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In Vitro Modulation of Keratinocyte-Derived Interleukin-1α (IL-1α) and Peripheral Blood Mononuclear Cell-Derived IL-1β Release in Response to Cutaneous Commensal Microorganisms

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The ability of a range of skin commensal microorganisms to modulate interleukin-1 (IL-1) release by cultured human keratinocytes and peripheral blood mononuclear cells (PBMCs) was investigated by a combination of enzyme-linked immunosorbent assays and bioassays. Three fractions (formaldehyde-treated whole cells, culture supernatants, and cellular fractions) were prepared from *Propionibacterium acnes*, *Propionibacterium granulosum*, *Staphylococcus epidermidis*, *Staphylococcus capitis*, *Staphylococcus hominis*, and *Malassezia furfur* serovar B. The levels of immunochemical IL-1α released by cultured keratinocytes during coincubations with these microbial fractions ranged from 0 to 136 pg/ml and were maximal after 72 h. No microbial fraction consistently upregulated immunochemical IL-1α release by freshly isolated keratinocytes from two donors and a transformed cell line, all of which produced the cytokine constitutively to various extents. Bioassays revealed that most of the IL-1 released was biologically inactive. In contrast, whole cells of formaldehyde-treated *P. granulosum* and *S. epidermidis* significantly stimulated release of IL-1β by PBMCs from three donors compared with the negative control (culture medium). Release was maximal at 24 h. Coincubation with intact cells of the yeast *M. furfur* significantly decreased levels of IL-1β below the values for the negative control by PBMCs from all three donors. There was good correlation between bioassay data and immunoassay data for IL-1β, and the depressive effect of *M. furfur* cells on cytokine production by all three cultures of PBMCs was mirrored in the levels of bioactive cytokine. This reduction in IL-1β release by PBMCs by *M. furfur* may provide an explanation why dermatoses thought to be caused by this yeast are essentially noninflammatory or only mildly inflammatory.

The two interleukin-1 (IL-1) agonist species (IL-1α and IL-1β) are thought to play a central role in the induction of an inflammatory response—a feature of many dermatoses. The primary source of IL-1α and so-called IL-1α-like material, detectable in significant levels within the normal and diseased epidermis (5, 7, 15), is the keratinocyte (24, 30, 39). It has been postulated that the release of keratinocyte-derived IL-1α may serve to recruit T lymphocytes into the epidermis in an antigen-independent manner and costimulate the expression of vascular endothelial adhesion molecules (2). IL-1β is also produced by keratinocytes but remains inactive (32). More importantly, IL-1β is a secreted product of the activated mononuclear phagocyte fraction of the blood (10) and as such may well be detectable within the cutaneous target regions of migrating monocytes/macrophages and contribute to the inflammatory IL-1 activity as a whole within the dermis, which may participate in the further activation of primed $CD4^+$ T cells. Histological investigations in inflammatory diseases such as acne vulgaris and psoriasis have confirmed the presence of $CD4^+$ T-cell infiltrates, increasing HLA-DR and intercellular adhesion molecule 1 expression (3, 26, 34).

Although the synthesis and abundant release of IL-1α by keratinocytes appears to be constitutive (14, 23, 24, 32), several physical and environmental agents have been shown to increase IL-1α mRNA and bioactive IL-1α production including UV B irradiation, retinoic acids, urushiol (a low-molecular-weight allergen), phorbol 12-myristate 13-acetate (PMA) either alone or in combination with lipopolysaccharide (LPS), and cytokines such as tumor necrosis factor alpha (TNF-α) (2, 25, 36, 39, 44). Mononuclear phagocyte production of IL-1β can be mediated by a range of signals which include complement, LPS, and other microbial products (10, 35).

The cutaneous commensal microflora includes *Propionibacterium*, *Staphylococcus*, and *Malassezia* species, which are readily isolated from the surface and pilosebaceous follicular ducts of normal and acne-affected skin (17, 27), and from the majority of open comedones in acne vulgaris (19). The continuous proximity of the microbial flora with the epidermis coupled with events, such as injury which may lead to the entry of microorganisms and their antigens into the tissue layers, has generated speculation, investigation, and conflicting evidence regarding the immunological localized and systemic effects of commensal microorganisms. The potent immunostimulatory effects of propionibacteria have been well documented (12). Monocytes incubated with heat-killed or formaldehyde-killed *Staphylococcus epidermidis* cells have been shown to release IL-1β (6) and TNF-α (43), but in contrast, glutaraldehyde-fixed *S. epidermidis* incubated with monocytes did not elicit any substantial IL-1β or TNF-α release (29). *Malassezia furfur*, although believed to be the causative agent in pityriasis versicolor and some episodes of catheter-related sepsis (31), has also been shown to possess adjuvant activity (41, 42). The

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capacity of *M. furfur* to stimulate cytokine release from mononuclear phagocytes has not been previously investigated.

In view of the potent immunostimulatory capacity of the cutaneous microflora, it was important in the study of inflammatory dermatoses to determine whether these microorganisms and their products could upregulate the in vitro release of the proinflammatory cytokine IL-1 by human keratinocytes and mononuclear phagocytes.

MATERIALS AND METHODS

Keratinocytes. Normal human keratinocytes (NHK) were isolated by aseptic technique from neonatal foreskins. The skin samples were washed in phosphate-buffered saline (PBS) three times, chopped finely, and immersed in Leibovitz medium (ICN Flow) supplemented with 2 mg of dispase per ml (Boehringer-Mannheim) for 18 h at room temperature. Epidermal layers were peeled from the dermal layers and incubated at 37°C for 10 min in trypsin-EDTA (Sigma) to disaggregate keratinocytes. The trypsin was inactivated, and the resulting cell suspension was washed in Dulbecco's modified Eagle's medium (DMEM; Life Technologies), supplemented with 0.06 μg of penicillin per ml (Britannia Pharmaceuticals), 100 μg of streptomycin per ml (Heyl), 10% (vol/vol) fetal calf serum (Life Technologies), 20 mM L-glutamine (Life Technologies), and 0.35% (wt/vol) $NaHCO_3$ (Life Technologies). NHK were seeded in flasks (Falcon) at a 1:1 ratio onto mitomycin-treated (Sigma) 3T3 mouse fibroblast feeder layers ($2.7 \times 10^4/cm^2$) and cultured for 2 or 3 passages in keratinocyte serum-free medium (K-SFM; Life Technologies), supplemented with 5 ng of epidermal growth factor (Life Technologies) per ml and 50 μg of bovine pituitary extract per ml (Life Technologies) at 37°C in 5% (vol/vol) CO_2 in air.

Virus-transformed keratinocytes of the cell line SVK14, were cultured in DMEM culture medium. Cells were trypsinized, washed, and resuspended in K-SFM or DMEM culture medium.

PBMCs. Peripheral blood mononuclear cell (PBMC) fractions were prepared from blood samples from three healthy volunteers (donors I, II, and III). Heparinized whole blood was added to equal volumes of Macrodex (Nycomed) and incubated at 37°C for 1 h to allow the formation of erythrocytic rouleaux. The supernatant was removed, centrifuged at $500 \times g$ (Mistral 3000i; MSE) for 5 min, and the leukocytes were resuspended in RPMI 1640 (Life Technologies) supplemented with 0.06 μg of penicillin per ml, 100 μg of streptomycin per ml, 20 mM (vol/vol) *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (HEPES; Life Technologies), and 5% (vol/vol) fetal calf serum for separation on Lymphoprep (Nycomed) by density centrifugation ($800 \times g$ for 20 min), after which PBMCs were removed. The cells were washed and resuspended in RPMI culture medium (RPMI supplemented with penicillin, streptomycin, 20 mM HEPES, 10% [vol/vol] fetal calf serum, and 2 mM [wt/vol] L-glutamine).

Microbial fractions. One isolate of *Propionibacterium acnes*, *Propionibacterium granulosum*, *S. epidermidis*, *Staphylococcus capitis*, *Staphylococcus hominis*, and *M. furfur* serovar B was obtained by swabbing the skin of the faces or necks of normal volunteers with a sterile swab moistened in wash fluid (PBS with 0.05 [vol/vol] Triton X-100 [Sigma], pH 7.9). After species confirmation by typing, batch cultures of not less than 1 liter were prepared of the six microorganisms: propionibacteria were grown at 37°C anaerobically in 2% (wt/vol) tryptone, 1% (wt/vol) yeast extract, and 0.5% (wt/vol) glucose medium (TYEG; Unipath Ltd.) for 49 h (*P. acnes*) and 120 h (*P. granulosum*). *S. epidermidis* and *S. capitis* were grown aerobically (25 and 28 h, respectively) at 37°C, in synthetic staphylococcal (SS) medium modified from that of Cove et al. (8) containing the following (concentrations in grams per liter): potassium phosphate, 15.2; Casamino Acids, 10; citric acid, 9.15; glucose, 10; ammonium sulfate, 2; sodium chloride, 1.5; tryptophan, 0.3; magnesium sulfate, 0.2; nictotinic acid, 0.05; pyridoxine hydrochloride, 0.0125; pantothenic acid, 0.0025; thiamine hydrochloride, 0.025; biotin, 0.001, ferrous sulfate, 0.02; manganese sulfate, 0.02; calcium chloride, 0.02; zinc chloride, 0.005; cobalt chloride, 0.005; and copper sulfate, 0.001. Casamino Acids were obtained from Difco, vitamins; ammonium sulfate, sodium chloride, and glucose were supplied by Sigma; all other reagents were obtained from Merck. *S. hominis* was grown aerobically in tryptone yeast extract (TYE) medium for 23 h at 37°C. *M. furfur* was grown aerobically for 114 h in modified milk (MM) medium (28) at 34°C. Cultures were harvested during stationary phase when the optical density was maximal. Culture purity was determined after batch preparations and during the subsequent processing. Intact microbial cells were washed and either treated with formaldehyde overnight (1% [vol/vol] formaldehyde [Merck] in PBS) at 4°C, rewashed in PBS and resuspended in RPMI culture medium, or after storage at −70°C, were sheared by vortexing with sterile glass beads in ice-cold PBS. Cell wall debris was removed by centrifugation at 12,000 $\times g$ (Sorval; Dupont) for 10 min, the remaining soluble cellular fraction was assayed for total protein content (Bio-Rad Microassay), and freeze-dried. The microbial medium supernatant was adjusted to pH 7.4, filter sterilized, assayed for protein content, and freeze-dried. Both freeze-dried fractions were reconstituted in RPMI, K-SFM, or DMEM culture medium. Growth medium controls (TYEG, SS, TYE, and MM) were prepared by the method described for the medium supernatant.

Coincubation of keratinocytes or PBMCs with microbial fractions. Keratinocytes were seeded in 96-flat-well plates (Falcon) at 1.5×10^4/well in volumes of 200 μl in K-SFM (NHK strains) or DMEM culture medium (SVK14) and incubated overnight in 5% (vol/vol) CO_2 in air in a humidified modular incubator (ICN) at 37°C to allow attachment. K-SFM or DMEM was then aspirated from the wells and replaced with triplicate 200-μl volumes of the appropriate medium containing microbial fractions. PBMCs were seeded into 96-U-well plates at 10^5 cells per well in volumes of 100 μl of RPMI culture medium. A further 100 μl of RPMI culture medium containing microbial fractions was added to wells in triplicate. Final concentrations of microbial cells were 6×10^5/well (with keratinocytes) or 2×10^6/well (with PBMCs), medium supernatants and cellular fractionates were 1 μg of protein per ml. The microbial cell to keratinocyte/PBMC ratio and concentrations of medium supernatants and cellular fractions were previously determined to be optimal. In extensive preliminary experiments, U937 cells and SVK14 cells were tested with a range of concentrations of microbial cells and microbial products from mid-log and stationary-phase cultures and cytokine release was measured. Those results then determined the conditions for this study. Appropriate microbial growth medium controls were incorporated (1 μg of protein per ml). K-SFM or DMEM with keratinocytes served as negative controls. PMA (10 ng/ml; Sigma) was also incubated with keratinocytes, since it has been reported to enhance cytokine release by these cells in vitro (4, 44). For PBMCs, LPS (from *Escherichia coli*; Sigma) at 10 ng/ml and RPMI culture medium, served as positive and negative controls, respectively. Plates were incubated at 37°C for 72 h in 5% (vol/vol) CO_2 in air in a humidified modular incubator. Coincubation supernatants (200 μl) were harvested at 0, 24, and 72 h from the wells and centrifuged at $8,000 \times g$ (Microcentaur; MSE) for 3 min. Aliquots of each triplicate supernatant were stored at −20°C for IL-1 assays. Growth and viability of keratinocytes and PBMCs were estimated at 0, 24, and 72 h by an adaptation of the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) cleavage assay (33). A_{570} were determined (reference wavelength, 630 nm; MR7000 platereader; Dynatech).

ELISAs. Keratinocyte supernatants were assayed for IL-1α by an enzyme-linked immunosorbant assay (ELISA) (Biotrak IL-1α; Amersham International). PBMC supernatants were assayed for IL-1β by an ELISA (Cistron IL-1β; Lab Impex). Levels of IL-1 were expressed in units of specific activity (picogram of cytokine per optical density at 570 nm).

IL-1 bioassays. Keratinocyte and PBMC supernatants were measured for their levels of biologically active IL-1α and IL-1β by a murine proliferation assay adapted from that of Falk et al. (13), as previously described (19). Specificity was confirmed with anti-human IL-1α and IL-1β neutralizing antibodies (British Biotechnology). The IL-1 levels were expressed as picograms per milliliter.

Statistical analysis. Following analysis of variance of the ELISA data (picograms of cytokine per optical density at 570 nm), the minimum significant differences (MSD) between means ($n = 3$) were calculated by the T method (40).

RESULTS

Immunochemical IL-1α release by cultured human keratinocytes in response to microbial stimuli. To investigate the capacity of a range of cutaneous microorganisms to modulate IL-1 release in vitro by cultured human keratinocytes, we measured the levels of IL-1α present in coincubation supernatants at 0, 24, and 72 h by an ELISA. Biological activity was also measured on selected samples (see below).

The levels of immunochemical IL-1α released by keratinocytes in response to coincubation with microbial fractions ranged from 0 to 136 pg/ml and were generally maximal at 72 h. MTT cleavage by the keratinocytes did not display substantial variation between 0 and 72 h, indicating that no loss of viability occurred with prolonged incubation. The amounts of IL-1α in units of specific activity for the negative controls incorporated into each keratinocyte experiment were as follows: NHK strain I, 0.23 ± 0.09; NHK strain II, 0.12 ± 0.10; and SVK14, 0.05 ± 0.01. PMA failed to enhance IL-1α release by keratinocytes in these experiments. However, when coincubated with microbial fractions, IL-1α levels increased, an indication of the responsive capacity of the keratinocytes. The response of NHK strain I was greater than NHK strain II or SVK14—whose values of IL-1α specific activity were very low (≤0.1 irrespective of the microbial stimulus). Compared with the negative control, only three statistically significant increases (MSD $P < 0.01$) were observed in the specific activities of IL-1α released by keratinocytes; NHK strain I coincubated with intact cells of *S. epidermidis* (Fig. 1), NHK strain II coincubated with cellular fraction of *M. furfur* (data not shown),

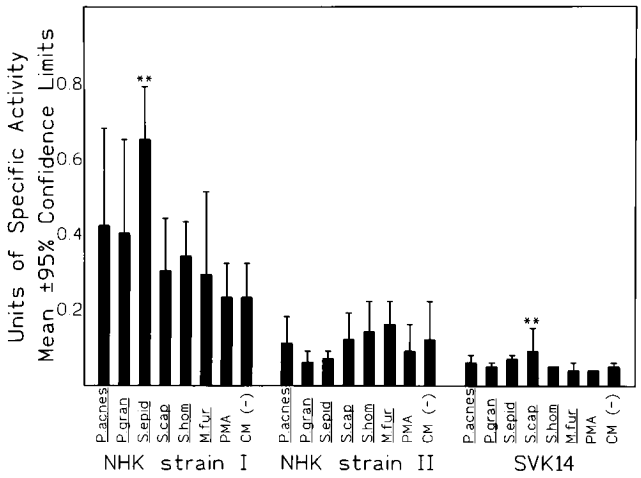

FIG. 1. Specific activities of immunochemical IL-1α released by NHK strains I and II and SVK14 coincubated for 72 h with microbial cells. PMA and negative controls [CM (−)] are also shown. Significant increases (MSD $P < 0.01$ [**]) in comparison with the negative control are indicated. *P. acnes*, *P. acnes*; *P.gran*, *P. granulosum*; *S.epid*, *S. epidermidis*; *S.cap*, *S. capitis*; *S.hom*, *S. hominis*; *M.fur*, *M. furfur.*

and SVK14 cells coincubated with intact cells of *S. capitis* (Fig. 1). Only coincubation of *P. granulosum* supernatant and cellular fractionate with NHK strain II produced significant decreases (MSD $P < 0.05$) in specific IL-1α activity compared with the negative control (data not shown). All significant changes were observed after 72 h of coincubation.

Bioactive IL-1α release by cultured human keratinocytes in response to microbial stimuli. Bioactive IL-1α was measured in the majority of keratinocyte supernatants at 0, 24, and 72 h. Of these samples, the IL-1α levels in 74% were undetectable (≤1.5 pg/ml), and the remainder contained up to 464 pg/ml (data not shown), so it was not possible to apply analysis of variance to these data. Consistent with the immunochemical data, maximal levels of bioactivity were most frequently observed after 72 h of coincubation, and once again, PMA did not significantly stimulate cytokine release. There was no correlation between the levels of bioactive IL-1α in the 26% positive samples and the corresponding values for immunochemical IL-1α (Pearson's product moment correlation coefficient $r = 0.0199$, P was nonsignificant).

Immunochemical IL-1β release by PBMCs in response to microbial stimuli. The levels of immunochemical IL-1β measured in mononuclear cell supernatants ranged from 15 (the lower limit of detection of the ELISA) to 11,400 pg/ml and were generally maximal at 24 h. MTT conversion levels did not vary substantially between 0 and 72 h, again indicating that the PBMCs remained viable throughout the coincubation period. The LPS positive control showed statistically significant increases compared with the negative control in six of nine determinations (two of three occasions with PBMCs from each of the three donors, Fig. 2 to 4).

Compared with the negative control, coincubation of PBMCs with intact cells of *P. granulosum* and *S. epidermidis* significantly increased (MSD $P < 0.01$) specific IL-1β activity in all three cultures of donor cells (Fig. 2). Coincubation with intact cells of *S. capitis*, *S. hominis*, and *P. acnes* also significantly increased PBMC IL-1β specific activity but in only one of the donor cultures (Fig. 3 and 4). Surprisingly, in all three PBMC cultures, there was a significant decrease (MSD $P < 0.01$ for donors I and II and MSD $P < 0.05$ for donor III)

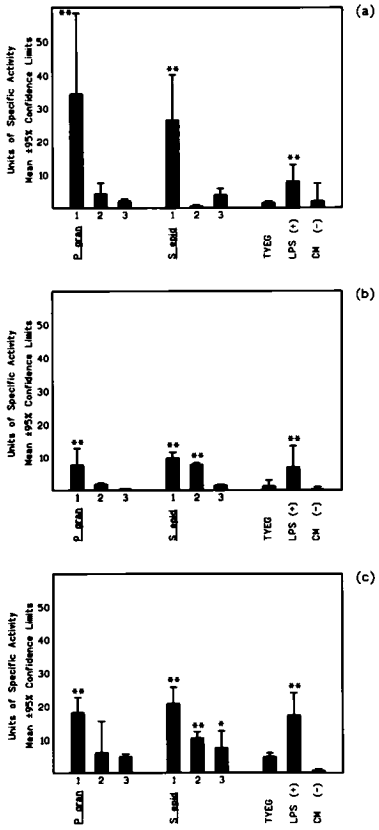

FIG. 2. Specific activities of immunochemical IL-1β released by PBMCs from donors I (a), II (b), and III (c) coincubated for 24 h with microbial cells (bars 1), microbial culture supernatants (bars 2), and microbial cellular fractions (bars 3). Culture medium control (TYEG) and positive [LPS (+)] and negative [CM (−)] controls are also shown. Significant increases (MSD $P < 0.01$ [**] and MSD $P < 0.05$ [*]) in comparison with the negative control are indicated. *P. gran*, *P. granulosum*; *S. epid*, *S. epidermidis.*

compared with the negative control in specific IL-1β activity during coincubation with intact cells of *M. furfur* (Fig. 4).

In addition to these effects of intact microbial cells, there were other significant increases or decreases in IL-1β specific activity observed in one or two but not all three PBMC cultures during coincubation with *S. epidermidis* supernatant, *S. epidermidis* cellular fractionate, *P. acnes* supernatant (increases), or the uninoculated growth medium of *P. acnes* (decrease).

Bioactive IL-1β release by PBMCs in response to microbial stimuli. Bioactive IL-1β was measured in a selection of PBMC supernatant triplicate samples harvested at 24 h, as determined by a preliminary screening of samples. The levels of bioactive IL-1β in these samples ranged from 0.15 to 9.51 ng/ml (data not shown) and showed a highly significant degree of correlation with the corresponding immunochemical values (Pearson's product moment correlation coefficient $r = 0.577$; $P < 0.001$). Furthermore, these bioactivity data confirmed the pronounced depression of IL-1β release by all three donor PBMC cultures coincubated with whole cells of *M. furfur* compared with culture medium and LPS (Table 1).

DISCUSSION

In this study, we have examined in vitro IL-1α release by keratinocytes and IL-1β release by PBMCs coincubated with whole cells and fractions of cutaneous microorganisms. Our

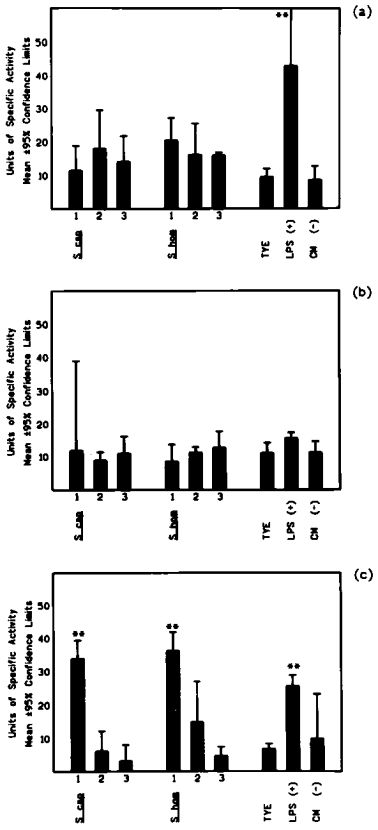

FIG. 3. Specific activities of immunochemical IL-1β released by PBMCs from donors I (a), II (b), and III (c) coincubated for 24 h with microbial cells (bars 1), microbial culture supernatants (bars 2), and microbial cellular fractions (bars 3). Culture medium control (TYE) and positive [LPS (+)] and negative [CM (−)] controls are also shown. Significant increases (MSD $P < 0.01$ [**]) in comparison with the negative control are indicated. *S cap*, *S. capitis*; *S hom*, *S. hominis.*

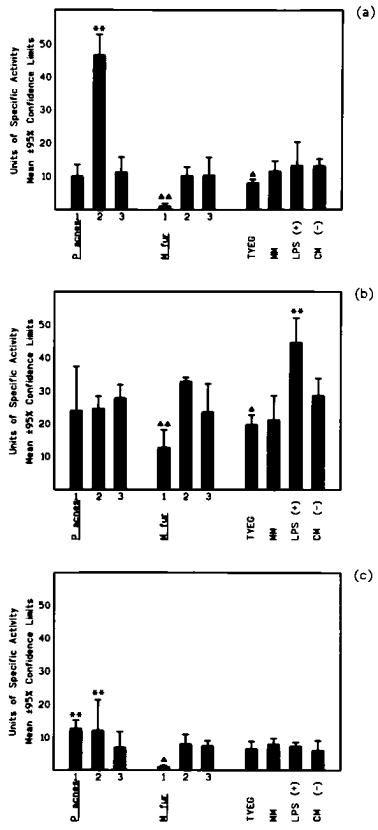

FIG. 4. Specific activities of immunochemical IL-1β released by PBMCs from donors I (a), II (b), and III (c) coincubated for 24 h with microbial cells (bars 1), microbial culture supernatants (bars 2), and microbial cellular fractions (bars 3). Culture medium controls (TYEG and MM) and positive [LPS (+)] and negative [CM (−)] controls are also shown. Significant increases (MSD $P < 0.01$ [**]) in comparison with the negative control are indicated. Significant decreases (MSD $P < 0.01$ [▲]; MSD $P < 0.05$ [▲▲]) in comparison with the negative control are indicated. *P. acnes*, *P. acnes*; *M. fur*, *M. furfur.*

data indicate that low levels of IL-1α are released constitutively by cultured human keratinocytes. Although isolated significant increases in specific IL-1α activity in response to whole cells of *S. epidermidis* and *S. capitis* treated with formaldehyde and the cellular fraction of *M. furfur* were detected, these results did not support the hypothesis that cutaneous microorganisms and their products will consistently upregulate IL-1α release by keratinocytes. There were no uniform keratinocyte responses, indicating inherent donor-dependent biological variation. The data demonstrate the importance of using different strains of keratinocytes, and especially NHK, in experimental work of this nature.

In this study, no significant increases in specific immunochemical IL-1α activity or bioactive IL-1α release were associated with exposure to PMA. Other studies have apparently shown that similar levels of PMA enhance transcription of keratinocyte IL-1α and β mRNA or increase cell-associated bioactive IL-1, but not the released functional protein (2, 4, 25). One published study, a comparison of the in vitro effects of PMA and 13-*cis* retinoic acid on bioactive IL-1α release showed a significant increase over the control value with PMA (44). To our knowledge, apart from this report, only PMA combined with LPS has been adequately demonstrated to enhance keratinocyte IL-1α secretion (36).

The association between bioactive IL-1α and immunochemical IL-1α release was also examined in this study through the use of a highly sensitive bioassay in addition to an ELISA. Immunochemical IL-1α released appeared to be generally biologically inactive. However, many factors, such as cytokine inhibitors, antagonists, or even synergists, may have been present in the samples assayed, which could be reflected in the results (46). Furthermore, the microflora of the epidermis is undoubtedly present among a host of other factors which may provide independent or costimulatory signals for the production and release of bioactive keratinocyte cytokines in vivo.

Coincubation of microorganisms and their fractions with PBMCs from three donors produced various results as determined by the release of IL-1β. The time scale of the IL-1β

TABLE 1. Levels of bioactive IL-1β released by PBMCs during coincubation with intact cells of *M. furfur*

Stimulus	Amt (ng/ml) of bioactive IL-1β released by PBMCs from donor:		
	I	II	III
M. furfur (intact cells)	0.22 ± 0.09	2.20 ± 1.67	0.20 ± 0.12
Culture medium[a]	2.02 ± 3.35	5.06 ± 1.83	1.36 ± 2.13
LPS[b]	5.45 ± 4.83	6.65 ± 6.21	2.71 ± 1.13

[a] Negative control.
[b] Positive control.

release was in agreement with the observations of others in that maximal cytokine release occurred after 24 h (16). Whole cells of *P. granulosum* and *S. epidermidis* treated with formaldehyde consistently enhanced IL-1β production by PBMCs from all three donors, while their culture filtrates and cellular fractions were less active. Interestingly, formaldehyde-treated whole cells of the closely related coagulase-negative staphylococci, *S. capitis* and *S. hominis*, and of the predominant *Propionibacterium* species on human skin, *P. acnes*, only significantly enhanced IL-1β release by PBMCs from donor III. The variability between different PBMC cultures in response to all stimuli, including LPS, which may have reflected the individual donor immune responses to their own cutaneous microflora and interdonor variations, rather than a group effect, should be anticipated. Although the culture system contained both lymphocytes and monocytes together with the microorganisms, each donor was known to have differential counts of these cells within the normal ranges. The finding that not all cutaneous species of propionibacteria and staphylococci universally enhance IL-1β production by PBMCs questions the conventional wisdom that bacteria invariably enhance proinflammatory cytokine release by mononuclear cells. This merits further investigation, since it may have important implications in our understanding of the normal relationship between the commensal cutaneous microflora and host defense mechanisms. The conflicting evidence in the literature of the effects of *S. epidermidis* on cytokine production by mononuclear phagocytes (6, 29, 43) may be explained by the results of this study which indicated variation in the responses of PBMCs from different donors to different staphylococci. *S. epidermidis* is a very heterogeneous species, and it is likely that different biotypes will have differing potencies with respect to their ability to stimulate mononuclear cells. Some strains are covered with exocellular slime which is likely to interfere with phagocytosis and hence the ability of the organisms to promote cytokine release.

It is interesting that those organisms which consistently stimulated release of IL-1β from PBMCs did not also stimulate release of IL-1α by cultured keratinocytes. This discrepancy has been noted before. *E. coli* has been shown to upregulate the production of a wider range of cytokines by PBMCs than any of several epithelial cell lines (1). Thus, the lack of effect of skin microorganisms on IL-1 release may not reflect their effect on the production of other cytokines.

In this study, *P. acnes* failed to enhance IL-1β release by PBMCs from two of the three donors. This is surprising, given the extensive literature on the immunopotentiating properties of propionibacteria (*P. acnes* is synonymous with *Corynebacterium parvum*; 9, 38). The difference may simply reflect the fact that the strain of *P. acnes* used in this study was a fresh isolate, whereas those used by other investigators were established laboratory strains selected specifically for their immunopotentiating properties and perhaps not representative of wild-type *P. acnes.*

The most significant observation from this study was that irrespective of PBMC donor, both immunochemical and bioactive IL-1β release by PBMCs was consistently and significantly decreased below the negative-control levels with whole cells of *M. furfur* serovar B treated with formaldehyde. As far as we are aware, this is the first time that a microorganism has been shown to depress the production of a cytokine. *M. furfur* forms part of the skin flora in the normal adult population but has also been implicated in a range of dermatoses including pityriasis versicolor (22), seborrhoeic dermatitis (21), and dandruff (45). In these diseases, the cellular infiltrate is minimal compared with the fungal load. The presence of *M. furfur* and concurrent decrease in proinflammatory cytokine release by mononuclear phagocytes could account for the essential noninflammatory nature of these dermatoses. The depressive effect of this yeast on IL-1β production was demonstrated with whole cells and was not therefore caused by exocellular products such as azelaic acid. The exact mechanism mediating the depressive effect is not known. It is possible that the lipid content of the yeast cell wall (10 to 20%; 18) enables the yeast cell to evade stimulating the mononuclear cells to enhance IL-1β production, but this does not explain why the levels of cytokine are decreased below the constitutive level. The phenomenon is not a general property of all yeasts, because *Candida albicans* has been shown to stimulate the production of a range of cytokines including IL-1 by monocytes and/or macrophages (11, 20, 37). It is possible that *M. furfur* expresses IL-1 receptors or that IL-1 is nonspecifically adsorbed by the cells, but our subsequent investigations to test this possibility provide no evidence to support this. Nevertheless, the ability to depress production of a proinflammatory cytokine may be a novel pathogenic mechanism possessed by *M. furfur* to help it to evade detection by the immune system.

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