ACTIVATED HUMAN LANGERHANS CELLS EXPRESS mRNA FOR INTERLEUKIN-1α AND INTERLEUKIN-1β AND PRODUCE THESE CYTOKINES BUT DO NOT SECRETE THEM#

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Langerhans cells (LC) are bone-marrow derived dendritic cells that constitutively express class II major histocompatibility antigens (HLA-DR in humans, 1-3). LC remain in the epidermis for a period and migrate through lymphatics to the paracortical areas of lymph nodes, where their dendritic extensions are closely opposed to T-lymphocytes; in this site they are known as interdigitating cells (4). Dendritic cells of the LC lineage efficiently induce contact hypersensitivity (5). The number of LC in the skin is increased in humans with contact dermatitis (6). These findings have led to the suggestion that LC are the principal antigen-presenting cells (APC) in the skin (1-5).

Expression of class II MHC glycoproteins is one requirement for APC; the second is that they produce appropriate cytokines (7). It has been reported that LC p- duce IL-1 detectable by the thymocyte co-mitogenic assay (8). However, this assay is now known to be responsive to other cytokines, including IL-6 (9) and IL-7 (10). It is also apparent that the expression of IL-1α and IL-1β varies in different cell types: in monocytes the predominant form is IL-1β (11) whereas in keratinocytes it is IL-1α (12,13). To confirm that LC do, in fact, produce IL-1, as well as to ascertain which types of the cytokine are present and whether they are secreted, we have investigated both the expression of the IL-1α and IL-1β genes and their secretion, using specific cDNA probes and immunoassays.

MATERIALS AND METHODS

Preparation of LC. Single-cell suspensions of normal skin were prepared from skin obtained at female cosmetic surgery as described previously (14). Briefly, trimmed skin was cut into 1x5 cm strips and split-cut at 0.1 mm

Castroviejo keratotome (Storz, St. Louis, MO). The resulting slices were treated for 45 min at 37°C with 0.3% trypsin (M.A. Biproducts, Walkersville, MD) and 0.1% EDTA in GNK (0.8% NaCl, 0.04% KCl, 0.1% glucose, 0.084% $NaHCO_3$, pH 7.3). Dispersed cells were suspended in Dulbecco's minimum essential medium (Gibco, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (FCS, Hyclone Labs, Logan, UT), 50 µg/ml gentamycin and 2 mM L-glutamine. Viability as determined by trypan blue exclusion immediately after trypsinization was 80% or better. LC enrichment was achieved by a previously described panning technique (14). Petri dishes were coated with affinity-purified goat anti-mouse IgG (Zymed Labs, Inc., South San Francisco, CA). Epidermal cells were incubated with monoclonal anti-CD1 antibody (OKT6, Ortho Pharmaceuticals, Raritan, NY), washed and layered on the IgG-coated Petri dishes. Supernatants containing the unattached cells (LC-depleted) were collected. The cells attached to the plastic surface (LC-enriched) were scraped off using a rubber policeman or blown off by vigorous pipetting and cultured overnight in RPMI-1640 plus 10% serum and antibiotics. The culture supernatants were collected and tested for IL-1 (see below).

Extraction and analysis of RNA in LC. Cells were treated as indicated in the figure legends. Cell lysates for RNA dot blots were prepared according to procedures described by White and Bancroft (15). Alternatively, total RNA was extracted in a solution of RNAzol® (Cinna/Biotecx, Friendswood, TX) containing guanidine isothiocyanate and phenol (16). One-tenth volume of chloroform was added and the mixture was incubated at 4°C for at least 15 min. RNA was isolated from the aqueous phase of this extraction by centrifugation at 10,000xg and precipitated with 1 volume of isopropanol. The RNA pellet was washed in 70% ethanol, dried,

and heat-denatured in 7.5% formaldehyde and 6x SSC (20x SSC = 3 M NaCl, 0.3 M Na citrate) at 60°C for 15 min and rapidly cooled to 4°C. RNA was blotted onto Nytran paper (Schleicher and Schuell Inc., Keene, NH). Blots were baked for 30-120 min at 80°C in a vacuum chamber. Prehybridization and hybridization mixtures were composed of 5x Denhardt's solution (0.1% BSA, 0.1% Ficoll, Sigma Chemical Co., St. Louis, MO), 5x PIPES (0.75% M NaCl, 25 mM piperazine-N, N'-bis-2-ethane-sulfonic acid (PIPES, Sigma) and 0.25 mM EDTA), 50% formamide, 0.2% SDS, 50 mM Na phosphate, 100 µg/ml yeast tRNA (Sigma), 100 µg/ml salmon sperm DNA (Sigma), and carried out at 42°C in a water bath for 4-24 h. cDNA probes were labeled with ^{32}P by nick-translation (BRL, Rockville, MD) to a specific activity of at least 10^8 cpm/µg. Blots were hybridized with at least 10^6 cpm/ml for 17-24 h, washed for two 1 h periods in 2x SSC/0.2% SDS at 42°C, for 1 h in 0.2x SSC/0.2% SDS at 65°C, and exposed at -70°C on Kodak XAR films with an intensifying screen. The intensity of hybridization of cDNA to RNA in dot blots was quantified by scanning with an optical densitometer (Hoefer Scientific Co., San Francisco, CA). Data was collected using a Macintosh computer with a data acquisition software (Dynamax, Rainin Instrument Co., Woburn, MA) and normalized using β-actin as the internal control. Purified RNA was submitted to northern-blot analysis as previously described (17).

cDNA probes, cytokines and drugs. Human IL-1β and IL-1α, TGF-α and TGF-β, and β-actin cDNA were kindly provided by Immunex Corporation (Seattle, WA), R. Derynck (Genentech, South San Francisco, CA), and B. Endlich (University of California, San Francisco, CA), respectively. GM-CSF, IL-1α and IL-1β were obtained from Immunex Corporation. IL-6 was a gift from P. Sehgal (Rockefeller University, New York, NY).

Lipopolysaccharide (LPS, E. coli O111:B4), cycloheximide (Chx) and phorbol 12-myristate 13-acetate (PMA) were purchased from Sigma.

IL-1 assays. LC were washed with PBS and lysed with NP-40 as described (18). IL-1β and IL-1α in LC lysates and culture supernatants were assayed in a two-site sandwich ELISA as described by Kenney et al. (18,19).

RESULTS AND DISCUSSION

LC-enriched and -depleted cell fractions were cultured for 17-24 h and analyzed for IL-1 mRNA expression. Unstimulated LC did not spontaneously express IL-1β or IL-1α mRNAs. Following stimulation with phorbol myristate acetate (PMA, 100 nM), IL-1β mRNA was expressed (fig. 1A). FCS either at 2 or 10% did not affect IL-1β RNA expression. Further investigation showed the presence of both IL-1α and IL-1β mRNAs in PMA-stimulated LC (fig. 1B). While this pattern of IL-1 expression is similar to that of PMA-activated monocytic cells, IL-6 and TGF-β mRNAs, which are present in activated monocytes, were not detected in activated-LC. A variety of agents commonly used to activate IL-1 production in peripheral blood monocytes were tested. LPS, a potent stimulus for peripheral blood monocytes, did not induce IL-1 mRNA in LC. Ionomycin alone or in combination with LPS also had little effect (fig. 1B). While LPS synergizes with PMA in the expression of IL-1β mRNA in the promonocyte U937 cell line (17), LPS and PMA cotreatment of LC did not show any significant enhancement over expression induced by PMA stimulation alone (data not shown).

In an attempt to identify a physiologic stimulus which could induce IL-1 mRNA in LC, we incubated LC with insulin-like growth factor-1 (IGF-1,

100 ng/ml), granulocyte-monocyte colony stimulating factor (GM-CSF, 10 ng/ml) and interleukin-6 (IL-6, 50 U/ml). However, none of these cytokines induced IL-1 mRNA (data not shown). Furthermore, stimulation of LC with conditioned media from the cell lines 5637, GCT or MOLT, either alone or in combinations with LPS, did not result in IL-1 mRNA expression. Since LC have been shown to be efficient antigen-presenting cells in the skin, and muramyl dipeptide (MDP) and analogs have been used in subcutaneously administered vaccines to augment immune responses (20), we tested the effect of MDP treatment on LC. MDP did not activate LC to express IL-1 mRNA (fig. 1C). Moreover, MDP did not increase the level of IL-1 mRNA induced by PMA (data not shown). MDP induces the formation of IL-1 in human monocytes (20).

We have previously described the down-regulation of IL-1 mRNA by glucocorticoids in cells of the monocyte lineage (15). Dex at a concentration which effectively inhibits expression of IL-1 in induced monocytes, did not significantly decrease the IL-1β mRNA level in LC by northern blot analysis (fig. 2). In parallel studies, northern blot analysis of LC-depleted cell fractions which contained mainly keratinocytes did not show any IL-1 mRNA expression under the conditions used (fig. 2). The mechanism by which IL-1 genes are induced and regulated in LC <u>in vivo</u> has not yet been defined, but is probably different from that active in monocytes.

To confirm that the mRNA for IL-1 is translated into protein, and to ascertain whether it is secreted, cell lysates and culture supernatants were analysed using specific immunoassays for IL-1α and IL-1β. As shown in table I, both IL-1α and IL-1β were detected in unstimulated LC and the levels of both were greatly increased following induction by PMA. In the

experiment shown basal and induced levels of IL 1α were about three times higher than those of IL-1β, but in other experiments the concentrations of the two were nearly equal. In neither of two experiments was IL-1α or IL-1β detectable in supernatants of induced or uninduced LC cultures. The absence of IL-1 in supernatants is at variance with a previous report that culture medium of LC incubated for 72 h contained IL-1 activity (7). However, the thymocyte co-mitogenic assay used in now known to respond to other cytokines including IL-6 and IL-7.

Our observation that IL-1β is found within LC but is not secreted suggests that these cells may lack the specific neutral proteinase that converts the biologically inactive 31K primary gene product into the biologically active and secreted 17.5K form (21). This processing enzyme is found in cells of the monocyte-macrophage lineage but not in fibroblasts; when fibroblasts are transfected with a plasmid expressing the IL-1β gene they produce the 31K form of the cytokine which is neither processed nor secreted (21).

Evidence is accumulating that IL-1α associated with a plasma membrane may play a role in activating antigen-presenting cells in the initiation of immune responses (22). Monoclonal antibodies against IL-1α were found to activate peripheral blood monocytes and B-lymphocytes so that T-lymphocytes co-cultured with them were induced to proliferate (22). In view of the relative abundance of IL-1α in LC, membrane associated IL-1α in these cells could play a similar role, which we are currently investigating.

In contrast to the lack of secretion of IL-1 by activated LC is their capacity to release TNF-α (23). The absence of IL-6 mRNA induction in LC which we have observed is also remarkable, since IL-6 is produced by a wide range of cell types exposed to a wide range of inducers (24). In

PMA-treated LC c-fos is expressed (data not shown), and the fos protein is known to bind to nucleotide sequences in the promoter region of the IL-6 gene and act as a trans-repressor (25).

SUMMARY

Human Langerhans cells (LC) were isolated from epidermal cell preparations by panning with mouse anti-CD1 monoclonal antibody. RNA was prepared and probed for the presence of mRNAs for various cytokines using radiolabeled cDNAs. After stimulation with phorbol myristate acetate LC express RNA for IL-1α and IL-1β and produce proteins but do not secrete them at detectable levels. LC-associated IL-1, particularly IL-1α, may play a role in antigen presentation. PMA did not induce IL-6 expression in LC. The addition of lipopolysaccharide, a muramyl dipeptide analog, ionomycin, insulin-like growth factor 1 or IL-6 did not induce IL-1 mRNA in LC. Glucocorticoids did not detectably affect IL-1α or IL-1β mRNA levels following PMA induction. Thus the inducers and regulators of IL-1 formation in human LC and monocytes are not identical.

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Table I. IL-1 protein expression in Langerhans cells.

	IL-1 Protein (mean ± SD, pg) per 10^5 cells			
	Supernatants		Lysates	
Treatment	IL-1β	IL-1α	IL-1β	IL-1α
Control	ND*	ND	0.98 ± 0.49	3.46 ± 1.18
PMA (100 nM)	ND	ND	18.31 ± 1.16	52.19 ± 3.29

*, not detected.

FIGURE LEGENDS

Figure 1. Expression of IL-1 mRNA in LC. A, LC cultured in RPMI with either 2 or 10% FCS were treated with 100 nM PMA. After 17 h of incubation, RNA was extracted using RNAzol® and blotted onto Nytran paper. Probe 1, β-actin; 2, IL-1β. B. cells were cultured in RPMI with human serum and treated with PMA (100 nM), LPS (10 μg/ml), and/or ionomycin (100 nM) for 17 h. RNA dot blot was prepared as described in fig. 1A. Probe 1, β-actin; 2, IL-1β; 3, IL-1α; 4, TGF-β; 5, IL-6; 6, TGF-α. C, cells were cultured in RPMI-1640 with human serum and treated with MDP (10 μg/ml) and PMA (100 nM) for 17 h. Three-fold dilutions of cell lysates were blotted onto Nytran paper (left panel, 1,2,3; right panel, 4,5,6) and hybridized to β-actin (left panel) and IL-1β (right panel) cDNAs. Neither IL-1β nor IL-1α mRNA was detected in LC-depleted epidermal cells in all the experiments described here (data not shown).

Figure 2. Northern blot analysis of RNA from activated LC and LC-depleted epidermal cells. LC ($8x10^5$ cells) and LC-depleted epidermal cells ($2.3x10^6$ cells) were treated with PMA or PMA and Dex for 17 h. Total RNA were extracted as described in fig. 1A. Top panel shows blot hybridized to IL-1β cDNA probe. Bottom panel shows ethidium bromide staining of similar amounts of RNA from control and treated samples detected on the agarose gel. Lane 1, control; lane 2, PMA (100 nM) and lane 3, PMA (100 nM) + Dex (1 μM).

TABLE LEGEND

Table I. IL-1 expression in LC. Samples were analysed in triplicate and the sensitivity of the tests was about 10 pg/ml. Data were expressed as IL-1 (pg) per 100,000 cells.

Fig. 1

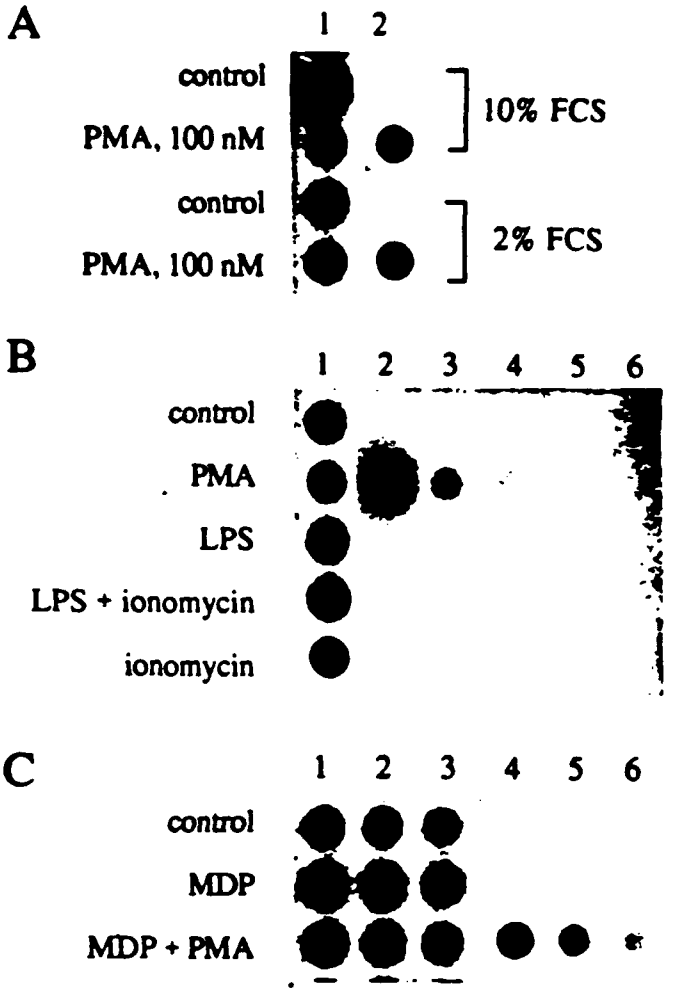
A
1 2
control
PMA, 100 nM
10% FCS
control
PMA, 100 nM
2% FCS
B
1 2 3 4 5 6
control
PMA
LPS
LPS + ionomycin
ionomycin
C
1 2 3 4 5 6
control
MDP
MDP + PMA

Fig 2