Keratinocyte Membrane-Associated Epidermal Cell-Derived Thymocyte-Activating Factor (ETAF)*

David Goldminz, M.D., Thomas S. Kupper, M.D., and Joseph McGuire, M.D.

Department of Dermatology and Howard Hughes Medical Institute (TK), Yale University School of Medicine, New Haven, Connecticut, U.S.A.

This study describes the association between secreted keratinocyte interleukin 1 (IL-1) and its presence on the keratinocyte cell surface. These properties were studied in normal and transformed human keratinocytes as well as in transformed murine keratinocytes. We will present evidence that the secretion of IL-1 by human and murine keratinocytes is associated with the presence of IL-1 on the keratinocyte membrane. In addition, although transformed murine keratinocytes secrete other cytokines, namely keratinocyte T-cell growth factor (KTGF) and IL-3, no KTGF or IL-3 activity can be demonstrated on the cell surface. *J Invest Dermatol 88:97–100, 1987*

In recent years, the epidermis has been increasingly identified as an organ with immunologically active cells that can mediate active processes designed to recognize and respond to foreign antigens [1]. Specifically, keratinocytes have been shown to produce factors that influence T lymphocytes, namely ETAF/IL-1, KTGF, and IL-3. Epidermal cell-derived thymocyte-activating factor (ETAF), according to its biochemical and functional properties, is indistinguishable from macrophage-derived interleukin 1 (IL-1) [2–4]. Keratinocyte T-cell growth factor (KTGF) is able to sustain the growth of an "IL-2 dependent" T helper cell line [5]. Finally, it has been shown recently that epidermal cells synthesize a cytokine with properties similar to IL-3 derived from T cells [6].

Membranes of paraformaldehyde-fixed macrophages have been recently shown to have IL-1 associated molecules. Extensive work by others has demonstrated that paraformaldehyde fixation is a useful procedure to detect cell surface activity of IL-1 [7,8]. Macrophages have been shown to present antigens to T cells in the context of Ia and soluble IL-1. More recently, a modification has been postulated. It has been shown that membrane-associated IL-1 in fixed macrophages can replace soluble IL-1 as a second signal for T-cell activation. Moreover, it has been suggested that this membrane-associated IL-1, and not soluble IL-1, is the more relevant compartment involved in antigen presentation [7,8]. We designed this study to investigate whether keratinocyte IL-1 is membrane associated, as has been shown for macrophage IL-1.

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Reprint requests to: Joseph McGuire, M.D., Department of Dermatology, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510.

Abbreviations:
CS: calf serum
DMEM: Dulbecco's minimal essential medium
ETAF: epidermal cell-derived thymocyte-activating factor
FCS: fetal calf serum
HFEC: human foreskin epidermal culture(s)
IL-1,-2,-3: interleukin 1, 2, 3
KTGF: keratinocyte T-cell growth factor

MATERIALS AND METHODS

Cell Cultures Human foreskin epidermal cultures (HFEC), long-term cultures of normal neonatal human foreskin keratinocytes, were obtained as previously described and maintained in Dulbecco's minimal essential medium (DMEM) with 20% fetal calf serum (FCS) [9]. A431, a cell line of transformed human keratinocytes derived from vulvar carcinoma, was provided by L. King (Vanderbilt University, Nashville, Tennessee) and maintained in DMEM with 10% calf serum (CS) [10]. PAM 212, a spontaneously transformed murine keratinocyte cell line, was provided by P. Hawley-Nelson (National Cancer Institute, Bethesda, Maryland) and maintained in DMEM with 10% FCS [11]. 3T3, a murine fibroblast cell line (CC_L 93), was purchased from American Type Culture Collection, Rockville, Maryland and maintained in DMEM with 10% CS.

Conditioned Media Conditioned media were obtained from confluent cultures of HFEC, A431, PAM 212, and 3T3 maintained in their respective complete media for 48 h.

Fixation of Cell Cultures and Preparation of Cell Fractions All cell fractions were fixed and prepared as previously described in detail for macrophages [7]. Briefly, confluent cultures were rinsed, fixed with 1% paraformaldehyde, rinsed, and incubated in complete media for 24 h. Cultures were rinsed again and then assayed for cytokine activity as fixed intact cells, sonicated fixed cells, and fixed cell membranes. All cell materials were prepared at a density of 10^5 cells per milliliter and assayed in serial dilutions. Cells were mechanically removed from tissue culture plates, spun in a clinical centrifuge, and the pellet representing fixed intact cells was assayed for cytokine activity. Fixed intact cells were resuspended in phosphate-buffered saline and sonicated. The sonicate, representing sonicated fixed cells which consist of membrane fragments and intracellular material, was assayed for cytokine activity. Finally, sonicated fixed cells were ultracentrifuged, rinsed, and the pellet, representing fixed cell membranes, was assayed for cytokine activity.

Lymphocyte Cell Lines D10.G4.1 cells and 3D3 antibody were provided by C. Janeway, Jr. (Yale University, New Haven, Connecticut) and were maintained as previously described [12]. HT-2 cells were obtained from P. Marrack (National Jewish Hospital, Denver, Colorado) and were maintained as previously described [13]. DA-1 cells were provided by J. Ihle (National Cancer

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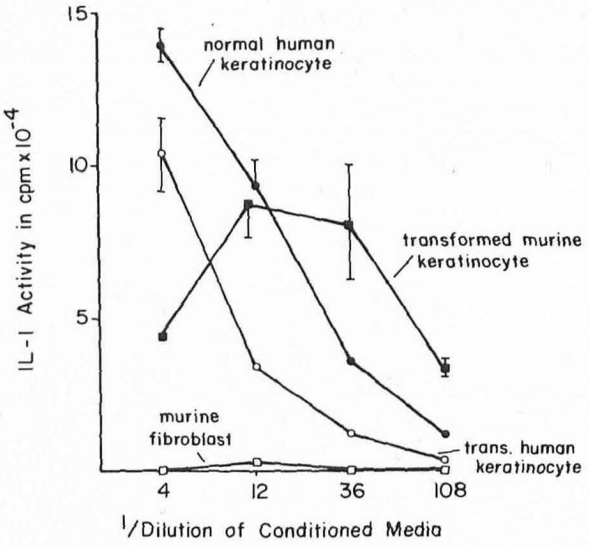

Figure 1. Interleukin 1 activity in conditioned media. Mean values ± SEM are plotted.

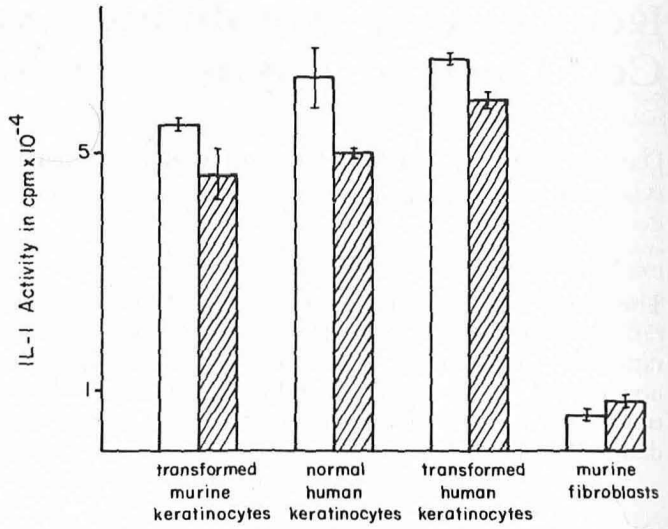

Figure 3. Interleukin 1 activity in cellular fractions. Sonicates (*open bars*) and membranes (*hatched bars*) at 10^5 cells/ml. Mean values ± SEM are plotted.

Institute, Frederick, Maryland) and were also maintained as previously described [14].

Keratinocyte Cytokine Assays The assay to detect IL-1 activity using the cloned T helper cell D10.G4.1 (D10) has been previously described [5,12]. Briefly, 2×10^4 D10 cells, a 1:1000 dilution of 3D3 hybridoma supernatant, and the experimental sample were cocultured in triplicate in Clicks medium with 5% FCS and incubated for 72 h at 37°C in a 5% CO_2/95% air incubator. Six hours prior to harvest, 1 μCi/well of [^{3}H] thymidine was added. Cultures were harvested on a MASH (multiple automated sample harvester) (Cambridge Technologies) and counted on a Beckman scintillation spectrometer. Keratinocyte T-cell growth factor activity was measured with the T helper cell line HT-2. Cells were diluted to 2×10^5 cells/ml in complete Clicks medium cultured in triplicate for 48 h. Cells were pulsed with [^{3}H] thymidine and harvested in an identical fashion to the IL-1 assay. Interleukin 3 activity was measured using the IL-3 dependent cell line, DA-1. Cells were diluted in RPMI 1640 with 10% FCS, mixed with experimental samples in triplicate, incubated, pulsed, and harvested as described above.

RESULTS

We selected the paraformaldehyde-fixed keratinocyte cell culture system because it allowed us to study the presence of IL-1 in previously active cells that have been rendered metabolically inert. Although extensive work by others has shown that fixation of macrophages neutralizes the cell's metabolic processes [15], we studied IL-1 activity in the media of keratinocytes (PAM 212) prior to paraformaldehyde fixation and then again subsequent to fixation. There was no IL-1 activity in the media of cells incubated for 48 h after fixation, whereas a significant amount of activity was present in the media prior to fixation (data not shown). Therefore, keratinocytes were rendered metabolically inert with regard to IL-1 secretion after fixation. As shown in Fig 1, keratinocyte-conditioned media derived from HFEC, A431, and PAM 212 cells, all contained specific and titratable IL-1 activity. In contrast, 3T3-conditioned media had no significant IL-1 activity. In Fig 2, the IL-1 activity of fixed intact PAM 212 keratinocytes and fixed sonicated PAM 212 keratinocytes containing intracel-

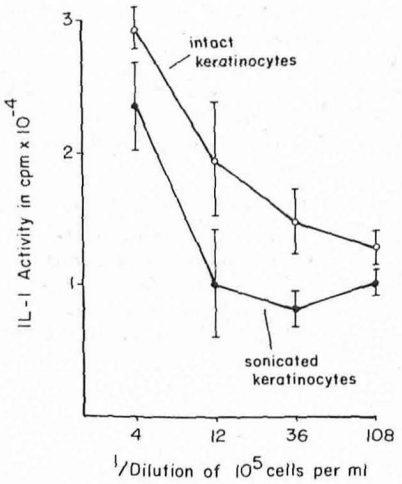

Figure 2. Interleukin 1 activity in PAM 212 keratinocytes. Mean values ± SEM are plotted.

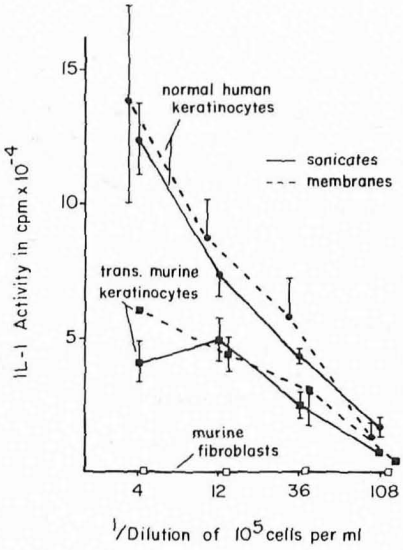

Figure 4. Interleukin 1 activity in cellular fractions. Mean values ± SEM are plotted.

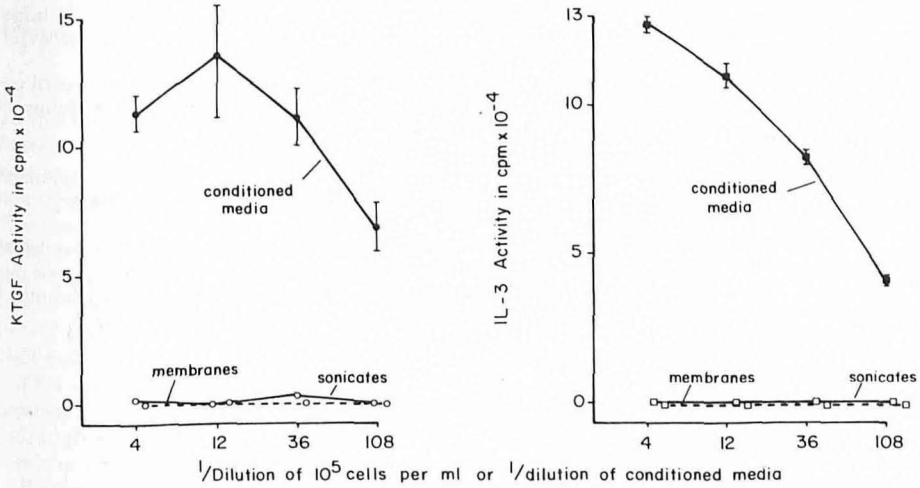

Figure 5. Cytokine activity in PAM 212 keratinocytes and their conditioned media. Mean values ± SEM are plotted.

lular materials and membrane fragments was compared. Both intact cells and sonicated cells possessed significant IL-1 activity. The half-maximal activation observed for fixed intact keratinocytes was only slightly higher than the half-maximal activation observed for sonicated cells. These preliminary experiments suggested to us that a significant component of the IL-1 activity associated with keratinocytes is associated with the cell surface. A further study showed that the sonification process itself did not alter the observed IL-1 activity (data not shown). As shown in Fig 3, virtually all the IL-1 activity in the unfractionated cell sonicate was cell membrane associated. This was true for PAM 212, HFEC, and A431 keratinocytes. In contrast, no IL-1 activity was found in either 3T3 sonicated fixed cells or fixed cell membranes. Importantly, no significant IL-1 activity was found even when these fibroblasts were preincubated with conditioned keratinocyte media (PAM 212), and paraformaldehyde fixed in the presence of keratinocyte conditioned media. In Fig 4, sonicates and membranes derived from HFEC keratinocytes contained significant IL-1 activity. Likewise, sonicates and membranes derived from PAM 212 keratinocytes demonstrated significant IL-1 activity. Similar results were obtained for A431 keratinocytes. In contrast, cell sonicates derived from 3T3 cells had no detectable IL-1 activity.

We next asked whether other cytokines known to be secreted by keratinocytes were also present on the keratinocyte cell membranes. Accordingly, we examined the PAM 212 keratinocyte cell cultures for KTGF and IL-3 activity. As shown in Fig 5, conditioned media contained significant KTGF and IL-3 activity. However, when keratinocyte cell fractions were tested, no KTGF activity could be demonstrated either in cell sonicates or in cell membranes. Similarly, no IL-3 activity was demonstrated either in cell sonicates or in cell membranes. Sonification of conditioned keratinocyte media (PAM 212) did not affect KTGF or IL-3 activity (data not shown). These observations were particularly interesting because they indicate that paraformaldehyde fixation does not bind cytokines to keratinocyte cell membranes in a nonspecific manner.

DISCUSSION

In summary, this study presents evidence that the secretion of IL-1/ETAF by human and murine keratinocytes is associated with the presence of IL-1/ETAF on the keratinocyte membrane. In addition, while transformed murine keratinocytes secrete other cytokines, their activity cannot be demonstrated on the cell surface. This latter observation is important for two reasons. First, it indicates that keratinocyte membrane-associated IL-1 activity resembles soluble IL-1 and does not appear to possess activity of other cytokines. Second, it suggests that paraformaldehyde fixation of cells does not make cytokines bind to cell membranes in a nonspecific manner. This is reinforced by the demonstration that fixation of cells known to lack membrane-associated IL-1 (3T3) in the presence of soluble IL-1 did not result in membrane-bound IL-1 activity. This again suggests that the fixation process itself does not yield nonspecific association of IL-1 to cell membranes.

The presence of secreted IL-1 in keratinocyte cultures substantiates the work of others [2–4]. The novel finding reported here of a membrane-associated IL-1/ETAF in keratinocytes further strengthens the functional parallel between keratinocytes and macrophages. Immunologically, these observations suggest that keratinocytes may be able to interact with T cells in a fashion similar to macrophages. It has been suggested that the relevant fraction of macrophage IL-1 involved in antigen presentation to T cells is membrane associated [7,8]. Under some circumstances (e.g., contact hypersensitivity), keratinocytes have been shown to express Ia antigens [16]. In this study, we have shown that keratinocytes have a membrane-associated IL-1; therefore, it is not implausible that keratinocytes, under certain circumstances, may be capable of presenting antigens to T cells in an immunogenic fashion.

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