HUMAN KERATINOCYTES CONTAIN mRNA INDISTINGUISHABLE FROM MONOCYTE INTERLEUKIN 1α AND β mRNA

Keratinocyte Epidermal Cell–derived Thymocyte-activating Factor Is Identical to Interleukin 1

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IL-1 is now recognized as a family of polypeptides that mediate a wide variety of biological responses to injury and infection (1). Two biochemically distinct forms of IL-1 are produced by monocytes and macrophages; these two forms have thus far been found in every species studied, and have now also been characterized by cDNA cloning experiments, using mRNA from both human and murine monocytes as the starting material (2–4). The cDNAs have been expressed in *Escherichia coli* and the rIL-1 proteins have been purified to homogeneity (2–4). IL-1β (pI 7.0) is the predominant species of IL-1 in activated human monocytes, and mRNA coding for this molecule composes ≥90% of total monocyte IL-1 mRNA (3, 4). IL-1α (pI 5.0) is correspondingly less abundant in human monocytes (4). The secreted forms of both IL-1β and IL-1α gene products appear to mediate similar if not identical biological activities on a wide variety of target tissues (5).

Recently (1, 6), it has become evident that secretion of polypeptides with IL-1-like activity is not limited to cells of the monocyte/macrophage lineage. Notably, keratinocytes have been shown to produce an epidermal cell–derived thymocyte activating factor (ETAF) that is functionally and biochemically similar to IL-1 (7). However, a recent report (8) has raised the possibility that ETAF and IL-1 may differ by certain criteria. It has also become clear that the epidermis represents an enormous reservoir of IL-1 activity; 1 gm of human stratum corneum contains 10^6 units of IL-1 activity (9). In our hands, this is comparable to the amount of IL-1 found in 10 liters of conditioned medium from LPS-stimulated human monocytes (our unpublished observations). When IL-1 activity in the viable layers of epidermis is also taken into account, it would appear that epidermis contains quantities of IL-1 activity that are comparable to those in the entire reticuloendothelial system. The present study was designed to compare

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monocyte-derived IL-1 and keratinocyte-derived ETAF at the molecular level. Evidence is presented that strongly suggests that IL-1 and ETAF are identical. In addition, keratinocytes appear to produce predominantly IL-1α, in contrast to LPS-stimulated human peripheral blood monocytes that produce predominantly IL-1β.

Materials and Methods

Keratinocytes. A431, a cell line derived from an epidermal carcinoma, was obtained from Lloyd King (Vanderbilt University, Nashville, TN) and was maintained in DMEM containing 10% FCS. Human foreskin keratinocytes (HFK) were grown by the method of Rheinwald and Green (10). For all studies, confluent cultures of HFK were maintained in the absence of the 10^{-6} M hydrocortisone normally included in the standard culture medium (10).

Antibodies. A rabbit antiserum to human monocyte IL-1 primarily active against IL-1β (11) was provided by Charles Dinarello (Tufts University School of Medicine, Boston, MA). An IgG fraction of rabbit antiserum to human monocyte IL-1 that neutralizes both IL-1α and IL-1β activity (R. Newton, personal communication) was provided by Robert Newton (DuPont Co., Glenolden, PA).

IL-1. HFK- and A431-conditioned media were obtained after incubation of fresh tissue culture medium with confluent cultures of the appropriate cell line for 48 h. Affinity-purified human monocyte IL-1β (11) was provided by Charles Dinarello.

IL-1 Assay. The assay to detect IL-1 activity uses the T cell clone D10 and the clonotype-specific mAb 3D3 or Con A, and has been described in detail (12). 6 h before harvest, [^{3}H]thymidine (1 μCi per well; 1 Ci = 37 GBq) was added. Cultures were harvested on an automated sample harvester (Cambridge Technology, Inc., Cambridge, MA) and radioactivity was measured with a scintillation spectrometer (Beckman Instruments, Inc. Fullerton, CA).

S1 Nuclease Protection Assay. An S1 nuclease protection assay requires the use of an end-labeled probe complementary to the mRNA to be detected (13). To achieve this, cDNA clones for human IL-1α (14) and IL-1β (3) were first modified by deleting one of the homopolymeric tails used in the original cloning. S1 probes, free of homopolymeric tails, were then generated for IL-1α by labeling a 960-bp Hind III–Pst I fragment at the Hind III site in the strand complementary to the mRNA, and for IL-1β by labeling an 855-bp Hind III–Taq I fragment at the Hind III site in the strand complementary to the mRNA. The specific activities of these probes were determined for each preparation and were usually on the order of $1-2 \times 10^4$ dpm/fmol. 2–5 fmol of end-labeled α and β probes were hybridized separately to 5–50 μg of total RNA sample in 80% deionized formamide, 0.4 M NaCl, 1 mM EDTA, 40 mM Pipes (pH 6.8) at 52°C overnight. After this incubation, each sample was digested for 60 min at 37°C with 100 units of S1 nuclease (15). The products were subsequently analyzed on a 5% sequencing gel. The sizes of the protected fragments in this assay are 210 nucleotides for IL-1α (corresponding to the coding region for amino acids 64–134 of the complete IL-1α precursor) and 525 nucleotides for IL-1β (corresponding to the coding region for amino acids 133–269 of the complete IL-1β precursor plus 116 nucleotides of the 3′ untranslated region).

To determine relative mRNA levels, films were scanned at 550 nm and areas under the peaks were cut out and weighed. The values obtained were corrected for the fact that the specific activity of the IL-1α probe was 1.9 times that of the IL-1β probe.

Results and Discussion

Affinity-purified human monocyte IL-1β induces the proliferation of D10 cells in the presence of 3D3 antibody, shown in Fig. 1*A*. This activity can be substantially blocked by an antibody specific for human IL-1β (11). Similarly, a second polyclonal antibody which neutralizes both IL-1α and β with equal

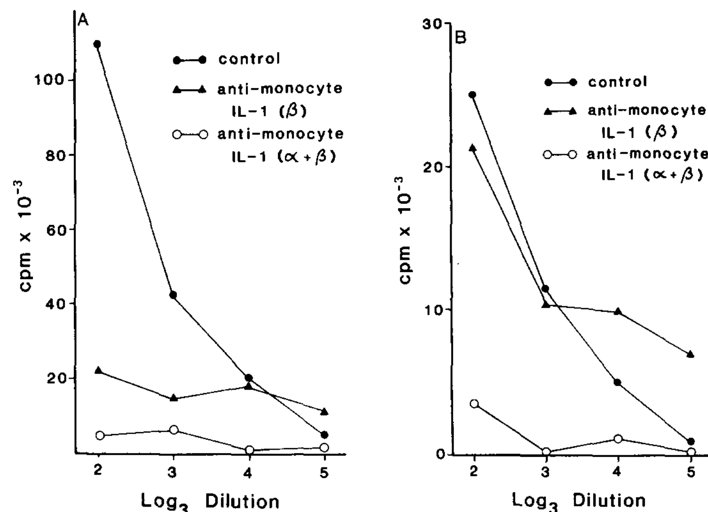

FIGURE 1. (*A*) Monocyte IL-1β is neutralized by two different antibodies to IL-1. Serial dilutions of 25 μ/ml of affinity-purified monocyte IL-1β (11) were added to 2 × 10^4 D10 cells in the presence of a 1:1,000 dilution of 3D3 hybridoma supernatant. In addition, 1% nonimmune rabbit serum (*filled circle*), 1% immune rabbit serum active against IL-1β (*filled triangle*), or 125 μg/ml of immune rabbit serum (IgG fraction) that neutralizes both IL-1α and β (Newton, R., personal communication) (*open circle*) were added. Both antibodies neutralize monocyte IL-1 activity. (*B*) Keratinocyte ETAF IL-1 activity is neutralized by an antibody that neutralizes IL-1α and IL-1β, but not by an antibody that neutralizes only IL-1β. Serial dilution of a 25% conditioned medium from HFKs were added to 2 × 10^4 cells in the presence of 1:1,000 dilution of 3D3 hybridoma supernatant. Antibody additions are identical to those in Fig. 1*A* (see legend).

efficency (Newton, R., personal communication) also blocks this activity. These antibodies had no effect on the IL-2-induced proliferation of D10 cells, indicating both specificity and lack of nonspecific cytotoxicity (data not shown). Fig. 1*B* shows that conditioned media from HFK also have significant IL-1 activity, as measured by proliferation of D10 cells in the presence of 3D3 antibody. In contrast, this activity cannot be neutralized by the antibody specific for monocyte IL-1β. The antibody that blocks both IL-1α and IL-1β activity, however, neutralizes this IL-1 activity completely. These data suggest that the IL-1 activity present in human keratinocyte–conditioned medium more closely resembles IL-1α than IL-1β.

HFK and A431 cells represent homogeneous populations of normal and malignant keratinocytes, respectively. A comparison of RNA extracted from these cell lines with RNA from LPS-stimulated human peripheral blood leukocytes was made by an S1 nuclease protection assay, using cDNA probes for human monocyte IL-1α and β. Such an assay permits the inference of identity if protection from S1 digestion occurs, since the S1 enzyme digests single-stranded but not double-stranded RNA or DNA. While complete homology between cDNA and mRNA results in a perfectly matched double strand resistant to S1 digestion, even single base mismatches in the cDNA/mRNA hybrids would result in the degradation of the labeled probe by S1 nuclease.

Fig. 2, *A* and *B* shows an S1 nuclease protection assay using both the α and β

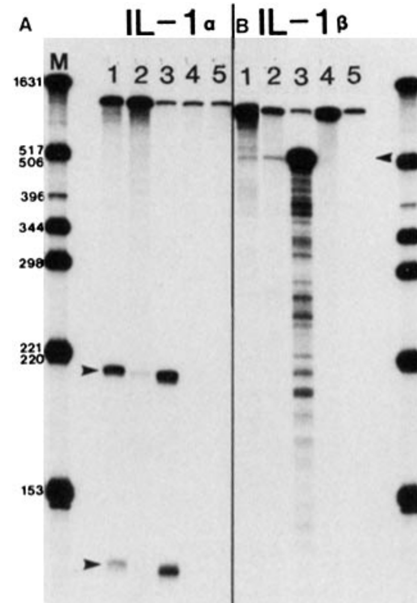

FIGURE 2. S1 nuclease protection assay of total RNAs from HFKs, A431, LPS-stimulated peripheral blood leukocytes, and pig intestine using ^{32}P-labeled cDNA probes for human monocytic IL-1α (*A*) and IL-1β (*B*). The protected fragments are indicated by *arrowheads*. See text for further explanations. Samples are: lane *1*, uninduced HFKs (20 μg); lane *2*, A 431 (16 μg); lane *3*, LPS-stimulated peripheral blood leukocytes (8 μg, positive control); lane *4*, pig intestine (30 μg, negative control); lane *5*, no RNA. *M*, marker (pBR322-Hinf I digest); *N*, sizes in nucleotides. The films were scanned at 550 nm and the relative ratios of IL-1α to IL-1β RNAs were determined from the areas under the peaks (determined by weighing), correcting for the fact that the specific activity of the α probe was about twofold higher than for the β probe. See Results.

probes, respectively. A comparison of the first lanes in Fig. 2, *A* and *B* indicates that normal unstimulated HFK contain mRNAs that protect IL-1α and IL-1β cDNA probes from S1 digestion. The reason that the IL-1α probe gives rise to two protected fragments even for the homologous RNA from LPS-stimulated peripheral blood leukocytes (positive control) is due to the presence of an S1-sensitive site rich in A-T basepairs located ~125 nucleotides away from the labeled end of the probe. This observation lends further support to the notion that protection from S1 digestion in such an assay is a stringent criterion for completed nucleic acid homology. Two to three times more mRNA for IL-1α than β is present in HFKs; in contrast, LPS-stimulated monocytes (lane *3*) express 10–20-fold more IL-1β mRNA than IL-1α mRNA. A431 cells appear to contain substantially less IL-1 mRNA per total RNA, and contain approximately five times more IL-1β than α mRNA (lane *2*). Porcine intestine RNA does not hybridize with either probe (lane *4*), and no spontaneous S1-mediated digestion of labeled probe is seen when RNA is not included in the reaction (lane *5*).

The data presented above indicate that, by several criteria, ETAF and IL-1 are identical. As shown by S1 nuclease protection analysis, mRNAs from both a malignant human keratinocyte line (A431) and HFK protect cDNA probes for monocytic IL-1α and IL-1β from S1 digestion. Given the stringency of the S1

assay, it can be concluded that keratinocytes, like monocytes, contain IL-1α and IL-1β mRNAs. This conclusion is further supported by the finding that keratinocyte ETAF activity is completely blocked in vitro by antibodies to monocytic IL-1. Keratinocytes contain more IL-1α mRNA than IL-1β mRNA in the absence of apparent stimulation. This relative abundance of IL-1α mRNA is also seen after UVB irradiation in vitro, which significantly enhances IL-1 gene expression.[1] IL-1 protein secreted by keratinocytes also consists predominantly of IL-1α, since an antibody that blocks IL-1β does not neutralize this activity. In contrast, monocyte IL-1β mRNA and protein is at least 10 times more abundant than IL-1α (3, 4).

Unstimulated human monocytes contain very small quantities of IL-1α and β mRNA (reference 4 and our unpublished observations); however, after stimulation with LPS, significant levels of these mRNAs can be detected. While we cannot exclude the possibility that picograms of LPS are responsible for the apparent constitutive production of IL-1 mRNA and protein in cultures of HFK, we cannot detect LPS in our cultures by the limulus assay, and the addition of polymixin B (16) has no effect on resting keratinocyte production of IL-1 activity (not shown). Therefore, we consider this possibility unlikely.

Both IL-1α and IL-1β appear to act through the same receptor to induce the same biological effects (5). Keratinocyte IL-1 (produces more α than β) and monocyte IL-1 (produces more β than α), however, may play different biological roles. Skin is the principal barrier between the external environment and the body. The release of preformed IL-1 from injured skin may thus provide the initial signal to the immune system that disruption or penetration of the integument has occurred. The IL-1-rich environment of the wound may provide an adjuvant effect on immune responses to foreign antigens encountered in this setting. In the absence of injury, the normal desquamation of terminally differentiated, nonviable keratinocytes provides a satisfactory mode for the excretion of this predominantly cell-associated factor. This mechanism may enable the keratinocyte to produce IL-1 constitutively without causing systemic effects.

Summary

Keratinocytes produce an IL-1 like factor termed epidermal cell–derived thymocyte-activating factor (ETAF). In this study, we show that ETAF and IL-1 are identical by the following criteria: (*a*) Both normal and malignant human keratinocytes contain mRNAs identical to monocytic IL-1α and IL-1β mRNA, as determined by an S1 nuclease protection assay; and (*b*) IL-1 activity in medium conditioned by these cells can be neutralized by antibodies specific for human IL-1. The IL-1α and IL-1β mRNAs can be identified in cultured human keratinocytes in the absence of identifiable stimulation; this basal level of mRNA can be further induced to accumulate with certain defined stimuli. Cultured normal human keratinocytes (HFKs) contain 2–4 times more IL-1α than IL-1β mRNA; in contrast, human peripheral blood monocytes contain 10–20 times more IL-1β than IL-1α mRNA. The IL-1 activity released by these HFK can be neutralized by an antibody that neutralizes both α and β IL-1, but not by an antibody

[1] Kupper, T. S., U. Gubler, A. Chua, J. S. McGuire, P. Flood. Interleukin 1 gene expression in human keratinocytes is augmented by ultraviolet (UVB) irradiation. Submitted for publication.

that neutralizes only IL-1β. While human monocytes produce a large excess of IL-1β after appropriate stimulation, these data suggest that IL-1α is a major (and may be the predominant) form of IL-1 produced by human keratinocytes.

Received for publication 18 July 1986 and in revised form 8 September 1986.

References

1. Dinarello, C. A. 1984. Interleukin 1. *Rev. Infect. Dis.* 6:51.
2. Lomedico, P. T., U. Gubler, C. P. Hellmann, M. Dukovich, J. G. Giri, Y.-C. E. Pan, K. J. Collier, R. Semionow, A. O. Chua, and S. B. Mizel. 1984. Cloning and expression of murine interleukin 1 cDNA in E. coli. *Nature (Lond.).* 312:458.
3. Auron, P. E., A. C. Webb, L. J. Rosenwasser, S. F. Mucci, A. Rich, S. M. Wolff, and C. A. Dinarello. 1984. Nucleotide sequence of human monocyte interleukin 1 precursor cDNA. *Proc. Natl. Acad. Sci. USA.* 81:7907.
4. March, C. J., B. Mosely, A. Larsen, D. P. Cerretti, G. Braedt, U. Price, S. Gillis, C. S. Henney, S. R. Kronheim, K. Grabstein, P. J. Conlon, T. P. Hopp, and D. Cosman. 1985. Cloning, sequencing, and expression of two distinct human interleukin 1 complementary DNA. *Nature (Lond.).* 315:641.
5. Kilian, P. L., K. L. Kaffka, A. S. Stern, D. Woehle, W. R. Benjamin, T. M. DeChiara, U. Gubler, J. J. Farrar, S. B. Mizel, and P. J. Lomedico. 1986. Interleukin-1α and interleukin-1β bind to the same receptor on T cells. *J. Immunol.* 136:4509.
6. Durum, S. K., J. A. Schmidt, and J. J. Oppenheim. 1985. Interleukin 1: an immunological perspective. *Annu. Rev. Immunol.* 3:263.
7. Luger, T. A., B. M. Stadler, S. I. Katz, and J. J. Oppenheim. 1981. Epidermal cell (keratinocyte) derived thymocyte activating factor. *J. Immunol.* 127:1493.
8. Sauder, D. N. 1985. Biologic properties of epidermal cell thymocyte activity factor (ETAF). *J. Invest. Dermatol.* 85:1765.
9. Gahring, L. C., A. Buckley, and R. A. Daynes. 1985. The presence of ETAF/IL-1 in normal human stratum corneum. *J. Clin. Invest.* 76:1585.
10. Rheinwald, J. G., and H. Green. 1975. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. *Cell.* 6:331.
11. Dinarello, C. A., L. Renfer, and S. M. Wolff. 1974. The production of an antibody against human leukocyte pyrogen. *J. Clin. Invest.* 60:465.
12. Kaye, J., and C. J. Janeway. 1984. Induction of receptors for interleukin 2 requires T cell-antigen-Ia receptor crosslinking and interleukin 1. *Lymphokine Res.* 3:175.
13 Berk, A. J., and P. A. Sharp. 1977. Sizing and mapping of early adeno virus mRNA's by gel electrophoresis of S1 endonuclease digested hybrids. *Cell.* 12:721.
14. Gubler, U., A. O. Chua, A. S. Stern, C. P. Hellmann, M. D. Vitek, T. M. DeChiara, W. R. Benjamin, K. J. Collier, M. Dukovich, P. C. Familletti, C. Feidler-Nagy, J. Jensen, K. Kaffka, P. L. Kilian, D. Stremlo, B. H. Wittreich, D. Woehle, S. B. Mizel, and P. T. Lomedico. 1986. Recombinant human interleukin 1α: purification and biological characterization. *J. Immunol.* 136:2492.
15. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
16. Duff, G. W., and E. Atkins. 1982. The detection of endotoxin by *in vitro* production of endogenous pyrogen: Comparison with limulus amebocyte lysate gelation. *J. Immunol. Methods.* 52:323.