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150-kDa Proteins in Dog Serum Bind 1.5-kDa Growth-Promoting Factors for Androgen-Independent Canine Prostatic Epithelial Cells

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ABSTRACT: Fractions obtained by gel filtration or ultrafiltration of dog serum were tested for their mitogenic activity on canine prostatic epithelial cells: two prostatic growth factor (PGF) entities were found, a major one of 150 kDa (PGF-I) and a minor one of 1.5–2.0 kDa (PGF-II). Treatment and/or extraction with acetic acid, hydrochloric acid, or acidified-ethanol of preparations enriched in PGF-I obtained either by ion-exchange chromatography, acetone precipitation, or retention by ultrafiltration membrane (cut-off 30 kDa) resulted, upon gel filtration, in the detection of a mitogenic activity eluting mainly at the position of PGF-II. Acid hydrolysis and proteolysis of PGF-II led to a loss of activity. It is proposed that, in dog serum, mitogenic peptides for prostatic epithelial cells of 1.5 kDa (PGF-II) are found in their free form and/or in association with proteins of 150 kDa (PGF-I).

Key words: Growth factor, epithelial cell proliferation, prostate cancer, benign prostatic hyperplasia, dog.

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Since the early reports by Holley (1975) and Barnes and Sato (1980) on the control of mammalian cell division in culture, isolation and characterization of polypeptide growth factors (GFs) from serum and extracts of animal tissues have been pursued in order to understand their role in normal and pathological situations. The observation that the dog, like man, develops a hyperplastic prostate (BPH) as well as prostatic adenocarcinoma with age (Moore, 1944) has led to the frequent use of this animal as a model to study the hormonal regulation of this gland (De Klerk et al, 1979; Aumüller et al, 1982). In most instances, human and dog epithelial cells isolated from normal and/or hyperplastic prostates, when cultured in primary monolayers, fail to respond to those steroids that stimulate their growth *in vivo* (Schroeder et al, 1971; Walsh and Wilson, 1976; De Klerk et al, 1979; Rohr et al, 1980; Eaton and Pierrepoint, 1982; Mistry et al, 1982; Syms et al, 1982; Chevalier et al, 1984; Peehl and Stamey, 1986a,b). In this respect, the hypothesis that GFs directly modulate their proliferation has been substantiated by the demonstration that purified and/or enriched GF preparations are mitogenic for human and canine prostatic epithelial cells in culture (Chevalier et al, 1984; Chapronière and McKeehan, 1986; Peehl and Stamey, 1986a,b). These findings are of interest and suggest that the relapse of prostate cancer after endocrine therapies, attributed to the growth of androgen-independent cells (Isaacs et al, 1980), may be the result of the proliferation of such cells already present in the adult gland.

In the canine model, dog serum is highly mitogenic for prostatic epithelial cells, whereas it has no significant activity on homologous prostatic fibroblasts (Chapdelaine and Chevalier, 1985; Chevalier et al, 1991). In addition, this effect cannot be mimicked by known GFs from commercial sources (Chevalier et al, 1991). In fact, a major entity of 150 kDa, referred to as prostatic growth factor-I (PGF-I), and a minor one, PGF-II, of low M_r (1.5 kDa), are indeed responsible for the mitogenic effect of dog serum (Chapdelaine and Chevalier, 1985; Chevalier et al, 1991). Interestingly, such a PGF-II entity is also present in dog prostatic tissue (Chevalier et al, 1991). In the present investigation, the possibility that these PGF-I and -II activities could be interrelated was studied by applying different extraction procedures to enriched preparations of PGF-I with the subsequent testing for the presence of PGF-II. The latter entity was also submitted to acid hydrolysis and proteolysis to ascertain its peptidic nature.

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Materials and Methods

Materials

Solvents and chemicals were of analytical grade. Sephacryl S-300 and DEAE-Sephadex were purchased from Pharmacia Fine Chemicals and Bio-Gels (P-100, P-30, P-2) from Bio-Rad. Several reference protein standards and peptides including GFs such as epidermal growth factor (EGF), insulin, and insulin-like growth factor-I (IGF-I) were used for column calibrations; in most instances, they were purchased from Bio-Rad or Sigma with the exception of IGF-I, which, in its radioiodinated form, was kindly provided by Dr. P. Manjunath (Maisonneuve-Rosemont Research Center, Montreal, Canada). All other reagents for cell isolation and culture were obtained from Gibco, Canada. Tissue culture plasticware was purchased from Corning or Gibco, Canada. Filtering units for sterilization of chromatographic fractions (0.22 μm) were obtained from Millipore; PM-30 and UM-10 membranes for ultrafiltration were from Amicon. Carboxypeptidase Y (bakers yeast), leucine aminopeptidase (porcine kidney), and trypsin (bovine pancreas) were purchased from Sigma. Hydrochloric acid (6 N HCl, constant boiling) used for PGF-II hydrolysis was obtained from Pierce.

The serum used for fractionation was prepared from blood drawn from adult mongrel dogs sacrificed to isolate prostatic cells. Preparations were kept frozen at −20°C until use.

Bioassay: Proliferation of Canine Prostatic Epithelial Cells

Our canine prostate cell culture system has already been characterized with respect to the isolation, separation, conditions for selective attachment, and growth of nonsecretory (basal) and secretory epithelial cell types as well as fibroblasts in both steroid- and serum-deprived or supplemented culture medium (Chevalier et al, 1980a, 1981, 1984, 1991; Chevalier and Chapdelaine, 1988, 1991). The isolation of prostatic cells by collagenase digestion and the establishment of primary monolayers of epithelial cells were performed as previously described (Chevalier et al, 1980a, 1984). Briefly, 2.5–5.0 × 10^5 cells in 3 ml of minimum essential medium D-valine (MEM D-Val) supplemented with 10% (v/v) dialyzed fetal bovine serum and antibiotics (1% penicillin–streptomycin solution) were inoculated in 20-cm² tissue culture petri dishes and allowed to attach for 3 days at 37°C in the presence of air : CO_2 (95%:5%); under these conditions, epithelial cell attachment (10–30% of plated cells) is mainly regulated by serum vitronectin (Chevalier and Chapdelaine, 1991). The number of epithelial cells in monolayers was then determined in three dishes; unattached cells in suspension in the remaining ones were discarded, and cultures were replenished with 3 ml of MEM D-Val alone but containing antibiotics as described above. To optimize the sensitivity of the bioassay, low-density cultures ($<5 \times 10^4$ cells attached per dish) were used (Chevalier et al, 1991). The chromatographic fractions, solubilized in serum-free medium and sterilized by filtration, were then added in duplicate at concentrations corresponding to 5 or 10% dog serum. In all experiments, parallel cultures were supplemented with homologous serum at 10% (v/v) to evaluate the proliferative response of every prostatic cell preparation. All cultures were further incubated for 6 days. Total cells, i.e., those in suspension and those harvested from the monolayers by trypsinization were then counted with a Coulter Counter, model ZF, as already described (Chevalier et al, 1981). Mitogenic activities of fractions are expressed as the mean total cell number per dish (±SD) or as percentages of increase as compared to control cultures performed in serum-free medium. In some instances, medium 199, MEM, or Dulbecco were also used and led to similar results in terms of cell response. The purity of all cell cultures (>95% epithelial) was evaluated microscopically following attachment and then periodically throughout the culture period.

Protein concentrations were determined by the method of Bradford (1976) using bovine serum albumin (BSA) as the standard.

Preparation of PGF-I by Ion-Exchange Chromatography and Treatment with Hydrochloric Acid

Dog serum (2-ml aliquots; 90–100 mg protein) was applied to a DEAE-Sephadex column (1 cm × 20 cm) equilibrated with 10 mM ammonium acetate (pH 7.0). The column was washed with two bed volumes of the same solution (flow rate: 20 ml/hour) and eluted thereafter (2-ml fractions) with a linear gradient of ammonium acetate up to 2 M (100 ml). Following measurement of the A_{280}, aliquots were pooled, lyophilized, and assayed for their mitogenic activity (equivalent to 10% [v/v] serum). Fractions where activity was detected were then combined, lyophilized, dissolved in a minimal volume of 10 mM ammonium acetate (pH 7.0), and submitted to gel filtration on a Sephacryl S-300 column as described below.

Such fractions enriched in PGF-I (prepared from 10 ml of serum) have been treated with HCl which was slowly added (12 N HCl, 7 ml) at 4°C to a final concentration of 5 N. The suspension was stirred overnight at 4°C, centrifuged (1,800 × *g*/10 minutes), and the precipitate washed twice with 5 ml of 5 N HCl (10 minutes on ice). The supernatants were combined, neutralized with NaOH, and methanol was added at −20°C to obtain a final concentration of 90% (v/v). Upon standing overnight at −20°C, the salt precipitated out and was discarded; the methanolic solution (peptide fraction) was brought to dryness by evaporation, redissolved in 5 ml of 10 mM ammonium acetate (pH 7.0), and used for gel filtration on a Bio-Gel P-30 column.

Acetone Treatment

In order to precipitate proteins, 45 ml of cold acetone (−20°C) were rapidly added to 5 ml of dog serum (227 mg protein); the suspension was stirred on ice for 10 minutes and submitted to centrifugation (3,000 × *g*/10 minutes). Both fractions (supernatant and precipitate) were dried under nitrogen, resuspended in 5 ml water, clarified by centrifugation (3,000 × *g*/10 minutes), and tested for activity. The precipitate was resolubilized with water to the initial volume of serum, and aliquots were submitted to gel filtration on a Sephacryl S-300 column, as described below.

Serum Ultrafiltration and Extraction of the Retentate

Ultrafiltration of serum (50 ml) was performed at 4°C in an Amicon cell system (60 ml capacity) through a PM-30 membrane (cut-off 30 kDa). The ultrafiltrate (U-30) was freeze-dried, redissolved in a minimal volume of ammonium acetate, pH 7.0 (at indicated concentrations), and aliquots (equivalent of 10 ml of serum) were used for gel filtrations (Bio-Gel P-2 and I-60 high-performance liquid chromatography [HPLC] columns as described below).

The retentate (C-30), diluted to the original volume of serum with water and reconcentrated twice, was then used for acetic acid extraction (Jacobs et al, 1979) with or without a 10-minute preincubation at 100°C (Zapf et al, 1975): in brief, acetic acid was added to a final concentration of 6 M; the suspension was stirred at 4°C for 4 hours and clarified by centrifugation (1,800 × *g*/10 minutes). It was verified in the bioassay that no activity was associated with the pellet (lyophilized and then dissolved in the original serum volume) when assayed at a concentration equivalent to 10% dog serum (v/v). The supernatants (extracts) were lyophilized, redissolved in a minimal volume of 0.1 M ammonium acetate, and chromatographed on a Bio-Gel P-100 column, which was selected to separate PGF-I from PGF-II and elute the PGF-II activity within the fractionation range of the gel. An untreated C-30 fraction was used as a control.

A C-30 fraction (10 ml) was also treated with nine volumes of cold-acidified ethanol (ethanol:acetic acid 98:2), which was added dropwise while stirring on ice; proteins were allowed to precipitate over a 1-hour period, and the preparation was centrifuged at 5,000 × *g*/15 minutes. The supernatant was evaporated to dryness under a stream of nitrogen, redissolved in a minimal volume of 10 mM ammonium acetate, pH 7.0, and loaded onto a Bio-Gel P-2 column.

Gel Filtrations

Sephacryl S-300—dog serum and preparations enriched in PGF-I, obtained either after ion-exchange chromatography or acetone precipitation (as described above), were applied onto Sephacryl S-300 columns (1.6 × 100 cm or 2.6 × 60 cm) equilibrated in 10 mM ammonium acetate (pH 7.0). Fractions of 3–5 ml were collected at a flow rate of 40 ml/hour.

Bio-Gels—Samples were dissolved in a minimal volume of 10 mM ammonium acetate (pH 7.0) and chromatographed either on columns filled with Bio-Gel P-100 (2.6 × 70 cm, 25 ml/hour), Bio-Gel P-30 (2.6 × 70 cm, 35 ml/hour), or Bio-Gel P-2 (1.6 × 60 cm, 15 ml/hour). All columns were equilibrated and eluted (fractions of 2–5 ml) in 10 mM ammonium acetate, pH 7.0. The elution of proteins and/or of peptides was generally monitored by the measurement of absorbance (A) at both 280 nm (aromatic amino acids) and 235 nm (peptide bonds), which, in all instances, yielded similar profiles even though the A_{235} was higher than A_{280}. Fractions were then pooled, lyophilized, resuspended in serum-free culture medium, sterilized, and tested for their mitogenic activity.

Acid Hydrolysis and Proteolysis

A fraction enriched in PGF-II was obtained by chromatography of the serum ultrafiltrate (U-30) (20 ml) on a Bio-Gel P-30 column as described previously (Chapdelaine and Chevalier, 1985) and used after freeze-drying for acid hydrolysis and proteolysis.

HCl (6 N) was added to aliquots of the freeze-dried fraction containing PGF-II, and the sealed and evacuated tube was heated at 110°C for 24 hours. The acid was then evaporated under a stream of nitrogen, and the sample, resuspended in serum-free medium, was assayed for its mitogenic activity, which was compared to that of an untreated sample.

For proteolysis, trypsin (440 U), carboxypeptidase Y (120 U), or leucine aminopeptidase (25 U) was added to the PGF-II-enriched preparation (in 0.1 M ammonium acetate, pH 7.0) and incubated overnight at 23°C. The mixture was then submitted to chromatography on a Bio-Gel P-30 column or to ultrafiltration through a UM-10 membrane (cut-off 10 kDa) to eliminate the proteolytic enzymes; the mitogenic activity was either tested on fractions eluting at the position of PGF-II (Bio-Gel P-30) or in the ultrafiltrate.

Estimation of the Apparent M_r of PGF-II by Gel Filtration Using HPLC

Ultrafiltration of 20 ml of serum was performed; the U-30 was lyophilized and redissolved in a minimal volume of 0.2 M ammonium acetate, pH 7.0, and chromatographed onto an I-60 column (7.8 mm × 30 cm) adapted to an HPLC system (Waters Associates). Elution was carried out with 0.2 M ammonium acetate at a flow rate of 1 ml/minute. The A_{235} was monitored and fractions (1 ml) were freeze-dried, redissolved in serum-free medium, and tested for PGF activity at concentrations corresponding to 10% of serum (v/v).

Results

Physico-Chemical Properties of PGF-I

When dog serum was chromatographed on DEAE-Sephadex (Fig. 1A), a major peak of PGF activity was retained on the column and eluted at a concentration of 1.5 M ammonium acetate, and a minor peak was observed in the void volume. To ascertain that the major peak of PGF activity did correspond to PGF-I (Chapdelaine and Chevalier, 1985; Chevalier et al, 1991), a pool of its fractions was applied onto a Sephacryl S-300 column; as shown in Figure 1B, most of the activity migrated to the position of PGF-I with an apparent M_r of 150 kDa (insert), as determined from the relationship between K_{av} and log M_r of standards. A minor peak of PGF-II activity was also eluted in the total volume of the column, corresponding to the position of the 1.4-kDa standard.

When serum proteins were precipitated with cold acetone and the mitogenicity of the resulting fractions assayed, most of the PGF activity was found to be associated with the redissolved precipitate (Table 1). The PGF activity of this material was eluted at 1.5 M ammonium acetate on DEAE-Sephadex and had an apparent M_r of 150 kDa on Sephacryl S-300 (not shown); these data indicate that the mitogenic activity present in the acetone precipitate was due to PGF-I. Minimal mitogenic activity was associated with the acetone supernatant (Table 1), which, as verified by thin-layer chromatography, contained most of the serum lipids (not shown).

Determination of the Apparent M_r of PGF-II

As illustrated in Figure 2A and in agreement with our previous report (Chevalier et al, 1991), when the U-30

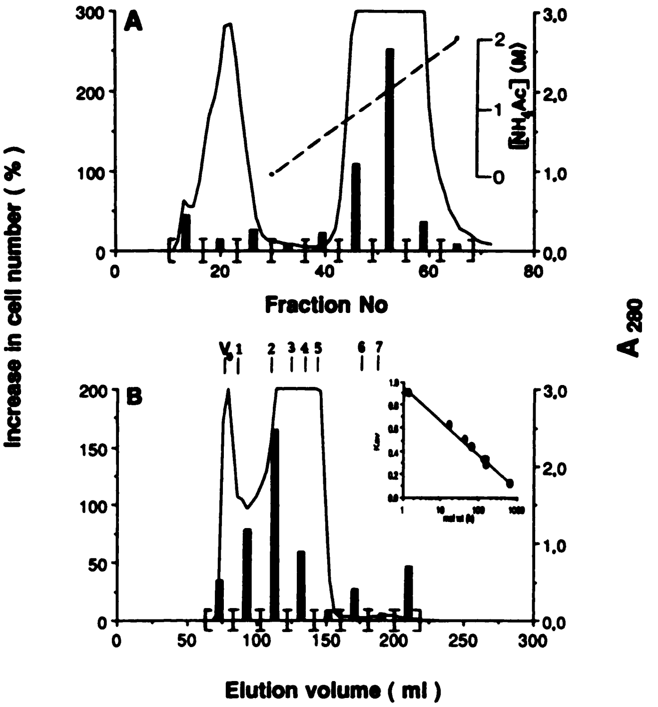

FIG. 1. Mitogenic activity of dog serum after ion-exchange and gel-filtration chromatographies. (A) Two milliliters of dog serum were applied onto a DEAE-Sephadex column (1 × 20 cm) equilibrated in 10 mM ammonium acetate, pH 7.0; elution was performed with a gradient of ammonium acetate [NH_4Ac] (– – –). The A_{280} (———) was recorded, and pooled fractions (indicated in brackets) were assayed for PGF activity (% increase in cell number, closed histograms) at a concentration equivalent to 10% (v/v) dog serum as described in the Materials and Methods section. (B) Fractions eluted with a gradient (between 1.2 and 1.6 M ammonium acetate) as in A were freeze-dried, redissolved in 10 mM ammonium acetate, pH 7.0, and chromatographed on a Sephacryl S-300 column (1.6 × 100 cm) equilibrated in 10 mM ammonium acetate. The A_{280} (———) was recorded and the PGF activity of pooled fractions (indicated in brackets) was determined. Elution volumes of dextran blue (V_o) and of standards (1: thyroglobulin, 670 kDa; 2: γ-globulin, 158 kDa; 3: albumin, 66 kDa; 4: ovalbumin, 44 kDa; 5: myoglobin, 17 kDa; 6: bacitracin, 1.5 kDa; and 7: vitamin B-12, 1.4 kDa) were determined and used to establish the relationship between K_{av} and log M_r (insert) in order to estimate the apparent M_r (o) of PGF-I.

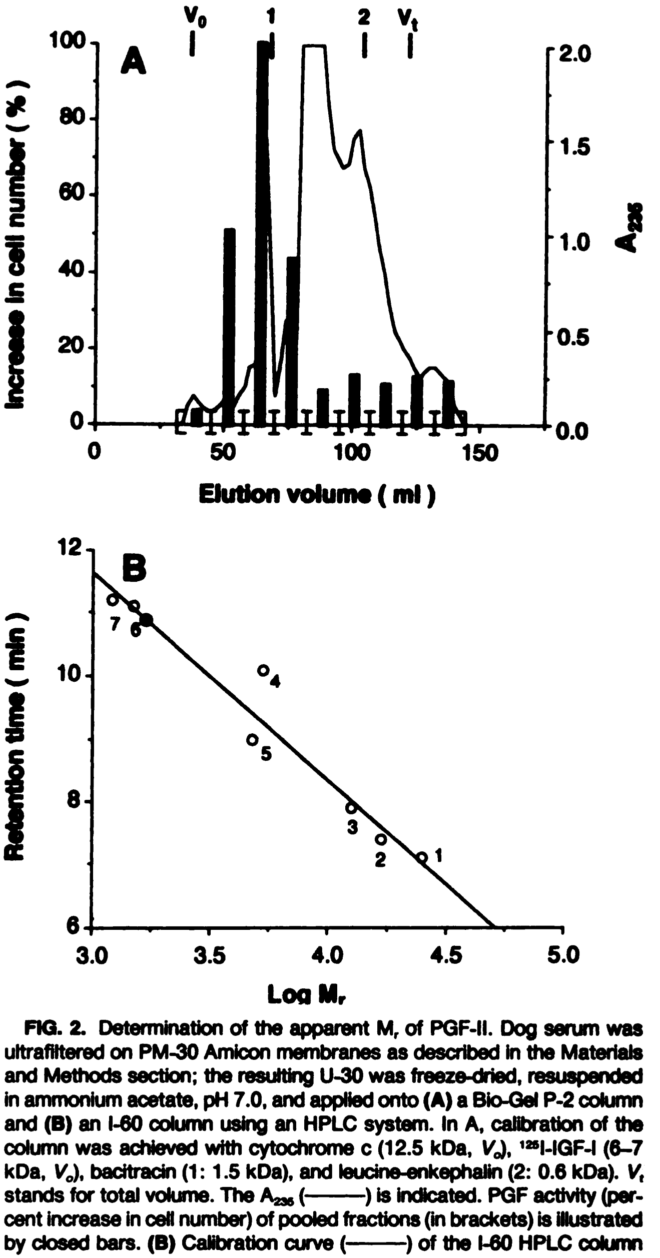

FIG. 2. Determination of the apparent M_r of PGF-II. Dog serum was ultrafiltered on PM-30 Amicon membranes as described in the Materials and Methods section; the resulting U-30 was freeze-dried, resuspended in ammonium acetate, pH 7.0, and applied onto (A) a Bio-Gel P-2 column and (B) an I-60 column using an HPLC system. In A, calibration of the column was achieved with cytochrome c (12.5 kDa, V_o), ^{125}I-IGF-I (6–7 kDa, V_o), bacitracin (1: 1.5 kDa), and leucine-enkephalin (2: 0.6 kDa). V_t stands for total volume. The A_{235} (———) is indicated. PGF activity (percent increase in cell number) of pooled fractions (in brackets) is illustrated by closed bars. (B) Calibration curve (———) of the I-60 HPLC column with reference proteins and peptides—1: chymotrypsinogen, 25 kDa; 2: myoglobin, 17 kDa; 3: cytochrome c, 12.5 kDa; 4: EGF, 5.3 kDa; 5: ACTH fragment, 4.8 kDa; 6: bacitracin, 1.5 kDa; 7: LHRH, 1.2 kDa. Elution of PGF-II activity is indicated (●).

Table 1. *PGF activity after acetone treatment of dog serum**

Supplementation of serum-free medium	Cell number (cells per dish)	Increase in number (%)
—	39,970 ± 3,730	0
Dog serum	112,068 ± 17,166	180 ± 43
Acetone precipitate	128,535 ± 827	222 ± 5
Acetone supernatant	48,270 ± 3,012	21 ± 8

* Dog serum and its subfractions obtained after acetone treatment were assayed in triplicate at a concentration corresponding to 10% (v/v) serum in serum-free culture medium as described in the Materials and Methods section. Three different preparations were evaluated. Results (±SD) of a typical experiment are presented.

from dog serum was submitted to size-exclusion chromatography on a Bio-Gel P-2 column, the mitogenic activity was eluted at the position of the 1.5-kDa standard. When the same fraction was run on an I-60 HPLC column (Fig. 2B), PGF-II had an apparent M_r of 2.0 kDa as calculated from the relationship between retention time and log M_r.

Hydrolysis of PGF-II

Acid hydrolysis and treatment of PGF-II with leucine aminopeptidase or carboxypeptidase Y strongly reduced its mitogenic activity, whereas the addition of trypsin was ineffective in that respect (Table 2).

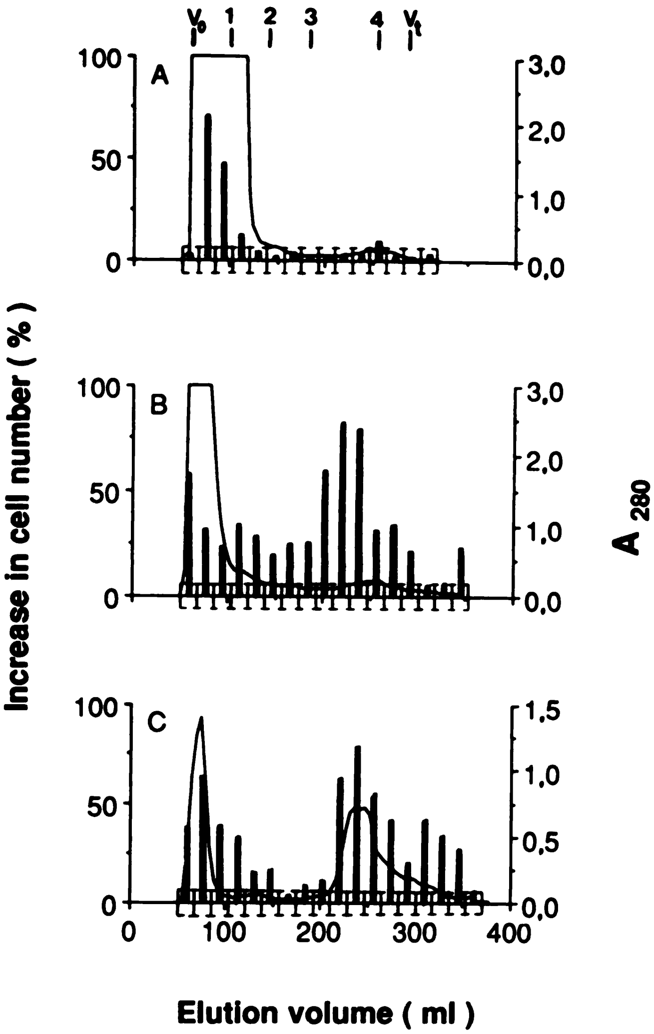

FIG. 3. Effect of acetic acid and heat treatments applied to PGF-I on the M_r of entities displaying mitogenic activity. (**A**) Control C-30 fraction; (**B**) C-30 fraction heated at 100°C (10 minutes) and then treated with 6 M acetic acid; (**C**) C-30 fraction treated with 6 M acetic acid. Gel filtrations were performed on a Bio-Gel P-100 column as described in the Materials and Methods section. The elution volumes of dextran blue (V_0) and of standards—ovalbumin (1: 44 kDa), chymotrypsinogen A (2: 25 kDa), ribonuclease A (3: 13.7 kDa), and bacitracin (4: 1.5 kDa)—are indicated, as well as the A_{280} (———). V_t indicates the total volume of the column. The PGF activity (percent increase in cell number, closed bars) of pooled fractions (in brackets) was assayed as described in the Materials and Methods section.

Table 2. *Effects of acid hydrolysis and proteolysis on PGF-II activity*

Treatment applied to PGF-II*	Mitogenic activity (% control ± SD)
No treatment	100 ± 17
Acid hydrolysis,	
HCl, 24 hours/110°C	−4 ± 4
No treatment	100 ± 5
Protease addition	
Carboxypeptidase Y	7 ± 15
Leucine aminopeptidase	29 ± 15
Trypsin	114 ± 44

* PGF-II was prepared by ultrafiltration of dog serum on PM-30 Amicon membranes and chromatography of U-30 on a Bio-Gel P-30 column as described in the Materials and Methods.

Interrelationship between PGF-I and PGF-II

Results of the experiments performed to study the possibility that PGF-I and -II were interrelated are presented in Figures 3 and 4. In Figure 3, C-30 fractions were treated with 6 M acetic acid with or without a preincubation at 100°C, and the resulting supernatants were submitted to gel filtration: the control preparation showed only the presence of PGF-I (Fig. 3A), whereas, after treatments, a portion of the PGF activity was obtained in the form of a low M_r entity corresponding to that of PGF-II (Fig. 3B,C).

PGF-II was also obtained from preparations enriched in PGF-I (peak from DEAE-Sephadex, as in Fig. 1A) after extraction with HCl. Figure 4 shows that the mitogenic activity of such an HCl extract was eluted as a 1.5-kDa entity on a Bio-Gel P-30 column. Similarly, the presence of mitogenic activity was observed at the position of PGF-II after filtration on Bio-Gel when PGF-I preparations, obtained either by precipitation with acetone or retention on PM-30 Amicon membranes, were extracted with HCl or acidified ethanol (not shown).

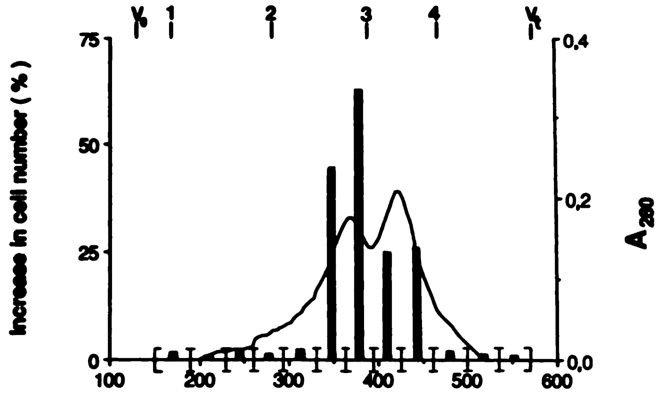

FIG. 4. Treatment of PGF-I with HCl. A fraction enriched in PGF-I, prepared by chromatography of dog serum (10 ml) on a DEAE-Sephadex column (as in Fig. 1A), was treated with 5 N HCl and the extract processed as described in the Materials and Methods section. It was then chromatographed on a Bio-Gel P-30 column in 10 mM ammonium acetate, pH 7.0. The elution profile is indicated by the A_{280} (———), and the proliferative response of prostatic epithelial cells (increase in cell number) of pooled fractions (in brackets) is illustrated by closed bars. Elution volumes of standards—albumin (V_0), cytochrome c (1: 12.5 kDa), ^{125}I-IGF-I (2: 6–7 kDa), aprotinin (2: 6.5 kDa), bacitracin (3: 1.5 kDa), and oxidized glutathione (4: 0.6 kDa)—are indicated. V_t indicates the total volume.

Discussion

The use of *in vitro* model systems is a prerequisite to elucidate the mechanisms that regulate cell division at the molecular level. Primary cultures of normal epithelial cells isolated from adult human and dog prostates appear to be of great value to the study of prostatic cancer and benign hyperplasia, since these diseases naturally affect both species but not rodents. In addition, the use of such primary cultures for studies of GFs should mimic the *in vivo* situation more closely than prostatic–embryonic, prepubertal, and neoplastic cell lines, or nonprostatic cells. We have selected and characterized the canine model for several reasons: (1) the efficiency of enzymatic digestion of prostatic slices is by far superior for the dog than for the human prostate; (2) the cell yields per gram of tissue are also higher; (3) because the fibromuscular compartment is a minor component of the canine prostate tissue, freshly dispersed cells are mainly of the epithelial type with no distinct band of prostatic fibroblasts in Percoll gradients; and (4) upon attachment in selective conditions (dialyzed fetal bovine serum and MEM D-Val medium), dog prostatic epithelial cell monolayers show a high degree of purity; primarily fibroblastic cell populations are obtained when subcultures are maintained in nonselective conditions (Chevalier et al, 1980a,b).

As previously reported, dog serum stimulates the proliferation of epithelial cells isolated from adult dog prostate and has no effect on canine prostatic fibroblasts (Chapdelaine and Chevalier, 1985; Chevalier et al, 1991). This activity is not due to endogenous steroids (Chevalier et al, 1984) and cannot be elicited by exogenous steroids or by known GFs such as EGF, insulin, fibroblastic growth factors (a and bFGF), IGF-I, as well as several other defined mitogens or peptides (tissue culture serum factor, calpeptide, arginine-vasopressin, bombesin, bradykinins, gonadotropin releasing hormone and thyroid releasing hormone) (Chevalier et al, 1991). In addition, gel filtration experiments revealed that the stimulation of epithelial cell proliferation is due to two entities, respectively, of high (PGF-I) and low (PGF-II) M_r. Interestingly, a PGF-II entity of 1.5 kDa can also be extracted from the canine prostatic tissue (Chevalier et al, 1991). In the present study, PGF-I activity, in the form of a 150-kDa entity, was retained on DEAE-Sephadex at pH 7.0 and remained active after acetone precipitation of serum proteins and removal of lipids. In addition, when various extraction procedures were applied to preparations enriched in PGF-I, a portion of the mitogenic activity eluted with an apparent M_r of 1.5–2.0 kDa, corresponding to that of free PGF-II present in serum and/or its ultrafiltrate. The sensitivity of free PGF-II to proteases such as leucine aminopeptidase and carboxypeptidase Y as well as to acid hydrolysis indicates that free PGF-II is a peptide.

An extraction protocol for PGF-II has been established, and the purification of this growth factor is now underway. We are also in the process of defining an arbitrary unit in order to quantify PGF-II recovery throughout our purification scheme. This has been a very difficult task since consecutive column fractions are tested on different primary cell cultures. As already reported, it is known that in this system, the plating efficiency of prostatic epithelial cells differs from one preparation to another (Chevalier and Chapdelaine, 1988). The mitogenic response of canine prostatic epithelial cells to dog serum is also inversely related to the number of cells in primary monolayers (Chevalier et al, 1991) and differs from one batch of dog serum to another. Interestingly, it appears that the serum withdrawn from the animal whose prostatic cells are used to establish primary cultures is always very active on its own cells. Whether this is of physiological significance is, however, still unknown. With these limitations, it is felt that the arbitrary unit for PGF could be the amount of peptide required to elicit a mitogenic response in relation to that observed with homologous serum.

Several types of gel matrices have been tested for their ability to retain PGF-II activity: hydrophobic supports and ion exchangers retain the activity while heparin-Sepharose does not. It may be concluded that, contrary to FGF and several other GFs, PGF-II does not belong to the heparin-binding-GF (HBGF) family. When column fractions were tested at different doses, only the PGF-II activity was observed; it showed a linear increase in mitogenic effect on prostatic epithelial cells, reaching a plateau at concentrations corresponding to an equivalent of 15–20% dog serum.

By analogy with several types of GFs, such as EGF, platelet-derived growth factor (PDGF), nerve growth factor (NGF), and the IGFs (Zapf et al, 1975; Berger and Shooter, 1977; Anundi et al, 1982; Huang et al, 1983; Noworytko et al, 1983), the high-M_r PGF-I could be considered as a carrier or a binding protein for PGF-II. However, the interaction between PGF-II and these 150-kDa proteins would differ from that described for the IGF system since, in the latter case, the dissociation of both entities can be achieved in 1 M acetic acid (Martin and Baxter, 1986). In fact, the use of more drastic extraction procedures (6 M acetic acid, heating) in the present study resulted in only a partial dissociation of PGF-II from PGF-I. Because in both humans and rodents, IGF-I is a 6–7-kDa peptide, it appears unlikely that PGF-II (of 1.5 kDa) activity in dog serum would be due to a canine IGF-I of such a low M_r. In addition, when human serum is similarly fractionated and assayed on canine prostatic epithelial cells, peaks of mitogenic activity are observed at 50 kDa and 1.5 kDa with no activity at the position of the human IGF-I carrier protein (Chapdelaine and Chevalier, 1985). Furthermore, the protein tyrosine kinase (PTK) expressed in the canine prostatic epithelial cells used for the assay of PGF appears to differ from those PTKs associated with known GF receptors (Bourassa et al, 1991), reinforcing the idea that the PGF activities evidenced with this bioassay are distinct from known GFs, including the IGFs.

Tight associations of some GFs (NGF, EGF, PDGF) with carrier proteins have already been reported (Berger and Shooter, 1977; Frey et al, 1979; Huang et al, 1983). In fact, the purification of EGF has been hampered by noncovalent binding to its kallikrein precursor even after its processing and also by the release of more than one biologically active form (Gray et al, 1983; Scott et al, 1983b). A similar situation, i.e., tight association with a carrier protein, also applies to NGF (Scott et al, 1983a; Ullrich et al, 1983). It is believed from the various extraction procedures used herein that PGF-II in serum is tightly but noncovalently associated with 150-kDa proteins. The fact that serum PGF activity is lost upon dialysis or by ammonium sulfate precipitation followed by dialysis (unpublished data) also supports this hypothesis. Because a PGF-II entity is also present in the prostate (Chevalier et al, 1991), the possibility that proteases such as arginine esterase (Chapdelaine et al, 1984) and prostatic-specific antigen (Dubé et al, 1986), respectively present in canine and human prostates, may locally be involved in its processing remains to be established.

The role of GF carriers or binding proteins for mitogens is still obscure; they may prolong the half-life of peptides in serum, prevent, and/or favor their action on target cells. It appears from our previous data (Chevalier et al, 1991) and from the results presented herein that, when tested at a concentration equivalent to 10% serum (v/v), the mitogenic activity of free PGF-II in dog serum is lower than that associated with PGF-I. However, the relative concentrations of PGFs and their specific activities cannot be quantified and determined yet. Because both entities, the free and extractable forms, are active on the same cells and have the same M_r, they are likely to be identical. Our data are thus strongly indicative of a relationship between PGF-I and PGF-II: the latter 1.5 kDa factors would be bound to but extractable from 150-kDa serum proteins. Indeed, the isolation procedure, the conditions used for protein precipitation and/or fractionation, may influence the affinity of PGF-II for such proteins. Physiological conditions could also affect the equilibrium between free and protein-bound PGFs.

In conclusion, serum PGFs that promote the androgen-independent proliferation of basal prostatic epithelial cells *in vitro,* both in the dog and in the human (Chevalier et al, 1984, 1991; Chapdelaine and Chevalier, 1985), may play a similar role *in vivo.* Interestingly, in the present system, Suramin, a GF antagonist actually used in the USA for the treatment of hormone-refractory metastatic prostate cancer (Mayers et al, 1992), can completely abrogate the effect of serum PGFs (unpublished data). The high incidence of proliferative prostatic diseases in both human and dog justifies the purification of these peptidic PGFs, also present in the gland, as well as the characterization of their carrier and/or binding proteins.

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