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Cloning and analysis of murine cDNA that encodes a fibrogenic lymphokine, fibrosin

(fibrosis/growth factor/fibroblast/lymphocyte)

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ABSTRACT **Tissue fibrosis that complicates chronic inflammation can be a cause of serious morbidity. The molecular links between inflammation and fibrosis appear to be a variety of proteins produced by activated chronic inflammatory cells. Collectively, these fibrogenic cytokines promote the growth of fibroblasts and the production of extracellular matrix that are the characteristic features of fibrotic tissue. In an attempt to clone cDNA for a fibrogenic lymphokine that we had isolated, we transfected COS-7 cells with a cDNA library derived from concanavalin A-stimulated lymphocyte line CDC25. Conditioned medium from the transfected COS-7 cells but not from sham-transfected cells stimulates fibroblast proliferation *in vitro*. We used heterologous expression in COS-7 cells of pools of CDC25 cDNA and screening for biological activity in conditioned medium to enrich for the cDNA clone(s) that encodes this activity. With this strategy of sib selection we isolated clone 2B3. The culture supernatants of 2B3-transfected COS-7 cells exert maximum growth-stimulating effects on fibroblasts at a dilution of 1:20,000. The isolated cDNA has one open reading frame (216 nucleotides) that has no significant homology with nucleotide sequences that encode other proteins. A synthetic peptide constructed from the deduced amino acid sequence is biologically active in picomolar concentrations, even though it may represent only a portion of the native fibrosin. This lymphokine, which we designate fibrosin, may play a role in regulating fibrogenesis in certain chronic inflammatory diseases.**

Tissue fibrosis (scarring) develops as a complication of a variety of diseases with diverse etiologies and can result in serious, sometimes life-threatening, morbidity (1). The realization that the scarring process (fibrogenesis) involves dynamic biological processes that include recruitment and proliferation of selected mesenchymal cells, such as fibroblasts, as well as increased net deposition of extracellular matrix constituents (most notably, collagen and glycosaminoglycans), has motivated a search for molecules that regulate these processes (2–4). Particular interest has focused on soluble fibrogenic mediators produced by mononuclear phagocytes (monocytes and macrophages) (4–6), and to a lesser extent also lymphocytes (7, 8), since such chronic inflammatory cells may infiltrate anatomic sites prior to scar formation in certain diseases.

We have studied the molecular links between chronic inflammation and fibrosis in a murine model of schistosomiasis. In this helminthic infection, *Schistosoma mansoni* eggs are deposited in the liver, where egg-derived antigens drive specific T-lymphocyte responses that lead to the periovular recruitment of chronic inflammatory cells and the formation of egg granulomas. Fibrosis around the granulomas—and in some patients, massive liver fibrosis—ensues. The granuloma inflammatory cells produce a variety of proteins with fibrogenic activities promoting chemotaxis (9), mitogenesis (10), and excessive extracellular matrix synthesis (11) in fibroblasts, suggesting that these are mediators of hepatic fibrosis in this infection.

We purified the fibroblast mitogen produced by the egg granulomas, designated fibroblast-stimulating factor 1 (FsF-1), and determined that it is a product of activated $CD4^+$ lymphocytes within the granulomas (12, 13). This lymphokine has certain characteristics that distinguish it from other known fibroblast mitogens and other lymphokines (ref. 13 and unpublished data). Our efforts to date to obtain amino acid sequence data have been frustrated because FsF-1 is blocked to Edman degradation and is also strikingly resistant to enzymatic proteolysis. Accordingly, as an alternative approach to establish its molecular identity, we sought to clone FsF-1 by heterologous expression of a $CD4^+$ lymphocyte-derived cDNA library in COS-7 cells. We now report that we have cloned and determined the sequence of a lymphocyte-derived cDNA that encodes fibrosin, a protein with potent fibroblast mitogenic activity. Its precise relationship to natural FsF-1 remains to be determined.§

MATERIALS AND METHODS

Fibroblast Proliferation Assay. Human diploid fibroblast cultures were established from newborn foreskins (14). Cells were grown in either RPMI 1640 or Iscove's modified Dulbecco's medium (GIBCO) supplemented with antibiotics (penicillin, 100 units/ml; streptomycin, 100 μg/ml) and 10% heat-inactivated fetal bovine serum (FBS; GIBCO). Cells were grown to confluence in 75-cm^2 tissue culture flasks (Costar) and passaged with 0.2% trypsin/0.1% EDTA (GIBCO). For the proliferation assay, 5–6 × 10^4 cells suspended in 1 ml of supplemented medium were seeded per well in 24-well tissue culture plates (Nuncalon, Rochester, NY). Cultures were incubated overnight at 37°C in an atmosphere of 5% CO_2/95% air and then washed twice with warm Hanks' balanced salts solution (HBSS) and replenished with serum-free medium supplemented with bovine serum albumin (BSA, 0.3 mg/ml). On the following day, 100 μl of test sample was added to each well. Twenty hours later, 1 μCi (37 kBq) of [*methyl*-^{3}H]thymidine (specific activity, 6.7 Ci/mmol; DuPont/NEN) was added to each well for 4 hr. Cells were then trypsinized and harvested onto glass fiber filters (Whatman) with a cell harvester (Titertek; Flow Laboratories). [^{3}H]Thymidine incorporation was quantitated by scintillation spectrometry (model LS 3801;

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Abbreviations: BSA, bovine serum albumin; FBS, fetal bovine serum; FsF-1, fibroblast-stimulating factor 1; IL-4, interleukin 4; NRIgG, nonimmune rabbit IgG.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. U20239).

Beckman). In some experiments, fibroblast growth was assessed by enumerating with a hemocytometer cells that had grown for 72 or 96 hr in the presence or absence of the test samples.

T-Cell Culture Supernatants. Several $CD4^+$ lymphocyte clones that had been established for other purposes were kindly provided as gifts from D. C. Parker (University of Oregon, Portland) and L. Glimcher (Harvard School of Public Health, Boston) and were screened for their ability to secrete fibroblast mitogenic activity into culture supernatants following *in vitro* stimulation. Cells were propagated in supplemented medium RPMI 1640 containing 10% FBS. Cells at midlogarithmic growth were washed extensively in HBSS and 10^6 cells were cultured in 1 ml of serum-free medium supplemented with BSA (0.3 mg/ml) and concanavalin A (Con A, 10 μg/ml; Sigma) for 24 hr at 37°C in a humidified atmosphere of 5% CO_2/95% air. Cell-free supernatants were retrieved by centrifugation at 200 *g* for 10 min and stored at −20°C until tested in a fibroblast proliferation assay. On the basis of our screening results, the CDC25 cell line, a $CD4^+$, T_H2 helper T-lymphocyte line (provided by D. C. Parker; ref. 15) was selected for further analysis. It was propagated biweekly as described (15) and used 2–3 weeks later for preparation of culture supernatants.

Antibody Neutralization of Fibrogenic Activity. Specific polyclonal IgG was prepared by immunizing rabbits with highly purified murine FsF-1, as described (12). This antibody preparation did not react (by dot blot ELISA) with a variety of recombinant murine lymphokines (12, 13). IgG purified from pooled sera of rabbits prior to immunization [nonimmune rabbit IgG (NRIgG)], which did not react with FsF-1, served as a control. Cell-free supernatants (undiluted) from lymphocytes or transfected COS-7 cells were incubated with IgG (2.5–7.5 μg/100 μl) at 37°C for 2–3 hr in polypropylene culture tubes (Falcon 2063; Becton-Dickinson Labware) that had been previously treated with BSA (1 mg/ml) and washed, to reduce nonspecific adsorption of proteins. Immune complexes were removed from selected mixtures with protein A coupled to Sepharose beads (Sigma). After incubation for 1 hr at 37°C, the beads were removed by centrifugation at 1000 × *g* for 10 min. The samples were then filter-sterilized (0.22-μm-diameter pore size; Millipore) and tested in a fibroblast proliferation assay.

cDNA Library and Expression Cloning. A cDNA library which we obtained from DNAX had been prepared for other purposes from mRNA isolated from Con A-stimulated cells of the CDC25 line. The library was constructed in the vector pcDSRα296, which contains a unique promoter that permits high-level, transient expression of the cDNA insert in COS-7 cells (16) and has been used previously in cloning of murine lymphokine cDNAs (17, 18).

Escherichia coli transformants containing the pcDSRα296 vectors were expanded in Luria–Bertani (LB) broth containing ampicillin (50 μg/ml), and plasmids were isolated on Qiagen columns (Qiagen, Chatsworth, CA). For transfection, 5×10^5 COS-7 cells (19) were seeded on 60-mm tissue culture dishes (Falcon) in supplemented Dulbecco's modified Eagle's medium (DMEM; GIBCO) containing 10% FBS (GIBCO). Cells were grown overnight to a density visually estimated to be 60–70% confluent. Cells were then washed with serum-free DMEM buffered with 50 mM Tris (pH 7.4), and plasmid DNA (1–5 μg) in serum-free medium (4 ml per plate) was added, followed by DEAE-dextran (200 μg/ml; Pharmacia). After coincubation with plasmid DNA for 4–5 hr, COS-7 cells were washed and treated for 2–3 hr with 100 μM chloroquine (Sigma) in the presence of 2% FBS. Cells were then washed and grown overnight in DMEM with 4% FBS. The following day, cells were washed and replenished with DMEM containing BSA (0.3 mg/ml). Forty-eight to seventy-two hours later, culture supernatants were collected and tested for their ability to stimulate fibroblast proliferation.

For controls, COS-7 cells were transfected with the pcDSRα296 plasmid containing a murine interleukin 4 (IL-4) cDNA insert (DNAX) and were subsequently maintained under the above conditions, with minor modifications. Supernatants of these cultures were harvested after 72 hr and tested for IL-4 activity on an indicator cell line [HT-2 (20)] and in the fibroblast proliferation assay.

DNA Nucleotide Sequence Analysis. Nucleotide sequences on both positive and negative cDNA strands were determined by the dideoxy chain-termination protocol with supercoiled DNA templates (21). Conditions were modified as needed to sequence G + C-rich areas with compressions. The nucleotide sequence was compared with sequences in GenBank.

Peptide Synthesis. A 71-aa oligopeptide (M_r 7620, as confirmed by mass spectrometry) with sequence corresponding to that of the cDNA (Arg^{26}–Leu^{96}; see Fig. 5) was synthesized by the methods of *tert*-butyloxycarbonyl/*N*-methylpyrrolidone chemistry using a Perkin–Elmer Applied Biosystems model 430A peptide synthesizer (22). The peptide was purified by HPLC on a Vydac C_4 column and eluted in a single fraction with a trifluoroacetic acid/acetonitrile gradient.

RESULTS

CDC25 Lymphocyte Line Produces Fibroblast Mitogen. Of the 30 lymphocyte lines and T–T hybridomas we tested, 5 elaborated detectable fibroblast mitogenic activity into culture supernatants following their *in vitro* stimulation with Con A for 24 hr. Con A had no intrinsic fibroblast mitogenic activity in our fibroblast assay. We chose line CDC25 for further analysis. Culture supernatants of CDC25 cells stimulated fibroblast [^{3}H]thymidine incorporation in a concentration-dependent manner; peak responses were detected at a concentration of 10% (the maximum tested; Fig. 1*A*). Anti-FsF-1 IgG reduced the activity of the CDC25 culture supernatant by 47% at a concentration of 2.5 μg/100 μl (Fig. 1*B*), relative to responses in the presence of the same concentrations of NRIgG, which we have established does not significantly alter the fibroblast responses to purified FsF-1 (12). Furthermore, in Western blots anti-FsF-1 IgG recognized a single protein band in CDC25 culture supernatants subjected to SDS/PAGE (apparent molecular mass, 50–60 kDa). NRIgG did not recognize this protein (data not shown).

CDC25 Library Contains cDNA That Encodes a Fibroblast Growth Factor. Culture supernatants of COS-7 cells transfected with the entire CDC25 library containing ≈10^6 clones

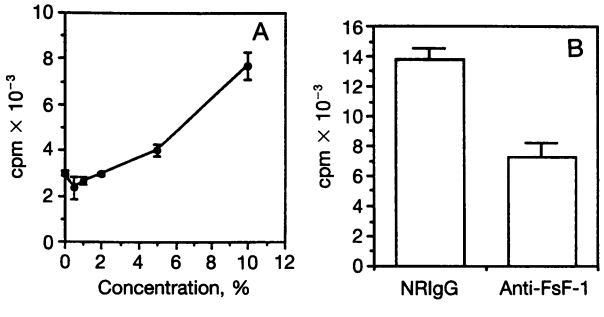

FIG. 1. Fibroblast [^{3}H]thymidine incorporation in response to culture supernatants of CDC25 cells stimulated with Con A for 24 hr. (*A*) Mean ± SEM of triplicate determinations is shown for each concentration (vol/vol) of culture supernatant tested. (*B*) Partial neutralization of the fibroblast mitogenic activity in CDC25 culture supernatant. Supernatants were incubated for 1 hr with either NRIgG or rabbit anti-FsF-1 IgG at 2.5 μg/ml. The mixture was tested at 5% (vol/vol) concentration. NRIgG did not affect the response to culture supernatant. Mean ± SEM of triplicate determinations is shown.

stimulated fibroblast [^{3}H]thymidine incorporation in a concentration-dependent manner (Fig. 2). Because culture supernatants from IL-4 cDNA transfectants, which contained IL-4 at 200–250 units/ml as judged from results in the HT-2 proliferation assay, had minimal effects on fibroblast proliferation, we concluded that the CDC25 library contained cDNA that specifically encoded a fibroblast mitogen that was not IL-4. We used sib selection as a strategy to clone this cDNA.

The CDC25 library was partitioned into pools of 10^3 clones that were seeded into separate wells of microtiter plates containing LB broth and ampicillin. Pools estimated to contain 10^4 clones were prepared by combining wells within a row. COS-7 cells were transfected with plasmid DNA prepared from these pools and the transfectant culture supernatants tested at various concentrations, to a maximum of 10%, were assayed for their ability to enhance fibroblast [^{3}H]thymidine uptake at least 2- to 3-fold above background at any concentration tested (positive transfectant).

Of the 20 pools initially prepared and tested, 3 (15%) were positive. One of the three pools (pool B) was selected because the biological activity in the supernatant of pool B transfectants could be neutralized with anti-FsF-1 IgG. Pool B was subdivided into 8 separate pools (each containing $\approx 10^3$ clones), and plasmid DNA prepared from each pool was used to transfect COS-7 cells. Conditioned medium from four of the transfectants (50%) had significant mitogenic activity. Based on antibody neutralization of bioactivity, 1 of the pools (2B) was selected. Pool 2B was then subdivided into 16 pools, each estimated to contain 100 clones. Five of the pools (31%) produced positive transfectants. Two of the positive pools were plated on solid agar, and 10 colonies were screened; 8 of these colonies produced positive transfectants.

One of the clones (2B3) was subcloned twice on solid agar and analyzed in detail. Supernatant of 2B3 transfectant was active in a fibroblast growth assay over a wide concentration range; the log-linear dose–response relationship was biphasic (Fig. 3*A*). Anti-FsF-1 IgG virtually abolished the fibrogenic activity of 2B3 transfectant supernatant (Fig. 3*B*). With each round of sib selection, the potency of transfectant culture supernatant increased 2000-fold from transfection with the whole library to transfection with 2B3 clone (Fig. 4).

Nucleotide Sequence of Clone 2B3. Clone 2B3 contains a cDNA insert of 216 bp and a single open reading frame starting with arginine (nt 76–79; Fig. 5) and terminating with leucine (nt 289–291) followed by the stop codon TAA. Thus, the open reading frame encodes 71 aa and is followed by 11 untranslated codons that precede the poly(A) tail. Since this single open reading frame does not contain an internal initiating methionine, we assume that polypeptide synthesis is initiated at the first in-phase ATG codon present in the vector's 16S splice

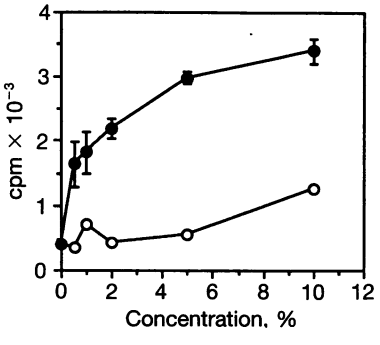

FIG. 2. Fibroblast [^{3}H]thymidine incorporation in response to culture supernatants from COS-7 cells transfected with plasmids DNA representing the whole CDC25 cDNA library (●) and from sham-transfected COS-7 cells (○). Mean ± SEM of triplicate determinations is shown for each concentration of culture supernatant shown.

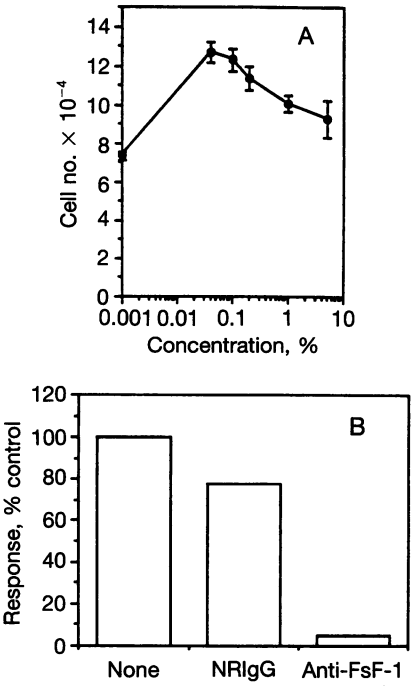

FIG. 3. Growth of fibroblasts in response to culture supernatants from COS-7 cells transfected with plasmid DNA containing the clone 2B3 cDNA insert. (*A*) Mean ± SEM of fibroblast cell number determined after 96 hr of incubation is shown for each concentration of transfectant culture supernatant tested (indicated on a logarithmic scale). Cultures of fibroblasts maintained for 96 hr in medium alone contained (6.7 ± 0.3) × 10^4 cells. (*B*) Neutralization of fibroblast-stimulating activity in culture supernatants of COS-7 cells transfected with clone 2B3-containing plasmid DNA. Supernatants were incubated for 1 hr with either normal rabbit IgG (NRIgG) or rabbit anti-FsF-1 IgG at 7.5μg/ml and then with protein A-Sepharose and were tested at a final concentration of 0.01% (vol/vol) for the ability to stimulate fibroblast [^{3}H]thymidine incorporation. The effect of treatments is shown relative to the response of fibroblasts to untreated transfectant culture supernatant tested at 0.01%.

junction area and that the cloned 2B3 peptide may represent a truncated form of the native protein. Based on this assumption, the total open reading frame that encodes the recombinant protein contains 288 nt. No significant homology of this

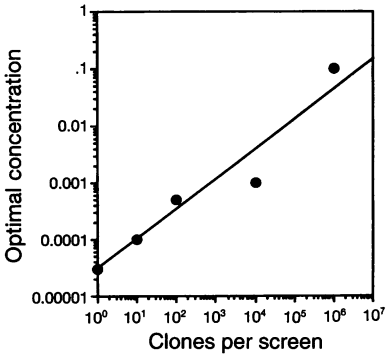

FIG. 4. Sib selection in the cloning of 2B3 cDNA that encodes a fibroblast mitogen. COS-7 cells were transfected with plasmid DNA and the transfectant supernatants were tested at various concentrations for their ability to stimulate fibroblast [^{3}H]thymidine incorporation. Following transfection with the entire library cDNA (10^6 clones), pools of clones were sequentially screened and selected for their ability to encode biologically active macromolecules. The inverse correlation between the number of clones per screen and the concentration of the corresponding transfectant culture supernatant that maximally stimulated fibroblast responses reflects the progressive enrichment of cDNA that encodes fibrogenic activity.

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ATG TTG CCT TTA CTT CTA GGC CTG TAC GGA AGT GTT ACT TCT GCT
Met Leu Pro Leu Leu Leu Gly Leu Tyr Gly Ser Val Thr Ser Ala  15
CTA AAA GCT GCT GCA CCC CCC CCC CCC CCA*AGG GCC TCT AGG CCC
Leu Lys Ala Ala Ala Pro Pro Pro Pro Pro Arg Ala Ser Arg Pro  30
TTG GCC TGC CTC CCC AAG GGC TCA CTA AGC CAG AGG CCA AAG TGC
Leu Ala Cys Leu Pro Lys Gly Ser Leu Ser Gln Arg Pro Lys Cys  45
CCC CCT CCC TTT CGC CTA CCA CCC AAG TTC TCA TGC CCT CCG AGG
Pro Pro Pro Phe Arg Leu Pro Pro Lys Phe Ser Cys Pro Pro Arg  60
GCT GAG GAA GGA GGA ACT AAA GGA ATA GGG GTT TCA TGT ACA TAT
Ala Glu Glu Gly Gly Thr Lys Gly Ile Gly Val Ser Cys Thr Tyr  75
TTA TCA CCC CTT CCA CAA ATC CCC CAG ACC TTT TGT ACA TTT TTA
Leu Ser Pro Leu Pro Gln Ile Pro Gln Thr Phe Cys Thr Phe Leu  90
CAG GGG TGC CCC TCC CTA TAA
Gln Gly Cys Pro Ser Leu STOP
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FIG. 5. Nucleotide sequence and predicted amino acid sequence of the 2B3 cDNA insert. Codons 1–25 are derived from the vector; the insert begins with codon AGG (star) and ends with the termination codon TAA (codon 97).

sequence with sequences in GenBank (as of November 16, 1994) could be identified.

Fibroblast Growth Stimulation by Synthetic Peptide. The 71-aa 2B3 peptide consisting of the amino acid sequence deduced from the open reading frame (arginine at Arg^{26}–Leu^{96}; Fig. 5) stimulated fibroblast proliferation (as judged by cell counting) in a concentration-dependent manner (Fig. 6). Notably, in 12 separate assays (6 in which cells were assayed after 72 hr of incubation and 6 after 96 hr of incubation), peak mitogenic activity was observed with peptide concentrations between 0.1 and 10 pM; a second peak of activity was detected at concentrations in the 1 μM range.

DISCUSSION

We previously identified FsF-1 as a fibroblast growth-stimulating lymphokine produced by $CD4^+$ lymphocytes (13). Using biological activity and its neutralization with anti-FsF-1 IgG antibody, we screened culture supernatants of established murine T-lymphocyte lines that had been stimulated with Con A. We identified a $CD4^+$ lymphocyte line CDC25 as one apparently able to produce FsF-1 (Fig. 1), and screened a CDC25 cDNA library by heterologous expression in COS-7 cells. The very high level of expression afforded by the design of the pcDSRα296 promoter elements permitted us to detect fibroblast mitogenic activity in conditioned medium from COS-7 cells transfected with the entire library (Fig. 2). Sib selection resulted in progressively enhanced potency of the fibroblast mitogenic activity in transfectant culture supernatants (Fig. 4) and ultimately resulted in the isolation of clone 2B3. 2B3cDNA encodes a protein with fibroblast mitogenic activity that is neutralized or adsorbed with anti-FsF-1 antibody (Fig. 3).

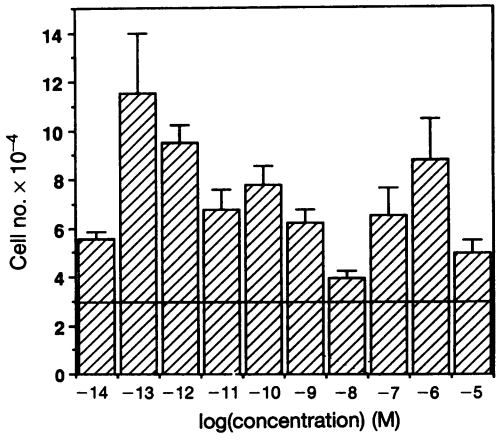

FIG. 6. Fibroblast growth in response to 72 hr of incubation with various doses of the 2B3 peptide in the absence of serum. Mean (and SEM) responses of triplicate determinations in a representative experiment are shown. The horizontal bar depicts the mean of fibroblast growth in medium alone (3.1 ± 0.78). In all, 12 experiments were performed; cells were enumerated at 72 hr in 6 and at 96 hr in 6. Maximum responses at picomolar concentrations were 3- to 4-fold baseline, and those at micromolar concentrations were 2- to 3-fold baseline.

Analysis of the nucleotide sequence of clone 2B3 cDNA indicates that it contains a single open reading frame. Since the deduced amino acid sequence of this open reading frame (Fig. 5) lacks a methionine residue, the translated product presumably is a fusion protein initiated by an in-frame methionine coded by a vector-derived ATG codon located 72 bp upstream from the 5′ end of the cDNA insert. The pcDSRα296 vector we employed, like the parental pcD vector originally designed by Okayama and Berg (23), allows a high level of expression of full-length cDNA inserts under the control of the simian virus 40 (SV40) early promoter. SV40-derived DNA fragments are arrayed in these vectors to permit transcription, splicing, and polyadenylylation of the cloned cDNA. The DNA contains both the SV40 early-region promoter and two introns normally used to splice the viral 16S and 19S late mRNAs (23) upstream of the cDNA cloning site. This ensures transcription and splicing of the cDNA transcripts. This system permits two alternative modes of splicing. Most often (60–70% of transcripts) splicing occurs at the 16S RNA intron junction and places the cDNA initiator ATG codon first in line from the 5′ end of the mRNA. When splicing occurs at the 19S RNA intron, it retains an ATG codon upstream of the cDNA in the processed mRNA. Therefore, if the clone contains an incomplete cDNA, translation from the upstream ATG codon may yield a fusion protein.

Analysis of the deduced amino acid sequence indicates that the amino terminus of the fusion protein is hydrophobic and may potentially function as a secretion signal sequence (24). If it does serve this function, cleavage would be expected at the alanine residue at position 15 or 20 of the fusion protein, resulting in a mature peptide containing 81 or 76 aa (predicted mass, 9–10 kDa). Alternative cleavage sites might exist. Since gel filtration chromatography of the transfectant culture supernatants gave fibroblast mitogenic activity in fractions corresponding to a molecular mass of 30 kDa (S.P., unpublished data), the protein may be further modified *in vivo* by glycosylation.

The 71-aa synthetic 2B3 peptide which was made from the deduced amino acid sequence of the clone was biologically active (Fig. 6), thus confirming the deduced sequence. The dose–response pattern we observed with the synthetic peptide is reminiscent of our experience with crude and purified natural FsF-1 (12, 14) as well as with supernatants of the 2B3 transfectant (Fig. 3). We have not had sufficient material to ascertain whether, like the 2B3 peptide, natural FsF-1 gives rise to a second peak of growth stimulation at higher concentrations. Although we currently have no explanation for the dose–response pattern, the possibility exists that it reflects multiple receptors with differing ligand affinities.

The 2B3 cDNA possesses no significant homology with other archived nucleotide sequences, and we have designated the corresponding protein fibrosin. The crossreactivity of polyclonal anti-FsF-1 antibody with fibrosin (Fig. 3*B*) suggests that the two lymphokines may be similar or identical.

Fibrosin differs in biological activity from IL-4 and transforming growth factor β, two known lymphokines with fibrogenic activity. Whereas transforming growth factor β and IL-4 are potent fibroblast chemoattractants (25, 26) and stimulate enhanced extracellular matrix synthesis (27, 28), they appear to have little direct mitogenic effect on fibroblasts in the absence of other growth factors (27, 29). In contrast, fibrosin is at least as potent as acidic and basic fibroblast growth factor when tested in our standard fibroblast proliferation assay (12). It can also be anticipated that fibrosin, like the other fibrogenic lymphokines, will prove to be multifunctional and may exert biological effects in addition to ones involved in fibrosis.

We thank Dr. David C. Parker for providing the CDC25 cell line and for several helpful suggestions; Dr. Frank Lee for providing the CDC25 cDNA library and expert advice; Dr. Laurie Glimcher for providing various lymphocyte cell lines; Dr. Charles Dinarello for helpful discussions; and the late Dr. Sheldon M. Wolff and Dr. Gerald T. Keusch for encouragement and support. We are grateful to Richard F. Cook and Heather LeBlanc (Massachusetts Institute of Technology) for synthesizing the peptide. The Newborn Nursery of St. Margaret's Hospital for Women (Dorchester, MA) and the Mother and Infant Unit of New England Medical Center Hospital assisted by providing foreskins for preparation of fibroblast cultures. Priscilla Rogers provided administrative support, and Patricia Turner and Sarah Wong prepared the manuscript. S.P. was the recipient of a Young Investigator Fellowship from Boehringer Ingelheim. The research was supported by a grant from the Whitaker Foundation, awarded to S.P., Grants AI17615 and DK34928 from the National Institutes of Health awarded to D.J.W., and American Cancer Society Research Professorship Grant ACS/PRP-55 awarded to P.W.R.

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