Hewlett-Packard (Palo Alto, CA). Analysis was performed using GeneChip Analysis Suite 5.0 software (Affymetrix). To highlight potential changes in global gene expression, no scaling or normalization of chip signals was performed.

### *Western-blot analysis of HBD-2*

Cultured human epidermis was harvested, homogenized, and extracted with 30% acetic acid overnight. The acid extractions were lyophilized and resuspended in 1 M HCl and 1% trifluoroacetic acid. After removing insoluble debris, supernatants were transferred to new tubes and aliquots of the solution were lyophilized completely. The resulting protein pellets were dissolved in SDS sample buffer overnight at 4°C. Samples and HBD-2 standard were boiled for 5 min and then loaded onto a 16.5% SDS-tricine polyacrylamide gel (23). Transfer to Immobilon-PSQ membranes (Millipore) was performed for 1 h at 0.18 A in 0.05 M sodium borate (pH 9.0) with 20% methanol and 0.05% SDS. Blots were fixed for 30 min with 0.5% glutaradehyde in TBS (500 mM NaCl and 20 mM Tris; pH 7.5), blocked for 30 min in 0.75% Blotto (nonfat powdered milk) in Blotto PBS (0.9% NaCl and 10 mM sodium phosphate buffer; pH 7.4), then incubated for 18 h in 1:1000 rabbit anti-HBD-2 serum (5) diluted in Ab dilution buffer (0.25% Blotto in PBS containing 0.01% thimerosal as a preservative). The blots were washed in 0.1% BSA in Blotto TBS (0.9% NaCl and 20 mM Tris-HCl; pH 4.5~5.0) three times for 10 min each. The membranes were incubated in a 1/2000 dilution of alkaline phosphatase (AP)-conjugated goat anti-rabbit IgG (Pierce, Rockford, IL) in Ab dilution buffer for 1 h, then washed three times as before and developed in AP development solution (bromochloroindolyl phosphate/nitroblue tetrazolium).

### *Immunocytochemistry*

Deparaffinized human epidermal sections were rehydrated and subsequently washed with TBS (500 mM NaCl and 20 mM Tris; pH 7.5). The slides were incubated with a 1/1000 dilution of rabbit polyclonal serum in 1% gelatin TBS, 0.05% Tween 20 (Sigma-Aldrich), and 0.01% thimerosal for 18 h at room temperature. After three 20-min washes in TBS with 0.05% Tween 20, the slides were incubated with AP-conjugated goat anti-rabbit IgG (Pierce) diluted 1/2000 in the same buffer as the first Ab and incubated for overnight followed three 20-min washes. Color development was conducted with Fast Red chromogen (Sigma-Aldrich) in Tris buffer for 3 min and then counterstained with Harris hematoxylin.

## Results

### *HBD-2 peptide and mRNA are induced by the secretions of LPS-stimulated MoDeC*

Human epidermal cultures (Epiderm; MatTek) formed all the morphological layers of human epidermis: a basal layer, a spinous layer, a granular layer, and a cornified layer (Fig. 1 and H&E stain, not shown). After exposure of the epidermis to medium conditioned by LPS-treated MoDeC (CM), HBD-2 was detected by immunostaining (4) in spinous and granular layers of epidermal cultures, in a pattern similar to that seen with IL-1-stimulated epidermal cultures (Fig. 1). Western blots of epidermal extracts (Fig. 2) confirmed the appropriate immunoreactive peptide band in CM-treated epidermis but not in unstimulated epidermis. HBD-2 peptide was detectable 12 h after stimulation with CM, and continued to accumulate thereafter. By comparison to standards, the

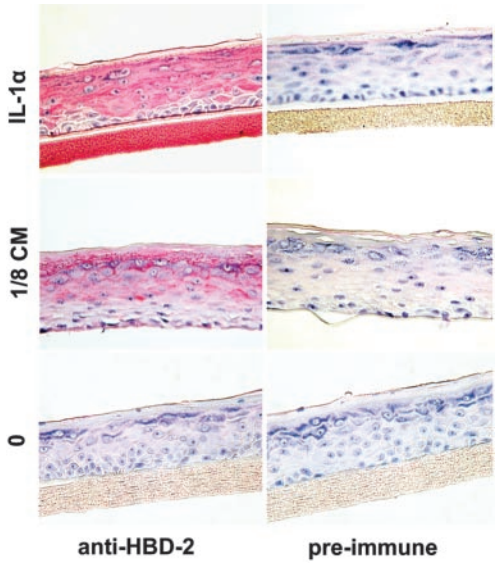


**FIGURE 1.** Induction of HBD-2 peptide expression in epidermal cultures. Epidermal cultures were stained for HBD-2 2 days after stimulation with IL-1$\alpha$ (50 ng/ml) or CM. Tissue sections with or without stimulus stained with preimmune serum were used as negative controls. The HBD-2 peptide is immunostained and developed with fast red chromogen and counterstained with Harris hematoxylin. Similar results were obtained in three independent experiments.

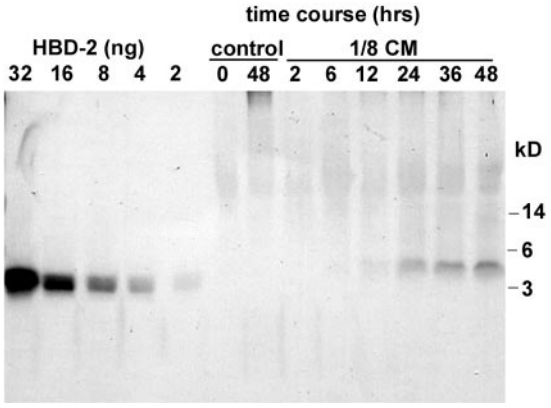


**FIGURE 2.** Western analysis of time course of HBD-2 induction. HBD-2 expression was analyzed at 2, 6, 12, 24, 36, and 48 h after stimulation with 1/8 dilution of CM. Groups of normal epidermal cultures not exposed to the stimulus were harvested at 0 and 48 h for controls. HBD-2 peptide was detectable at 12 h after CM stimulation and accumulated in keratinocytes thereafter.

amount of HBD-2 of stimulated epidermal culture after 48 h induction was estimated at 6~8 ng, i.e., ~10 $\mu$g/g of tissue, a concentration previously shown sufficient for antimicrobial activity (4). After stimulation with CM, there was a large increase in HBD-2 mRNA concentration, with a strong hybridization signal present already at 2 h (Fig. 3), peaking at 24 h and decreasing somewhat at 48 h. IL-1 was present in CM at active concentrations: IL-1$\alpha$, 380 $\pm$ 44 pg/ml, and IL-1$\beta$, 4649 $\pm$ 662 pg/ml (mean $\pm$ SD, $n = 3$ donors).

*Induction of HBD-2 by CM is blocked by IL-1Ra*

Substantial enhancement of HBD-2 mRNA induction (over the weak induction observed with LPS alone) was seen even at 1/32 dilution of CM in 1 ml of keratinocyte medium (Fig. 4), corresponding to the output of ~$4.8 \times 10^4$ monocytes/cm$^2$ of epidermis. The addition of IL-1Ra at a concentration of 200 ng/ml completely or nearly completely blocked HBD-2 mRNA induction

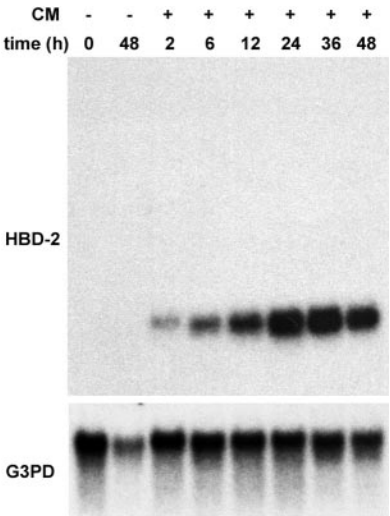


**FIGURE 3.** Northern blot analysis of HBD-2 expression in stimulated epidermal cultures. Epidermis stimulated by dilute (1/8) CM was analyzed for HBD-2 mRNA expression at 2, 6, 12, 24, 36, and 48 h after stimulation. Unstimulated epidermal cultures (−) were harvested at 0 and 48 h. Total RNA samples were loaded at 10 $\mu$g/lane. The blot was hybridized with $^{32}$P-labeled HBD-2 and G3PD cDNAs.

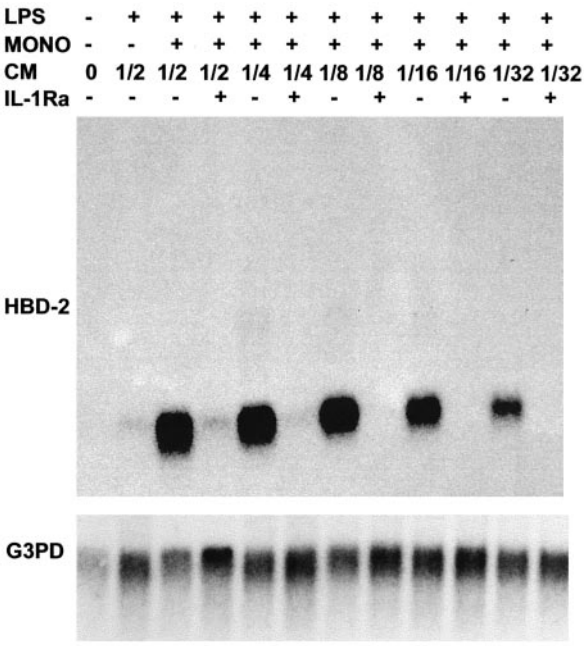


**FIGURE 4.** Effects of IL-1Ra on LPS-induced HBD-2 mRNA expression. CM-induced HBD-2 mRNA expression. Northern blot, film exposure time of 3 h. Epidermis stimulated for 48 h by diluted CM with or without IL-1Ra (200 ng/ml) was analyzed for HBD-2 mRNA expression. For comparison, epidermis exposed to LPS and culture medium for monocytes, but without monocytes, was harvested at 48 h. RNA from unstimulated epidermis cultures is shown as a control. HBD-2 mRNA was strongly induced by CM, and the induction was blocked by adding IL-1Ra.

(Fig. 4) indicating that IL-1 is the main inducer of HBD-2 expression in CM.

Direct LPS (100 ng/ml) stimulation of keratinocytes induced low or undetectable amounts of HBD-2 mRNA after 24-h incubation (Fig. 4 and data not shown). Unlike the induction of HBD-2 by IL-1 or CM, this effect was not consistent and two of four samples failed to generate detectable HBD-2 mRNA in response to LPS. In contrast to the CM-treated samples, this direct effect of LPS, when detectable, was not inhibited by the addition of IL-1Ra into the culture (data not shown), indicating that it did not depend on the intermediate generation of IL-1 by keratinocytes.

To confirm the expected effects of CM, LPS, and IL-1Ra on the levels of HBD-2 peptide, we performed Western analysis on human epidermal cultures. As shown in Fig. 5, a specific HBD-2 band was detectable in epidermal extracts after exposure to CM but the induction of HBD-2 peptide by CM was suppressed by IL-1Ra. Although in a parallel experiment we observed significant increase of HBD-2 mRNA after LPS induction, no HBD-2 peptide was detectable after 48 h of direct LPS stimulation. These data confirm that LPS alone is a weak stimulus for HBD-2 peptide synthesis in the epidermis.

*HBD-2 is among the most abundant mRNAs induced by IL-1$\alpha$ or CM*

Analysis of gene expression in epidermal cultures collected after 24 h of stimulation with 50 ng/ml of IL-1$\alpha$ showed that HBD-2 became one of the most abundant mRNAs in stimulated epidermis (Fig. 6). In CM-stimulated epidermis, HBD-2 is also among the most abundant inducible mRNAs (Fig. 6), and the induction was largely reversed by IL-1Ra. We confirmed the high abundance of HBD-2 mRNA after induction with CM or IL-1$\alpha$ in an independent experiment with CM from another donor and another sample

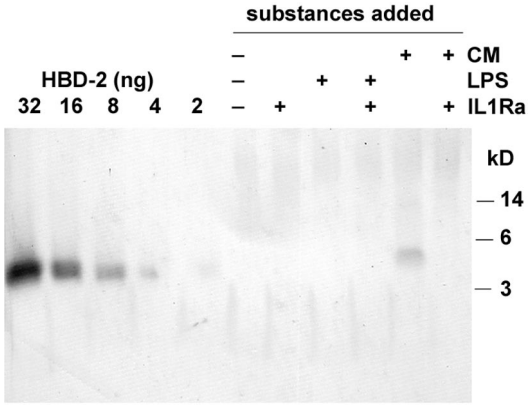


**FIGURE 5.** Western analysis of the effects of IL-1Ra, CM, and LPS on HBD-2 expression. Epidermis was harvested after 48 h of exposure to the stimuli. Unstimulated epidermal cultures (−) were harvested with the stimulated groups and used as controls. HBD-2 peptide expression was detected only in the epidermis stimulated by CM and completely inhibited by adding IL-1Ra.

of epidermis (Table I). In that experiment, global suppression of gene expression was seen after 24 h of CM and to a lesser extent after IL-1$\alpha$ stimulation (data not shown). The suppressive effect of CM on the expression of abundant genes was largely reversed by the addition of IL-1Ra (200 ng/ml) indicating that IL-1 was principally responsible for this effect as well. The biological determinants and reasons for the variability of the global suppressive effect of CM on gene expression remain to be determined.

Two other inducible mRNAs were similar in abundance to HBD-2 (Table I): psoriasin (also called S100A7, a member of the S100 calcium-binding protein family) and small proline-rich protein 2B (SPRR-2B) (Table I). In contrast to IL-1$\alpha$ and CM, LPS was a weak inducer of HBD-2 mRNA (Fig. 6), an observation consistent with Northern blot analysis (Fig. 4).

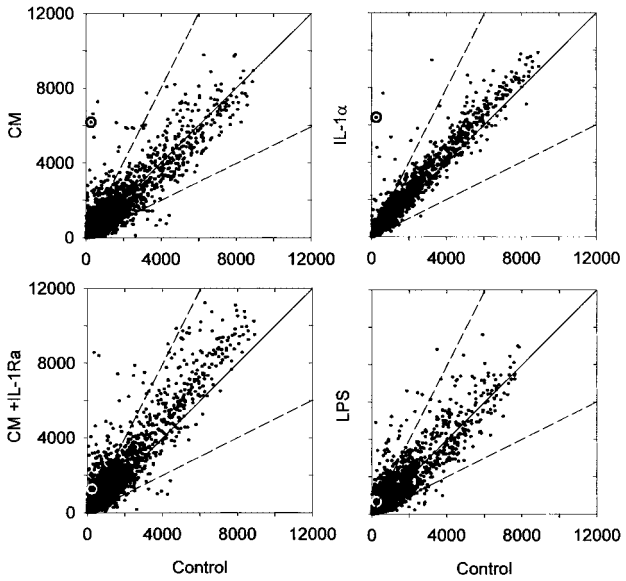


**FIGURE 6.** Gene expression changes induced by CM, IL-1$\alpha$, CM plus IL-1Ra, or LPS stimulation for 24 h. Each dot on the linear scatter plot compares the expression of a single gene in stimulated epidermis (vertical axis) to the baseline expression in unstimulated epidermis (horizontal axis). The signal from HBD-2 is marked by a circle. Signals from genes whose expression is unchanged line up on or near the diagonal (solid line). Twofold increase or decrease from control is shown by dashed lines.

Comparing the expression levels among known human antimicrobial peptides (defensins and the cathelicidin LL-37), Fig. 7 shows that HBD-2 is the only antimicrobial peptide that is robustly induced by IL-1 or CM. This finding strongly supports the hypothesis that, among the various antimicrobial peptides, HBD-2 plays a key biologic role in skin defense.

Even uninflamed human skin contains myeloid cells, chief among them Langerhans cells. Therefore, we examined whether LPS can induce HBD-2 mRNA in fresh uninflamed whole human skin and whether this response is also dependent on the intermediate generation of IL-1. In surgical specimens from three of three human donors, LPS (100 ng/ml) induced HBD-2 mRNA (Fig. 8), and this induction was abrogated by the simultaneous addition of IL-1Ra (200 ng/ml).

## Discussion

Evidence is accumulating that epithelia are not merely passive barriers to infection but that they possess the pathways that are required for the recognition of pathogen-associated molecules, and the subsequent activation of host defense effector mechanisms. Epithelial production of antimicrobial peptides is a prominent feature of such defensive responses, as first shown in the bovine tracheal epithelium exposed to LPS (24). In tracheal epithelial cells, LPS exposure induces the transcription of a bovine $\beta$-defensin, tracheal antimicrobial peptide, and the transcription is accompanied by NF-$\kappa$B activation and binding to the tracheal antimicrobial peptide promoter sequence (25). The local induction of defensin mRNA in response to local injury or infection was also observed in the keratinocytes that form the epithelium of the bovine tongue (26). In principle, the epithelial responses could be directly triggered by epithelial pattern recognition receptors such as the TLR. In support of this concept, TLRs have been detected on various epithelia by both functional and histological assays (11, 13, 14, 27). However, we noticed that the direct response to LPS, demonstrable in studies with epithelial cell lines transfected with NF-$\kappa$B reporter constructs, was relatively weak compared with the response of the same cells to cytokines such as IL-1 (28). In our organotypic epidermal cultures, the induction of HBD-2 mRNA in direct response to LPS was sometimes detectable, but IL-1 consistently induced much more HBD-2 production (4) than did relatively large doses of LPS. This raised the possibility that the epidermal responses to LPS in vivo could, under some circumstances, be amplified by other cell types that produce IL-1 in response to LPS. Such indirect mechanism would allow a graded response: colonization of the epidermal surface or minor disruption of epithelial integrity by pathogens would elicit low level activation, but a penetrating infection would expose nonepithelial cell types to the pathogens and would result in full activation of the epidermis. The molecular basis of the relative unresponsiveness of the epidermis to LPS was not directly examined in this study, but we anticipate that, like in recently studied epithelial cell lines (28), epidermal keratinocytes may express only low levels of TLRs and accessory molecules required for their function.

In earlier unpublished studies, we explored the effects of direct addition of LPS and monocytes to the apical surface of intact or abraded organotypic epidermal cultures (data not shown). In these experiments, we observed marked potentiation by monocytes of the LPS-mediated HBD-2 induction, but the magnitude of this effect was highly variable, probably due to the variable survival of monocytes under these conditions.

In the current study, we modeled the modulating effects of nonepithelial cell types on the epidermis by comparing the effects of LPS alone to those of LPS acting through the intermediate of the

Table I. *Abundant mRNAs >2-fold inducible by IL-1 and CM*[a]

| Stimulus, Expt. No. | HBD-2 Fold Induction | HBD-2 Signal Rank | Psoriasin Fold Induction | Psoriasin Signal Rank | SPRR-2B Fold Induction | SPRR-2B Signal Rank |
|---|---|---|---|---|---|---|
| IL-1$\alpha$, no. 1 | 42 | 2 | 30 | 1 | 2.2 | 3 |
| IL-1$\alpha$, no. 2 | 24.6 | 3 | 12.4 | 2 | 2.9 | 1 |
| CM, no. 1 | 10.6 | 3 | 4.4 | 4 | Suppressed | – |
| CM, no. 2 | 23.8 | 4 | 11.7 | 2 | 2.4 | 1 |

[a] Signal rank is determined relative to all mRNAs induced >2-fold.

LPS-MoDeC-CM. Compared with the effects of LPS alone, exposure of the epidermis to CM increased the induction of HBD-2 mRNA as well as of other transcripts, some of which are also implicated in host defense responses. The near complete inhibition of the effect of CM by IL-1Ra, and the presence of active range concentrations of IL-1$\alpha$ and IL-1$\beta$ in the CM supported our hypothesis that IL-1 is the key intermediate activator of the epidermal defensive response. Although we did not examine LPS-induced responses in other epithelia, our findings raise important questions about the relative magnitude, role, and mechanisms of direct vs indirect responses in all epithelia.

Like IL-1, CM induced the expression of mRNAs for HBD-2 as well as psoriasin, a nondefensin protein with a proposed role in host defense and inflammation (29). The mRNA for SPRR-2B was also abundant and inducible by both IL-1 and CM (Table I). Both psoriasin and SPRR-2B are located in a genomic cluster (30) encoding proteins involved in barrier properties of the skin. In view of the inducibility of these mRNAs by IL-1, the potential host defense role of these two proteins deserves further examination.

In the epidermis in vivo, both keratinocytes and Langerhans cells are capable of synthesizing IL-1 (31), but keratinocytes lack caspase-1 and thus cannot activate IL-1$\beta$ (32), while IL-1$\alpha$ is not readily released from keratinocytes except by mechanical stress or other injurious stimuli (33). Moreover, the release of IL-1$\alpha$ during mechanical strain is accompanied by the release of comparatively large amounts of IL-1Ra (33). Thus, it appears that Langerhans cells and, during later phases of the inflammatory response, recruited monocytes and macrophages, constitute significant sources of active IL-1 in the infected human epidermis. The density of Langerhans cells in normal human skin is $\sim 10^5/cm^2$ of epidermis (34), which is somewhat higher than the density of monocytes necessary for the potentiation of epidermal LPS response in our experiments. Even if Langerhans cells are less active producers of IL-1 than are monocytes (35), their IL-1 production in response to microbial stimulation could contribute to the activation of epidermis and the early induction of HBD-2 synthesis in infected epidermis.

In experiments with fresh whole human skin, we confirmed that normal skin responds to LPS by inducing HBD-2 mRNA and that this response is also dependent on the intermediate generation of IL-1. Because only keratinocytes produce high levels of HBD-2 mRNA, and several other abundant cell populations (e.g., fibroblasts and vascular endothelial cells) are present in the whole skin, we cannot directly compare the intensity of the response of the whole skin to that of the epidermis. The high concentration of HBD-2 in psoriatic skin (2) could be due to the higher IL-1 concentration generated in inflamed skin by myeloid cells (monocytes, macrophages, and neutrophils) recruited into the lesion.

Erdag and Morgan (36) recently reported that IL-1$\alpha$ or IL-6 stimulation enhanced epidermal antimicrobial properties of cultured keratinocyte grafts maintained on devitalized dermis. IL-1$\alpha$ was more effective than IL-6 against *E. coli* and *Pseudomonas aeruginosa* but was less effective against *Staphylococcus aureus*. RT-PCR assays of antimicrobial peptide mRNAs showed that IL-1$\alpha$ but not IL-6 treatment induced HBD-2 expression in that system. Because *S. aureus* is resistant to HBD-2 (2, 4), this antimicrobial profile would be expected if HBD-2 peptide contributed appreciably to the antimicrobial properties of epidermis. Further studies of the mechanisms of enhancement of antimicrobial properties of grafts by cytokines are certainly indicated.

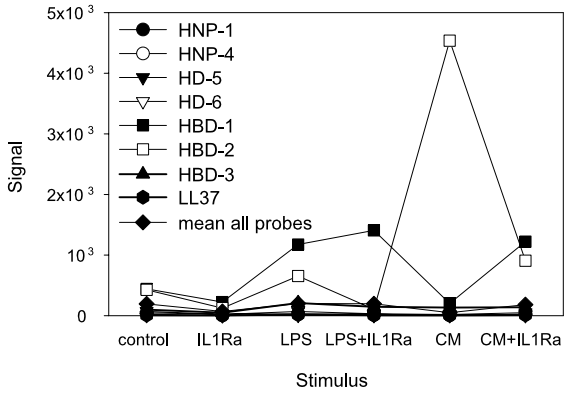


**FIGURE 7.** Effects of stimuli and inhibitors on the expression of antimicrobial peptide mRNAs. HBD-2 mRNA is the only antimicrobial peptide induced substantially by CM. The mean signal for all genes on the Affymetrix HG-U133A is also shown for comparison. Antimicrobial peptides include the $\alpha$-defensins: human neutrophil peptides (HNP)-1 and -4, human defensins (HD)-5 and -6; the $\beta$-defensins: HBD-1, -2, and -3; and the cathelicidin, LL-37. Similar results were obtained in a second experiment with CM from a second donor and another sample of epidermis.

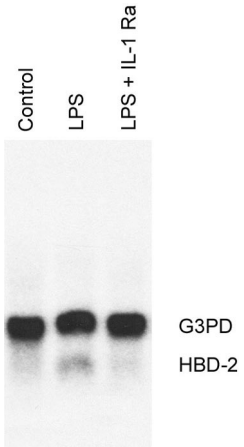


**FIGURE 8.** Induction of HBD-2 mRNA by LPS in whole human skin. Fresh whole human skin mRNA fragments were treated with solvent, LPS alone (100 ng/ml), or LPS with IL-1Ra (200 ng/ml). The Northern blot was probed with HBD-2 cDNA and G3PD cDNA. Similar results were obtained with skin samples from two other donors.

In epidermis stimulated by either IL-1 or CM, HBD-2 was among the most abundant induced transcripts detected by DNA microarrays and the only antimicrobial peptide that was substantially induced by these stimuli. In general, at least micromolar concentrations of antimicrobial peptides are required for activity against microbes. In neutrophils and Paneth cells, high concentrations of defensins are generated by release of preformed defensins from granules (37). Inside epidermal keratinocytes, HBD-2 is located predominantly in lamellar bodies that transport lipids and other substances to cell membranes and intercellular spaces (38). Compared with the granules of neutrophils and Paneth cells, lamellar bodies represent a very small storage compartment. In view of the limited storage compartment in the epidermis, rapid generation of high concentrations of peptides would require high rates of peptide synthesis and correspondingly high levels of peptide mRNAs. Based on its abundance, HBD-2 is a leading candidate among antimicrobial peptides as an effector of antimicrobial activity in the inflamed epidermis.

There is increasing evidence that antimicrobial peptides contribute significantly to the resistance of the skin to infections. Most recently, mice with ablation of the gene that encodes the murine cathelicidin cathelin-related antimicrobial peptide were found to have decreased resistance to cutaneous infection with group A streptococcus (39). In human dermatoses, the high level of HBD-2 expression in psoriatic lesions may explain the surprising resistance of these lesions to superinfection (40), and conversely, the lack of antimicrobial peptide expression in atopic dermatitis may predispose to infections (41). In the aggregate, the clinical and experimental observations point to the central role of IL-1-induced defensin HBD-2 in the host defense response of the epidermis.

## Acknowledgments

We thank Drs. Nelson and Chen in the University of California, Los Angeles, Microarray Core Facility for their constructive discussion and suggestions. We also gratefully acknowledge the assistance of Dr. Joe Kubilus at the MatTek Corporation, and Ping Fu at the Human Tissue Research Center at University of California, Los Angeles.

## References

1. Ganz, T. 2002. Epithelia: not just physical barriers. *Proc. Natl. Acad. Sci. USA 99:3357.*
2. Harder, J., J. Bartels, E. Christophers, and J.-M. Schroeder. 1997. A peptide antibiotic from human skin. *Nature 387:861.*
3. Singh, P. K., H. P. Jia, K. Wiles, J. Hesselberth, L. Liu, B. D. Conway, E. P. Greenberg, E. V. Valore, M. J. Welsh, T. Ganz, et al. 1998. Production of β-defensins by human airway epithelia. *Proc. Natl. Acad. Sci. USA 95:14961.*
4. Liu, A. Y., D. Destoumieux, A. V. Wong, C. H. Park, E. V. Valore, L. Liu, and T. Ganz. 2002. Human β-defensin-2 production in keratinocytes is regulated by interleukin-1, bacteria, and the state of differentiation. *J. Invest. Dermatol. 118: 275.*
5. Liu, L., L. Wang, H. P. Jia, C. Zhao, H. H. Q. Heng, B. C. Schutte, P. B. J. McCray, and T. Ganz. 1998. Structure and mapping of the human β-defensin *HBD-2* gene and its expression at sites of inflammation. *Gene 222:237.*
6. Raetz, C. R., R. J. Ulevitch, S. D. Wright, C. H. Sibley, A. Ding, and C. F. Nathan. 1991. Gram-negative endotoxin: an extraordinary lipid with profound effects on eukaryotic signal transduction. *FASEB J. 5:2652.*
7. Beutler, B. 2000. Endotoxin, Toll-like receptor 4, and the afferent limb of innate immunity. *Curr. Opin. Microbiol. 3:23.*
8. Poltorak, A., X. He, I. Smirnova, M. Y. Liu, C. V. Huffel, X. Du, D. Birdwell, E. Alejos, M. Silva, C. Galanos, et al. 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene. *Science 282:2085.*
9. Asai, Y., Y. Ohyama, K. Gen, and T. Ogawa. 2001. Bacterial fimbriae and their peptides activate human gingival epithelial cells through Toll-like receptor 2. *Infect. Immun. 69:7387.*
10. Cario, E., D. Brown, M. McKee, K. Lynch-Devaney, G. Gerken, and D. K. Podolsky. 2002. Commensal-associated molecular patterns induce selective Toll-like receptor-trafficking from apical membrane to cytoplasmic compartments in polarized intestinal epithelium. *Am. J. Pathol. 160:165.*
11. Cario, E., and D. K. Podolsky. 2000. Differential alteration in intestinal epithelial cell expression of Toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. *Infect. Immun. 68:7010.*
12. Fusunyan, R. D., N. N. Nanthakumar, M. E. Baldeon, and W. A. Walker. 2001. Evidence for an innate immune response in the immature human intestine: Toll-like receptors on fetal enterocytes. *Pediatr. Res. 49:589.*
13. Hornef, M. W., T. Frisan, A. Vandewalle, S. Normark, and A. Richter-Dahlfors. 2002. Toll-like receptor 4 resides in the Golgi apparatus and colocalizes with internalized lipopolysaccharide in intestinal epithelial cells. *J. Exp. Med. 195: 559.*
14. Song, P. I., T. A. Abraham, Y. Park, A. S. Zivony, B. Harten, H. F. Edelhauser, S. L. Ward, C. A. Armstrong, and J. C. Ansel. 2001. The expression of functional LPS receptor proteins CD14 and Toll-like receptor 4 in human corneal cells. *Invest. Ophthalmol. Visual Sci. 42:2867.*
15. Wolfs, T. G., W. A. Buurman, A. van Schadewijk, B. de Vries, M. A. Daemen, P. S. Hiemstra, and C. van't Veer. 2002. In vivo expression of Toll-like receptor 2 and 4 by renal epithelial cells: IFN-γ and TNF-α mediated up-regulation during inflammation. *J. Immunol. 168:1286.*
16. Dinarello, C. A. 1994. The biological properties of interleukin-1. *Eur. Cytokine Network 5:517.*
17. Dinarello, C. A. 1996. Biologic basis for interleukin-1 in disease. *Blood 87:2095.*
18. Murphy, J. E., C. Robert, and T. S. Kupper. 2000. Interleukin-1 and cutaneous inflammation: a crucial link between innate and acquired immunity. *J. Invest. Dermatol. 114:602.*
19. Debets, R., J. P. Hegmans, P. Croughs, R. J. Troost, J. B. Prins, R. Benner, and E. P. Prens. 1997. The IL-1 system in psoriatic skin: IL-1 antagonist sphere of influence in lesional psoriatic epidermis. *J. Immunol. 158:2955.*
20. Carter, D. B., M. R. Deibel, Jr., C. J. Dunn, C. S. Tomich, A. L. Laborde, J. L. Slightom, A. E. Berger, M. J. Bienkowski, F. F. Sun, R. N. McEwan, et al. 1990. Purification, cloning, expression and biological characterization of an interleukin-1 receptor antagonist protein. *Nature 344:633.*
21. Irikura, V. M., M. Lagraoui, and D. Hirsh. 2002. The epistatic interrelationships of IL-1, IL-1 receptor antagonist, and the type I IL-1 receptor. *J. Immunol. 169: 393.*
22. Freeman, B. D., and T. G. Buchman. 2001. Interleukin-1 receptor antagonist as therapy for inflammatory disorders. *Expert Opin. Biol. Ther. 1:301.*
23. Schagger, H., and G. von Jagow. 1987. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal. Biochem. 166:368.*
24. Diamond, G., J. P. Russell, and C. L. Bevins. 1996. Inducible expression of an antibiotic peptide gene in lipopolysaccharide-challenged tracheal epithelial cells. *Proc. Natl. Acad. Sci. USA 93:5156.*
25. Diamond, G., V. Kaiser, J. Rhodes, J. P. Russell, and C. L. Bevins. 2000. Transcriptional regulation of β-defensin gene expression in tracheal epithelial cells. *Infect. Immun. 68:113.*
26. Schonwetter, B. S., E. D. Stolzenberg, and M. A. Zasloff. 1995. Epithelial antibiotics induced at sites of inflammation. *Science 267:1645.*
27. Gewirtz, A. T., T. A. Navas, S. Lyons, P. J. Godowski, and J. L. Madara. 2001. Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression. *J. Immunol. 167:1882.*
28. Abreu, M. T., P. Vora, E. Faure, L. S. Thomas, E. T. Arnold, and M. Arditi. 2001. Decreased expression of Toll-like receptor-4 and MD-2 correlates with intestinal epithelial cell protection against dysregulated proinflammatory gene expression in response to bacterial lipopolysaccharide. *J. Immunol. 167:1609.*
29. Watson, P. H., E. R. Leygue, and L. C. Murphy. 1998. Psoriasin (S100A7). *Int. J. Biochem. Cell Biol. 30:567.*
30. Cabral, A., P. Voskamp, A. M. Cleton-Jansen, A. South, D. Nizetic, and C. Backendorf. 2001. Structural organization and regulation of the small proline-rich family of cornified envelope precursors suggest a role in adaptive barrier function. *J. Biol. Chem. 276:19231.*
31. Sauder, D. N., C. A. Dinarello, and V. B. Morhenn. 1984. Langerhans cell production of interleukin-1. *J. Invest. Dermatol. 82:605.*
32. Mizutani, H., R. Black, and T. S. Kupper. 1991. Human keratinocytes produce but do not process pro-interleukin-1 (IL-1)β: different strategies of IL-1 production and processing in monocytes and keratinocytes. *J. Clin. Invest. 87:1066.*
33. Lee, R. T., W. H. Briggs, G. C. Cheng, H. B. Rossiter, P. Libby, and T. Kupper. 1997. Mechanical deformation promotes secretion of IL-1α and IL-1 receptor antagonist. *J. Immunol. 159:5084.*
34. Cumberbatch, M., C. E. Griffiths, S. C. Tucker, R. J. Dearman, and I. Kimber. 1999. Tumour necrosis factor-α induces Langerhans cell migration in humans. *Br. J. Dermatol. 141:192.*
35. Rasanen, L., M. Lehto, C. Jansen, T. Reunala, and P. Leinikki. 1986. Human epidermal Langerhans cells and peripheral blood monocytes: accessory cell function, autoactivating and alloactivating capacity and ETAF/IL-1 production. *Scand. J. Immunol. 24:503.*
36. Erdag, G., and J. R. Morgan. 2002. Interleukin-1α and interleukin-6 enhance the antibacterial properties of cultured composite keratinocyte grafts. *Ann. Surg. 235: 113.*
37. Lehrer, R. I., A. K. Lichtenstein, and T. Ganz. 1993. Defensins: antimicrobial and cytotoxic peptides of mammalian cells. *Annu. Rev. Immunol. 11:105.*
38. Oren, A., T. Ganz, L. Liu, and T. Meerloo. In human epidermis, β-defensin-2 is packaged in lamellar bodies. *Exp. Mol. Pathol. In press.*
39. Nizet, V., T. Ohtake, X. Lauth, J. Trowbridge, J. Rudisill, R. A. Dorschner, V. Pestonjamasp, J. Piraino, K. Huttner, and R. L. Gallo. 2001. Innate antimicrobial peptide protects the skin from invasive bacterial infection. *Nature 414: 454.*
40. Christophers, E., and T. Henseler. 1987. Contrasting disease patterns in psoriasis and atopic dermatitis. *Arch. Dermatol. Res. 279(Suppl.):S48.*
41. Ong, P. Y., T. Ohtake, C. Brandt, I. Strickland, M. Boguniewicz, T. Ganz, R. L. Gallo, and D. Y. Leung. 2002. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N. Engl. J. Med. 347:1151.*