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Partial purification and characterization of a growth factor present in goat's colostrum

Similarities with platelet-derived growth factor

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A factor in goat's colostrum which stimulates DNA synthesis and cell proliferation in Swiss 3T3 fibroblasts has been purified approx. 350-fold by a sequence of acid precipitation, cation-exchange chromatography and gel filtration. The growth factor is a highly basic, heat stable (100°C for 5min) polypeptide with M_r approx. 35000. The polypeptide resists denaturation by guanidinium chloride or urea but is totally inactivated by treatment with reducing agents. The factor, which we have termed colostric basic growth factor (CBGF), inhibits the binding of ^{125}I-labelled epidermal growth factor (^{125}I-EGF) to Swiss 3T3 fibroblasts but does not inhibit ^{125}I-EGF binding to epidermoid A431 cells. CBGF interacts synergistically with plasma in stimulating DNA synthesis in quiescent Swiss 3T3 cells. The chemical and biological properties of CBGF are thus very similar to the properties reported for the human platelet-derived growth factor. Although high concentrations of CBGF are present in the colostrum of goats, cows, and sheep, the milk of these species contains little or no factor. The origin and possible functions of CBGF are unknown.

Colostrum from goats, sheep and cows stimulates the proliferation of mouse 3T3 fibroblasts in cell culture and inhibits the binding of ^{125}I-labelled EGF to its cellular receptors (Brown & Blakeley, 1983*a*,*b*). This raises the possibility that an EGF-related mitogen is present in the mammary secretions of these ruminants; EGF is already known to be present in mouse and human milk (Hirata & Orth, 1979*a*,*b*; Carpenter, 1980). However, the ^{125}I-EGF-inhibiting growth promoter in goat's colostrum is a basic polypeptide with $M_r > 20000$ (Brown & Blakeley, 1983*b*), and is therefore different from either mouse or human EGF, which are acidic polypeptides of M_r approx. 6000. Furthermore, the characteristics of the inhibition of ^{125}I-EGF binding by the colostric factor were different from those of the inhibition of binding produced by native unlabelled mouse EGF (Brown & Blakeley, 1983*b*). The ^{125}I-EGF-inhibiting growth factor in goat's colostrum has now been partially purified using acid decaseination, ion-exchange chromatography and gel filtration. The results indicate that the factor is chemically and functionally similar to a growth factor which has been isolated from human platelets (Heldin *et al.*, 1981*a*; Deuel *et al.*, 1981; Raines & Ross, 1982).

Abbreviations used: CBGF, colostric basic growth factor; DME, Dulbecco's modified Eagle's medium; EGF, epidermal growth factor; PDGF, platelet-derived growth factor.

Materials and methods

Materials

Culture medium, antibiotics, trypsin and newborn calf serum were from Flow Laboratories. Na^{125}I and [^{3}H]thymidine were from Amersham International. EGF was prepared from male mouse submaxillary gland by the method of Savage & Cohen (1972) and labelled by using lactoperoxidase (Thorell & Johansson, 1971) as previously described (Brown & Blakeley, 1983*b*). Platelet-poor plasma (goat) was prepared by the method of Rutherford & Ross (1976).

Cell cultures

Stock and experimental cultures of Swiss mouse 3T3 cells were grown in DME medium containing

10% (v/v) newborn calf serum and antibiotics as described previously (Brown & Blakeley, 1983*a*,*b*).

Partial purification of the colostric basic growth factor

General. All operations were carried out at 4°C except where otherwise stated. Protein determinations were made with the Bradford (1976) procedure with pig gamma globulin as standards.

Decaseination of goat's colostrum. Colostrum was obtained from a goat (number 565) within 1 h after parturition and frozen at −20°C until further processing. After thawing, 750 ml of colostrum was centrifuged at 22000$g_{av.}$ for 30 min and the aqueous phase was withdrawn from below the floating lipid. The pH of the defatted colostrum was lowered to 4.4 by the dropwise addition of 1 M-acetic acid. The acidified colostrum was stirred for 2 h and then left overnight without further stirring. The precipitated casein was removed by centrifugation as described above to yield decaseinated whey.

Cation-exchange chromatography. The decaseinated whey was dialysed (Spectrapor 3 dialysis tubing, M_r cut-off 3500) against 15 litres of 0.02 M-ammonium formate (pH 7.0) for approx. 60 h followed by dialysis against 15 litres of 0.02 M-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] (pH 7.0) for 48 h. The precipitate which formed was removed by centrifugation as described above and the clarified whey was applied to a column (10 cm × 5 cm) of carboxymethylcellulose (Whatman CM52) equilibrated with 0.02 M-Hepes (pH 7.0). After sample application, the column was washed with 460 ml of 0.02 M-Hepes (pH 7.0) followed by 1500 ml of 0.2 M-Hepes (pH 7.0). Finally the column was eluted with 300 ml of 0.2 M-Hepes (pH 7.0) containing 1 M-NaCl. Throughout these procedures the column was operated with a 40 cm head of pressure and a flow rate of approx. 150 ml/h. Bulk fractions with the volumes indicated in Fig. 1 were collected.

Gel filtration. The ^{125}I-EGF-inhibiting fraction eluted from the CM52 column by 0.2 M-Hepes containing 1 M-NaCl (Fig. 1) was concentrated from 100 ml to 4.4 ml by pressure ultrafiltration (Amicon PM10 membrane). The retentate was applied to a column (90 cm × 2.6 cm) of Ultrogel AcA 54 equilibrated and eluted with phosphate-buffered saline (pH 7.4) containing 1 M-NaCl. The flow rate was maintained at 20 ml/h using a peristaltic pump. Fractions (10 ml) were monitored manually with a Zeiss spectrophotometer at 280 nm.

Assays. The ability of samples to inhibit cellular ^{125}I-EGF binding and to stimulate DNA synthesis in quiescent cultures of Swiss mouse 3T3 cells was measured as described previously (Brown & Blakeley, 1983*b*).

Results

Partial purification

A cationic growth factor in goat's colostrum was partially purified by a sequence of acid decaseination, cation-exchange chromatography, and gel filtration. The purification was monitored by using two different assays; stimulation of DNA synthesis in quiescent 3T3 cell cultures and inhibition of ^{125}I-EGF binding to 3T3 cells. Based on the latter assay, an increase in specific activity of about 350-fold was achieved with an overall recovery of approx. 3% (Table 1).

The ^{125}I-EGF-inhibiting growth factor is present exclusively in the aqueous, non-casein (i.e. whey) fraction of colostrum since none of the inhibitory activity was lost on removal of fat (results not shown) or on acid-precipitation of casein (Table 1). The defatted, decaseinated whey was dialysed against 0.02 M-Hepes (pH 7.0) and applied to a CM52 column equilibrated with 0.02 M-Hepes (pH 7.0). Most of the ^{125}I-EGF-inhibiting activity was retained by the column and was not eluted by 0.2 M-Hepes but was desorbed by 0.2 M-Hepes containing 1 M-NaCl. The ^{125}I-EGF-inhibiting activity in approx. 1 litre of whey was collected in a concentrated form in 100 ml of eluate. This ^{125}I-EGF-inhibiting fraction was a potent stimulator of DNA synthesis in quiescent 3T3 cells (Fig. 1). The unretained material from the CM52 column and the material eluted with the first 500 ml of 0.2 M-Hepes also contained considerable cell growth-promoting activity (Fig. 1) but these fractions had little activity in the ^{125}I-EGF binding assay. This result suggests that other growth factors, in addition to the ^{125}I-EGF-inhibiting material, are present in the decaseinated colostric whey.

The ^{125}I-EGF-inhibiting activity eluted from the CM52 column was concentrated by ultrafiltra-

Table 1. *Partial purification of a basic growth factor from 750 ml of goat's colostrum*

A unit of activity is defined as the amount of protein required to produce 50% inhibition of ^{125}I-EGF binding to Swiss 3T3 cells under the conditions described in the legend to Fig. 1.

Stage	Volume (ml)	Total protein (mg)	Specific activity (units/mg)	Recovery (%)
Defatted	615	101475	1.9	100
Decaseinated	808	42016	4.9	108
Dialysed	1030	23805	2.7	33
CM52	100	775	10.1	4
PM10 ultra-filtration	4	631	16.7	5
AcA 54	38	9	649	3

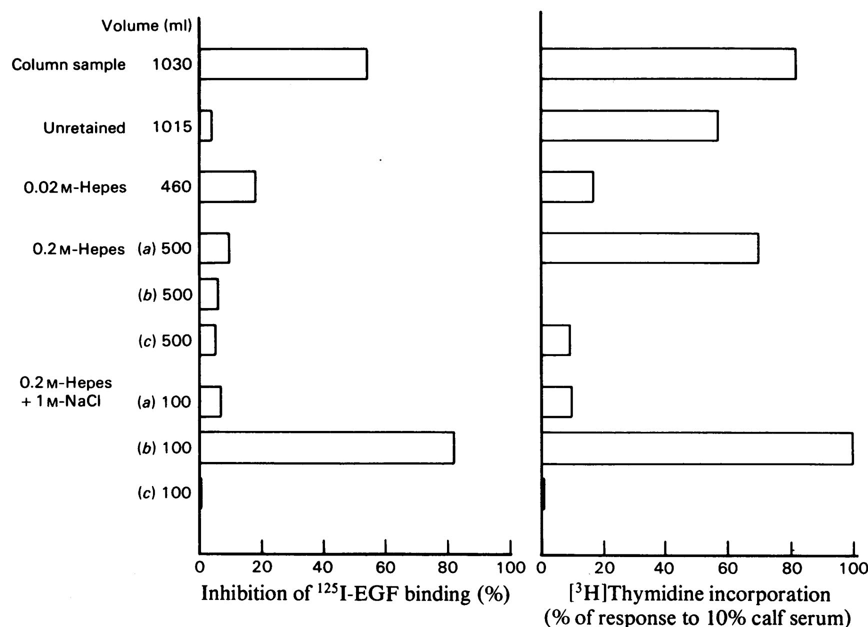

Fig. 1. *CM-cellulose chromatography of cell growth-promoting activity in colostric whey*
The column and column sample were prepared and the column was developed as described in the Materials and methods section. The indicated fractions were collected and tested for their ability to inhibit ^{125}I-EGF binding to Swiss 3T3 cells. Cells, grown in 24-well cluster trays (Nunc), were rinsed with 1–2 ml of binding medium {DME containing 0.1% crystalline bovine serum albumin, 0.1 μM-KI and 50 mM-Bes [*NN*-bis(2-hydroxyethyl)-2-aminoethanesulphonic acid] adjusted to pH 7.0}. Binding medium (0.5 ml/well) containing ^{125}I-EGF (1.3 ng/ml; 42000 c.p.m./ng) and samples of the column fractions (40 μl/ml) was added to the cells. After a 90 min incubation at 37°C, the cells were washed and cell-associated radioactivity was determined. Non-specific ^{125}I-EGF binding, measured as cell-associated radioactivity in the presence of unlabelled EGF (2 μg/ml), was less than 5% of the total under these conditions. The mean value (n = 2) for the percentage inhibition of ^{125}I-EGF binding relative to the binding to untreated, control cells is shown. The column fractions were also tested for their ability to stimulate DNA synthesis in quiescent cultures of Swiss 3T3 cells. Test fractions (100 μl/ml of medium) were added directly to the depleted growth medium of cells grown on 3 cm plastic dishes (Nunc). [^{3}H]Thymidine (20 μl) was added to give a final concentration of 1 μCi/ml (1×10^{-6} M). After incubation for 40 h at 37°C, the medium was removed from the dishes and the cells were washed twice with cold phosphate-buffered saline (pH 7.4). The cells were extracted for 30 min with 1–2 ml of cold 5% (w/v) trichloroacetic acid, rinsed with ethanol, and air dried. The cells were dissolved in 1 ml of 0.1 M-NaOH and 0.5 ml of this solution was mixed with 10 ml of acidified scintillation fluid for counting of ^{3}H. The mean value for duplicate determinations of [^{3}H]thymidine incorporation is expressed as a percentage of the stimulation obtained by adding 10% (v/v) calf serum to the cultures (1.69×10^5 c.p.m./dish). Unstimulated cultures incorporated 2.3×10^3 c.p.m./dish.

tion and the resulting clear, orange-brown coloured retentate was chromatographed on an Ultrogel AcA 54 column. The ^{125}I-EGF-inhibiting activity was eluted from the column (Fig. 2) after the void volume protein peak and the coloured material (lactoferrin). The indicated fractions (21–24; M_r 28000–40000) were pooled and shown to stimulate [^{3}H]thymidine incorporation into quiescent 3T3 cells; none of the other column fractions tested was active (Fig. 2). This post-AcA 54 pool was used for further chemical and biological characterization of the partially purified CBGF.

Stability

The effects of proteolytic enzymes, heat, denaturing conditions, and reduction on the ^{125}I-EGF-inhibiting activity and the cell growth-promoting activity of the CBGF were investigated. Both activities showed a similar response to all conditions, providing further evidence that the two effects are caused by a single compound. The factor is apparently a polypeptide since all activity is destroyed by incubation with proteolytic enzymes (Table 2). The activity of the factor is not affected by dissociating agents (6 M-urea, 4 M-guanidinium chloride) but activity is totally destroyed on reduction by 2-mercaptoethanol. The factor is stable to heat, with no loss of activity on heating to 100°C for 5 min (Table 2). These properties of CBGF, together with its apparent charge and M_r, closely resemble those reported for the polypeptide mitogen PDGF, which has been purified from human

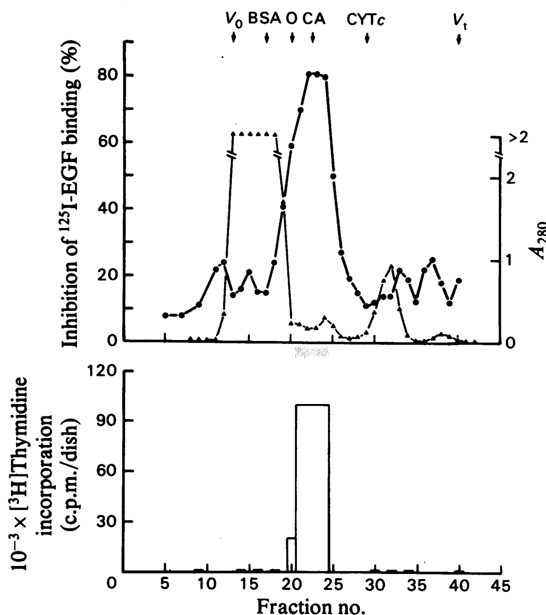

Fig. 2. *Gel filtration of the basic growth factor from goat's colostrum*

The ^{125}I-EGF-inhibiting fraction eluted from the CM52 column by elution with 0.2M-Hepes and 1M-NaCl was concentrated and chromatographed on a column of Ultrogel AcA 54 as described in the Materials and methods section. Column fractions were tested (100 μl/ml of binding medium) for their ability to inhibit ^{125}I-EGF binding to Swiss 3T3 cells (upper panel, ●) as described in the legend to Fig. 1. Fractions 21–24 were pooled and the pool, together with the indicated individual column fractions, were tested for the ability to stimulate [^{3}H]-thymidine incorporation in quiescent Swiss 3T3 cells (lower panel). In this experiment the addition of calf serum (10%, v/v) to the depleted growth medium produced an incorporation of 1.17×10^5 c.p.m./dish. The column void volume (V_0), total volume (V_t) and the elution positions of bovine serum albumin (BSA), ovalbumin (O), carbonic anhydrase (CA) and cytochrome *c* (CYT*c*) are indicated. ▲, A_{280}.

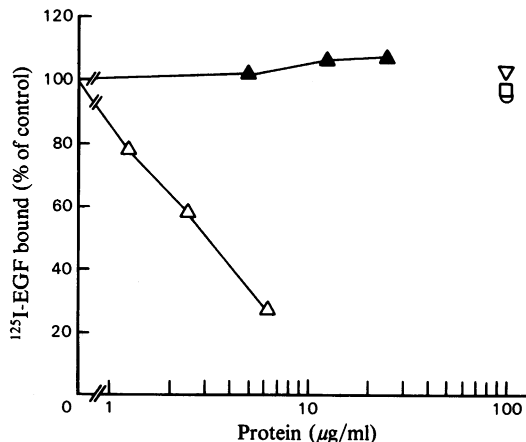

Fig. 3. *Dose–response for the inhibition of* ^{125}*I-EGF binding by CBGF*

The inhibition of ^{125}I-EGF binding to Swiss 3T3 cells by the indicated concentrations of CBGF (△), cytochrome *c* (○), lysozyme (□), or protamine sulphate (▽) was measured as described in the legend to Fig. 1. The failure of CBGF to inhibit ^{125}I-EGF binding to the human epidermoid cell line, A431 (▲) is also shown. Each point represents the mean value ($n = 2$ or 3) for ^{125}I-EGF binding expressed as a percentage of the mean control value for binding to untreated cells.

platelets (Heldin *et al.*, 1981*a*; Deuel *et al.*, 1981; Raines & Ross, 1982).

Inhibition of ^{125}*I-EGF binding*

The dose-dependent inhibition of ^{125}I-EGF binding to Swiss 3T3 cells at 37°C by CBGF is shown in Fig. 3. Half-maximal inhibition of binding was achieved at a protein concentration of approx. 3 μg/ml. This inhibition is not a non-specific action of basic proteins since other very basic proteins (lysozyme, protamine sulphate, cytochrome *c*), at concentrations up to 100 μg/ml, failed to inhibit ^{125}I-EGF binding to Swiss 3T3 cells (Fig. 3).

Unfractionated colostric whey inhibits ^{125}I-EGF binding to Swiss 3T3 cells by decreasing the apparent affinity of the cellular receptors for ^{125}I-EGF (Brown & Blakeley, 1983*b*). However, the colostrum-mediated inhibition of ^{125}I-EGF binding, although rapid and apparently competitive, was shown to differ in several important characteristics from the inhibition of binding induced by native, unlabelled EGF. Thus the inhibitory effect of colostrum is markedly decreased when the assay temperature is shifted from 37°C to 4°C, whereas unlabelled EGF is an equally effective competitive inhibitor at both temperatures. Incubation of cells with EGF causes a reduction in cell-surface EGF receptors, whereas exposure to colostrum does not induce EGF receptor down-regulation (Brown & Blakeley, 1983*b*). Identical results (not shown) have been obtained using the partially purified CBGF. These findings suggest that the CBGF does not bind directly to cellular EGF receptors but inhibits ^{125}I-EGF binding by an indirect mechanism. This is supported by the observation (Fig. 3) that ^{125}I-EGF binding to another cell line, the human epidermoid-carcinoma-derived A431, is not sensitive to inhibition by CBGF. This result also indicates that the CBGF-mediated inhibition

Table 2. *Sensitivity of the colostric basic growth factor to inactivation by proteolytic enzymes, denaturing conditions and reduction*

Aliquots of CBGF (post-AcA 54 pool) were exposed to phosphate-buffered saline, pH 7.4, 6M-urea, 4M-guanidinium chloride, or 2% β-mercaptoethanol for 24h at 4°C, then dialysed (exclusion limit 3500Da) against phosphate-buffered saline. The samples were subsequently tested for their ability to inhibit ^{125}I-EGF binding to Swiss 3T3 cells and to stimulate DNA synthesis in quiescent Swiss 3T3 cells. For each assay the remaining activity is expressed as a percentage of the activity in the phosphate buffered saline-treated controls. In a separate experiment, a sample of the post-AcA 54 pool was heated to 100°C for 5min and tested in the assays. The remaining activity is expressed as a percentage of the activity in unheated controls. In a third experiment, aliquots of the post-AcA 54 pool were incubated for 4h at 37°C with a mixture of trypsin and α-chymotrypsin (each at a concentration of 0.5mg/ml), a mixture of heat-inactivated trypsin and α-chymotrypsin (each at 0.5mg/ml), or phosphate-buffered saline without enzymes. At the end of the incubation, soya bean trypsin inhibitor (2mg/ml) was added and the remaining activity in the proteinase-treated samples was measured and is expressed as a percentage of the activity in the phosphate buffered saline-treated controls.

		Activity remaining (%)	
Expt.	Treatment	^{125}I-EGF-inhibiting activity	Stimulation of [^{3}H]thymidine incorporation
I	6M-Urea, 4°C, 24h	101	127
	4M-Guanidinium chloride, 4°C, 24h	95	109
	2% (v/v) 2-mercaptoethanol, 4°C, 24h	<1	<1
II	100°C, 5min	108	107
III	Trypsin + α-chymotrypsin, 37°C, 4h	<1	2
	Heat-inactivated trypsin + α-chymotrypsin, 37°C, 4h	98	99

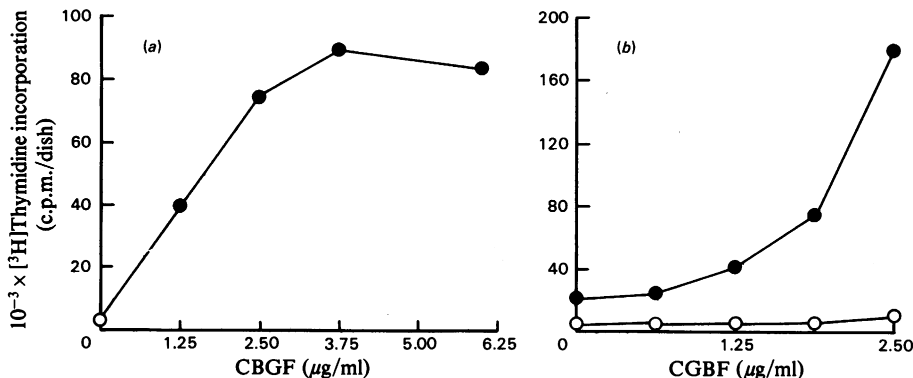

Fig. 4. *Stimulation of DNA synthesis in quiescent Swiss 3T3 cells by CBGF*

(*a*) 3T3 cells were seeded into 3cm plastic dishes (Nunc) and grown to confluence (7 days) without medium change. The indicated concentrations of CBGF together with [^{3}H]thymidine (20μl; final concentration 1μCi/ml and 1×10^{-6}M) were added directly to the depleted growth medium. After incubation for 40h at 37°C, the incorporation of radioactivity into acid-precipitable material was measured as described in the legend to Fig. 1. Each point represents the mean value for duplicate determinations of [^{3}H]thymidine incorporation. In this experiment the addition of fresh calf serum to the medium (10%, v/v) produced an incorporation of 1.14×10^{5}c.p.m./dish. (*b*) The growth medium on 7-day-old cultures of 3T3 cells was removed from the dishes, the cells were rinsed with serum-free DME and 2ml of this medium without (○) or with (●) 5% (v/v) platelet-poor plasma (goat) were added. The indicated concentrations of CBGF together with [^{3}H]thymidine were added to the medium and the dishes were incubated at 37°C for 40h and the incorporation of radioactivity into acid-precipitable material was measured. Each point represents the mean of duplicate determinations of [^{3}H]thymidine incorporation.

of ^{125}I-EGF binding is not due to proteinase contamination in the preparation or to binding of the ^{125}I-EGF ligand by the basic CBGF.

Stimulation of 3T3 cell growth

The dose–response for the stimulation of DNA synthesis in quiescent 3T3 cells by CBGF is shown in Fig. 4. In one experiment (panel *a*), CBGF was added directly to the depleted growth medium of 3T3 cells. A 27-fold stimulation of DNA synthesis was achieved with a maximal effect being produced by approx. 4μg of CBGF/ml of depleted

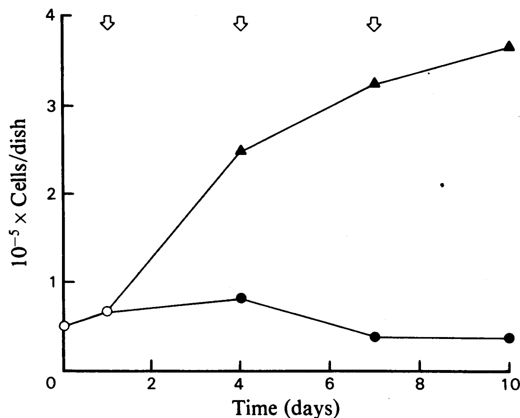

Fig. 5. *Effect of CBGF on the proliferation rate of Swiss 3T3 cells in low-serum medium*

Cells were seeded at the indicated density (○, day 0) in DME containing 10% calf serum, incubated at 37°C for 24h and cell numbers (○) were determined as described below. The remaining dishes of cells were rinsed with serum-free DME and 2ml of this medium containing 0.2% calf serum without (●) or with (▲) 7.5μg of CBGF were added per dish. The medium with or without CBGF was changed at 3 day intervals (arrows). At the indicated times, cells were detached from the dishes by incubation (5min at 37°C) with 2ml of Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline which contained 0.43mM-EDTA and 0.05% trypsin. The cells were dispersed into a suspension of single cells by gentle pipetting and an aliquot of the cell suspension was diluted into Isoton and cells were counted using a Coulter Counter. Each point represents the mean value for cell number obtained from three dishes of cells.

medium. It has been shown (Pledger *et al.*, 1977, 1978; Vogel *et al.*, 1978) that low concentrations of PDGF induce 'competence' in treated cells but that additional growth factor(s), present in platelet-poor plasma, are necessary for 'progression' through the cell cyle. We have observed a similar effect using CBGF, which produced only a small stimulation of 3T3 cell DNA synthesis when added alone to serum-free medium (Fig. 4*b*). However, the CBGF stimulated cells synergistically in combination with 5% (v/v) platelet-poor plasma (goat), which by itself had little stimulatory activity (Fig. 4*b*). This result indicates that depleted medium contains residual 'progression' factors which allow the expression of the 'competence'-inducing activity of CBGF in the experiment shown in Fig. 4(*a*).

The effect of CBGF on the proliferation rate of Swiss 3T3 cells is shown in Fig. 5. Cells placed in low-serum medium (DME+0.2% calf serum) showed an initial small increase in number but this was followed by a decrease in number. In contrast, the addition of 3.75μg of CBGF/ml of low-serum medium led to rapid cell proliferation with a minimal population doubling time of approx. 24h (Fig. 5). An attempt was made to grow 3T3 cells in DME with added CBGF in the complete absence of calf serum supplement. Initially the cells attached to the plastic dish, but by 24h after seeding all of the cells had rounded and detached. After 10 days, the cultures in unsupplemented, serum-free DME remained isolated, unattached, and appeared nonviable (Fig. 6*a*). In contrast, the cells in serum-free medium supplemented with unfractionated colostric whey (Fig. 6*b*) or with the post-AcA 54 pool of CBGF (Fig. 6*c*) had formed floating cell colonies. The colonies ranged in size from a few cells to several hundred cells with the largest colonies in the dishes supplemented with unfractionated colostric whey. These colonies were composed of viable cells (not aggregates of dead cells) since, on adding calf serum to the cultures, the colonies attached to the substratum and cells migrated from the colonies (Figs. 6*e* and 6*f*). In contrast, isolated unattached cells present in unsupplemented medium failed to attach when calf serum was added (Fig. 6*d*).

Discussion

We have characterized a major growth factor (colostric basic growth factor, CBGF) in the colostrum of goats and have demonstrated that it is a basic polypeptide with M_r approx. 35000 which inhibits the cellular binding of ^{125}I-EGF. CBGF differs chemically from either mouse or human EGF, which are acid polypeptides of M_r 6000. Our results indicate that CBGF is indeed much more closely related to the growth factor isolated from human platelets, PDGF (Heldin *et al.*, 1981*a*; Deuel *et al.*, 1981; Raines & Ross, 1982). Firstly, CBGF and PDGF are highly basic proteins with very similar M_r. Furthermore, CBGF (Table 2) and PDGF (see Ross, 1981) exhibit a similar resistance to denaturation by heat, urea and guanidinium chloride, while both are completely inactivated by exposure to reducing agents. In addition, the factors display a functional similarity in acting as 'competence' factors (Pledger *et al.*, 1977, 1978; Vogel *et al.*, 1978) synergistically interacting with platelet-poor plasma to stimulate cell proliferation. Finally, we have recently found (Brown *et al.*, 1983) that human PDGF inhibits ^{125}I-EGF binding to Swiss 3T3 cells by an indirect, temperature-sensitive mechanism similar to that found for the inhibition of ^{125}I-EGF binding by colostrum (Brown & Blakeley, 1983*b*). Neither CBGF nor PDGF, however, inhibits ^{125}I-EGF binding to human epidermoid A431 cells, which have been shown to lack PDGF receptors (Heldin *et al.*,

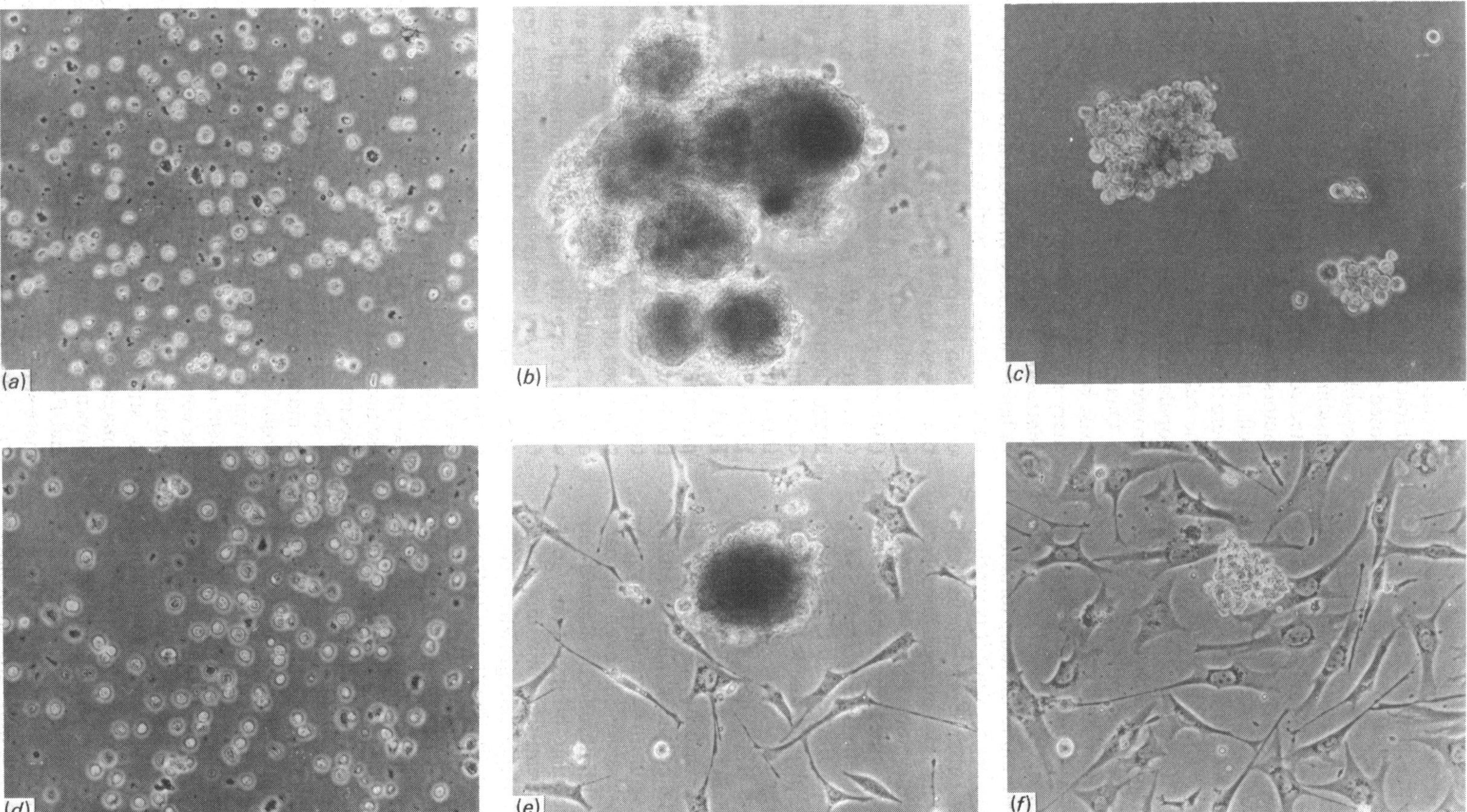

Fig. 6. *Growth of Swiss 3T3 cells in serum-free medium supplemented with decaseinated colostric whey or CBGF*

Stock cells were dispersed into 1 ml of Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline containing 0.43 mM-EDTA and 0.05% trypsin. Soya bean trypsin inhibitor (1 ml of a 0.25% solution) was added, the cells were centrifuged (150 **g**, 5 min) and resuspended in serum-free DME. Aliquots (35 μl containing 9×10^4 cells) of the suspension were seeded into DME containing either no addition (*a*, *d*), 10% (v/v) decaseinated colostric whey (*b*, *e*) or 2.5 μg of CBGF/ml of medium (*c*, *f*). After 9 days, calf serum (10%, v/v) was added to some cultures (*d*–*f*) while others (*a*–*c*) remained serum-free. After a further 3 days the cultures were photographed unfixed and unstained.

1981*b*; Bowen-Pope & Ross, 1982; Huang *et al.*, 1982).

Human PDGF is present in platelet α-granules (Kaplan *et al.*, 1979) and is probably synthesized in megakaryocytes in conjunction with α-granule formation (Chernoff *et al.*, 1980). The origin of CBGF is unknown but the close similarity with PDGF raises the possibility that the colostric factor is derived from goat platelets. A recent phylogenetic analysis of PDGF by radioreceptor assay (Singh *et al.*, 1982) showed that sera from all mammals tested contained PDGF activity. Furthermore, we have found (results not shown) that release-products from freshly prepared goat platelets contain a potent, cationic mitogen for 3T3 cells. It is possible, therefore, that CBGF accumulates in colostrum as a consequence of platelet degranulation. However, it has recently been shown that another α-granule protein, thrombospondin, is not platelet-specific since it is synthesized and secreted by cultured endothelial cells (Mosher *et al.*, 1982) and by cultured fibroblasts (Jaffe *et al.*, 1983). Furthermore, PDGF-like polypeptides are produced by some transformed cells in culture (Heldin *et al.*, 1980; Dicker *et al.*, 1981). These results raise the possibility that CBGF may be derived from a non-platelet source, perhaps in the mammary gland itself.

Steimer & Klagsbrun (1981) have reported that bovine colostrum is deficient in cell attachment factors when compared with bovine serum. Untransformed fibroblastic cell lines failed to attach to the substratum in serum-free medium supplemented with bovine colostrum, although precoating the culture dishes with fibronectin allowed cell attachment and subsequent proliferation. Colostrum-supplemented serum-free medium supported the anchorage-independent growth of colonies of virus-transformed fibroblasts, but untransformed cell lines did not show a similar response. The Swiss 3T3 cells used in the present work are an untransformed cell line in that they have a high serum requirement for proliferation and a low saturation density (approx. 5×10^4 cells/cm^2) in medium supplemented with 10% calf serum. In addition, the cells do not form colonies in agarose culture in medium supplemented with 10% calf serum. Nevertheless, we have observed that goat colostrum (Fig. 6*b*) is able to promote the anchorage-independent formation of colonies of these cells. Furthermore, this effect is mediated, at least in part, by CBGF, which also promoted the formation of viable, floating cell colonies (Fig. 6*c*). This ability of CBGF to stimulate colony formation in the complete absence of other exogenous factors is somewhat puzzling in view of our finding (Fig. 5*b*) that the full expression of CBGF growth-promoting activity requires a synergistic interaction with other growth factors. However, several points are worth noting. The number and size of the colonies formed were far greater in the cultures treated with unfractionated colostrum than in those cultures exposed to CBGF. This effect may be due to the possible presence of 'progression' factors (e.g. insulin, insulin-like growth factors) in unfractionated colostrum. The requirement for 'progression' factors may be reduced in unattached cells or in cells at a low population density. Finally, colonies were formed from only a minority (approx. 30%) of the cells seeded and these may represent a subpopulation of cells with a reduced requirement for progression factors.

Qualitative as well as quantitative differences exist between the growth factor content of mammary secretions from different species. The concentration of EGF (measured by homologous radioimmunoassay) in mouse milk has been reported to increase from birth to reach a maximal concentration of approx. 430 ng/ml at day 8 of lactation. These high EGF concentrations were maintained until day 17 and the approach of weaning (Beardmore & Richards, 1983). Human milk (donated 5 weeks post-partum) was shown to be highly active in stimulating DNA synthesis in quiescent human fibroblasts, and approx. 70% of this activity was blocked by antibodies to human EGF (Carpenter, 1980). These results indicate that EGF is the major cell growth factor of human (and probably mouse) mammary secretions and high levels of the factor are found in mature milk obtained in mid-lactation. In contrast, the mammary secretions of the domestic ruminants show a very different pattern of cell growth-promoting activity, with high activity in pre-colostrum and colostrum falling to very low levels within the first few days after parturition (Brown & Blakeley, 1983*a*,*b*). Furthermore, the colostrum of goats (the present work) or cows and sheep (K. D. Brown & D. M. Blakeley, unpublished results) contains a PDGF-like growth factor, but EGF-related polypeptides are not found in the mammary secretions of these species.

The physiological function of cell growth factors in mammary secretions is unknown. It has been postulated (Brown & Blakeley, 1983*b*) that these factors may play a role in the growth and development of the mammary gland of the mother and/or the control of cell proliferation in the intestinal epithelium of the neonate. Since EGF has been shown to stimulate the proliferation of mammary (Imagawa *et al.*, 1982) and intestinal (Blay & Brown, 1983) epithelial cells *in vitro* it is possible that EGF in mouse and human milk might act at these sites *in vivo*. In contrast, PDGF is active only on connective tissue-derived cells and not on epithelial cells, which appear to lack PDGF receptors

(Heldin *et al.*, 1981*b*). The close biochemical similarity of CBGF with PDGF suggests that this factor will also fail to stimulate epithelial cells and preliminary experiments (results not shown) support this hypothesis. Thus, any effect of the colostric factor on the growth of mammary or intestinal epithelium would have to occur indirectly via an effect on stromal cells. However, a stimulation of the growth of epithelial cells by unfractionated bovine colostrum has been reported (Klagsbrun, 1980). It is possible that the uncharacterized growth factors, distinct from CBGF and shown to be present in the unfractionated colostrum (Fig. 1), produce this effect.

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