

# Cold-Insoluble Globulin (Fibronectin) in Connective Tissues of Adult Human Lung and in Trophoblast Basement Membrane

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**ABSTRACT** Cold-insoluble globulin (CIG), which is immunochemically indistinguishable from the fibroblast surface protein known as large external transformation-sensitive glycoprotein and fibronectin, was detected immunologically in connective tissue fractions from adult human lung. The fractions tested were (a) intact parenchyma, (b) acidic structural glycoproteins (ASG) extracted from lung parenchyma with 0.3 M acetic acid, and (c) isolated alveolar basement membrane (ABM). For comparison with ABM, preparations of human glomerular basement membrane and human trophoblast basement membrane (TBM) were tested. CIG was not detected in glomerular basement membrane but was present in large amounts in TBM. The CIG antigen could be solubilized from the parenchyma and from ABM by collagenase digestion, which indicates that CIG occurs in lung connective tissue in association with collagen. Fibrinogen antigenic determinants were present in the ASG fraction, but the question of whether CIG and fibrin(ogen) are associated in lung connective tissue requires further study. When CIG was quantified by electroimmunoassay, intact lung parenchyma contained  $\approx 0.4\%$  CIG, ASG contained 3–4.5% CIG, ABM contained 0.1–0.9% CIG, and TBM contained 1.5–7.2% CIG. The evidence suggests that CIG is a chemical constituent of lung connective tissue matrix where it may influence the function of alveoli.

## INTRODUCTION

Since the early descriptions (1–5) of a major surface-glycoprotein of fibroblasts, which is transformation sensitive, this substance has been designated in a variety of ways. These include large external transformation sensitive glycoprotein, cell surface protein, “galactoprotein a”, and fibronectin. Recently, cold-

insoluble globulin (CIG)<sup>1</sup> of human plasma (6, 7) has been shown to be immunochemically indistinguishable from the fibroblast surface antigen (8) and has been called plasma fibronectin. The terms CIG and fibronectin are used interchangeably in this paper.

Fibronectin appears to interact with collagen on the cell surface (9), and was earlier shown to mediate the adhesion of fibroblasts to collagen (10). CIG also binds to collagen (11). Both CIG and the fibroblast antigen act as substrates for plasma transglutaminase (fibrin stabilizing factor) with the formation of high molecular weight multimers (12, 13). This transglutaminase also covalently links CIG to fibrin.

The cell culture form of fibronectin exists as insoluble aggregates of high molecular weight on the cell surface. Although the nature of this aggregation is incompletely understood at present, it is known that disulfide bonding (14–16) is essential to its integrity. After isolation from the cell surface, the glycoprotein is insoluble in isotonic solutions at physiological pH (15). Because these reactions of fibronectin with collagen and fibrin or other fibronectin molecules provide mechanisms for the synthesis of high molecular weight networks or aggregates, it is reasonable to assume that the protein may influence the organization of the connective tissue matrix and of basement membranes in organs such as the lung and placenta.

The object of this study was to examine intact, adult, human lung parenchyma and tissue fractions isolated from it for the presence of fibronectin with a specific antiserum to CIG. Alveolar basement membrane (ABM) (17) was one of the fractions studied and human glomerular basement membrane (GBM) as well as human trophoblast basement membrane (TBM) (18)

<sup>1</sup>Abbreviations used in this paper: ABM, alveolar basement membrane; ASG, acidic structural glycoprotein(s); CIG, cold-insoluble globulin; GBM, glomerular basement membrane; TBM, trophoblast basement membrane.

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have been examined for comparison. A soluble fraction was prepared from intact lung parenchyma by extraction with 0.3 M acetic acid (19), a procedure which had been shown to be effective in solubilizing placental villi (18). Soluble proteins could be recovered from these extracts by reversible isoelectric precipitation at pH 4.7. This soluble fraction has been designated an acidic structural glycoprotein (ASG) fraction on the basis of its similarity to ASG fractions from lung (20) and from a variety of other tissues (21).

## METHODS

**Preparation of connective tissue fractions.** Lungs collected at autopsy were stored at  $-70^{\circ}\text{C}$ . Each preparation was made from the lungs of a single individual who was clinically free of lung disease, except where noted, and lung tissue was normal grossly and histologically. The parenchyma was first freed of pleural membranes, large blood vessels, and bronchi, and then was washed repeatedly with 0.15 M sodium chloride followed by water to free it of blood. Aliquots were lyophilized for collagenase digestion experiments but the wet tissue was used immediately for ASG (19) or ABM (17) preparations. Two other human basement membranes, GBM and TBM (18), were studied for comparison. Each preparation was made from tissue of a single individual.

**ABM.** Details of the preparation of ABM by sieving and sonication of lung parenchyma as well as methods used to solubilize it have been described (17). Likewise, the membrane preparations from terminal villi of placenta (TBM) and from renal cortex (GBM) have been described (18).

**ASG.** The ASG were extracted from washed (blood-free) lung parenchyma by stirring with 0.3 M acetic acid (6 ml/g) at  $4^{\circ}\text{C}$  for 24 h. Four extractions were carried out. The insoluble residue was lyophilized (acetic acid insoluble parenchyma). A measured aliquot of each extract was dialyzed and lyophilized to determine the weight of parenchyma solubilized. The ASG were recovered from extracts by adjusting the pH to 4.7 either with 1 N NaOH or by sequential dialysis against 0.02 M  $\text{NaH}_2\text{PO}_4$  (pH 4.7) and 0.1 M sodium acetate buffer (pH 4.7). The precipitate (ASG) was collected by centrifugation and was dissolved in 0.2 M glycine (pH 8.0). Before analysis, the solution was dialyzed and lyophilized.

**Collagenase soluble parenchyma.** Intact, washed parenchyma and parenchyma which had been extracted with 0.3 M acetic acid (*vide infra*) were both digested with purified collagenase to determine how much CIG could be solubilized. The batch of collagenase used (form III, Advance Biofactures Corp., Lynbrook, N. Y.) showed no evidence of contaminating proteases when assayed in this laboratory on [ $^3\text{H}$ ]tryptophan-labeled chick embryo protein (22). Only 3 cpm were detected in the TCA-soluble fraction after digestion with 25 U of collagenase. Under exactly the same conditions, 10  $\mu\text{g}$  of trypsin produced 1,429 cpm.

Washed, lyophilized parenchyma (1.1 mg) was suspended in 0.055 ml of pH 7.5 Tris buffer (23) of final concentration of 10 mM Tris which contained KCl (10 mM),  $\text{MgCl}_2$  (1.5 mM), and  $\text{CaCl}_2$  (10 mM) with 69 U collagenase. To prevent bacterial contamination, a strip of Whatman 3MM filter paper (Whatman, Inc., Clifton, N. J.) saturated with toluene was suspended in the tube which was sealed with several layers of parafilm and incubated at  $37^{\circ}\text{C}$  for 19 h. The supernate was analyzed directly by the Laurell technique (24) with an antiserum specific to CIG (6). Plasma of known CIG concentration was used as a standard. The percent CIG was calculated on the basis of the weight of lyophilized parenchyma.

In a large-scale experiment, 2 g (dry weight) of acetic acid insoluble parenchyma was digested with 250 U collagenase in 100 ml buffer (23) at  $37^{\circ}\text{C}$  under toluene for 220 h. The undigested residue was removed by centrifugation at 16,000 g for 20 min and was washed once with buffer and repeatedly with water until it was salt free. The supernate, buffer wash, and first water wash were combined, dialyzed until salt free, and lyophilized. The lyophilized material was soluble in 0.087 M barbital buffer, pH 8.6 and was analyzed for CIG content by the Laurell technique (24) and for glycosaminoglycans (*vide infra*).

**Solubilization of CIG from basement membranes.** Plasmin as well as collagenase (*vide supra*) was used to solubilize the basement membranes. Plasmin activated with matrix-bound activator was obtained from A. B. Kabi, Stockholm, Sweden, and was used in phosphate-buffered saline, pH 7.4. Weighed aliquots of the membranes (1 mg range) were suspended at a concentration of 10 mg/ml in the appropriate buffer which contained either 125 U of collagenase or 0.5 caseinolytic U of plasmin/mg of basement membrane. Filter paper saturated with toluene was suspended in the tube which was then tightly covered and incubated at  $37^{\circ}\text{C}$  for 3 days. The undigested residue was centrifuged out and the supernates were used directly in double diffusion tests (25) or were analyzed for CIG content by the Laurell technique (24) with plasma of known CIG content as a standard. The percent CIG was calculated on the basis of the original weight of the basement membrane.

Dilute alkali (0.01 N NaOH) was also used to solubilize CIG from TBM. The membranes were suspended at a concentration of 5 mg/ml in 0.01 N NaOH and the suspension was stirred at  $25^{\circ}\text{C}$  for 24 h. The undigested residue was centrifuged out and the clear supernates were analyzed for CIG by the Laurell technique (24).

**Analytical methods.** The amount of protein in the supernates from the enzymatic digests was estimated by the method of Lowry et al. (26). The amino acid analyses on the basement membranes (17, 18) and on the ASG fractions were performed on weighed samples (1–2 mg) which had been hydrolyzed *in vacuo* (2 ml 6 N HCl, 24 h,  $110^{\circ}\text{C}$ ) and taken to dryness in a vacuum desiccator in the presence of NaOH. The analyses were performed by the automatic technique of Spackman et al. (27) on a Spinco 120B amino acid analyzer with type PA 35 resin in the short column and type AA 15 resin in the long column (Beckman Instruments Inc., Spinco Div., Palo Alto, Calif.). Modifications of the long column run (28) were made to allow separation of hydroxyproline from aspartic acid at  $55^{\circ}\text{C}$  and for the determination of desmosine and isodesmosine. The thiobarbituric acid reaction (29) was used for the determination of sialic acid after hydrolysis for 1 h at  $80^{\circ}\text{C}$  with 0.1 N  $\text{H}_2\text{SO}_4$ . Hexosamines were determined by the manual procedure of Swann and Balazs (30) after hydrolysis for 16 h at  $100^{\circ}\text{C}$  in 4 N HCl. The phenol-sulfuric acid method of Dubois et al. (31) was used to estimate total hexose. For the detection of glycosaminoglycans in the collagenase-soluble, nondialyzable parenchyma, the sample was analyzed for uronic acid by the method of Bitter and Muir (32). Individual glycosaminoglycans were separated by electrophoresis on Sepharose III (Gelman Instrument Co., Ann Arbor, Mich.) and detected by staining with Alcian blue (Matheson, Coleman and Bell, Norwood, Ohio). Two different buffer systems, 1 M acetic acid adjusted to pH 3.5 with pyridine, and 0.1 M barium acetate (pH 8), were used.

The antisera to CIG and to fibrinogen were reacted with tissue antigens by the double diffusion technique (25) in agarose gels in Petri dishes. The gels consisted of 0.7% agarose (Seakem, Marine Colloids, Inc., Springfield, N. J.) in pH 7.6 barbital buffer (0.05 M), saline (0.15 M) which contained

glycine (0.33 M), and Merthiolate (Eli Lilly and Company, Indianapolis, Ind.) (0.01%). Immunoelectrophoresis was carried out by the microtechnique of Scheidegger (33). Quantitation of CIG was carried out on microscope slides by the electroimmunoassay technique (24) in 1% agarose gel (Seakem) which contained barbital buffer (0.044 M, pH 8.6), EDTA (0.005 M), and sodium azide 0.05%. Plasma of known CIG content was used for a standard.

**Reference CIG.** Reference CIG was prepared by clotting 100 mg Cohn fraction I in 2 ml phosphate-buffered saline, pH 7.4, with 50 U bovine Thrombin (Parke, Davis & Company, Detroit, Mich.). After 5 min, the clot was withdrawn and the liquid which contained the CIG was centrifuged to remove clot fragments. The reference CIG was shown to be free of fibrinogen by its failure to react with antifibrinogen in double diffusion and immunoelectrophoresis experiments.

**Antisera.** Rabbit antiserum to human CIG was generously made available by Dr. M. W. Mosesson, Downstate Medical Center, Brooklyn, N. Y. The antiserum had been made specific for CIG by absorption with the supernate of Cohn fraction I (6) and contained fibrinogen as a result of this absorption (Fig. 3). The rabbit antiserum to human fibrinogen, which did not react with CIG, and the goat antiserum to human serum, were kindly provided by Dr. Konrad C. Hsu of Columbia University, College of Physicians & Surgeons, New York. The rabbit antiserum to human  $\alpha_2$ -macroglobulin was obtained from Behring Diagnostics, American Hoechst Corp., Somerville, N. J.

## RESULTS

**Qualitative observations with a specific antiserum to CIG.** The presence of CIG in lung parenchyma is demonstrated in Fig. 1 where the precipitin line given by collagenase-solubilized parenchyma (well 1) gave a reaction of identity with reference CIG (well 2). The highly purified collagenase (Methods) also solubilized CIG from ABM (Fig. 2). Again, a reaction of identity with reference CIG was formed.

As shown in Fig. 3, the presence of CIG in each of the ASG preparations from four different lungs (wells 2 and 4-6) was demonstrated by the clear precipitin lines

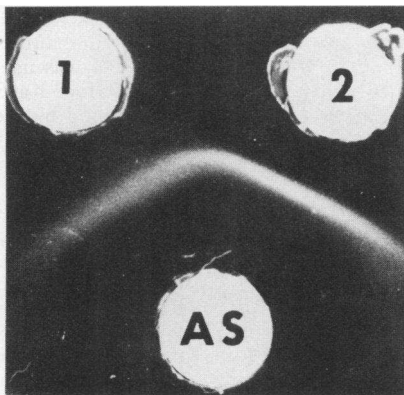


FIGURE 1 Immunodiffusion reactions in agarose between anti-CIG antiserum (AS) and collagenase-solubilized fragments of lung parenchyma. Wells contain: (1) Collagenase-solubilized lung parenchyma at 3 mg/ml (This hole was filled twice.); (2) Reference CIG.

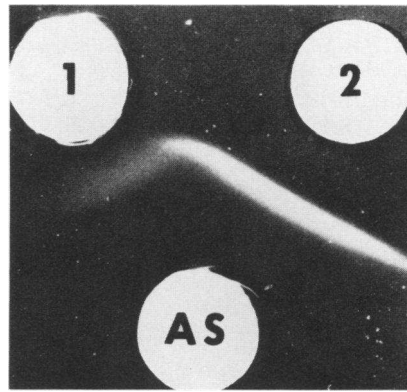


FIGURE 2 Immunodiffusion reactions in agarose between anti-CIG antiserum (AS) and collagenase-solubilized fragments of ABM. Wells contain: (1) ABM (2.6 mg protein/ml); (2) Reference CIG.

which occurred with the antiserum to CIG. These were reactions of identity with reference CIG (well 1). In this figure, the ASG in wells 2, 4, and 6 were from three different normal lungs, whereas the ASG in well 5 was from a human lung which had severe interstitial fibrosis resulting from X-irradiation. No distinctions in precipitin reaction were demonstrated among these specimens. However, the ASG from two of the normal lungs and from the fibrotic lung show a double precipitin line which suggests the presence of two molecular species carrying the relevant determinants.

In well 3, antifibrinogen antiserum is reacting with soluble fibrinogen in the anti-CIG serum. The presence

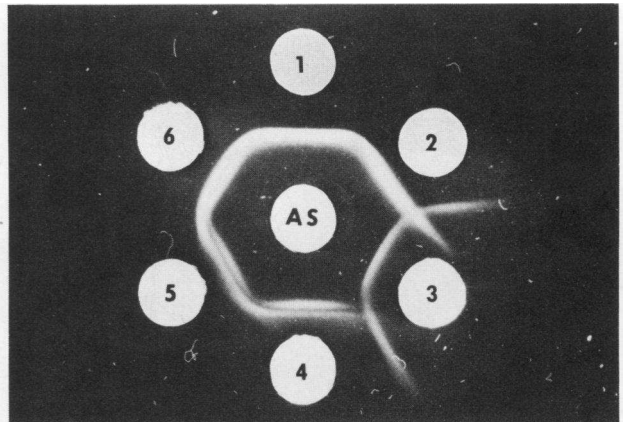


FIGURE 3 Immunodiffusion reactions in agarose between anti-CIG antiserum (AS) and ASG preparations from four different lungs. Wells contain: (1) Reference CIG; (2) ASG fraction from lung no. 1 (normal lung); (3) Anti-fibrinogen antiserum; (4) ASG fraction from lung no. 2 (normal lung); (5) ASG fraction from lung no. 3 (This lung demonstrated severe interstitial fibrosis from X-irradiation.); (6) ASG fraction from lung no. 4 (normal lung). The ASG were dissolved in 0.2 M glycine buffer, pH 8.0 at a concentration of 5 mg/ml.

of fibrinogen in the anti-CIG serum is the result of absorption (6). Antifibrinogen also reacted with the ASG fractions placed next to it in wells 2 and 4 (normal lungs). In the specimen in well 2, the CIG determinants and the fibrinogen determinants were clearly independent. However, in the sample in well 4, this independence was not apparent.

The two ASG fractions (wells 2 and 4) were examined for the presence of fibrinogen by immunoelectrophoresis. The pattern seen in Fig. 4 was obtained in both cases. The same sample used in well 4 of Fig. 3 appears in the lower well of Fig. 4. It was electrophoresed at pH 8.6 along with human plasma diluted 1 → 5 (upper well). Antifibrinogen was placed in the center trough. After 48 h, plasma gave an arc of the expected shape at the origin. However, the fibrinogen antigenic determinants in the ASG fractions were present on molecules which were more negatively charged than intact fibrinogen.

*Quantitative results obtained with a specific anti-serum to CIG.* The electroimmunoassay results obtained on the human lung connective tissue fractions are shown in Table I. Also shown for comparison are results obtained on human GBM and TBM. The amount of CIG in lung parenchyma which can be solubilized by collagenase digestion is 0.4% by weight. Sample 1 was digested directly with collagenase and the CIG content was 0.38%. Essentially the same result was obtained on sample 2 from which ASG were prepared by acetic acid extraction before analysis. The ASG fraction which accounted for 3% of the parenchyma contained 4.5% CIG (Table I, ASG sample 2). Therefore, the CIG which was recovered in the ASG fraction was 0.14% of the intact parenchyma. 2 g of the acetic acid insoluble parenchyma was digested with collagenase, whereupon 40% of it was solubilized. The collagen peptides and salt were removed by dialysis and the

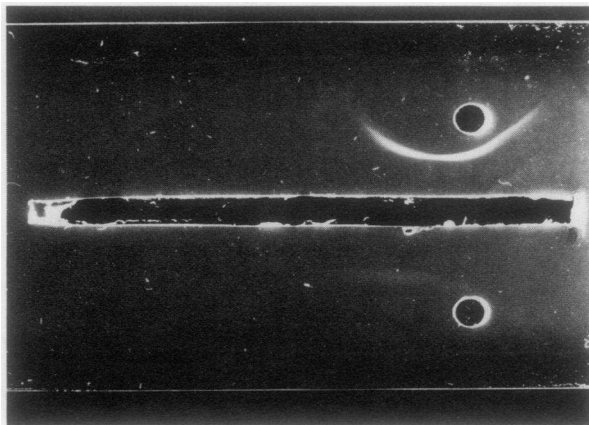


FIGURE 4 Immunoelectrophoresis of a 1 → 5 dilution of human plasma (top) and lung ASG (5 mg/ml) (bottom) against antifibrinogen antiserum (center trough).

TABLE I  
Percent CIG in Connective Tissue Fractions from Normal Human Lung and from TBM and GBM

Fraction	Sample no.	Method of solubilization			
		Buffer pH 8.6	Collagenase digestion	Plasmin digestion	Alkali extraction
		%	%	%	%
Lung parenchyma	1		0.38*		
	2		0.44†		
Lung ASG	1	3.0			
	2	4.5			
	3	3.9			
ABM	1		0.86	0.75	
	2		0.35		
	3		0.1		
TBM	1			5.1	3.8
	2			7.2	
	3				1.5
GBM	1		<0.1		
	2‡		<0.1	<0.1	

\* 0.38% was obtained by digesting washed parenchyma directly with collagenase.

† 0.44% is the sum of CIG in ASG and in acetic acid insoluble parenchyma.

‡ Canine GBM.

nondialyzable fraction was lyophilized. This fraction weighed 120 mg, or 6% of the 2 g digested. This fraction contained 5% CIG, and the CIG content of the acetic acid insoluble parenchyma was, therefore, 0.3%. The sum of CIG in the ASG and in the acetic acid insoluble parenchyma was 0.44% (Table I).

Two other preparations of lung ASG were analyzed and found to contain 3.0 and 3.9% CIG, respectively (Table I).

CIG was present in the three ABM preparations tested, but the amounts were small and variable (0.1%, 0.35%, and 0.86%). The highest concentration of CIG was present in TBM (up to 7.2%) (Table I).

CIG was not detected in two different preparations of human GBM (one of the two was tested by immunodiffusion only) nor in canine GBM (Table I).

*Factors which affect the quantification.* (a) The shape of the precipitin arcs given by the tissue samples (Fig. 5) differs from that given by plasma CIG. (b) None of the solubilization procedures solubilized the entire parenchyma. The four sequential extractions with 0.3 M acetic acid solubilized 6%, 2–3%, 1%, and 1%, respectively, or a total of 10% of lung parenchyma. Collagenase digestion of the acetic acid-insoluble residue solubilized an additional 40%. (c) The CIG antigenic determinants in the ASG fractions occur on at least two

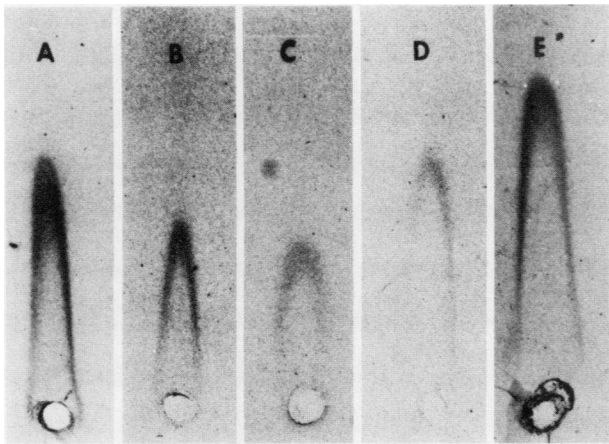


FIGURE 5 Precipitin patterns obtained when plasma and tissue fractions were electrophoresed into agarose which contained anti-CIG. (A) Standard plasma (1 → 4 dilution; 0.082 mg CIG/ml). (B) ABM solubilized by collagenase: 0.95 mg ABM was digested at 10 mg/ml in buffer (23) which contained 119 U collagenase under toluene at 37°C for 64 h. (C) Collagenase-solubilized lung parenchyma: acetic acid-insoluble lung parenchyma (2 g) was digested with collagenase (250 U) in 100 ml buffer (23) at 37°C under toluene for 220 h. The nondialyzable, collagenase-soluble fraction was dissolved in 0.087 M barbital buffer (pH 8.6) at a concentration of 2 mg/ml. (D) TBM solubilized by plasmin: 0.93 mg TBM was digested for 64 h under toluene at 10 mg/ml in phosphate-buffered saline (pH 7.4), which contained 0.5 caseinolytic U of plasmin. The sample was diluted 1 → 2 with 0.087 M barbital buffer (pH 8.6) just before electrophoresis. (E) ASG from normal human lung was dissolved in 0.087 M barbital buffer (pH 8.6) at a concentration of 5 mg/ml.

different molecular species. This can be seen in Fig. 3 where a double line was given with the anti-CIG serum by three of the preparations.

#### Partial characterization of connective tissue fractions

**Basement membranes.** As reported (17, 18), both ABM and TBM had chemical compositions which differed from GBM prepared by the same methods in the same laboratory. The amount of collagen as estimated by the hydroxyproline analyses was much less in TBM and intermediate in ABM. Large amounts of an acidic glycoprotein were present in TBM. Elastin, as evidenced by the presence of desmosine and isodesmosine, was a consistent component of ABM.

**Collagenase-solubilized lung parenchyma.** The nondialyzable, collagenase-solubilized fraction which contained 5% CIG (*vide supra*) was examined for the presence of glycosaminoglycans. Both hyaluronic acid and dermatan sulfate were detected by Alcian blue staining of samples which had been electrophoresed on cellulose acetate. The fraction contained 2.3% uronic acid (32) which would indicate a glycosaminoglycan content of  $\approx 7\%$ .

**Acidic structural glycoproteins.** The four sequential extractions with 0.3 M acetic acid solubilized 6%, 2–3%, 1%, and 1%, respectively, of lung parenchyma. These fractions contained little or no collagen as evidenced by the absence or trace quantities of hydroxyproline detected in the dialyzed, lyophilized extracts. The material recovered from the extracts by precipitation at pH 4.7 comprised  $\approx 30\%$  of the extracts or 3% of the parenchyma. The CIG content of the ASG fractions was 3–4.5% or 0.09–0.14% of washed parenchyma. These fractions were designated ASG fractions because the material underwent reversible isoelectric precipitation at pH 4.7, migrated with a mobility identical with or slightly lower than human  $\alpha_1$  globulin upon electrophoresis at pH 8.6, and contained carbohydrate as well as large amounts of cysteine. In Table II, an analysis of a typical ASG fraction extracted from human lung with 0.3 M acetic acid and recovered by isoelectric precipitation at pH 4.7 is

TABLE II  
Amino Acid and Carbohydrate Analyses of ASG Preparations from Lung Parenchyma

Amino acids	Human	Bovine*
	<i>residues/1000 residues</i>	
Hydroxylysine	1	Trace
Lysine	57.5	47.4
Histidine	22.7	19.8
Arginine	56.1	56.4
Hydroxyproline	†	Trace
Aspartic acid	94.8	106
Threonine	47.2	50.2
Serine	66.8	61.0
Glutamic acid	131	137
Proline	39	54.2
Glycine	133	113
Alanine	71.4	74.5
Valine	55.6	64.0
Half cystine	14	14.2
Methionine	18.2	15.9
Isoleucine	39.2	44.5
Leucine	93.1	94.9
Tyrosine	23.5	17.4
Phenylalanine	35.0	29.8
Desmosines	†	—
Carbohydrate		
	%	
Hexose	2.8	3.08
N-Ac-hexosamine	1.1	0.55
Sialic acid	0.55	0.48

\* These data are from the paper of Francis and Thomas (20) and are analyses on a fraction prepared by extracting bovine parenchyma with 8 M urea-0.1 M NaBH<sub>4</sub>.

† Not detected.

shown compared with the analysis of an ASG fraction extracted from bovine lung parenchyma by Francis and Thomas (20) with 0.1 M NaBH<sub>4</sub> in 8 M urea. Both preparations contained large amounts of aspartic acid, glutamic acid, leucine, and half-cystine; and small amounts of hexose, hexosamine, and sialic acid. Hydroxyproline, hydroxylysine, desmosine, and isodesmosine were either not detected in the preparations or were present in trace amounts. Therefore, collagen and elastin were not present.

Only one serum protein was detected in ASG by double diffusion experiments and it was presumably fibronectin. This was corroborated by immunoelectrophoresis of ASG and human serum. Whereas human serum diluted 1 → 5 gave the expected pattern of arcs with goat anti-human serum, the ASG fractions produced one arc. The ASG fractions also failed to react with specific antiserum to human  $\alpha_2$ -macroglobulin in double diffusion experiments. Antigenic determinants of fibrinogen, but not the fibrinogen molecule, were present in these ASG fractions (Figs. 3 and 4).

## DISCUSSION

In this study, fibronectin has been demonstrated in several fractions of adult human lung, i.e., intact parenchyma, ABM, and ASG from parenchyma. Also, an association of lung fibronectin with collagen has been demonstrated because degradation of insoluble collagen with collagenase also solubilized fibronectin and proteoglycans. The presence of CIG and its association with collagen and possibly with proteoglycans was anticipated from observations that cultured human fetal lung fibroblasts which synthesize collagen (34) and proteoglycans (35) also synthesize fibronectin (36, 37).

Possible functions for fibronectin in lung interstitium can only be speculative. Studies of fibroblasts in culture (38, 39) indicate a role for fibronectin in fibroblast attachment to a substratum, in spreading, and in the organization of the cytoskeleton. Bornstein and Ash (9) envision a model in which collagenous proteins and fibronectin form a fibrillar network on the surface of the fibroblasts and propose that this network influences the attachment of cells to a substratum. Such functions for fibronectin are consistent with the 0.4% fibronectin found in lung parenchyma in this study which is small compared to amounts of recognized structural fibrillar proteins such as collagen and elastin. However, in view of the factors which affect the quantification, 0.4% fibronectin is proposed as an approximate value.

Acidic structural glycoproteins are associated with collagen and elastin in a variety of connective tissues (21, 40) and it has been suggested they are structural components of the elastic fiber microfibrils (41). In bovine lung parenchyma they comprise 15.5% of the dry weight of salt-extracted, delipidated parenchyma

(20). The methods now in use for the preparation of ASG, which include extraction with 8 M urea, reduction and alkylation of disulfide bonds (42), proteolytic digestion, treatment with sodium borohydride in 8 M urea, and even extraction with strong alkali, result in denatured and/or degraded proteins. Wolff et al. (43) have shown that these ASG preparations are heterogeneous by electrophoresis in polyacrylamide gels containing dodecylsulfate and that certain antigens are destroyed by reduction with mercaptoethanol in urea. The alternative procedure used in the present study, extraction with 0.3 M acetic acid, is simple, does not require previous removal of collagen or of lipids, and the antigenicity of the fractions is retained. In our studies, 0.3 M acetic acid effectively extracted ASG from lung parenchyma but did not extract the collagen, unlike the results obtained by Kefalides and Denduchis on dog lung (44). The isolation and characterization of various components of the ASG fraction, especially those which have been shown in this study to carry the antigenic determinants of fibrinogen, will be the object of a future study.

The finding that GBM does not contain fibronectin was a surprising one in view of an earlier report (45) which demonstrated the presence of fibronectin in all limiting membranes of the developing chick embryo by use of the indirect immunofluorescence technique. However, the data are in complete agreement with more recent immunofluorescence results from two different laboratories (46–48) which show an absence of fibronectin from human GBM. The fibronectin content of ABM and TBM in this study showed wide variation among preparations, each from a separate lung or placenta. This variation is, at present, unexplained. It may reflect the activity of proteases and inhibitors in the individual organs. Alternatively, it could mean that fibronectin is not an integral part of ABM or TBM, but is peripherally associated in a structure which is variably removed by the preparative procedures used, e.g., sonication.

While it is recognized that fibronectin combines with fibrin, the nature of its association with fibrinogen and collagen remains unknown. However, these associations and its ability to form multimers in the presence of fibrin-stabilizing factor suggest a functional significance in membranes. The basement membranes of lung alveoli, alveolar capillaries, and of placenta are selective in membrane transport. The relative abundance of fibronectin in the membranes of alveoli and placenta in contrast to its absence in GBM may have important functional as well as structural significance for these different organs.

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