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Purification and Characterization of a Silica-Induced Bronchoalveolar Lavage Protein With Fibroblast Growth-Promoting Activity

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Abstract Experimentally induced silicosis provides a good model for chronic interstitial pulmonary inflammation and fibrosis. In the present study, a specific single polypeptide with an apparent molecular mass of 58,000 and a pI of 4.5 was purified and characterized from the bronchoalveolar lavage fluid of silicotic rats. The same protein was also isolated from both the extract and conditioned medium of alveolar macrophages of silicotic rats. Therefore, this protein was termed an inducible silicotic (rat) bronchoalveolar lavage protein-p^{58} (iSBLP^{58}) or an inducible silicotic (rat) pulmonary macrophage factor (iSPMF-p^{58}). iSBLP^{58} has been purified to homogeneity by a combination of gel permeation, Mono Q ion exchange, and reverse-phase high performance liquid chromatography. This polypeptide displayed a potent fibroblast growth-promoting activity in vitro. The sequence of the first 15 NH_2-terminal amino acids was determined and was found to have high sequence homology with members of the mammalian chitinase-like protein family, which includes human cartilage gp39, mammalian oviduct-specific glycoprotein, and a secretory protein from activated mouse macrophages. J. Cell. Biochem. 67:257–264, 1997.

Key words: silicosis; bronchoalveolar lavage protein; fibroblast proliferation-promoting factor; inducible macrophage factor; fibrosis

It is known that chronically inflamed and fibrotic lung tissue can actively express different genes and produce a number of cytokines and growth factors as part of pathological process resulting in a variety of physiological, pathological, and even anatomic changes through a series of cascading cellular interactions [Gauldie et al., 1993]. The cellular and molecular events of silicosis, resulting from the inhalation of respirable mineral particles, have been previously investigated, and a great deal of attention has been paid to this project. Silicosis is a disease whose incidence and prevalence in developing countries are high and appear to be rising, while it has not been completely stamped out even in industrialized countries. Not only is this research necessary for the prevention and therapy of silicosis but also is available as a good model for the study of chronic interstitial pulmonary inflammation and fibrosis [Li, 1995]. The interaction between macrophages and fibroblasts by macrophage-derived mediators play a critical role in silicotic fibrosis. Since Heppleston and Styles [1967] proposed the fibrogenic factor hypothesis, a series of cytokines or growth factors have been proven to be related to the silicotic process, including interleukin 1 (IL-1), tumor necrosis factor (TNF), fibroblast growth factor (FGF), and platelet-

Abbreviations: AMCM, alveolar macrophage-conditioned medium; AME, alveolar macrophage freeze-thawed extract; BALF, the supernatant of bronchoalveolar lavage fluids; FGPF, fibroblast growth-promoting factor; IEF, isoelectric focusing; iSBLP^{58}, inducible silicotic (rat) bronchoalveolar lavage protein-p^{58}; iSPMF-p^{58}, inducible silicotic (rat) pulmonary macrophage factor p^{58}; MeCN, acetonnitril; MTT, 3-(4,5-dimethylthiazol-zyl)-2,5 diphenyl tetrazdium bromide; TFA, trifluoroacetic acid.

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derived growth factor (PDGF) as well as others [Vanhee et al., 1995; Schmidt et al., 1984; Li et al., 1987; Marinelli et al., 1991; Kovacs, 1991; Gosset et al., 1991]. Some proteins with fibrogenic activity have also been characterized, including fibronectin and a silica-induced macrophage factor (Mr 16,000–18,000), which is well known among postulated fibrogenic factors although its amino acid sequence has not yet been determined [Aalto and Kulonen, 1980; Aalto et al., 1988]. However, no specific silicotic fibrogenic factors have been purified to homogeneity and characterized further. Since the 1980s, we have been paying close attention to a protein with Mr 60,000 or so. The content of this protein rises significantly in the bronchoalveolar lavage fluid and pulmonary macrophages of silicotic rats, and this protein has been found to be capable of stimulating fibroblast proliferation [Cai et al., 1983, 1984; Chen and Li, 1991].

In the present study, we purified and characterized a protein of Mr 58,000 from the bronchoalveolar lavage fluid of silicotic rats, which acts as a potent fibroblast growth-promoting factor (FGPF) and is identified to be most likely derived from alveolar macrophages. Here, this polypeptide is referred to as an inducible silicotic (rat) bronchoalveolar lavage protein (iSBLP^{58}) or an inducible silicotic (rat) pulmonary macrophage factor (iSPMF-p^{58}).

MATERIALS AND METHODS

Preparation of Bronchoalveolar Lavage of Silicotic Rats and the Cultivation of Alveolar Macrophages

Wistar male rats (180–200 g) received an intratracheal instillation of 50 mg silica (SiO_2 content more than 97%; diameter less than 5 μm) suspended in 1 ml saline. The silica-instilled rats were raised for 3 weeks along with the normal rats, which imitatively received silica-free saline under the same condition. Both groups of rats were killed by bloodletting, and the lungs were removed. Bronchoalveolar washing was then performed with 8 ml of Ca^{2+}, Mg^{2+}-free Hanks solution (CMF-Hanks) five times, yielding 40 ml of bronchoalveolar lavage fluid which was harvested from each rat.

The lavage fluid was centrifuged for 5 min at 1,000 rpm, and both the supernatant and pelleted cells were collected separately. The supernatant of the lavage fluid is abbreviated as BALF. The pelleted cells were then washed twice with CMF-Hanks and resuspended in serum-free RPMI-1640 medium at 4°C. The cells were plated into 100 ml flat cell culture flasks at 3×10^7 cells per flask, allowed to attach for 2 h at 37°C, 5% CO_2, rewashed to remove nonadherent cells, and continuously incubated in 12 ml of fresh media for 14–16 h; the medium was then collected (referred to as alveolar macrophage-conditioned media [AMCM]). The adherent macrophages were disrupted by means of a repeated freeze-thawing process in an appropriate volume of Ca^{2+}, Mg^{2+}-free phosphate buffered saline (PBS^-), and the freeze-thawed cell extract was then centrifuged for 10 min at 10,000 rpm, 4°C; the supernatant was reserved (referred to as alveolar macrophage freeze-thawed extract [AME]). For the silicotic group, the BALF, AMCM, and AME have been specifically termed BALFs, AMCMs, and AMEs, respectively.

The collected supernatant of BALFs, AMCMs, and AMEs were desalted and their solvents changed to 10 mM phosphate buffer (PB) using a PD-10 desalting Sephadex G-25 column (Pharmacia, Uppsala, Sweden). These samples were dried by lyophilization for bioassay and the following purification process.

Bioassay of Fibroblast Growth-Promoting Factor (FGPF) Activity

Stock cultures of 2BS fibroblasts, a diploid cell strain from human embryonic lung (purchased from Beijing Institute of Biological Products) were subcultured in MEM medium (GIBCO) containing 10% fetal bovine serum (FBS). For the bioassay of FGPF activity, MEM medium containing 5% FBS, 0.05% lactalbumin hydrolysate, 50 μg/ml ascorbic acid, and 100 μg/ml proline was used. The cells were trypsinized and seeded into 96-well culture plates at a density of 1×10^4 cells/well with 200 μl medium containing the test materials. Cells were incubated in a humidified atmosphere of 95% air/5% CO_2 at 37°C for 60 h. Cell proliferation was then measured directly in the 96-well culture plates using the MTT colorimetric assay according to Mosmann's method [Mosmann, 1983]. The cells, stained by 3-(4,5-dimethylthiazol-zyl)-2,5 biphenyl tetrazdium bromide (MTT) (Sigma, St. Louis, MO) in the 96-well culture plates, were read at 570 nm on an ELISA reader. The percent stimulation for each sample was

calculated using the formula

$$\% \text{ stimulation} = \frac{A_{570} \text{ in test sample well}}{A_{570} \text{ in control medium well}} \times 100.$$

Purification of Fibroblast Growth-Promoting Activity

Gel permeation chromatography (GPC). Lyophilized BALF, AMCM, and AME samples were dissolved in 75 mM PB (pH 7.4) and were each loaded onto a Superose 12 HR 10/30 column (300 × 10 mm; Pharmacia) preequilibrated with PBS^- and eluted at a flow rate of 0.5 ml/min with the same buffer at room temperature. An appropriate amount of sample was applied in each cycle of GPC (2–3 mg of protein of AMCMs or AMEs, 10–15 mg of protein of BALFs). Half-milliliter fractions were collected, and three to four tubes were pooled on the basis of eluted protein peak. The eight fractions obtained were then desalted for bioassay of FGPF activity and the next step of purification. To determine the molecular mass, we precalibrated the column with the following molecular weight marker proteins (Pharmacia): TMV capsid protein (17,500), carbonic anhydrase (30,000), actin (43,000), bovine serum albumin (67,000), and phosphorylase b (94,000).

Mono Q ion-exchange chromatography (IEC). The active fraction from GPC of BALF was applied to a Mono Q HR 5/5 anion exchange column (Pharmacia) previously equilibrated with 50 mM NaCl in 20 mM Tris-HCl (pH 7.4). Protein (1–2 mg) in preequilibration buffer was loaded per cycle of IEC and eluted at a flow rate of 0.5 ml/min with a linear gradient of NaCl from 50 mM to 450 mM over 30 min. Half-milliliter fractions were collected, and then one to two tubers were pooled; the ten resulting fractions were desalted using a PD-10 column and lyophilized for storage. The lyophilized fractions were redissolved in PBS^- for the bioassay and RP-HPLC.

Reverse-phase high performance liquid chromatography (RP-HPLC). The active fractions from GPC of AMCMs and AMEs and from IEC of BACFs were acidified with 0.1% (v/v) trifluoroacetic acid (TFA) and loaded onto a Vydac protein C_4 column (Type 214 TP 54; The Separation Group, Hesperia, CA) previously equilibrated with 20% acetonitrile (MeCN) and 0.1% TFA for 2 min. Protein samples (100–300 μg) were loaded per cycle of RP-HPLC. The column was washed with the equilibration buffer for 30 min and was eluted using two linear gradient of MeCN, first from 20% to 29.6% over 8 min and then from 29.6% to 45.6% over 32 min. Half-milliliter fractions were collected, dried by lyophilization, and then redissolved with PBS^- for the bioassay.

Amino-Terminal Amino Acid Sequence Analysis

The final purified active fraction (50 pM) from RP-HPLC of BALFs was subjected to automated amino acid sequence analysis using a model 476A amino acid sequencer (Applied Biosystems, Inc., Foster City, CA) under the following conditions: smooth degree 9, integration limits 0.00–30.00 min, baseline limits 0.10–1.00 min, peak width 0.10 min, peak separation 0.05 min, searching length 0.20 min, RT factor 75, and sensitivity 1.00.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was achieved using a 7.5% or 12% polyacrylamide gel with a 4% stacking gel. After electrophoresis, bands of proteins were detected by Coomassie brilliant blue R-250 staining. The molecular weight was calculated on account of the mobility of marker proteins (Bio-Rad Lab, Richmond, CA) including rabbit muscle phosphorylase b (Mr 97,400), bovine serum albumin (Mr 66,200), hen egg white ovalbumin (Mr 42,700), bovine carbonic anhydrase (Mr 31,000), and soybean trypsin inhibitor (Mr 21,500).

Isoelectric Focusing (IEF)

The isoelectric point (pI) of iSBLP[58] was determined by an IEF on a thin-layer (0.5 mm) polyacrylamide gel (T = 4%) with ampholyte (pH 3.5–10) (Sigma). Twenty micrograms of the purified iSBLP[58] was loaded onto the gel, and IEF was performed at 30 w for 4 h using 1 M NaOH and 40 mM glutamic acid as the catholyte and anolyte, respectively. After electrophoresis, protein bands were visualized by Coomassie brilliant blue R-250 staining. The pH gradient in the gel was measured by cutting an unfixed part of the gel into 5 mm pieces after focusing. Each piece was immersed in 2 ml of distilled water for 5 h, and the pHs of the extracts were measured. The pI was estimated by comparison with the pH gradient in the gel.

RESULTS

The present study confirmed that the bronchoalveolar lavage fluid (BALFs), alveolar macrophage-conditioned media (AMCMs), and alveolar macrophage (AMEs) from silicotic rats are capable of stimulating fibroblast proliferation. The proliferation of the fibroblasts induced by adding BALFs, AMCMs, or AMEs was 71.0%, 43.1%, and 55.2% higher than that of the control fibroblasts, respectively ($P < 0.01$). Further, a polypeptide with an apparent molecular weight of 58,000 was isolated and characterized on the basis of FGPF activity in vitro. This polypeptide has been purified to homogeneity from BALFs by a combination of GPC, IEC, and RP-HPLC and from AMCMs and AMEs by a combination of GPC and RP-HPLC.

Purification of iSBLP^{58} From BALFs

The concentrated BALFs was subjected to GPC. The FGPF activity was prominently present in fraction 4 near Mr 60,000 (Fig. 1A), which contained 13.5% of the total protein applied. Fraction 4 of GPC (GP 4) was used for IEC. Fraction 2 (IE 2) eluted at 0.18 M NaCl from the Mono Q column (Fig. 1B). Fraction IE 2 contained the majority of FGPF activity among the eight fractions obtained and contained 24.4% of the total protein of GP 4 loaded onto the IEC. The final step of purification was achieved using a Delta-pak C_4 reverse-phase high performance liquid chromatography. The main FGPF activity was found in fraction 2 (RP 2) at 35% MeCN (Fig. 1C). This fraction represented 7.1% of protein in the IE 2 fraction. A single band detected by both Coomassie brilliant blue R-250 staining (Fig. 2, lane 3) and silver nitrate staining (data not shown) on SDS-PAGE indicated that the resulting fraction RP 2 had been purified to homogeneous and had an apparent molecular weight of 58,000 under reducing condition. On the basis of IEF, a pI of 4.5 was determined for this polypeptide which was termed an inducible silicotic (rat) bronchoalveolar lavage protein-p^{58} (iSBLP^{58}).

A summary of iSBLP^{58} purification from BALFs is shown in Table I. The iSBLP^{58} (117 µg) was harvested from the bronchoalveolar lavage fluid of 20 rats. The purification ratio (the half-maximal activity dose of BALFs/the half-maximal activity dose of iSBLP^{58}) was 167-fold, and the overall yield of FGPF activity was 39.0%.

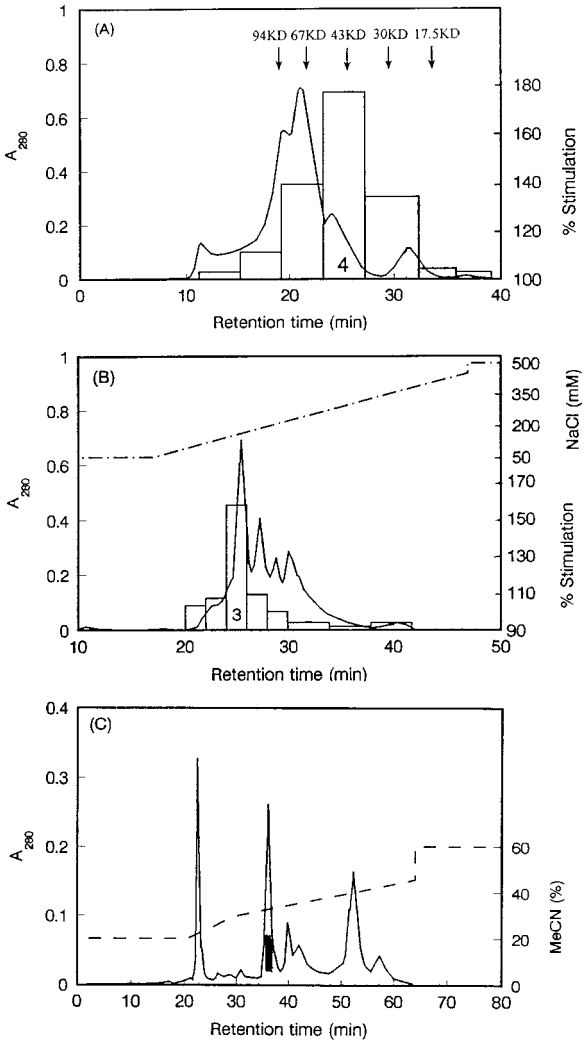

Fig. 1. Isolation of iSBLP^{58} from bronchoalveolar lavage fluid of silicotic rats. **A:** Gel permeation chromatography (GPC). Ten to fifteen milligrams of protein of BALFs in 0.15 ml of 75 mM PB (pH 7.4) was loaded on a Superose 12 HR 10/30 column (300 × 10 mm) preequilibrated with PBS$^-$ and eluted at a flow rate of 0.5 ml/min with the same buffer at room temperature. Aliquots (20 µl) of each pooled fraction were tested for bioassay of FGPF activity; the percent stimulation of each fraction was expressed as *open columns.* **B:** Mono Q ion-exchange chromatography (Mono Q IEC). Active GPC fraction 4 (2–3 mg) was applied onto a Mono Q HR 5/5 anion exchange column previously equilibrated with 50 mM NaCl in 20 mM Tris-HCl (pH 7.4) and eluted at a flow rate of 0.5 ml/min with a linear gradient of NaCl from 50 mM to 450 mM over 30 min. Aliquots (20 µl) of individual pooled fractions were examined for bioassay of FGPF activity. The percent stimulation of each fraction was expressed as open columns. **C:** RP-HPLC. Active Mono Q IEC fraction 3 (200–300 µg) was loaded on a Vydac protein C_4 column (Type 214 TP 54) previously equilibrated with 20% acetonitrile (MeCN) and 0.1% TFA and eluted using two steps of linear gradient of MeCN from 20% to 29.6% over 8 min and then from 29.6% to 45.6% over 32 min. Aliquots (20 µl) of each fraction were tested for FGPF activity. The *black column* represents the active protein fraction.

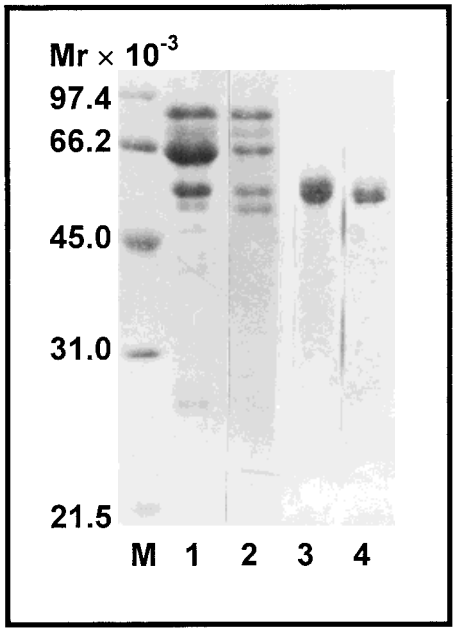

Fig. 2. SDS-PAGE of iSBLP[58] under reducing condition. Bands of proteins were detected by Coomassie brilliant blue R-250 staining. **Lane 1:** The fraction GP4 of GPC for BALFs from silicotic rats. **Lane 2:** The fraction GP4 of GPC for BALF from normal rats. **Lane 3:** The final purified iSBLP[58] from BALFs by RP-HPLC. **Lane 4:** The final purified iSBLP[58] from AMEs by RP-HPLC. **Lane M:** Molecular mass marker proteins.

Purification of iSBLP[58] From AMCMs and AMEs

By omitting the Mono Q IEC, we used an isolation protocol similar to BALFs separation in the purification of iSBLP[58] from AMCMs and AMEs. Only the GPC and RP-HPLC patterns of AMCMs were shown in Figure 3, since the GPC and RP-HPLC patterns of both AMCMs and AMEs resemble each other. A fraction with FGPF activity (referred as an inducible silicotic (rat) pulmonary macrophage factor [iSPMF-p[58]]) was obtained at the 35% MeCN gradient position in RP-HPLC similar to the iSBLP[58] from BALFs and only one band also appeared with an apparent molecular weight of 58,000 on SDS-PAGE which was identical to that of iSBLP[58] from BALFs (Fig. 2, lanes 3,4). So, iSPMF-p[58] is logically identical with iSBLP[58]. The summary of the purification of iSBLP[58] from AMCMs and AMEs demonstrated that the iSBLP[58] was purified 33.3-fold, with an overall yield of 18.8% and 17.6% from AMCMs and AMEs (Table I).

Amino-Terminal Amino Acid Sequence of iSBLP[58]

The first 15 amino-terminal amino acid sequence of the finally purified iSBLP[58] by RP-HPLC from BALFs was determined by the model 476A microsequencer. A comprehensive search of the Swiss-Prot and GenBank™ database was unable to locate a known N-terminal amino acid sequence that completely conformed to that of purified iSBLP[58] (Fig. 4). However, the determined residues of iSBLP[58] showed significant homology with members of the mammalian chitinase-like protein family, which includes the mature hamster oviduct-specific glycoprotein (HOGP) whose apparent molecular mass is larger than 200,000, human cartilage gp-39 (HCgp-39), which is a major secretory product of articular chondrocytes and synovial cells, and a secretory protein from activated mouse macrophage (AMMSP). In comparison with the N-terminal 15 residues of iSBLP[58], there is a high proportion of identical amino acid residues: 80% for HOGP, 67% for HCgp-39, and 60% for AMMSP; also, there are some conserved amino acid residues: 6.7% for HOGP, 20% for HCgp-39, and 13% for AMMSP. In addition, the determined residues of iSBLP[58] also revealed considerable homology with the chitinase of the nematode *B. malayi* (Chit. Nem) (67% identity) and chitinase of manse (Chit. Man) (67% identity).

Effect of Purified iSBLP[58] on 2BS Fibroblast Proliferation

The dose-dependent stimulation of iSBLP[58] on 2BS cell proliferation in the low serum (5% FBS) medium is illustrated in Figure 5. The iSBLP[58] was able to promote the proliferation of 2BS fibroblasts at a concentration as low as 0.5 μg/ml and had significant effects at an optimal concentration range of 2–6 μg/ml ($P < 0.01$). In the presence of 5 μg/ml iSBLP[58], the proliferation of 2BS fibroblasts was nearly 100% greater than that of the control after 60 h incubation.

DISCUSSION

In the present study, an inducible silicotic (rat) bronchoalveolar lavage protein-p[58] (iSBLP[58]) with an apparent molecular mass of 58,000 and a pI of 4.5 has been purified to homogeneity by a combination of gel permeation, Mono Q ion exchange, and reverse-phase high performance liquid chromatography. The same protein was also isolated from both the extract and conditioned medium of alveolar macrophages of silicotic rats and was termed an inducible silicotic (rat) pulmonary macrophage factor (iSPMF-p[58]). iSBLP[58] has been

TABLE I. Summary of Purification of iSBLP[58] From BALFs, AMCMs, and AMEs*

Sample source	Purification step	Total protein		MD_{50}[a] (μg/ml)	Yield (%)	Purification rate[b]
		Milligrams	%			
BALFs	BALFs	50	100	250	100	1
	GPC	6.75	135	ND	ND	ND
	Mono Q	1.65	3.3	ND	ND	ND
	RP-HPLC	0.117	0.23	1.5	39.0	167
AMCMs	AMCMs	11.5	100	50	100	1
	GPC	0.87	7.6	ND	ND	ND
	RP-HPLC	0.065	0.57	1.5	18.8	33.3
AMEs	AMEs	16.3	100	50	100	1
	GPC	0.92	5.6	ND	ND	ND
	RP-HPLC	0.086	0.52	1.5	17.6	33.3

*Twenty rats were used. ND, not determined.
[a]MD_{50}: the half-maximal activity dose (milligrams per well).
[b]Purification rate: the half-maximal activity dose of BALFs/the half-maximal activity dose of iSBLP[58].

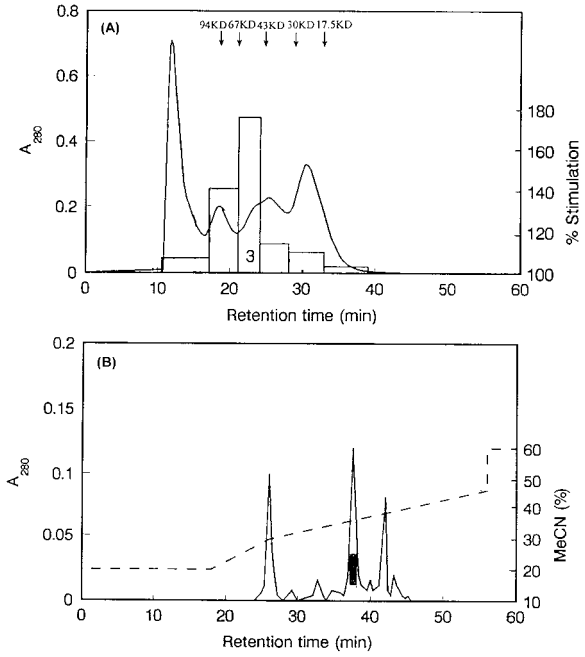

Fig. 3. Isolation of iSBLP[58] from AMCMs. **A:** Gel permeation chromatography (GPC). Protein (1–2 mg) of AMCMs was loaded onto the Superose 12 HR 10/30 column. The running and elution conditions and symbols are the same as for Fig. 1A. **B:** RT-HPLC. One hundred micrograms of the active fraction GP3 from GPC of AMCMs was loaded onto a Vydac protein C_4 column. The running and elution condition and symbols are the same as for Fig. 1C.

characterized as a fibroblast growth-promoting factor and is most likely secreted by alveolar macrophages. The bronchoalveolar lavage fluid and the extract or conditioned medium of alveolar macrophages from sham-exposed normal rats could yet promote the proliferation of fibroblast, but its activity was much weaker than that of dusted rats (data not shown). The mass production and secretion of the powerful iSBLP[58] by alveolar macrophage was obviously inducible and was responsible for bronchoalveolar dusting.

There are two possible sources for the production of iSBLP[58]. It may be produced and secreted by pulmonary macrophages bearing silica as Heppleston's hypothesis or by pulmonary macrophages activated by some cytokines or other cells responding to inhaled silica. In our previous study, a low molecular weight protein fraction (Mr 12,000–18,000) and a protein fraction of approximately Mr 58,000 were isolated from the conditioned medium of peritoneal macrophages silica-treated in vitro by gel permeation HPLC. Both fractions could significantly increase incorporation of ^{3}H-proline into collagen in 2BS fibroblasts [Cai et al., 1984]. But in vivo, silicotic pathological processes are more complex. It was reported that lymphocytes responding to pulmonary deposition of silica were able to induce secretion of a fibroblast growth factor(s) by pulmonary macrophages [Kaelin et al., 1988; Li et al., 1992; Wiedermann et al., 1988].

A search of the Swiss-Prot and GenBank database revealed that, on the basis of its N-terminal amino acid sequence, this newly purified iSBLP[58] had not been reported in previous publications but is significantly homologous with mammal oviduct-specific glycoproteins, with human cartilage gp-39, and with a cDNA sequence coding for a secretory protein from activated mouse macrophages [Sendai et al., 1994; Suzuki et al., 1995; Rejman and Hurley, 1988; Hakala et al., 1993]. These mammalian

ISBLP[58]	Y	N	L	V	C	Y	F	T	N	W	A	Q	Y	R	P	15
HOGP	Y	K	L	V	C	Y	F	T	N	W	A	H	S	R	P	15
HCgp39	Y	K	L	V	C	Y	Y	T	S	W	S	Q	Y	R	E	36
AMMSP	Y	Q	L	M	C	Y	Y	T	S	W	A	K	D	R	P	36
Chit.Nem	Y	V	R	G	C	Y	Y	T	N	W	A	Q	Y	R	D	37
Chit.Man	A	R	I	V	C	Y	F	S	N	W	A	V	Y	R	P	37

Fig. 4. Amino acid sequences and optimal alignment of iSBLP[58] with several mammalian proteins and proteins of a chitinase family. The amino acid sequence of hamster oviduct-specific glycoprotein (HOGP) was from Suzuki et al. [1995]. The amino acid sequences of human cartilage gp-39 (HCgp-39) (GenBank™ accession no. M 80927), a secretory protein from mouse macrophage (AMMSP) (GenBank™ accession no. M 94584), chitinases of the nematode *B. malayi* (Chit. Nem.; GenBank™ accession no. M 73689), and the chitinase-manse (Chit. Man.; Swiss-Prot P 36362) were from the comprehensive search of the Swiss-Prot and Genbank™ database. The unboxed residues represent identical amino acids with iSBLP[58]. The shaded residues indicate conserved amino acid residues with iSBLP[58]. The black-boxed residues were not homological residues with iSBLP[58].

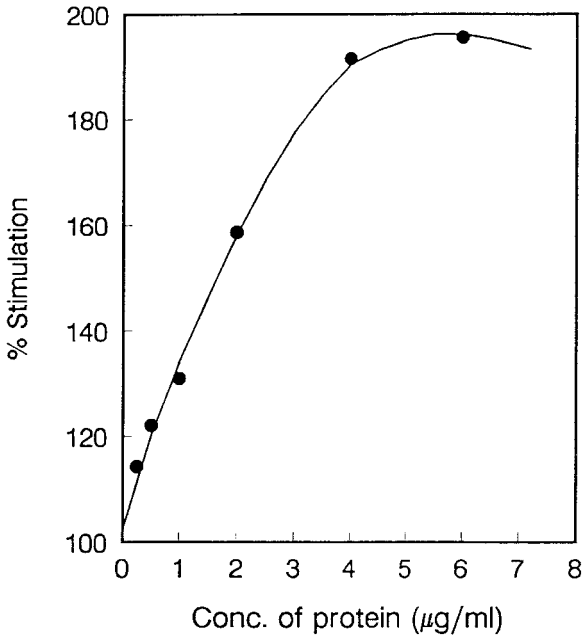

Fig. 5. Dose-dependent stimulation activity of iSBLP[58] on 2BS fibroblast proliferation. 2BS cells were seeded into a 96-well plate at a density of 1×10^4 cell/well with 200 μl of a control medium containing the indicated concentrations of iSBLP[58] and were incubated for 60 h. Cell proliferation was then measured using an MTT colorimetric assay to calculate percent stimulation at the different doses of iSBLP[58].

proteins seem to have no inherent relationship with each other in regard to molecular size, cellular sources, possible functions, etc. These mammalian proteins appear quite homologous with sequences of bacterial, fungal, and other chitinase. However, no endo- or exoglycosidase (such as chitinase) activity has been demonstrated to be associate with HCgp-39 and other mammalian proteins. Whether iSBLP[58] possesses chitinase-like activity is unknown. Related homologous sequences of these mammal proteins indicate the existence of a protein family widely distributed in mammals which have possibly a similar site(s) binding to some ligands, like β 1,4-N-GlcNAc in chitin [Hakala et al., 1993].

Until now, none of the functions of these mammalian proteins has been clearly known. However, a significant feature is that the synthesis and secretion of these mammalian proteins appear to correlate with physiologic and pathological changes of the cell environment and tissue remodeling. For instance, a considerable amount of human cartilage gp-39 may be produced in the synovium and cartilage under inflammatory or degenerative conditions, and the oviduct-specific glycoprotein is estrogen-inducible in the oviduct prior to and during ovulation. In the same manner, pulmonary macrophages synthesize and secrete iSBLP[58] in response to pulmonary deposition of silica. Therefore, we suggest that this mammalian protein family, including iSPMF-[58], may play important roles in the response of cells to changes in their environment and are involved in the cascades of cell-cell and cell-cytokine interaction in normal physiological homeostasis as well as in pathologic processes relating to tissue remodeling or deterioration. iSBLP[58], for example, may promote fibroblast proliferation in silicotic fibrosis. The molecular cloning of iSBLP[58] is in progress to investigate its complete molecular structure and function.

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