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Pure brain-derived acidic fibroblast growth factor is a potent angiogenic vascular endothelial cell mitogen with sequence homology to interleukin 1

(blood vessel growth/heparin/tumor angiogenesis factor/protein sequence/neurobiology)

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ABSTRACT **Pure bovine brain-derived acidic fibroblast growth factor is a very potent mitogen for vascular endothelial cells in culture and, in the presence of heparin, induces blood vessel growth *in vivo*. Partial amino acid sequence determinations confirm that this mitogen is a unique protein having amino acid sequence homology with human interleukin 1.**

Protein growth factors are molecules that induce growth, usually including cell division, in their target cells. They act by binding to specific plasma membrane receptors, thereby triggering an only partially characterized cascade of events (1). Since virtually all known activities of growth factors have been identified with cell culture systems, relatively little is understood about the physiological significance of the molecules *in vivo*.

Fibroblast growth factor (FGF) was originally identified in both pituitary (2) and whole brain (3). The growth factor activity was partially purified based on its ability to stimulate DNA synthesis in the immortal BALB/c 3T3 fibroblast cell line (4). Two mitogens, one acidic (5) and one basic (6), were subsequently identified in the partially purified brain FGF preparations. We previously reported the purification to apparent homogeneity and initial characterization of the approximately 17-kDa acidic FGF (aFGF) from bovine brain (7). A protein that may be similar, or identical, to the basic FGF found in brain has been purified from bovine pituitary (8, 9).

With partially purified material, the presence of the acidic mitogenic activity for BALB/c 3T3 cells was observed to correlate with mitogenicity for vascular endothelial cells (6). We report here that aFGF is a potent mitogen for vascular endothelial cells in culture and induces the growth of blood vessels *in vivo*. Protein sequence data are also presented that uniquely identify this molecule and reveal homology with one type of interleukin 1 (IL-1).

MATERIALS AND METHODS

Mitogenic Assays. Fetal bovine thoracic aortic endothelial cells (AG4762, National Institute of Aging Cell Repository, Institute for Medical Research, Camden, NJ) were assayed after 38 cumulative population doublings *in vitro*. The cells were plated in 6-well tissue culture dishes (Costar, Cambridge, MA) at 2×10^3 cells per cm^2 in 1 ml of 20% heat-inactivated calf serum (GIBCO) in Dulbecco's modified Eagle's medium (DMEM; GIBCO) per well and changed to 1% serum 18 hr later. All media were supplemented with glutamine and penicillin/streptomycin as described (5). Either pure mitogen (7) diluted in 100 μl of 1 mg of bovine serum albumin (Sigma) per ml of DMEM or serum samples were added to each well along with 1.6 μCi of [*methyl*-^{3}H]thymidine (20.0 Ci/mmol, New England Nuclear; 1 Ci = 37 GBq) and 45 μg of unlabeled thymidine (Sigma) in 40 μl of DMEM. After a 48-hr incorporation period, the cells were washed and lysed, and 75% of the trichloroacetic acid-insoluble radiolabeled DNA was counted as described (5). The pure mitogen was found to be stable in the 7 mM trifluoroacetic acid/33% acetonitrile HPLC elution solvent (7) at −20°C under argon but lost substantial activity when lyophilized. Therefore, in all mitogenic and angiogenic assays, the pure mitogen was diluted from this solvent. Control assays showed that equivalent amounts of HPLC solvent components were innocuous.

Mouse lung capillary endothelial cells (from T. Maciag, Revlon) were plated at 2.6×10^4 cells per cm^2 in 24-well dishes (Costar) and grown to confluence in 0.5 ml of 10% charcoal-treated calf serum (HyClone, Logan, UT) in DMEM per well, lowered to 0.5% serum after 72 hr, and allowed to become quiescent over 48 hr. Either serum or the pure mitogen was added in 50 μl as described above, followed 18 hr later by a 4-hr pulse of [*methyl*-^{3}H]thymidine (20 μl containing 100 μCi of radiolabeled thymidine in Dulbecco's phosphate buffered saline). The cells were processed, and radioactivity was measured as described above.

Chicken Egg Angiogenesis Bioassay. Three-day-old chicken embryos were removed from their shells and grown in Handiwrap pouches suspended inside paper cups. The tops of the cups were covered with Handiwrap, and the eggs were incubated at 37°C in a tissue culture incubator for 5–6 days (10). Either 1 μg of pure mitogen in about 30 μl of the HPLC elution solvent (7) or an identical HPLC solvent control solution were mixed with an equal volume of 2% low-gelling-temperature agarose (Miles) dissolved in lactated Ringer's solution (Abbott) containing 10 μg of heparin (from porcine intestinal mucosa; Sigma grade 1). Droplets (60 μl) were allowed to gel on the center of sterile plastic 1.3-cm-diameter Thermanox tissue culture coverslips (Miles), and at least part of the volatile acetonitrile evaporated by aeration for 15–30 min under a plenum of sterile air in a tissue culture hood. The coverslips were positioned, pellet down, over the chorioallantoic membrane of the eggs and incubated for 3 days. Eggs containing large white focal regions under the coverslips at

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Abbreviations: FGF, fibroblast growth factor; aFGF, acidic FGF; DMEM; Dulbecco's modified Eagle medium; IL-1, interleukin 1.

the end of the assay, presumably formed by inflammatory cells, were discarded. The chorioallantoic membranes were examined microscopically and scored for the proliferation of fine capillaries under the center of the coverslips by observers who did not know the contents of the agarose pellets.

Amino Acid Sequence Determination. Brain-derived aFGF was purified as described (7), and 4.2 nmol were dried in 50-μl aliquots in a 6 × 50 mm glass tube by vacuum centrifugation. The protein sample was dissolved in 100 μl of 0.1 M Tris·HCl (pH 9.5) made 6.0 M in guanidinium chloride (Heico, Delaware Water Gap, PA) and 0.1% in Na_2EDTA and containing 1.7 μmol of D,L-dithiothreitol (Sigma). The reaction mixture was covered with argon and maintained at 50°C for 2 hr. The reduced protein was alkylated under argon in the dark for 35 min at 20–22°C with 13.5 μmol of iodoacetic acid (Sigma; recrystallized from petroleum ether) containing 100 μCi of iodo[2-^{14}C]acetic acid (55.4 mCi/mmol; Amersham) dissolved in 100 μl of 0.7 M Tris·HCl (pH 7.8) made 6.0 M in guanidinium chloride and 0.1% in Na_2EDTA. The reaction mixture was injected on a Vydac C_4 reversed-phase HPLC column (The Separations Group, Hesperia, CA) and was eluted as described for the unreduced protein (7).

Pure reduced and [^{14}C]carboxymethylated mitogen (2.5 nmol) was digested with a 1:100 mass ratio of HPLC-purified (11) L-(tosylamido-2-phenyl)ethyl chloromethyl ketone-treated bovine pancreatic trypsin (Worthington) to substrate for 6 hr at 37°C in 200 μl of 0.1 M ammonium bicarbonate (pH 8.3). The digestion mixture was loaded directly on a 4.6 mm × 25 cm Vydac C_{18} reversed-phase HPLC column (particle size, 5 μm; pore size, 330 Å) equilibrated in 10 mM trifluoroacetic acid (Aldrich) and was eluted at a flow rate of 0.5 ml/min with a 3-hr linear gradient of acetonitrile from 0–67% (by volume) at 20–22°C, with absorption continuously monitored at 210 nm. The amino acid compositions of 10–25% by volume of the individual peptide peaks were determined after hydrolysis in constant boiling HCl (Pierce) containing 0.1% phenol (Ultrapure, BRL) at 110°C for 20 hr, followed by quantitation on a high-sensitivity phenylisothiocyanate-based analyzer (12). All peptides reported here were recovered in at least 50% yield. Protein or peptide samples were sequenced on an Applied Biosystems 470A microsequencer using Polybrene-coated filters. Phenylthiohydantoin-amino acids were identified and quantitated by HPLC analysis.

Homologies of the bovine aFGF partial amino acid sequence with catalogued protein sequences from the 1984 update of the Protein Identification Resource (Dayhoff) compendium of protein primary structures were evaluated with the Align computer program (13). After manual alignment of the amino- and carboxyl-terminal ends of aFGF with IL-1, the two ends of aFGF and equivalent regions of IL-1 were each fused to make single chains with gap positions frozen by insertion of "X"-residues. Given the percentage of sequence identity, a mutation matrix based on 152 accepted point mutations was used. The BIAS and GAP parameters were iteratively optimized to 16 and 5, respectively. The statistical significance of the homology was evaluated by comparing it to the similarity of one sequence with 500 random permutations of the second.

RESULTS

Mitogenic Activity. Based on previous results that the partially purified brain-derived aFGF was mitogenic for vascular endothelial cells (6), the mitogenic activity of the pure protein was measured by using fetal bovine aortic endothelial cells. The protein is a potent mitogen for these cells, with a half-maximal stimulation of DNA synthesis at about 620 pg/ml (37 pM) (Fig. 1*A*). Furthermore, the response of these cells to serum shows that the pure mitogen elicits a significantly greater maximal response than that of the 10% calf serum control. Acidic mitogen was also tested on a culture of mouse lung capillary endothelial cells (Fig. 1*B*) and observed to elicit a half-maximal stimulation of DNA synthesis at 250 pg/ml (15 pM). The pure protein is about equally mitogenic for human vascular endothelial cells (data not shown).

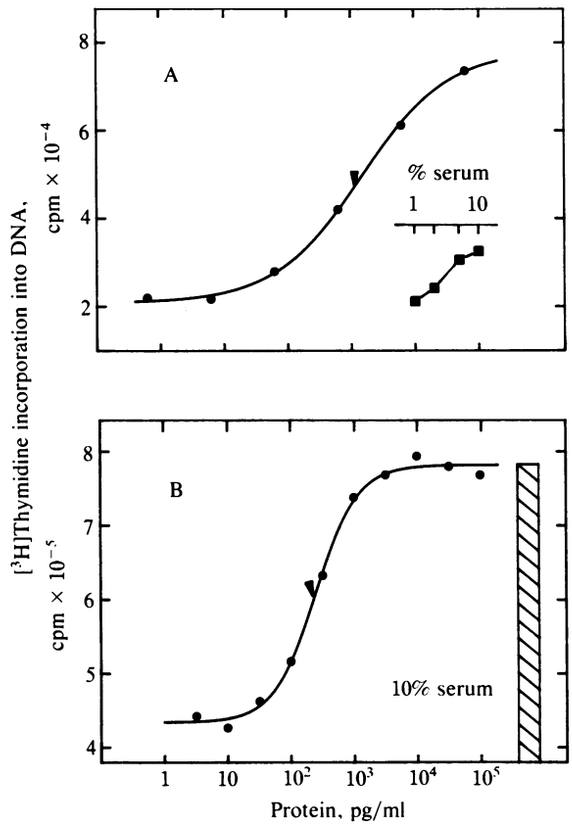

FIG. 1. Mitogenic response of vascular endothelial cells and fibroblasts to pure aFGF. The dose–response of aFGF-induced incorporation of [*methyl*-^{3}H]thymidine into DNA (-●-) is shown for fetal bovine aortic arch endothelial cells (*A*) and mouse lung capillary endothelial cells (*B*). The concentrations of aFGF that elicit half-maximal responses (arrowheads) of the endothelial cells are: 620 pg/ml in *A* and 240 pg/ml in *B*. Serum controls are shown either as a function of dose (-■-) in *A* or as a bar at a single 10% concentration in *B*.

Angiogenic Activity. During sustained vascular growth, endothelial cells are observed to actively proliferate (14). Therefore, we tested the ability of the purified mitogen to induce blood vessel growth in the chicken egg chorioallantoic membrane angiogenesis assay. Based on previous reports that crude tumor angiogenesis factor was significantly more active with coadministered heparin (15), we tested the vascularization response of heparin alone and heparin plus pure aFGF. A 10-μg dose of heparin per egg was inactive but the same amount of heparin plus 1 μg of aFGF per egg

Table 1. Angiogenic activity of aFGF

Sample contents	Angiogenic response	
	Negative	Positive
Control	15	0
aFGF	2	10

These data are a composite of three separate experiments. Using *t*-distribution statistics, the group of mitogen-stimulated eggs was calculated to be different from the control population with a confidence level of >99.9%.

appeared to enhance the growth of small capillaries[§] at the site of application with no sign of inflammation (Table 1). The assay is reproducible, the results being a composite of three separate assays with different samples of aFGF. Control (Fig. 2*A*) and positive angiogenic responses (Fig. 2*B*) show the extent of capillary proliferation induced by aFGF. The mitogen is, therefore, a potent angiogenic protein in the presence of heparin.

Amino Acid Sequence. Two microheterogeneous forms of the pure protein with nearly identical masses have been seen on electrophoresis in $NaDodSO_4$/polyacrylamide gels (7) and, after separation by shallow gradient elution from a reversed-phase C_4 HPLC column, were found to have very similar amino acid compositions. Sequence determinations of reduced and carboxymethylated proteins revealed two amino termini (Fig. 3). The longer sequence, aFGF-1, contains 6 amino-terminal residues not found on the shorter aFGF-2 form. The relative amounts of these two offset amino termini vary from one preparation to another but are closely correlated to the relative abundance of the two very close bands of protein seen after electrophoresis in $NaDodSO_4$ gels (7). The amino-terminal sequence of one sample that was almost entirely composed of the higher mass and longer amino-terminal form (aFGF-1) corroborated the assignments made from the mixed sequence determinations.

Peptides of aFGF, generated by digestion with trypsin, confirmed most of the amino-terminal sequence (Fig. 3). A tryptic peptide devoid of both arginine and lysine was generated and sequenced; considering the specificity of trypsin to cleave peptide bonds following lysine and arginine residues, this peptide was derived from the carboxyl-terminal end of the protein.

Amino Acid Sequence Homology. In a search of the Dayhoff protein data bank (13), aFGF appears to be unique compared to the approximately 2000 protein sequences contained in this list. Its lack of similarity to epidermal growth factor, platelet-derived growth factor, and insulin-like growth factor II is consistent with its inability to compete with these proteins for binding to their receptors (unpublished data).

Similarity was observed, however, between the amino- and carboxyl-terminal ends of aFGF and segments of human (17) IL-1, deduced from the cDNA sequence, as shown in Fig. 3. The aFGF amino-terminal sequence aligns with a human IL-1 region that begins 122 residues from the putative initiation methionine residue. The carboxyl-terminal end of aFGF aligns with the carboxyl-terminal end of human IL-1 predicted from the cDNA sequence. As aligned, these regions are over 30% identical. The 148-residue polypeptide chain between the beginning of the amino-terminal and end of the carboxyl-terminal homologous regions of human IL-1 is almost the same length as the ≈149-residue aFGF (7). Furthermore, the size of this segment of the IL-1 polypeptide chain agrees well with the 15–20 kDa mass estimated for the processed human protein. The gap positions, chosen to optimize the number of amino acid identities, occur at or adjacent to glycine or proline residues in the amino terminus. These two residues often appear at turns in the tertiary structure of proteins, regions in which insertions and deletions are most common (18). The estimated significance of the

[§]The "spoke-wheel" pattern formed by the convergence of large vessels on the chicken egg chorioallantoic membrane at the site of sample application, proposed to be a measure of angiogenesis (16), was not seen on application of aFGF. We confirmed such vascular patterns in response to male mouse submaxillary gland homogenates as listed in Table IV of this reference. On partial purification, "spoke-wheel"-generating activity that was eluted from G-100 Sephadex gel filtration chromatography paralleled arginine peptidase activity, so we tested a variety of pure proteases including trypsin, chymotrypsin, plasmin, γ-nerve growth factor subunit, and epidermal growth factor binding protein, the latter two proteins being among the principal arginine peptidases of the male mouse submaxillary gland. All of these proteases generated the "spoke-wheel" pattern. On further characterization, we observed this response to be generated within hours, seemingly too fast to be the result of neovascularization. A grid, composed of tiny pieces of filter paper placed on the chorioallantoic membrane, showed that the rapid convergence of large vessels was generated by contraction of the elastic membrane toward the site of sample application. We conclude, therefore, that this pattern of large blood vessel convergence does not represent angiogenesis.

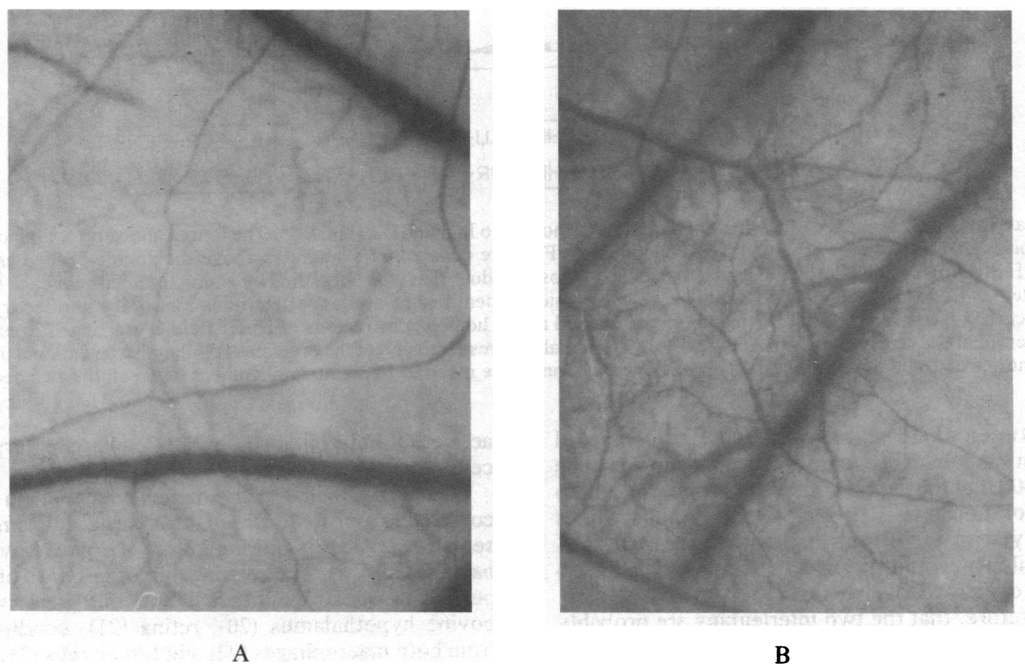

FIG. 2. Angiogenic response of chicken egg chorioallantoic membrane. Membranes of living chicken embryos were photographed at ×40 magnification 3 days after application of either control solution containing 10 μg of heparin (*A*) or coadministration of 1 μg of aFGF with 10 μg of heparin (*B*).

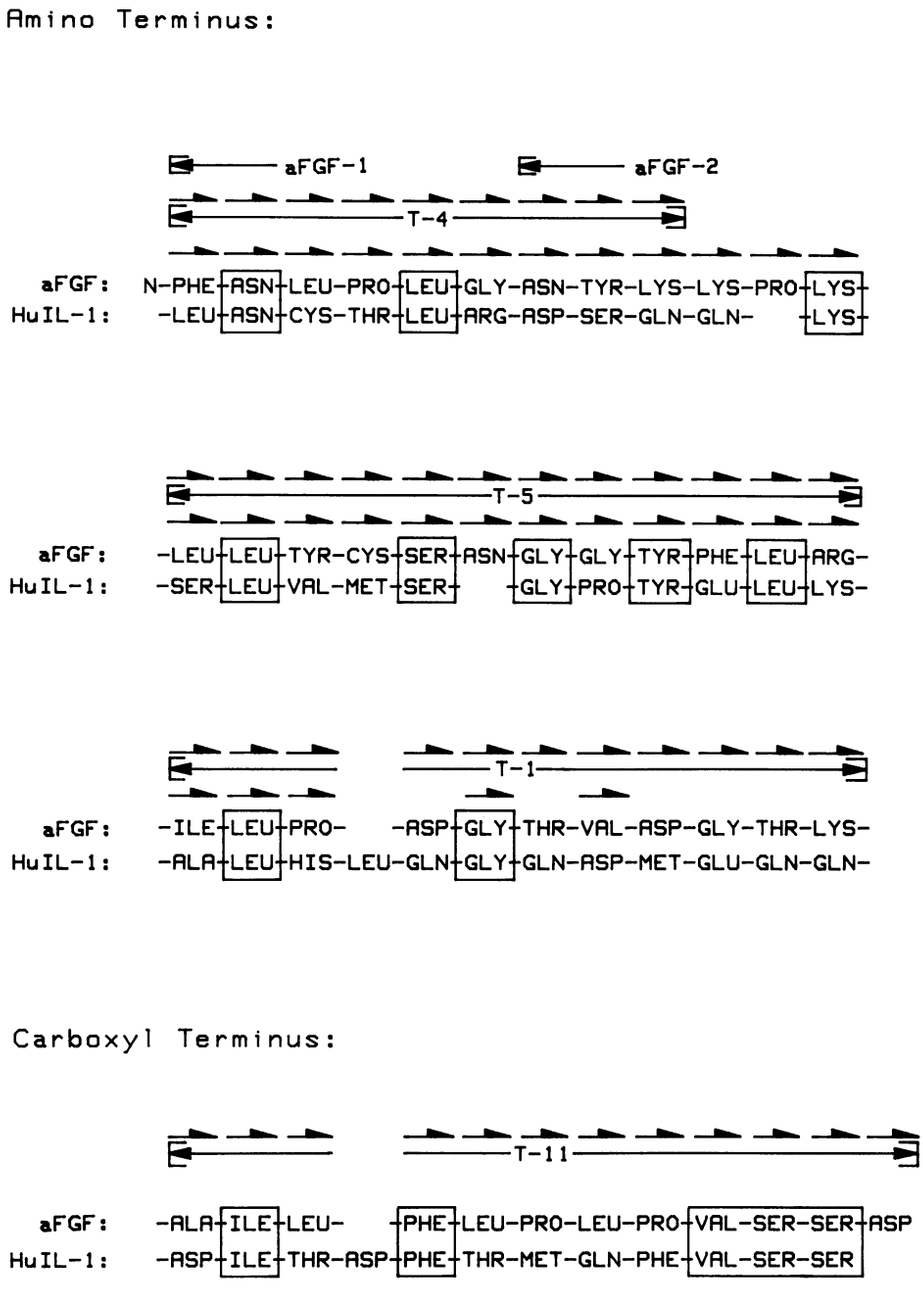

FIG. 3. Partial amino acid sequence of bovine aFGF and its homology to human IL-1 (HuIL-1). The longer and shorter amino-terminal ends of aFGF are denoted -1 and -2, respectively. Tryptic peptides of aFGF are denoted by T-numbers above the double-headed arrows denoting their full length. Half-arrows above the amino acid name indicate those residues that were identified by amino terminal sequence determinations of the entire protein, whereas those above the T-numbers signify residues identified by sequence determinations of the purified tryptic peptides. The amino- and carboxyl-terminal ends of bovine aFGF are aligned above homologous regions of HuIL-1 either starting 122 residues from the putative amino-terminal end or 12 residues from the carboxyl-terminal end, respectively, of the predicted full-length translational product. Amino acid residue identities are enclosed in boxes. Blank residue positions were inserted to maintain alignment between the two sequences.

relatedness between aFGF and human IL-1 is 3.6 standard deviations from random. This value corresponds to a chance of about 1 in 3000 that the alignments are random. The amino acid sequence of mouse IL-1 (19), on the other hand, aligns poorly not only with aFGF but also with human IL-1. A substantial similarity would be expected between the amino acid sequences of equivalent proteins from humans and mice. It appears, therefore, that the two interleukins are probably members of different classes of IL-1s.

DISCUSSION

Pure bovine aFGF is clearly a very potent mitogen for vascular endothelial cells in culture. The growth factor is active not only on aortic but also with capillary endothelial cells.

Other endothelial cell mitogens have been partially or completely purified. Based on charge and amino-terminal sequence, aFGF is different from the pure bovine pituitary basic FGF (8, 9). The relationship of aFGF to other partially purified vascular endothelial cell mitogens isolated from bovine hypothalamus (20), retina (21), conditioned media from both macrophages (22) and tumor cells (23, 24), and the mitogens purified from human brain (25) and extracellular matrix deposited by chondrosarcoma cells in culture (26) remains to be determined. An alternative purification scheme for aFGF from brain using heparin chromatography recently

has been reported (27). The purified protein has the same amino acid composition as previously described (7) and was demonstrated to be a mitogen both for BALB/c 3T3 and for bovine capillary endothelial cells.

Blood vessel growth originates primarily by sprouting of new capillaries from existing capillaries and venules (14, 28). Vascular endothelial cells that form growing capillaries divide during sustained angiogenesis (14, 29). Therefore, we tested the ability of the pure mitogen to induce the growth of capillaries *in vivo*. Heparin, a mixture of sulfated polysaccharides produced by mast cells that are often seen to precede growing capillary sprouts, has been shown to enhance the angiogenic activity of crude tumor angiogenesis factor (15), a substance that is proposed to be made and released by tumor cells *in vivo*. Pure aFGF, in the presence of heparin, elicited a substantial proliferation of capillaries on the chicken egg extraembryonic chorioallantoic membrane bioassay, whereas heparin alone was inactive. Preliminary results indicate that the angiogenic activity of aFGF appears to be diminished in the absence of heparin.

The ability of solid tumors to become vascularized by the host and thereby grow beyond a few mm in diameter has been attributed to tumor angiogenesis factor. This hypothesis has received support from the observation that conditioned media from tumor cells grown in culture cause neovascularization in bioassays (30). The mechanism of heparin augmentation of tumor angiogenic activity is unknown but could be related not only to its tight binding *in vitro* to both this protein (unpublished results; ref. 27) and other potentially similar growth factors (20, 21, 25, 26) but also to its ability to potentiate the activity of mitogens for endothelial cells in culture (31). Furthermore, conditioned media from tumor cells contain mitogenic activity for vascular endothelial cells (24). These properties are in common with aFGF, indicating a possible similarity or identity between the pure mitogen and tumor angiogenesis factor.

The homology between the amino acid sequences of the bovine brain-derived endothelial cell mitogen aFGF and the human macrophage product IL-1 is unexpected. The resemblance indicates that the two classes of proteins probably diverged long ago. Substantially less similarity is observed between the same regions of mouse IL-1 and both bovine aFGF and human IL-1. The difference between the two ILs is very surprising and probably indicates that they represent unique families of IL-1s. Although a few regions of limited sequence similarity could be found between aFGF and human IL-2 (32), the homology of IL-2 with aFGF or either human or mouse IL-1 was not statistically significant. Bovine aFGF is inactive in either IL-1 thymocyte or IL-2 T-cell proliferation assays (unpublished results).

The similarity between bovine aFGF and human IL-1 indicates that these proteins are members of a new homologous family of growth factor proteins that is different from platelet-derived growth factor and from both the epidermal growth factor (33) and insulin (34) families. Although macrophages produce endothelial cell mitogenic (22) and angiogenic (35) substances, it is not known if they are identical to, or different from, aFGF. The cell source of aFGF is also unknown so the immunological significance, if any, of its homology with either IL-1 or other immune cell mediators remains to be established.

Note Added in Proof. The form of human IL-1 that we have shown to be homologous to aFGF has recently been denoted IL-1β to distinguish it from a second type named IL-1α. Based on the complete amino acid sequence of aFGF, it is homologous to both forms of IL-1 and brain-derived basic FGF.

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1. Thomas, K. A. (1985) *Comments Mol. Cell. Biophys.* **3.** 1–13.
2. Gospodarowicz, D. (1978) *J. Biol. Chem.* **250,** 2515–2520.
3. Gospodarowicz, D. (1974) *Nature (London)* **249,** 123–127.
4. Gospodarowicz, D., Bialecki, H. & Greenburg, G. (1978) *J. Biol. Chem.* **253,** 3736–3743.
5. Thomas, K. A., Riley, M. C., Lemmon, S. K., Baglan, N. C. & Bradshaw, R. A. (1980) *J. Biol. Chem.* **255,** 5517–5520.
6. Lemmon, S. K., Riley, M. C., Thomas, K. A., Hoover, G. A., Maciag, T. & Bradshaw, R. A. (1982) *J. Cell Biol.* **95,** 162–169.
7. Thomas, K. A., Rios-Candelore, M. & Fitzpatrick, S. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 357–361.
8. Lemmon, S. K. & Bradshaw, R. A. (1983) *J. Cell. Biochem.* **21,** 195–208.
9. Bohlen, P., Baird, A., Esch, F., Ling, N. & Gospodarowicz, D. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5364–5368.
10. Dunn, B. E., Fitzharris, T. P. & Barnett, B. D. (1981) *Anat. Rec.* **199,** 33–43.
11. Titani, K., Sasagawa, T., Resing, K. & Walsh, K. A. (1982) *Anal. Biochem.* **123,** 408–412.
12. Heinrikson, R. L. & Meredith, S. C. (1984) *Anal. Biochem.* **136,** 65–74.
13. Dayhoff, M. O. (1978) *Atlas of Protein Sequence and Structure* (National Biomedical Research Foundation, Washington, DC), Vol. 5, Suppl. 3, pp. 345–358.
14. Ausprunk, D. H. & Folkman, J. (1977) *Microvasc. Res.* **14,** 53–65.
15. Folkman, J., Taylor, S. & Spillberg, C. (1983) in *Development of the Vascular System*, Ciba Foundation Symposium 100, eds. Nugent, J. & O'Conner, M. (Pitman, London), pp. 132–149.
16. Folkman, J. & Cotran, R. (1976) *Int. Rev. Exp. Pathol.* **16,** 207–248.
17. Auron, P. E., Webb, A. C., Rosenwasser, L. J., Mucci, S. F., Rich, A., Wolff, S. M. & Dinarello, C. A. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7907–7911.
18. Thomas, K. A. & Schechter, A. N. (1980) in *Biological Regulation and Development*, ed. Goldberger, R. F. (Plenum, New York), Vol. 2, pp. 43–100.
19. Lomedico, P. T., Gubler, U., Hellmann, C. P., Dukuvich, M., Giri, J. G., Collier, K., Semionow, R., Chua, A. O. & Mizel, S. B. (1984) *Nature (London)* **312,** 458–462.
20. Maciag, T., Mehlman, T., Friesel, R. & Schreiber, A. B. (1984) *Science* **225,** 932–935.
21. D'Amore, P. A. & Klagsbrun, M. (1984) *J. Cell Biol.* **99,** 1545–1549.
22. Mostafa, L. K., Jones, D. B. & Wright, D. H. (1980) *J. Pathol.* **132,** 207–216.
23. Fenselau, A. & Mello, R. J. (1976) *Cancer Res.* **36,** 3269–3273.
24. Orlander, J. V., Marasa, J. C., Kimes, R. C., Johnston, G. M. & Feder, J. (1982) *In Vitro* **18,** 99–107.
25. Conn, G. & Hatcher, V. B. (1984) *Biochem. Biophys. Res. Commun.* **124,** 262–268.
26. Shing, Y., Folkman, J., Sullivan, R., Butterfield, C., Murray, J. & Klagsbrun, M. (1984) *Science* **223,** 1296–1299.
27. Lobb, R. R. & Fett, J. W. (1984) *Biochemistry* **23,** 6295–6299.
28. Burger, P. C., Chandler, B. S. & Klintworth, G. K. (1983) *Lab. Invest.* **48,** 169–180.
29. Hobson, B. & Denekamp, J. (1984) *Br. J. Cancer* **49,** 405–413.
30. Folkman, J. & Cotran, R. (1976) *Int. Rev. Exp. Pathol.* **16,** 207–248.
31. Thornton, S. C., Mueller, S. N. & Levine, E. M. (1983) *Science* **222,** 623–625.
32. Clark, S. C., Arya, S. K., Wong-Staal, F., Matsumoto-Kobayashi, M., Kay, R. M., Kaufman, R. J., Brown, E. L., Shoemaker, C., Copeland, S., Oroszlan, S., Smith, K., Sarngadharan, M. G., Lindner, S. & Gallo, R. C. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2543–2547.
33. Marquardt, H., Hunkapiller, M. W., Hood, L. E., Twardzik, D. R., De Larco, J. E., Stephenson, J. R. & Todaro, G. J. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4684–4688.
34. James, R. & Bradshaw, R. A. (1984) *Annu. Rev. Biochem.* **53,** 259–292.
35. Polverini, P. J., Cotran, R. S., Gimbrone, M. A., Jr., & Unanue, E. R. (1977) *Nature (London)* **269,** 804–806.