Biologic Properties of the Thymocyte-Activating Factor (CETAF) Produced by a Rabbit Corneal Cell Line (SIRC)

Günther Grabner, Thomas A. Luger,* Bia M. Luger,* Gilbert Smolin, and Jang O. Oh

Rabbit corneal epithelial cell cultures produce a cytokine (CETAF) that greatly enhances the proliferation of C3H/HeJ mouse thymocytes. The rabbit corneal cell line SIRC was used to generate CETAF activity in the culture supernatant. CETAF was then partially purified by Sephacryl S-200 gel filtration, where peaks of activity eluted in a molecular weight range of 95,000–55,000 (CETAF I) and 30,000–15,000 (CETAF II). Similar to the epidermal cell-derived thymocyte-activating factor (ETAF), CETAF (I and II) stimulated the growth of a human dermal fibroblast line (CRL 1445) in a dose-dependent manner, but failed to enhance the proliferation of an Interleukin 2 (IL 2)-dependent T-cell line (CT 6). Although CETAF did not exhibit any IL 2 activity, it clearly enhanced the IL 2 production by C3H/HeJ mouse splenocytes stimulated with suboptimal doses of lectins. Crude SIRC supernatants as well as the partially purified CETAF preparations showed a marked inhibition of polymorphonuclear neutrophil migration at high concentrations, but were significantly chemotactic when diluted samples were tested. CETAF release by SIRC cells was increased by stimulation with mitomycin C, phorbolmyristate acetate, hydroxyurea, silica, lipopolysaccaride B, and when the cells were cultured under serum-free conditions. These observations suggest that corneal epithelial cells may not only interact with the immune system in a way similar to keratinocytes, but may also stimulate corneal stromal cell through the production of CETAF. Invest Ophthalmol Vis Sci 24:589–595, 1983

Rabbit corneal epithelial cell cultures produce a cytokine (CETAF) that greatly enhances the growth of thymocytes and, therefore, was named corneal epithelial cell-derived thymocyte-activating factor (CETAF).1 This hormone-like factor is produced not only by corneal epithelial cells in primary tissue culture, but also by an established rabbit corneal cell line (SIRC). It is similar in its molecular weight and biologic activities to human and murine epidermal cell-derived thymocyte-activating factor (ETAF) and to Interleukin 1 (IL 1), which is produced by human and murine macrophages as well as rabbit alveolar macrophages.2-7

Therefore, we investigated whether partially purified CETAF, produced by SIRC cells, would exhibit some of the other biologic properties attributed to ETAF and IL 1, such as enhancement of fibroblast proliferation,6,8 chemotactic activity for polymorphonuclear neutrophils (PMN),6 and enhancement of Interleukin 2 (IL 2) production by cultured splenocytes.4,6 Additionally, in order to obtain increased levels of CETAF in the culture supernatants, we tested whether SIRC cells could be stimulated by a variety of agents known to promote the production of cytokines by other cell types.6,9,10

Materials and Methods

Generation and Purification of CETAF

For the generation of CETAF, SIRC cells (American Type Culture Collection, Rockville, MD) where trypsinized and resuspended in serum-free Eagle’s medium containing penicillin (100 U/ml), streptomycin (100 μg/ml) (Gibco, Grand Island, NY) and amphotericin B (2.5 μg/ml) (Squibb and Sons, Princeton, NJ) at a cell density of 1 × 10^5 cells/ml. Fifty milliliters of the cell suspension were then incubated in 150 cm² tissue culture flasks (Corning Glass Works, Corning, NY) at 37 C in a humidified 5% CO₂ containing atmosphere. The supernatants were harvested after 72 hrs of incubation and concentrated tenfold by using
Amicon ultrafiltration cells with UM-10 Diaflo membranes (Amicon Corporation, Lexington, MA). No significant loss of biologic activity occurred during this procedure. Twenty milliliters of this concentrated SIRC cell supernatant were then lyophilized, resuspended in 5 ml phosphate-buffered saline (PBS), sterilized by filtration with a Nalgene 0.45-micron filter, and tested in the thymocyte proliferation assay, which has been described in detail elsewhere. This preparation was designated “Crude CETAF” (C-CETAF) and was found to contain an activity of 2,258 U CETAF/ml, determined as previously described.1

To obtain partially purified CETAF, concentrated supernatants were subjected to gel filtration on a Sephacryl S-200 column (2.5 X 95 cm) (Pharmacia Fine Chemicals, Piscataway, NJ) as previously described.4 Peaks of CETAF activity eluted from this column in the 95,000–55,000 molecular weight (mw) and 30,000–15,000 mw ranges.1 The fractions of the high mw peak (35 ml) were pooled, lyophilized, resuspended in 5 ml phosphate-buffered saline (PBS), sterilized by filtration, and tested for CETAF activity. This preparation was designated “CETAF I” and contained 1846 U/ml. Fractions of the low mw peak (17 ml) were processed identically and designated “CETAF II”. It contained a final concentration of 420 U/ml.

Stimulation of CETAF Release by SIRC Cells

Following the trypsinization of SIRC monolayers as outlined above, the cells were incubated at a cell density of 5 X 10⁵ cells/ml in 24 well tissue culture plates (Costar, Cambridge, MA). To the medium (MEM, supplemented with penicillin, streptomycin, fungizone, and 2.5% FCS) one of the following agents: mitomycin C, phorbol myristate acetate (PMA), hydroxyurea, silica (0.014-micron particle size) (all from Sigma, St. Louis, MO), or lipopolysaccaride B, Escherichia coli 055:B5 (LPS) (Difco Laboratories, Detroit, MI) were added at various concentrations (given in detail in Table 1). Triplicate control cultures were plated at cell densities of 2.5 X 10⁵, 5 X 10⁵ and 1 X 10⁶ cells/ml (in serum-free medium). The effect of the addition of 2.5%, 5%, or 10% FCS was tested at a cell concentration of 5 X 10⁵ cells/ml. Supernatants of the cultures were harvested after 48 hrs of incubation in the presence of the different agents, diluted 1:2 with supplemented MEM, dialyzed for 24 hr in a 100 fold greater volume of PBS (at 4 C), sterilized by filtration (Nalgene, 0.45-micron filter), and tested in the thymocyte proliferation assay. Furthermore, the density of the cultures and their viability (by trypan blue exclusion) was determined for each well at the end of the incubation period. Si-

Table 1. Effects of different agents on the production of CETAF by SIRC cells

<table>
<thead>
<tr>
<th>Stimulant</th>
<th>Dose</th>
<th>CETAF (U/ml)*</th>
<th>P value†</th>
<th>Cells/ml (X10⁵)‡</th>
<th>Viability (%)§</th>
<th>³H-TdR uptake (cpm ± SD)¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>—</td>
<td>8.6 ± 1.5</td>
<td>—</td>
<td>9.0 ± 0.97</td>
<td>98</td>
<td>57,003 ± 6,391</td>
</tr>
<tr>
<td>Mitomycin</td>
<td>100 µg/ml</td>
<td>37.3 ± 5.1</td>
<td>&lt;0.0005</td>
<td>1.28 ± 0.9</td>
<td>75</td>
<td>52 ± 9.5</td>
</tr>
<tr>
<td></td>
<td>10 µg/ml</td>
<td>28.0 ± 4.4</td>
<td>&lt;0.002</td>
<td>2.76 ± 0.6</td>
<td>89</td>
<td>88 ± 19</td>
</tr>
<tr>
<td></td>
<td>1 µg/ml</td>
<td>20.8 ± 0.7</td>
<td>&lt;0.005</td>
<td>4.5 ± 0.32</td>
<td>93</td>
<td>349 ± 27</td>
</tr>
<tr>
<td></td>
<td>0.1 µg/ml</td>
<td>17.0 ± 2.6</td>
<td>&lt;0.005</td>
<td>5.25 ± 0.18</td>
<td>98</td>
<td>4,436 ± 143</td>
</tr>
<tr>
<td>PMA</td>
<td>10 µg/ml</td>
<td>17.7 ± 2.5</td>
<td>&lt;0.005</td>
<td>7.44 ± 0.52</td>
<td>96</td>
<td>46,897 ± 6,189</td>
</tr>
<tr>
<td></td>
<td>1 µg/ml</td>
<td>20.0 ± 1.4</td>
<td>&lt;0.002</td>
<td>9.14 ± 0.92</td>
<td>99</td>
<td>51,200 ± 10,657</td>
</tr>
<tr>
<td></td>
<td>0.1 µg/ml</td>
<td>29.7 ± 2.5</td>
<td>&lt;0.0005</td>
<td>9.12 ± 0.47</td>
<td>97</td>
<td>48,368 ± 5,496</td>
</tr>
<tr>
<td></td>
<td>0.01 µg/ml</td>
<td>18.3 ± 4.1</td>
<td>&lt;0.01</td>
<td>7.64 ± 0.46</td>
<td>98.5</td>
<td>48,505 ± 7,991</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>20 mM</td>
<td>14.7 ± 2</td>
<td>&lt;0.01</td>
<td>3.94 ± 0.25</td>
<td>95</td>
<td>567 ± 60</td>
</tr>
<tr>
<td></td>
<td>2 mM</td>
<td>18.0 ± 4.2</td>
<td>&lt;0.01</td>
<td>5.32 ± 0.36</td>
<td>95</td>
<td>963 ± 25</td>
</tr>
<tr>
<td></td>
<td>0.2 mM</td>
<td>14.0 ± 1.7</td>
<td>&lt;0.01</td>
<td>4.56 ± 0.16</td>
<td>97.5</td>
<td>60,225 ± 3,643</td>
</tr>
<tr>
<td>Silica</td>
<td>100 µg/ml</td>
<td>21.7 ± 3</td>
<td>&lt;0.002</td>
<td>2.06 ± 0.5</td>
<td>92</td>
<td>8,903 ± 372</td>
</tr>
<tr>
<td></td>
<td>50 µg/ml</td>
<td>18.7 ± 3</td>
<td>&lt;0.02</td>
<td>3.48 ± 0.22</td>
<td>94</td>
<td>28,883 ± 3,147</td>
</tr>
<tr>
<td></td>
<td>10 µg/ml</td>
<td>13.7 ± 2.5</td>
<td>&lt;0.02</td>
<td>7.94 ± 0.9</td>
<td>97</td>
<td>45,869 ± 5,394</td>
</tr>
<tr>
<td>LPS</td>
<td>100 µg/ml</td>
<td>26.3 ± 0.5</td>
<td>&lt;0.005</td>
<td>8.19 ± 0.95</td>
<td>99.5</td>
<td>40,154 ± 9,512</td>
</tr>
<tr>
<td></td>
<td>50 µg/ml</td>
<td>32.3 ± 3.5</td>
<td>&lt;0.005</td>
<td>9.18 ± 0.68</td>
<td>99.5</td>
<td>41,586 ± 7,206</td>
</tr>
<tr>
<td></td>
<td>10 µg/ml</td>
<td>21.6 ± 4</td>
<td>&lt;0.005</td>
<td>8.46 ± 0.33</td>
<td>98.5</td>
<td>59,393 ± 5,890</td>
</tr>
<tr>
<td></td>
<td>1 µg/ml</td>
<td>10.0 ± 3.5</td>
<td>&lt;NS</td>
<td>10.28 ± 0.74</td>
<td>99</td>
<td>59,541 ± 8,017</td>
</tr>
</tbody>
</table>

* Mean ± SD of CETAF activities (U/ml) in supernatants of triplicate SIRC cultures (5 X 10⁵ cells/ml) after 48 hrs of incubation.
† Statistical significance, CETAF supernatant activities of stimulated SIRC cultures vs control cultures, (t-test).
‡ Cell counts ± SD of the triplicate cultures after 48 hours of incubation.
§ Viability of the SIRC cultures after 48 hours of incubation (trypan blue exclusion). The variance of median % trypan blue exclusion was ±5%.
¶ ³H-thymidine uptake (cpm ± SD) by stimulated and control SIRC cells.
multaneously, triplicate cultures of SIRC cells were plated under identical conditions in 96-well tissue culture plates (Falcon, Oxnard, CA), pulsed with 3H-TdR for 5 hr after 43 hr of incubation (1 µCi), harvested following trypsinization (Mash II, Microbiological Associates, Walkersville, MD), and counted in a liquid scintillation counter.

**Thymocyte Proliferation Assay**

Different preparations of CETAF, ETAF (produced by the PAM 212 murine keratinocyte cell line), and a human IL 2 preparation were tested at dilutions of 1:4 to 1:32 in the thymocyte costimulator assay as previously described. The results were expressed in Units (U) of CETAF activity per milliliters compared to an ETAF standard preparation.

**Fibroblast Proliferation Assay**

The effect of these cytokines on the proliferation of human dermal fibroblasts (CRL 1445) was tested as described previously by Schmidt et al.

**Biologic Assay for IL 2**

These cytokines were also tested for their ability to maintain the growth of the IL 2 dependent C57 BL/6 mouse-derived T-cell line (CT6 cells).

**Induction of IL 2 Activity**

Spleen cell suspensions from C3H/HeJ mice were prepared as already described. The cells were washed with RPMI 1640 medium and resuspended to a density of 2 x 10^6 cells/ml. IL 2 supernatant activity was generated in 200 µl cultures containing 1 x 10^5 cells/ml in 96-well tissue culture plates (Costar, Cambridge, MA) in the presence of 2 µg/ml Concanavalin A (Con A) (Calbiochem, San Diego, CA), 5 ng/ml phorbol myristate acetate (PMA) (Sigma Chemical Co., St. Louis, MO), and different preparations of CETAF. After 48 hr of incubation at 37 C and 5% CO_2, supernatants were tested in the biologic assay for IL 2.

**Chemotaxis Assay**

To assay the chemotactic activity of C-CETAF, CETAF I, and CETAF II for rabbit PMNs, a modified Boyden's chemotaxis chamber (Bio-Rad Laboratories, Richmond, CA) was used. In each test positive (C5 fragments generated in fresh rabbit serum by incubating with zymosan) and negative controls (RPMI 1640) were run at the same time. In addition, the buffer solution used in the gel chromatography was tested as a control.

**Results**

**Stimulation of CETAF Release**

All substances tested (mitomycin C, PMA, hydroxyurea, silica, LPS) were found to increase significantly the release of CETAF activity into the supernatants. This increase was detected both in cultures with a high cell viability and normal proliferation (as measured by 3H-TdR uptake) after 48 hr of incubation (with LPS and PMA) as well as in those cultures where the stimulants (mitomycin C, hydroxyurea, silica) significantly damaged the SIRC cells (Table 1).

Culturing SIRC cells in serum-free medium significantly increased the CETAF level in the supernatant, with the relatively highest activity (6 U/10^5 cells) appearing in the cultures seeded at the lowest concentration (2.5 x 10^5 cells/ml) (Table 2). This closely parallels our earlier observations that under serum-free conditions low cell densities yield the highest levels of thymocyte-stimulating activity. Without addition of FCS, SIRC cells adhere only minimally to the plastic surface and have a tendency to aggregate in the medium. Different concentrations

<table>
<thead>
<tr>
<th>FCS (%)</th>
<th>Seeding density (×10^5 cells/ml)</th>
<th>CETAF (U/ml)</th>
<th>P value</th>
<th>Cells/ml (×10^6)</th>
<th>Viability (%)</th>
<th>3H-TdR-uptake (cpm ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>2.5</td>
<td>15.0 ± 3.0</td>
<td>&lt;0.05</td>
<td>—</td>
<td>—</td>
<td>8,657 ± 445</td>
</tr>
<tr>
<td>0%</td>
<td>5</td>
<td>24.6 ± 4.6</td>
<td>&lt;0.002</td>
<td>—</td>
<td>—</td>
<td>2,430 ± 756</td>
</tr>
<tr>
<td>0%</td>
<td>10</td>
<td>21.0 ± 4.3</td>
<td>&lt;0.005</td>
<td>—</td>
<td>—</td>
<td>641 ± 89</td>
</tr>
<tr>
<td>2.5%</td>
<td>5</td>
<td>8.6 ± 1.5</td>
<td>—</td>
<td>9.0 ± 0.97</td>
<td>98</td>
<td>57,003 ± 6,391</td>
</tr>
<tr>
<td>5%</td>
<td>5</td>
<td>9.0 ± 1.7</td>
<td>N.S.</td>
<td>9.36 ± 0.57</td>
<td>99</td>
<td>11,776 ± 2,214</td>
</tr>
<tr>
<td>10%</td>
<td>5</td>
<td>8.7 ± 1.5</td>
<td>N.S.</td>
<td>9.32 ± 1.19</td>
<td>98</td>
<td>10,516 ± 1,847</td>
</tr>
<tr>
<td>5%</td>
<td>0 (control)</td>
<td>0</td>
<td>—</td>
<td>159 ± 112.6</td>
<td></td>
<td>—</td>
</tr>
</tbody>
</table>

* Mean ± SD of CETAF activities (U/ml) in supernatants of triplicate SIRC cultures after 48 hrs of incubation.
† Statistical significance. CETAF supernatant activities of the cultures were compared with the culture seeded at a cell density of 5 x 10^5 cells/ml in 2.5% FCS (t-test).
Fig. 1. Biologic effects of various cytokines on different target cells. CETAF I: pooled activity of S-200 gel filtration, mw ~65,000; CETAF II: pooled activity of S-200 gel filtration, mw ~22,000; ETAF: serum-free PAM 212 cell line supernatant, after 48 hr of incubation; IL 2: supernatant of 2×10^6 peripheral blood leukocytes incubated with 10 μg/ml Con A and 10 μg/ml PMA for 48 hr. The results are expressed as Δcpm (cpm of test sample minus background cpm). Background 3H-TdR incorporation (cpm ± SD) in these assays were: for C3H/HeJ thymocytes, incubated with Con A (1 μg/ml) alone: 6,392 ± 449 cpm; for CRL fibroblasts alone: 10,470 ± 614 cpm. CT6 cells incorporated 2,190 ± 78 cpm. An IL 1 preparation (detailed values not shown for clarity) gave results very similar to those of ETAF.

Effect of CETAF on Different Target Cells

It was recently shown that ETAF, like IL 1, is able to enhance the proliferative activity of thymocytes and is mitogenic for fibroblasts. In contrast, both ETAF and IL 1 have no effect on the proliferation of the IL 2-dependent CT6 cell line. In order to demonstrate the close relationship between IL 1, ETAF, and CETAF, different preparations of partially purified CETAF were tested for their effects on C3H/HeJ thymocytes, a human dermal fibroblast line (CRL 1445) and the IL 2-dependent CT6 cell line. Like ETAF, both CETAF I and CETAF II were able to enhance the proliferation of thymocytes and to stimulate fibroblast growth in a dose dependent manner. In contrast both CETAF preparations failed to stimulate CT6 cells. An IL 2 standard preparation that was tested as a control significantly increased the proliferation of CT6 cells and thymocytes, but was not active on fibroblasts. Therefore, CETAF, like ETAF and IL 1, affects thymocytes and fibroblasts, but has no effect on CT6, indicating that CETAF does not contain IL 2 activity (Fig. 1). Therefore, we tested whether CETAF, like IL 1 and ETAF, can enhance IL 2 production by splenocytes stimulated with suboptimal doses of lectins. Addition of CETAF I and CETAF II further increased the supernatant IL 2 activity of these lectin-stimulated spleen cell cultures (Table 3). Although CETAF does not exhibit any IL 2 activity, it clearly enhances IL 2 production by spleen cells and can thereby activate the immune system.

Chemotaxis Assay

All three CETAF preparations (C-CETAF, CETAF I, CETAF II) showed a marked inhibition of PMN migration when undiluted samples or dilutions up to 1:5 were tested and PMNs were found to aggregate on the upper surface of the membrane. Dilutions 1:10 or higher, however, were found to increase significantly the migration of PMNs when compared to the diluent (Fig. 2). Repeated experiments gave identical results. Separate tests indicated that the increased migration of PMNs is due to specific chemotactic activity of CETAF rather than to a mere chemokinetic activity.

Discussion

CETAF, a hormone-like mediator is produced both by primary rabbit corneal epithelial cell cultures,
as well as by a rabbit corneal cell line (SIRC). The activity found in the supernatants of SIRC cell cultures was partially purified by gel chromatography and observed to consist of a high mw (95,000–55,000 = CETAF I) and a low mw peak (30,000–15,000 = CETAF II).

A wide variety of agents and treatments (such as B- and T-cell mitogens, inducers of phagocytosis, and immunologic adjuvants) induce leukocyte cultures, containing both lymphocytes and monocytes, to produce IL 1 (for review see ref. 9). The mechanism of macrophage activation, however, is as yet undefined. A recent study by Gery et al.15 shows that various injurious agents may significantly increase the intracellular and/or extracellular IL 1 activities of murine peritoneal macrophages. Since a direct correlation was observed between the level of injury induced by silica and glucocerebrosides and IL 1 production and release, the authors suggest, that the two processes are related. In a similar way ETAF release by the PAM 212 cell line16 and CETAF release by SIRC cells, as shown above, can be stimulated by a variety of substances and by the disruption of a confluent monolayer.14 In the presence of mitomycin C and silica, an increased CETAF production appeared to be associated with a decrease in cell proliferation and viability. Addition of hydroxyurea, a G1/S blocker16 increased the supernatant ETAF and CETAF activity while it concomitantly blocked the proliferation of PAM 212 and SIRC cells. This suggests that these factors are released mainly during G1 phase of the cell cycle, since all the signals that induce greater ETAF production increase the number of cells in this phase, either by prolongation of G1 or by shifting the cells into G1.14 A similar mechanism can be postulated for SIRC cells.

Similar to human and murine IL 1 and ETAF, cytokines produced by macrophages and keratinocytes, respectively, CETAF I (and II) stimulates thymocyte proliferation, but fails to promote the growth of an IL 2-dependent T-cell line.4,6 However, when added to spleen cell cultures stimulated with suboptimal doses of Con A and PMA, CETAF I and CETAF II clearly increased the supernatant IL 2 activity. This finding is of importance because IL 2 has been demonstrated to have a variety of crucial effects on immunocompetent cells. IL 2 promotes the growth of T-cell lines,17,18 generates cytotoxic T-cells,19 enhances the antibody production by B-cells,20 and induces interferon (Y).21 Therefore, corneal epithelial cells may have the ability of enhancing the immune system through the production of CETAF.

The role of the corneal epithelium in corneal wound healing has been extensively investigated.22 Epithelia with persistent defects (as in the case of alkali burns) have long been held responsible for the dissolution of the underlying stroma. In human ulcers of diverse etiologies, however, keratocytes and stromal collagen fibrils, but not corneal epithelium stain with antiserum to skin collagenase (Gordon et al., cited by ref. 22). Blood mononuclear cells have been shown to stimulate serially passaged stromal cells from normal or alkali-burned rabbit cornea to produce collagenase, but the responsible mediator has not been identified.23 Direct cell contact or close proximity of epithelial with stromal cells have been suggested as the mode of regulation of corneal collagenase production, with the stromal cell identified as the source of enzyme and the epithelial cell as the stimulator.24 Recent evidence suggests that lymphokines can regulate fibroblast function by stimulating fibroblast pro-
liferation and collagen synthesis and by initiating di-
rected migration of fibroblasts in vitro.25-27 In addi-
tion, activated macrophages and a factor in 
supernatants of human secondary mixed leukocyte 
reactions (with the biochemical and biologic char-
acteristics of IL 1) have been shown to stimulate in 
vitro fibroblast function.8,28,29 Mizel et al have also 
demonstrated that partially purified IL 1 stimulates 
collagenase and prostaglandin production of fibro-
blast-like rheumatoid synovial cells.30

Our observation that CETAF, just like human and 
purified murine ETAF,10 significantly increases the 
proliferative rate of a fibroblast cell line in the absence 
of any other signal, suggests a possible in vivo role 
of CETAF in corneal wound healing. It remains to 
be established, however, whether CETAF also in-
duces keratocytes to produce inflammatory products 
such as collagenase and prostaglandins.30

Earlier findings by Wiemar suggest that the injured 
corneal epithelium releases a substance that is che-
moattractant for PMNs.31 Both, the stromal ulceration 
of the alkali-burned rabbit cornea and the infiltration 
by active PMN, which is invariably associated with 
it, can be prevented by a glued-on contact lens that 
blocks re-epithelization. From these experimental 
data and observations made by others in patients,32 
Kenyon et al raised the possibility that the epithelium 
stimulates infiltration of the stroma by PMNs.33 Tear 
fluid samples obtained 20 min after a complete cor-
nal denudation have been found to be chemotactic 
for PMNs in other uninjured eyes.34 We have pre-
viously observed that ETAF, produced by a human 
squamous cell carcinoma line, has chemotactic prop-
erties for PMNs in vitro.4 In the present study we 
have found CETAF to possess identical properties. 
The fact that crude CETAF was less chemotactically 
active when compared to partially purified CETAF 
on a U/ml basis may be due to the presence of in-
hibitory factors in the unpurified SIRC supernatants, 
as found in supernatants of keratinocyte cell lines.10 
Therefore CETAF, released by corneal epithelial cells, 
might be a factor that, among others, may cause im-
migration of PMNs into the corneal stroma in vivo 
following a variety of injuries.

At present we do not know, however, why a high 
concentration of CETAF leads to the aggregation of 
PMN in vitro and actually interferes with cell mo-
bility. It is tempting to speculate that CETAF, similar 
to human leukocyte pyrogen, which cannot be se-
parated by chromatographic or electrophoretic pro-
cedures from IL 1,32 might induce the release of 
specific granule contents from the PMNs.36 These 
cytosplasmic granules contain chemoattractant-
inactivating proteases, including elastase and 
cathepsin G. Furthermore, stimulated human PMNs 
inactivate peptide chemoattractants by a myeloperox-
didase-catalyzed oxidation of thioethers that may 
constitute an inflammatory control mechanism.37-39

Our findings suggest that CETAF produced by the 
SIRC cell line is quite pleotropic in its biologic ac-
tivities, affecting not only thymocytes but also fibro-
blasts and PMNs. There are several cautionary notes, 
however, that must be added to the previous discus-
sion. The purification of CETAF requires large 
amounts of supernatants, so that the corneal SIRC 
cell line has been used to produce this cytokine, just 
as macrophage, lymphocyte and keratinocyte lines 
have been used for production of IL 1, IL 2 and 
ETAF.4,40,41 Cells kept in culture for such extended 
periods of time may give results different in quantity 
and/or quality from normal primary cultures. Fur-
thermore, it must be stated that all these hypothesis 
regarding the possible in vivo role of CETAF are 
based on in vitro experimental models and that there 
is as yet no direct evidence that any mitogenic factors 
actually contribute to in vivo immune responses. In 
addition, it is quite possible that further biochemical 
analysis and purification of corneal epithelial cell-de-
ved mediators that are involved in the aforementioned 
activities may in fact reveal them to be a quite 
distinct group of factors.

Biochemical analysis and animal experiments are 
currently in progress to elucidate the potential role 
of CETAF in corneal wound healing and diseases of 
different etiology.

Key words: SIRC cell line, corneal epithelial cell-derived 
lymphocyte-activating factor (CETAF), dermal fibroblast 
stimulation, IL 2 induction, chemotaxis for PMNs, stim-
ulation of CETAF release.

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