# Eye-derived growth factor from retina and epidermal growth factor are immunologically distinct and bind to different receptors on human foreskin fibroblasts

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## 1. INTRODUCTION

Ocular tissues contain potent mitogens that stimulate the in vitro proliferation of BEL cells [1,2] as well as cells from non-ocular tissues from different species [3]. These mitogens fulfilled the criteria proposed for the definition of a cell growth factor [4] and were named eye-derived growth factor (EDGF) [3]. Retinal EDGF, purified >1000-fold, induced maximum cell growth at doses similar to those used with EGF and FGF [3]. Although the $M_r$ estimates of EDGF differed from EGF and FGF [3,5], EDGF and EGF had identical isoelectric points at pH 4.5 ± 0.5 and comparative studies of their biological properties on several target cells were similar [3]. Both factors stimulated corneal endothelium [6,7], chondrocytes [3,7] and epidermal cells [8,9]. EDGF stimulated BEL cells to divide, but EGF did not, even though receptors for EGF were detected on BEL cells [3,6,10]. In contrast, we found that HF cells were not stimulated by EDGF [9]. HF cells have EGF receptors and consistently respond to its mitogenic effect [11,12]. We now extend our studies and show that HF cells also have receptors for EDGF and, by competition experiments, the EDGF receptors are distinct from those of EGF. Furthermore, to determine that the mitogenic activity found in our EDGF preparations was not due to EGF contamination, we used a radioimmunoassay for EGF to screen our EDGF preparations. Mitogenic levels of EGF were not detected.

*Abbreviations:* BEL cells, bovine epithelial lens cells; HF, human foreskin fibroblasts; EGF, epidermal growth factor; EDGF, eye-derived growth factor; PBS, phosphate-buffered saline

## 2. MATERIALS AND METHODS

### 2.1. *Cell cultures*

HF cells were routinely grown in Dulbecco's modified Eagle's medium with 10% fetal calf serum [12]. Two days prior to radioreceptor assay, cells were plated in 24 well culture dishes (2 $cm^2$/well) at $5 \times 10^4$ cells/$cm^2$. The medium was changed just before the assay and 0.45 ml fresh serum-free medium was placed into each well.

### 2.2. *Growth factor preparation*

mEGF was isolated from mouse submaxillary glands following the procedure in [13] or obtained from Collaborative Research Labs. Both preparations induced maximum stimulation of HF cells at 10 ng/ml culture medium. EDGF was purified from adult bovine retina. Retina were homogenized in PBS and centrifuged at 20 000 × *g* for 30 min and at 100 000 × *g* for 16 h. Ammonium sulfate was added to the supernatant to bring the salt to 20%, and after centrifugation at 20 000 × *g* for 30 min, protein components were further concentrated by bringing the salt to 60% ammonium sulphate. The precipitate was resuspended in 10 vol. PBS and dialysed overnight against 0.1 N acetic acid. The supernatant obtained after a centrifugation at 20 000 × *g* for 30 min was then dialysed against PBS. The

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precipitations yielded a 50-fold purification of EDGF. Further purification, ~1000-fold, was achieved by high-pressure liquid chromatography on silicium-grafted columns (I-125, Waters Assoc.). EDGF was found in the fractions eluted between 15 000–20 000 $M_r$. This material induced maximal [$^3$H]thymidine incorporation into DNA of BEL cells at 100 ng/ml culture medium (in preparation). Protein concentration was measured using bovine serum albumin as standard as in [14].

### 2.3. *$^{125}$I-mEGF and $^{125}$I-EDGF*

Iodination was carried out using $^{125}$I-Na carrier-free (Amersham) and the chloramine T procedure [15]. Specific activities of $^{125}$I-mEGF and $^{125}$I-EDGF were 30–50 μCi/μg and 80–100 μCi/μg, respectively.

### 2.4. *Radioimmunoassay*

Radioimmunoassays were performed in 0.025 M sodium barbital buffer (pH 7.9); 0.1 ml rabbit anti-mEGF diluted 1:2000 (Collab. Res.) was added to various amounts of unlabeled mEGF or EDGF. After 2 h incubation at 37°C, $^{125}$I-mEGF was added to 0.22 × $10^6$ cpm/ml and the reaction mixture was centrifuged. Second antibody (goat antiserum against rabbit) was then added for another 2 h at 37°C and the immunocomplex precipitated recovered after centrifugation.

### 2.4. *$^{125}$I-EDGF binding*

Increasing concentrations of $^{125}$I-labeled EDGF (1–1000 ng/ml) were added to cultures. Cells were incubated at 37°C for 1 h and the reaction was stopped by washing each dish 4 times with cold PBS. Cells were trypsinized (1 ml trypsin, 0.25%) and lysed with 1 ml lysing buffer [12]. Radioactivity was measured with a Beckman Biogamma II counter. Non-specific binding was measured by adding excess unlabeled EDGF (5 μg/dish) to a duplicate set of reaction plates at each point.

### 2.5. *Competitive binding studies*

Competition between $^{125}$I-mEGF and unlabeled EGF or EDGF or a mixture of both was done as follows. To each well containing 440 μl culture medium, 10 μl $^{125}$I-mEGF ($10^6$ cpm) was added and 50 μl of the appropriate amount of unlabeled mEGF or EDGF was added.

## 3. RESULTS

### 3.1. *Cross-immunoreactivity between EGF and EDGF*

Immunological cross-reactivity between EGF and EDGF was tested in a radioimmunoassay (fig.1). Large amounts of EDGF, up to 50 μg/ml, a 500-fold greater dose than needed for maximum stimulation of DNA synthesis in BEL cells, showed no detectable cross-reactivity with EGF. Over 95% of the labeled EGF remained free when only 35 ng/ml of unlabeled EGF was added. Since the limit of detection of EGF was ~0.1 ng/ml (inducing a displacement of 2–3% of the 100% of the control), the EDGF preparations contained less than this amount. If EGF contamination existed in our preparations, it would be unlikely that the maximum stimulation of BEL cells obtained with 200 ng EDGF could be due to contamination by ≤0.1 ng EGF. Maximum stimulation of HF cells was achieved at 10 ng EGF/ml (not shown) and minimal doses of stimulation by EGF was at 0.1 ng EGF.

### 3.2. *Specific EDGF binding to HF fibroblasts*

Fig.2 shows that EDGF binds to HF cells. Maximum binding was obtained at 750 ng EDGF/ml (after 60 min at 37°C). EDGF binding was enhanced by the addition of 10 ng EGF/ml. The shape of the binding curve was similar to the binding of

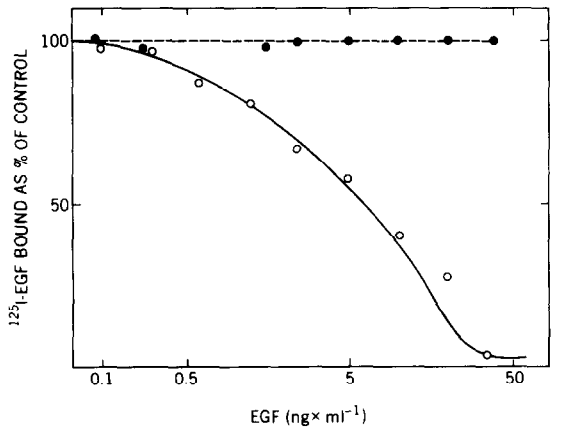


Fig.1. Radioimmunoassay for mEGF: Precipitation of $^{125}$I-mEGF–anti-EGF immunocomplex after antibody reaction with unlabeled EGF (o——o) and with unlabeled EDGF (•---•).

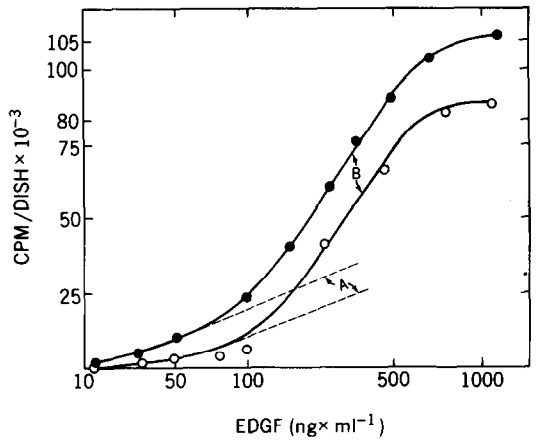


Fig.2. Binding of $^{125}$I-EDGF to human foreskin fibroblasts with (•——•) or without 10 ng mEGF/ml (○——○); see section 3.

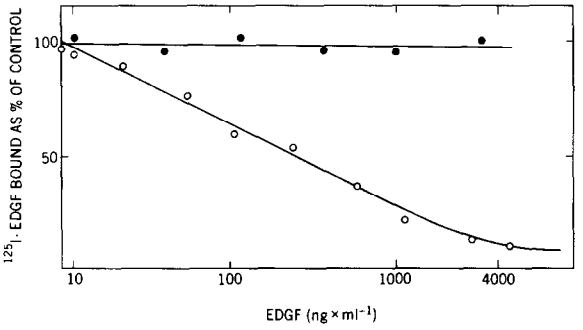


Fig.3. Radioreceptor assay for EDGF: Displacement of $^{125}$I-EDGF binding is shown for unlabeled EDGF (○——○) or unlabeled EGF (•——•).

mEGF and retinoids to target cells [16] and suggests 2 types of binding sites: one type with high affinity and low capacity (fig.2A) and another with lower affinity and increased capacity (fig.2B). The shape of the curves were identical in 5 separate expt. A radioreceptor assay using $^{125}$I-EDGF and cold EDGF was performed (fig.3); 50% competition was obtained at ~200 ng/ml and maximal competition was achieved at ~3000 ng/ml.

### 3.3. *mEGF radioreceptor assay and EDGF competition*

Addition of cold mEGF or a mixture of EDGF

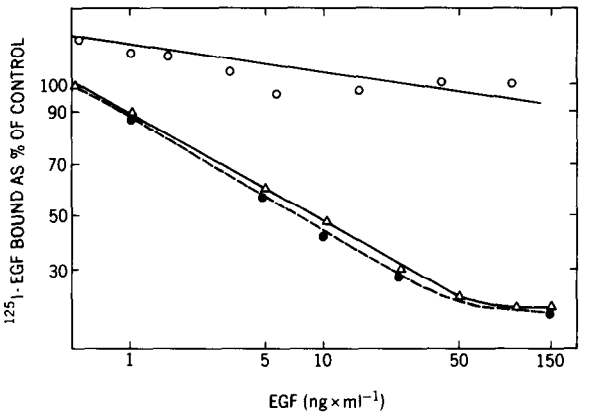


Fig.4. Radioreceptor assay for mEGF: Displacement of $^{125}$I-mEGF binding to its receptors on human foreskin fibroblasts was studied with various amounts of mEGF alone (△——△), EDGF alone (○——○) or mEGF plus EDGF (•---•).

and mEGF displaced $^{125}$I-mEGF; 50% competition was obtained at 10 ng mEGF/ml (fig.4). EDGF alone did not displace bound $^{125}$I-mEGF even at the high dose of 1000 ng/ml (not shown). Interestingly, at smaller doses of EDGF, mEGF binding was enhanced ~10–20%.

## 4. DISCUSSION

Our studies indicate that EDGF and mEGF are immunologically distinct. EDGF and mEGF may bind to different cell surface components and, in appropriate competition studies, do not compete for each other's specific receptors. Furthermore, our EDGF preparations did not contain EGF in amounts currently detectable by radioimmune or radioreceptor assays. This point is important because EGF has been detected in trace amounts in virtually all tissues studied. It is found in the peripheral circulation of mammals, particularly man and rodents, at ~1–5 ng/ml, concentrations that are potently mitogenic [17]. mEGF has highly specific and reproducible effects on the acceleration of eyelid opening in the neonatal mouse and has specific effects on epithelial and mesenchymal cells of the eye [13]. We were concerned that the mitogenic activity of the eye extracts could be accounted for by EGF. The mitogenic activity of somatomedin B was found to be due to EGF contamination [18]. Our

results show no evidence of EGF contamination in our EDGF preparations and further establish EDGF as a unique mitogenic agent.

Although receptors for both EDGF and EGF are found on a wide variety of cell types from different species, the presence of receptors does not apparently mean that the cells will show a mitogenic effect. BEL cells have receptors for both EDGF and EGF but only EDGF stimulated the BEL cells to divide in the presence of fetal calf serum. In contrast, HF cells responded to EGF and not to EDGF [3,6,10]. Small amounts of EGF did, in fact, potentiate the total amount of EDGF binding to both BEL and HF cells but the enhanced EDGF binding did not appear to translate into an enhanced mitogenic effect (unpublished). Synergistic effects between polypeptide growth factors, phorbol esters, retinoids and glucocorticoids on cultured cells have been observed [19–21]. Modulation of the specific binding of one growth factor by another may, in some instances, directly affect the mitogenic responsiveness of target cells [20]. The potentiating effect of these factors may be related to increased trans-membrane movement of metabolic precursors essential for cell division and not to a direct mitogenic stimulation [20,22]. Platelet-derived growth factor (PDGF) induced a transient down-regulation of EGF receptors and inhibited any further effect of EGF, suggesting some common relationship between EDGF and EGF receptors [23]. The effect of EGF on EDGF binding is analogous to the glucocorticoid effect on EGF binding [19]. It is apparent that growth factors may have multiple roles as conditioning agents as well as primary growth promoters.

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