Partially Degraded Fibrin(ogen) Stimulates Fibroblast Proliferation *In Vitro*

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The conversion by thrombin of soluble plasma fibrinogen to an insoluble fibrin matrix is central to hemostasis and subsequent wound healing. Fibroblasts adhere to and rapidly grow into fibrin clots, resulting in collagen deposition and, ultimately, scar formation. Although a number of soluble mediators have been implicated in this process, a role for fibrin(ogen) itself has not been described. The present study further investigated the nature of mitogenic activity remaining in solution after *in vitro* fibrin clot formation. Liquid expressed from a fibrin clot (clot supernatant) elicited a mitogenic response of up to 83 ± 4.7% above media control. Upon addition of a polyclonal fibrinogen antibody, this activity was reduced by 50%. The remaining activity was attributed to the presence of thrombin and was neutralized by the addition of a specific thrombin inhibitor. Fibrinogen cleavage products were separated by molecular sieve chromatography and the mitogenic potential of each fraction assessed. A peak of activity was observed in fractions containing proteins with apparent molecular weights of 200 to 300 kD. Enhanced chemiluminescence Western blotting of these fractions established the presence of several fibrin(ogen)-derived protein bands. It is therefore proposed that thrombin cleavage of fibrinogen, in addition to producing fibrin, generates high-molecular-weight soluble cleavage products that may play an important role during normal wound healing and in the pathogenesis of disease states associated with vascular leakage and fibrosis.

Fibrin deposition at the site of tissue injury is central to normal hemostasis and subsequent wound healing, providing both a hemostatic plug that prevents loss of cellular and protein plasma constituents and a three-dimensional substrata over which mesenchymal cells adhere and proliferate (1). Tissue repair is marked by the migration of fibroblasts and new blood vessels into the fibrin matrix, where they proliferate rapidly and synthesize extracellular matrix proteins (2). Such processes ultimately result in the formation of acellular scar tissue composed predominantly of dense collagen fibers (3).

Fibroblast replication is believed to be stimulated, initially by mitogens released from platelet-α granules (such as platelet-derived growth factor) and subsequently by factors released by macrophages that actively migrate into the fibrin matrix (4–6). In addition, components of the coagulation process may play a role in the promotion of proliferation and matrix deposition. For example, thrombin reversibly binds to the fibrin matrix (7), eliciting multiple effects including the stimulation of fibroblast and smooth muscle cell proliferation (8–10). Fibrinopeptides A and B, two small peptides cleaved by thrombin from the amino terminal end of the fibrinogen molecule, have also been shown to stimulate fibroblast chemotaxis and proliferation (11, 12).

The ability of fibrin(ogen) to directly influence events during hemostasis and wound healing has been investigated by a number of groups. Brown and colleagues (13) observed that fibroblasts actively migrate into fibrin clots derived from highly purified fibrinogen. In addition, several mesenchymal cell types possess integrin receptors that interact with the two RGD sequences located on the Aα chain of the fibrinogen molecule. The expression of such receptors is thought to play a critical role in cellular adhesion and migration during wound repair (14, 15).

Our group has previously investigated the ability of fibrinogen to directly stimulate mesenchymal cell proliferation. We reported that fibrinogen prior to clot formation does not stimulate fibroblast proliferation (11), but that proteins remaining in solution after *in vitro* clot formation (clot supernatant) are potent fibroblast mitogens (11). Such activity could not be accounted for by the presence of either the serine protease thrombin or the known thrombin cleavage products of fibrinogen, the fibrinopeptides A and B. In this study we investigated the nature of mitogenic activity present as components of clot supernatant and demonstrated that several partially degraded forms of soluble fibrin(ogen) are generated during clot formation, which are potent fibroblast mitogens.

Materials and Methods

Fibrin Clot Formation

Fibrin clots were generated *in vitro* by the interaction of human thrombin (Sigma Chemical Co., Pool, Dorset, UK) (final concentration 1.1×10^{-9} M) with human fibrinogen

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Abbreviations: Dulbecco's modified Eagle's medium, DMEM; enhanced chemiluminescence detection, ECL; newborn calf serum. FCS; phosphate-buffered saline, PBS; D-PHE-PRO-ARG-CH_2Cl^-, PPACK; sodium dodecyl sulfate-polyacrylamide gel electrophoresis, SDS-PAGE.

(Sigma Chemical Co.) (final concentration 1.5×10^{-6} M) in Dulbecco's modified Eagle's medium (DMEM), pH 7.4, at 20°C. Clot formation was allowed to proceed for 30 min. The newly formed fibrin clot was separated into solid and liquid phase. The liquid phase was collected in a polypropylene tube (clot supernatant) and the insoluble fibrin matrix discarded. To remove microscopic clot fragments and possible bacterial contaminants, clot supernatant was finally passed through a 0.2-μm filter.

Cell Culture

Cell culture was performed on a human fetal lung fibroblast line IMR-90 (American Type Culture Collection, Rockville, MD). Cells were maintained in DMEM supplemented with 5 to 10% newborn calf serum (NCS), in a humidified atmosphere of 10% CO_2 in air at 37°C. Cells were washed with calcium- and magnesium-free phosphate-buffered saline (PBS) and suspended in 0.02% EDTA/0.02% trypsin for 2 min. Trypsin was neutralized with 10% NCS and cells were washed again with calcium- and magnesium-free PBS and resuspended in serum-free medium. Cells were counted in a hemocytometer, and plated in DMEM as described subsequently.

Assay for Fibroblast Replication

Cell replication was assessed in 96-well culture dishes, using a colorimetic assay based on the uptake and subsequent release of methylene blue (16). Cells were plated at 5×10^3 cells/well in 50 μl of serum-free DMEM, either 24 h before the test solutions were added, or at the same time. A 50-μl sample of test solution in serum-free medium was serially diluted across the plate; 50 μl of serum-free medium was added to three columns representing medium controls. Plates were incubated for 48 h at 37°C in 10% CO_2 and 100% humidity. The colormetric assay was validated as a means of determining cell replication by direct cell counting. This was performed on cells stained with methylene blue prior to elution of the dye. A mean count was taken of four estimates made in several areas of each well.

Antibody and Chemical Blocking

Antibody-blocking experiments were performed using rabbit anti-human fibrinogen polyclonal antibodies (Dako Ltd., High Wycombe, Bucks, UK). Clot supernatant was incubated with antibody solutions ranging in concentration from 1:100× to 1:1,000× dilution. The antibody–antigen conjugation reaction proceeded for 30 min at 37°C in nonpyrogenic tubes prior to incorporation in the methylene blue assay.

D-PHE-PRO-ARG-CH_2Cl- (PPACK), final concentration 1×10^{-8} M, was incubated with clot supernatant or thrombin for 30 min at room temperature prior to incorporation in the methylene blue assay.

Sepharose 6B Gel Filtration Chromatography

Separation of the clot supernatant was achieved through molecular sieve chromatography. A column 1.6 cm in diameter and 92 cm in length was packed with 177 cm^3 of Sepharose 6B (Pharmacia, Uppsala, Sweden) at a flow rate not exceeding 0.3 ml min^{-1}. Two to three milligrams of clot supernatant was loaded onto the top of the column in a volume constituting no more than 2% of the column bed. Conditions used throughout these experiments were: eluent buffer 0.02 M $(NH_4)_2CO_3$, fraction size 2.5 ml and flow rate 0.11 ml min^{-1}. Fractions were freeze-dried and resuspended in 1 ml of DMEM. Each fraction was subsequently tested for mitogenic activity in the methylene blue assay.

Polyacrylamide Gel Electrophoresis

Protein constituents of each gel filtration fraction were assessed using gel electrophoresis. Polyacrylamide gels with stacking gel and running gel acrylamide concentrations of 3 and 9.5%, respectively, were prepared in glass cassettes (the gel dimensions were 140 × 100 × 1.5 mm). A 25-μl portion of sample was loaded into each well using a microsyringe, and stacking was allowed to occur at a current of 25 mA. Once the bromophenol blue marker had entered the running gel, the current was increased to 55 mA. When electrophoresis was complete, protein bands were visualized with silver stain.

Enhanced Chemiluminescence Detection (ECL)

Proteins were transferred from polyacrylamide gels onto Hybond-ECL (Amersham, Aylesbury, Bucks, UK). Potential nonspecific binding sites on the membrane were blocked for 1 to 2 h with 3% wt/vol dried milk and 0.05% vol/vol Tween-20 dissolved in PBS solution. Polyclonal fibrinogen antibody directly conjugated with horseradish peroxidase was incubated with the blocked transfer membrane for a minimum of 1 h. Finally, the antibody solution was decanted and the membrane washed with 400 ml of PBS solution containing 0.05% vol/vol Tween-20 for 30 min.

Labeled antigen–antibody complexes were visualized with an ECL Western blotting detection kit (Amersham) employing a nonradioactive light-emitting reaction. Blots were incubated with ECL detection reagents for 1 min, after which time excess reagent was drained and the blots wrapped in Saran Wrap™. A light-emitting signal was detected by exposure of blots to autoradiography film for 15 s to 3 min.

Statistics

To calculate growth activity, each absorbance value in the methylene blue assay was compared with the absorbance value of an adjacent control well. The control values varied little within a plate (standard deviations were 2 to 5% of the mean for each group). On different days, however, the variability between media control was 5%; thus, it was deemed appropriate to correct test absorbance values for the media control values on each day. These values were then given as percentage change from media control ± SEM. Thus, data from experiments performed on different days could be combined. The difference between growth activity of clot supernatant before and after incubation with antibodies or PPACK was analyzed using an unpaired *t*-test. The mean value of various parameters was said to be significantly different when the probability of the difference of that magnitude fell below 5% (i.e., $P < 0.05$). Where mean values are calculated, SEM are also given.

Results

Clot supernatant elicited an optimal mitogenic response on human fetal lung fibroblasts at a total protein concentration

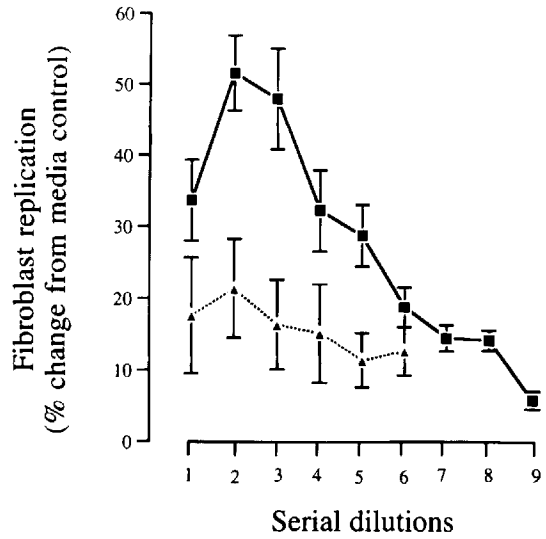

Figure 1. Fibroblast mitogenic activity in response to clot supernatant (*squares*) and human thrombin (*triangles*), assayed in serum-free conditions for 48 h. Both clot supernatant and thrombin were serially diluted across a 96-well tissue culture plate. Starting concentrations of clot supernatant and thrombin were 14 mg ml^{-1} (total protein) and 2.2×10^{-9} M, respectively. The growth curves for the clot supernatant and thrombin have been overlaid to show the approximate thrombin concentration and level of mitogenic activity attributable to thrombin in any one dilution of the clot supernatant.

of 7 μg ml^{-1}. In a series of highly reproducible experiments, values ranged from 50 to 80% above media control (Figures 1 through 4). No significant increase in the magnitude of response was observed at higher protein concentrations and activity diluted out in a dose-dependent manner, (Figures 1 and 2). In the same assay, 5% NCS, which represents optimal conditions for stimulating replication of this cell type, gave a simulation of between 100 and 150%.

To examine the effect of clot supernatant on plating efficiency, cells were preplated for 24 h prior to exposure to test solutions. There was no significant difference between the numbers of these cells (as shown Figure 1). and those of cells plated concurrently with clot supernatant (Figure 2). Further, no replication was seen when cells were coplated with test solution and incubated for 18 h, a time point at which there would be no significant level of replication in preplated cells. Thus, cell replication in response to clot supernatant was similar whether the cells were preplated, suggesting that test solution had no effect on plating efficiency.

Thrombin alone, in a series of experiments, elicited a mitogenic response of about 20% above media control at a concentration of 1.1×10^{-9} M (the concentration used to initiate fibrin clot formation) (Figure 1). It was important to determine the quantity of thrombin present in solution after clot formation. Fibrinogen was clotted with thrombin of which 50% was radiolabeled with ^{125}I. Four independent experiments were performed to determine the percentage of ^{125}I-labeled thrombin remaining in solution after clot formation. We sequestered $31.1 \pm 4.9\%$ of thrombin within the fibrin clot, and $68 \pm 4.9\%$ remained in solution. Thus, the mean concentration of thrombin present as a constituent element of clot supernatant at the peak response shown in Figure 1 was about 7.5×10^{-10} M.

To assess by independent method the contribution of thrombin to the mitogenic activity observed in the clot su-

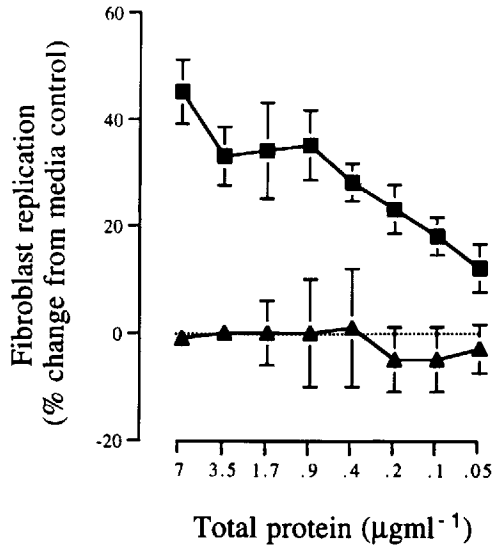

Figure 2. Mitogenic activity of preplated fibroblasts (plated 24 h prior to exposure to test solutions) in response to an optimal concentration of clot supernatant (*squares*). Mitogenic activity of fibroblasts assessed after an 18-h incubation with an optimally stimulatory concentration of clot supernatant (*triangles*). Both test solutions have been serially diluted across 96-well culture plates.

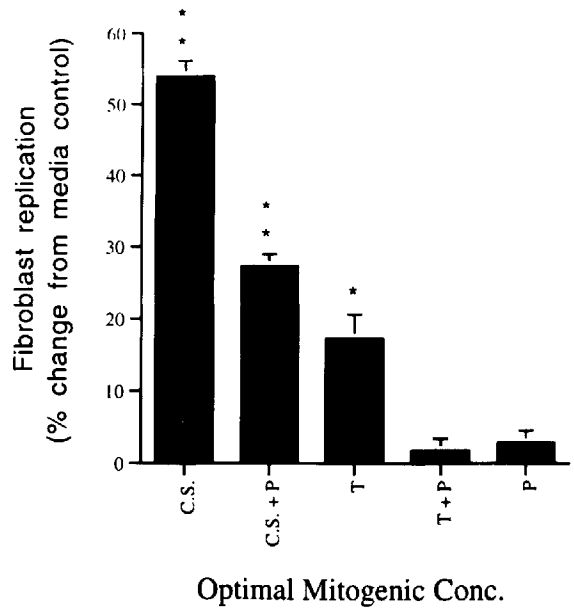

Figure 3. Fibroblast mitogenic activity in response to an optimal concentration of clot supernatant (7 μg ml^{-1}) (C.S.), an optimal concentration of clot supernatant incubated with 5.4×10^{-9} M PPACK (C.S. + P), thrombin alone (2.7×10^{-9} M) (T), thrombin (2.7×10^{-9} M) + PPACK (5.4×10^{-9} M) (T+P), and PPACK alone (5.4×10^{-9} M). Cell culture was performed for 48 h in the absence of serum. * $P < 0.01$, ** $P < 0.001$.

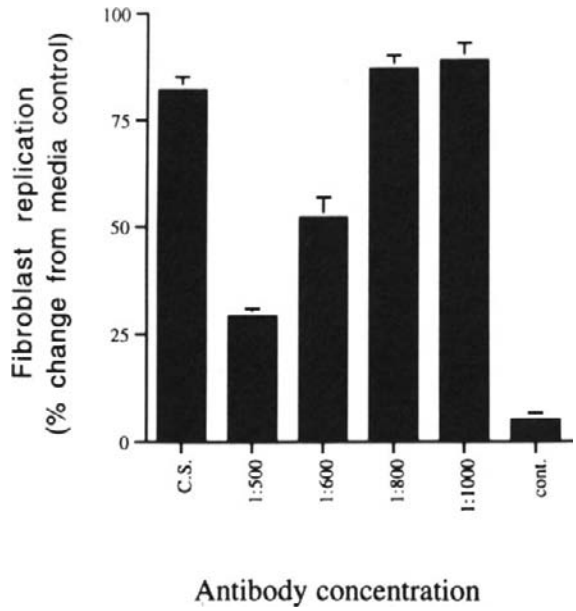

Figure 4. The ability of polyclonal fibrinogen antibodies to block the proliferative activity of clot supernatant (14 μg ml^{-1} total protein). Clot supernatant alone (C.S.). Clot supernatant incubated with varying concentrations of polyclonal human fibrinogen antibody (1:500 to 1:1000). cont. = antibody (1:500) + tissue culture medium.

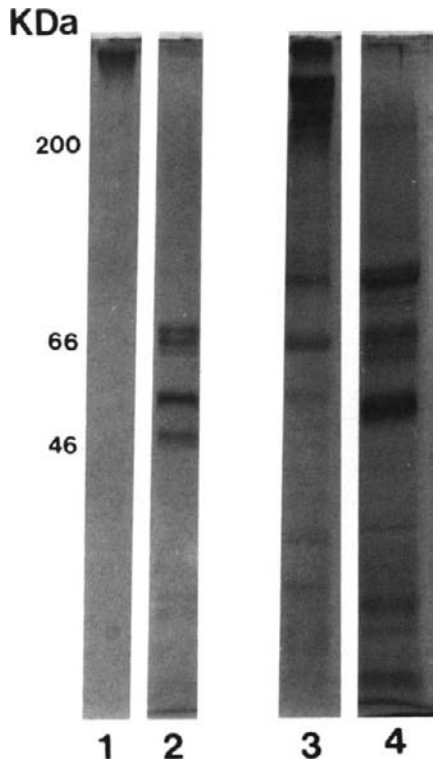

Figure 5. SDS-polyacrylamide gel (9.5%) of track 1 human fibrinogen (8 μg loaded); track 2 reduced human fibrinogen (8 μg loaded); track 3 clot supernatant (22 μg total protein loaded); and track 4 reduced clot supernatant.

pernatant, a specific thrombin inhibitor was employed. PPACK has been shown to block both the mitogenic and proteolytic action of thrombin (17), no matter whether it is free in solution or bound to fibrin(ogen) (18). The addition of PPACK to an optimally mitogenic concentration of thrombin blocked 100% of its activity (Figure 3; T+P). Further, addition of PPACK to an optimal mitogenic concentration of clot supernatant reduced the activity by about 50% (Figure 3; C.S.+P). PPACK alone was neither stimulatory nor inhibitory.

Figure 4 shows the ability of polyclonal fibrinogen antibodies to block the mitogenic activity of clot supernatant. Clot supernatant alone elicited a mitogenic response of about 80% above media control. On the addition of a 1:500 dilution of polyclonal antibody, this activity was reduced to about 30% above media control. Higher concentrations of antibody (1:100 and 1:200) did not further reduce mitogenic activity. At concentrations below 1:500 inhibitory effects were reduced in a dose-dependent fashion.

Prior to separation of clot supernatant by molecular sieve chromatography, both whole fibrinogen and clot supernatant were run on 9.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels to determine the purity of fibrinogen before clot formation and the number of protein moieties remaining in solution after clot formation. Fibrinogen was shown to be free of major contaminants (Figure 5, tracks 1 and 2). Multiple protein bands were observed in the solution remaining after clot formation, none of which appear to be present in the initial fibrinogen solution (Figure 5, tracks 3 and 4). Thrombin was also examined on SDS-PAGE gels and found to contain no contaminants (data not shown).

Following molecular sieve chromatography, active proteins eluted from the column in two distinct peaks (Figure 6). The first peak contained protein moieties eluting between 90 and 115 ml and displayed a maximal mitogenic response of about 50% above media control. The second peak contained protein moieties eluting between 127 and 135 ml, and also showed an optimal mitogenic response of about 50% above media control. Activity observed in the constituent fractions of both peaks diminished on serial dilution. In addition, fractions that were inhibitory to cells at the highest concentration tested were neither inhibitory nor stimulatory at lower concentrations.

Thrombin is known to be a mitogen and a component of clot supernatant (Figures 1 and 3). To determine the position at which thrombin eluted from the gel filtration column, a representative fraction from each peak was tested in the methylene blue assay with or without incubation with PPACK. In this sequence of experiments peptides constituting the first peak of mitogenic activity (Figure 6, peak A) elicited an optimal stimulus of about 60% above media control at an elution volume of 109 ml (Figure 1A; −PPACK). Peptides constituting the second peak of mitogenic activity (Figure 6, peak B) elicited an optimal mitogenic response of about 70% above media control at an elution volume of 138 ml (Figure 7B; −PPACK). After incubation with PPACK the activity in peak A was not significantly reduced (Figure 7A, +PPACK); in contrast, the activity in peak B was reduced to a value of about 20% stimulation above media control (Figure 7B, +PPACK).

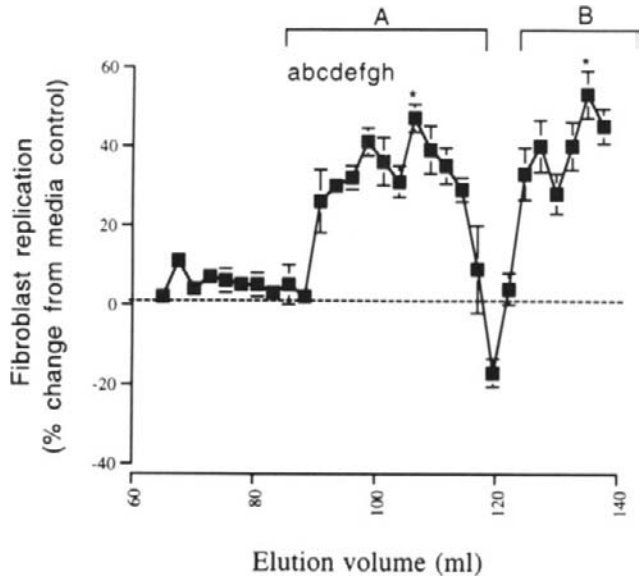

Figure 6. Fibroblast mitogenic activity in response to clot supernatant separated on a Sepharose 6B gel filtration column (fraction volume = 2.6 ml). Each fraction was lyophilized and reconstituted in 1 ml of tissue culture medium prior to assessment in the methylene blue assay. Fibroblast mitogenic activity eluted from the column in two peaks, peak A and peak B. (a to h) indicate column fractions used for SDS-PAGE analysis (Figure 8). * $P < 0.001$.

Protein components of fractions eluted from the gel filtration column were visualized employing SDS-PAGE analysis. Figure 8 (tracks a through h) show fractions collected between 88 and 109 ml, column eluent that contained mitogenic protein moieties that were insensitive to PPACK inactivation (Figure 7A). Active fractions contained about six protein moieties with molecular weights of 200 kD or greater. To investigate the nature of these proteins, ECL Western blotting employing a polyclonal fibrinogen antibody was performed. Figure 8A shows an ECL Western blot of protein moieties; these were also visualized by silver stain in Figure 8, track f. A strong signal was observed, indicating that such proteins were derived from the partial degradation of fibrin(ogen). Fractions constituting the second peak of mitogenic activity also contained partially degraded fibrin(ogen) but of a significantly lower molecular weight than that constituting the first peak. A band corresponding in molecular size to thrombin was also observed in fractions constituting the second peak of mitogenic activity (data not shown).

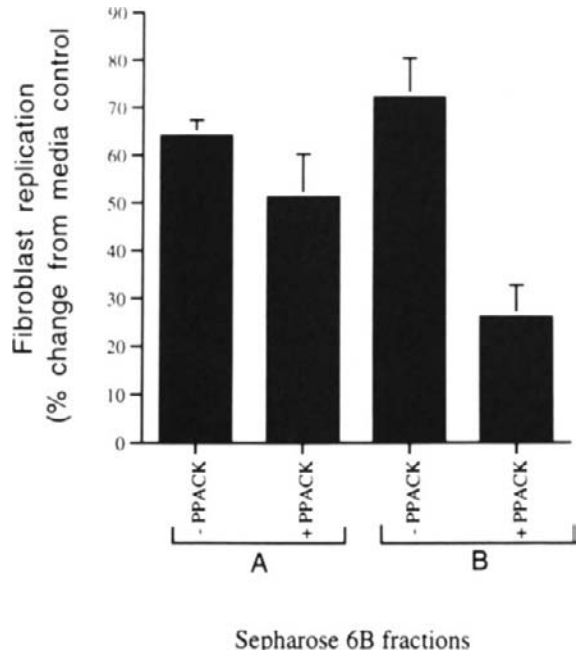

Figure 7. Fibroblast mitogenic activity in response to two representative fractions of clot supernatant eluting with peak A and peak B from a Sepharose gel filtration column (see Figure 6). Each fraction was tested before (−) and after (+) treatment with PPACK (5.4×10^{-9} M).

Discussion

This study shows that several forms of partially degraded fibrinogen remain in solution after fibrin clot formation and are potent fibroblast mitogens. Previous studies by our own and other groups have indicated that fibrinogen prior to clot formation does not stimulate fibroblast proliferation (11, 19). It is proposed that the serine protease thrombin, in addition to the production of an insoluble fibrin clot, generates several partially degraded forms of soluble fibrin(ogen) that are fibroblast mitogens.

Thrombin exhibits a number of diverse actions effecting mesenchymal cell function (8–10, 20). The ability of thrombin to simulate both fibroblast and smooth muscle cell proliferation has been described by several groups including our own (8, 11). It was therefore a concern of the present study that activity observed in the solute remaining after clot formation was not solely attributable to the mitogenic action of thrombin. The issue was examined considering the mitogenic dose response from thrombin, enzymatic inactivation of thrombin, blockage of clot supernatant activity by an antibody to fibrinogen, and separation of moieties in the clot supernatant according to molecular weight.

Thrombin optimally stimulated fibroblast replication at a concentration of 1.1×10^{-9} M, eliciting a mitogenic response that did not exceed 25% above media control in a series of experiments. In contrast, clot supernatant elicited a mitogenic response of between 50 and 80% above control in several experiments. On this basis the maximum mitogenic activity in the clot supernatant attributable to thrombin was usually less than about 50% of total clot supernatant activity. Thrombin binds both fibrinogen and fibrin; it was therefore important to quantify the concentration of thrombin present as a component of clot supernatant to determine the level of mitogenic activity that could be attributed to this protease. ^{125}I-labeling studies indicated that about two-thirds of thrombin used to produce a fibrin clot remained in solution. Considering the amount of thrombin bound to fibrin(ogen), free in solution, or both, the most each accounted for was < 50% of the mitogenic activity observed in the clot supernatant.

When the thrombin remaining in the supernatant after clot formation was neutralized with the specific thrombin inhibitor PPACK, mitogenic activity remained. It was, however, a concern that fibrin(ogen)-bound thrombin might be less susceptible to the inhibitory effects of PPACK than free

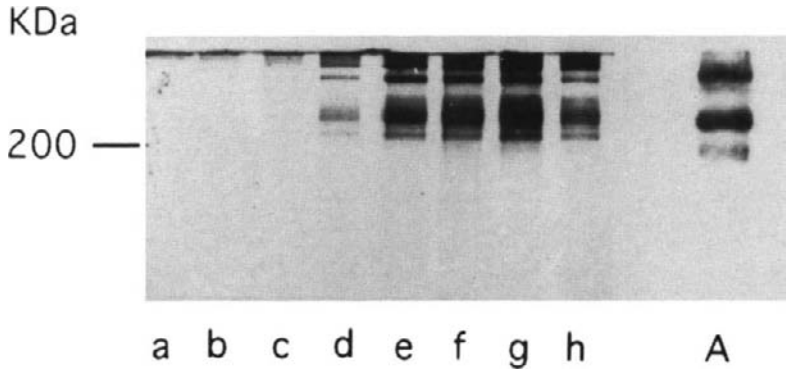

Figure 8. Silver-stained 9.5% SDS-polyacrylamide gel of clot supernatant fractions separated on a Sepharose 6B gel filtration column. Tracks (a through h) represent fractions eluting with mitogenic peak A (elution vol 88 to 109 ml; Figure 6). Marked along the left edge of the gel are relative molecular weight markers. Far right of the gel (column A) shows an autoradiograph of track f after incubation with HRP-labeled fibrinogen antibody (light emission was facilitated through enhanced chemiluminescence).

thrombin, and that the mitogenic effects observed in the clot supernatant were a result of such fibrin(ogen)-bound thrombin. Previous studies have shown that fibrin- and fibrinogen-bound thrombin, although less susceptible to inactivation by heparin–antithrombin III, remain highly susceptible to inactivation by hirugen and PPACK (18). If so, our results are compatible with the concept that thrombin was not responsible for all of the mitogenic activity in the clot supernatant.

The nature of mitogens constituting clot supernatant (other than thrombin) was investigated employing polyclonal antibodies to human fibrinogen. The activity of clot supernatant was reduced by about 50% with a 1:500 dilution of antibody, whereas antibody controls neither stimulated nor inhibited cellular replication. These experiments indicated that activity observed in the clot supernatant after the inactivation of thrombin was the result of fibrinogen-derived protein(s).

Results obtained from gel filtration chromatography and SDS-PAGE analysis indicated that proteins in the clot supernatant could be separated into two fractions containing higher versus lower molecular weights. The higher-molecular-weight fraction contained substantial mitogenic activity that was insensitive to the inhibitory action of PPACK, because the molecular weights were > 200 kD; the activity was insensitive to PPACK, and PPACK has been reported to inactivate both free and bound thrombin. The activity of this fraction was unlikely to be due to bound and/or free thrombin (mol wt = 39 kD). The nature of high-molecular-weight protein moieties as determined by enhanced chemiluminescence Western blotting showed the presence of several peptides derived from the partial degradation of fibrin(ogen). Although partially degraded fibrin(ogen) can be isolated from normal plasma (21), SDS-PAGE analysis suggested that in our study none of the degraded forms of fibrinogen described appeared to be present as components of fibrinogen preparations prior to their interaction with thrombin. In addition, fibrinogen elicits no mitogenic response prior to clot formation (11). These observations suggest that in addition to fibrin clot formation, several soluble fibrin(ogen) degradation products are generated by thrombin. Further, these products are potent fibroblast mitogens.

The lower-molecular-weight fraction obtained from gel filtration chromatography also contained substantial mitogenic activity, but the activity was largely blocked by PPACK. This, plus the presence of a protein band having a molecular weight expected for thrombin, suggests that thrombin was present in the fraction and accounted for some of the mitogenic activity. The significant mitogenic activity that remained after PPACK, although not specifically examined in the present study, was similar to that observed from the combined effects of the small A and B fibrinopeptides that are cleaved from fibrinogen by thrombin. If so, the activity in the lower molecular weight fraction could be attributed to thrombin plus the fibrinopeptides A and B. Given this evidence that clot supernatant contains high-molecular-weight proteins that are mitogenic, the question arises as to how such proteins are generated. Several studies suggest that the proteolytic action of thrombin is specific to the fibrinopeptides A and B, the removal of which initiates fibrin polymerization (22). There are, however, a number of reports indicating that the proteolytic action of thrombin is not site specific. Incubation of reduced fibrinogen chains with thrombin produces marked degradation of the Aα chain carboxyl terminus after about 20 min (23). It has also been observed that fibrin polymerization involves sites within the carboxyl portion of the Aα chain (24). It is therefore probable that degradation of the Aα carboxyl terminus inhibits polymerization. These observations may provide an explanation for the presence of soluble partially degraded fibrin(ogen) present as a component of the clot supernatant. We have previously investigated the nature of mitogenic sites within the fibrin(ogen) molecule. We have shown that after reduction and alkylation, the Aα and Bβ chains of fibrinogen are fibroblast mitogens (25). Furthermore, the removal of the fibrinopeptides significantly enhanced mitogenic activity of each chain. The present study has not investigated mitogenic sites within partially degraded fibrin(ogen). However, based on these previous data it is tempting to speculate that mitogenic sties may be located within the amino terminal domain of the molecule and become active on the removal of the fibrinopeptides and/or further degradation of the Aα or Bβ chain.

The observation that the action of thrombin on fibrinogen not only produces an insoluble fibrin matrix but also generates mitogenic activity has important implications for both normal wound healing and the pathogenesis of a number of disease states. Several studies have shown that fibroblasts rapidly invade and proliferate in a fibrin matrix, an action that may in part be sustained by the mitogens described in the present study. In addition, there are a number of disease states during which fibrin formation and mesenchymal cell proliferation occur in concert; examples include pulmonary vascular remodeling, atherosclerosis, and pulmonary fibrosis. The chronic generation of partially degraded fibrin(ogen) at the site of vascular injury may play a significant role in the pathogenesis of these disease states.

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