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A Fibrin Specific Monoclonal Antibody which Interferes with the Fibrinolytic Effect of Tissue Plasminogen Activator

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Key words

Fibrin specific antibody – Tissue plasminogen activator

Summary

Monoclonal antibodies to human fibrin have been prepared from stable hybridomas, obtained by fusion of a mouse myeloma cell line (NS-1) and spleen cells of Balb/c mice immunized with a suspension of human fibrin. One cell line, DG1, producing a monoclonal antibody of the IgG_1, κ subclass, reacted specifically with human fibrin (K_D = 1.2 nM). Western blotting analysis indicates that DG1 crossreacts with the fibrin fragment D-dimer. Using both a chromogenic and an ^{125}I-fibrin release assay it was illustrated that in the presence of the fibrin specific antibody the t-PA mediated generation of plasmin was significantly inhibited.

An animal model system, developed to monitor thrombosis and induced reactive fibrinolysis, was used to investigate the interference of plasminogen activation, by the antibody, *in vivo*.

This fibrin specific antibody prolonged the onset of reactive fibrinolysis in a dose dependent manner.

Introduction

Activation of the blood coagulation cascade results in the generation of thrombin which converts the plasma protein fibrinogen to fibrin monomer by proteolytic cleavage of fibrinopeptide A (FPA) from the amino terminus of fibrinogen A α-chains (1). The consequence of FPA cleavage is the activation of polymerization sites and the polymerization of fibrin monomers in a linear manner, forming protofibril strands. Thrombin cleavage of a second peptide, fibrinopeptide B (FPB) from the amino terminus of the B β-chain exposes another active site on the fibrin polymer which promotes lateral polymerization of the fibrin strands. Finally, in the presence of the transglutaminase, factor XIIIa, extensive covalent crosslinking of the fibrin polymer occurs resulting in the formation of insoluble fibrin (2, 3). The presence in plasma of elevated levels of fibrinopeptides and soluble fibrin polymers has long been recognized to be indicative of a number of vascular disorders such as deep vein thrombosis and disseminated intravascular coagulation (4). Other haemostatic processes such as fibrinolysis and fibrinogenolysis, which involve the plasmin degradation of fibrin(ogen), give rise to a range of fibrin and fibrinogen degradation products (FDPs). The blood levels of these FDPs can also be used in assessing vascular disorders (5, 6). Due to the importance of measuring the *in vivo* concentrations of fibrin(ogen) and its derivatives in the diagnosis and treatment of impaired haemostasis, much effort has gone into developing specific and accurate analytical methods for their determination. Many of the diagnostic methods devised are based on the production of polyclonal antisera to fibrin(ogen) derivatives (7–9). Although there have been reports of specific antisera (10–12) the inherent problem of antiserum to fibrin(ogen) derivatives is the lack of specificity due to the relatively small number of neoantigenic sites generated in fibrin and FDPs (13).

The development of hybridoma technology (14) has made available highly specific analytical probes in the form of monoclonal antibodies which can be directed at individual epitopes. The production of antibodies, specific for neoantigenic sites on fibrin and fibrin(ogen) derivatives is, however, complicated by the high level of covalent structure conservation of fibrinogen when it is converted to fibrin or degraded by plasmin to FDPs (15). There have been several recent reports of fibrin specific monoclonal antibodies (16–19). The strategy employed by two of these investigators (16, 17) was based on the hypothesis that neoantigenic sites are exposed on the amino terminus of fibrinogen when the fibrinopeptides are cleaved by thrombin. In both cases, monoclonal antibodies were produced to synthetic peptides representing the newly exposed amino terminals. Fibrin fragments have also been used as antigens to produce fibrin specific monoclonal antibodies (18, 19).

In this study we report the production of a fibrin specific monoclonal antibody using fibrin as immunogen. The fibrin specificity of the antibody was extensively studied. The inhibitory effect of the antibody on the tissue plasminogen activator-mediated generation of plasmin was also investigated.

Materials and Methods

Materials

All tissue culture reagents were purchased from M. A. Bioproducts (Walkinsville, MD) with the exception of RPMI 1640 medium, fetal bovine serum and horse serum, all of which were from Gibco Laboratories (Grand Island, NY). Bovine serum albumin and plasminogen-free bovine thrombin were both obtained from Miles Laboratories (Elkhart, IN). Human urokinase was from Calbiochem (La Jolla, CA) and the chromogenic substrate, H-D-valyl-L-leucyl-L-lysine-p-nitroanilide dihydrochloride (S-2251) was from KabiVitrum (Stockholm). ^{125}I-labelled fibrinogen was from Amersham (Arlington Heights, IL) and carrier free $Na^{125}I$ was from New England Nuclear (Boston, MA).

Preparation of Proteins

Plasminogen was purified from human plasma by affinity chromatography on Sepharose 4B-L-lysine (Pharmacia) using a modification of the Deutsch and Mertz (20) method as described by Brockway and Castellino (21). Human fibrinogen (grade L, Kabi) was made plasminogen free by affinity chromatography on Sepharose-L-lysine. Tissue plasminogen activator (t-PA) was isolated from the serum-free tissue culture fluid of a melanoma cell line (Bowes), generously provided by Dr. D. Rijken (Rockefeller University, New York). The activator was purified using a modification of the method described by Rijken and Collen (22). Briefly,

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tissue culture fluid from the cell line was chromatographed on two affinity columns, phenyl-Sepharose followed by Sepharose-concanavalin A. Final purification of the activator was performed by gel filtration on a Sephadex G-150 column.

Fibrinogen degradation products were prepared by incubating fibrinogen with plasmin, at 37 °C, for time periods ranging from 10 min to 3 h as described by Varadi and Patthy (23). At the desired time fibrinolysis was stopped by the addition of Trasylol (100 Kallikrein Inhibitor units/ml) and 20 mM ε-aminocaproic acid (EACA).

Fibrin degradation products were prepared by the addition of thrombin (4 NIH units/ml) to a fibrinogen solution (5 mg/ml) in Tris buffered saline (TBS, pH 7.4, 50 mM Tris-HCl, 150 mM NaCl), containing 10 mM $CaCl_2$, plasminogen (0.25 mg/ml) and urokinase (50 I.U./ml). The mixture was incubated at 37 °C and the plasmin digestion terminated at different time intervals as described above (21). Fibrinogen α, β, and γ chains were prepared by incubation of fibrinogen with 5% mercapoethanol at 90 °C for 5 min. Purified samples of fibrinogen fragments D (MWt = 90,000) and E were a gift from Dr. F. J. Castellino (University of Notre Dame).

Fibrinogen and the monoclonal antibodies were labelled with ^{125}I using Iodobeads (Pierce Chemical Co.) and carrier free $Na^{125}I$, as described by Markwell (24). The labelled protein was separated from free $Na^{125}I$ by gel filtration on a Sephadex G-150 column followed by dialysis against phosphate buffered saline, pH 7.4 (PBS).

Preparation of Solid-Fibrin-Phase

In those assays requiring a solid-fibrin-phase, microtiter assay plates were coated with 0.2 ml of a fibrinogen solution (0.05 mg/ml in borate/saline buffer) overnight at 4 °C. The fibrinogen coated onto each plastic well was converted to fibrin by incubation with 0.2 ml of a thrombin solution (2 NIH units/ml), containing 2 mM $CaCl_2$, for 1 h at 37 °C.

Spectrophotometric Assay for t-PA Activity

The rate of activation of plasminogen to plasmin by t-PA was measured by a chromogenic assay using the plasmin specific substrate, S-2251. The assay was performed using a modification of the method described by Drapier et al. (25). A TBS solution (pH 7.4) containing 0.5 mM S-2251, plasminogen (1.0 mg/ml), BSA (2.5 mg/ml) and t-PA (2.8 I.U./ml) was added to the fibrin coated wells of a microtiter plate. The plates were incubated at 37 °C and the rate of plasmin formation determined by measuring the change in absorbance of the solutions at 405 nM, as described by Drapier (25).

^{125}I-Fibrin Plate Assay

The fibrinolytic activity of t-PA was measured using a modified version of the ^{125}I-fibrin plate assay method, describing by Unkeless et al. (26). In brief, ^{125}I-labelled fibrinogen (1300 cpm/ng) was diluted with unlabelled fibrinogen so that approximately 6.5×10^5 cpm was added to each well of a 24-well tissue culture plate (Falcon). The fibrinogen coated on each well was converted to fibrin by incubation with 1 ml of a thrombin solution (2 NIH units/ml) for 1 h at 37 °C. The plates were allowed to dry at 37 °C for 48 h. In control experiments plasminogen (5 μg/ml) and t-PA were added in a total volume of 1 ml to the ^{125}I-fibrin coated wells. At 30 min intervals, aliquots (50 μl) were removed and the activity released was measured in a gamma counter (Beckman Model 5500). To determine the effect of the antibodies on the fibrinolytic activity of t-PA, saturating levels of the antibody were preincubated in the fibrin coated wells prior to the assay.

Production of Monoclonal Antibodies

Balb/c mice were immunized, intraperitoneally (i.p.) with a mixture of soluble and insoluble human fibrin in Freund's complete adjuvant. A booster injection was administered (i.p.) after 4 weeks using the same amount of antigen in Freund's incomplete adjuvant. A final booster was given intravenously 4 weeks later (3 days before fusion).

The fusion protocol was essentially as described by Oi and Herzenberg (27) utilizing polyethylene glycol 1000 (Baker) as the fusion agent. Culture supernatant from hybrid colonies were screened by ELISA for antibody production on fibrin and fibrinogen coated wells.

Monoclonal antibodies were isolated from tissue culture media by affinity chromatography using a Sepharose-rabbit anti-mouse IgG column. Antibody from ascites was purified by ion-exchange HPLC (28). Homogeneity of the purified antibodies utilizing affinity chromatography or ion exchange chromatography was verified by isoelectric focusing. Subclassing of the antibodies was assessed by a solid phase immunoassay (ELISA) using antigen coated wells and a standard reference kit (Zymed), containing rabbit anti-mouse IgG_1, IgG_{2a}, IgG_{2b}, IgM in addition to rabbit anti-mouse κ and λ light chains.

Immunospecificity Determination

The immunospecificity of the monoclonal antibodies to fibrin, fibrinogen and their respective degradation products was first assessed using the ELISA technique. An immunoblotting technique was also employed in which samples of fibrinogen and fibrin degradation products were electrophoresed on 5% polyacrylamide gels. Protein transfer from the gel to nitrocellulose sheets was performed for 48 h at 4 °C utilizing an electroblotting cell (Bio Rad). Subsequent formation and detection of the antigen-antibody complex was essentially as described by Law and Lingwood (29). The avidity of the monoclonal antibodies to fibrin and fibrinogen was determined by solid phase radioimmunoassays using the method described by Frankel and Gerhard (30) with modifications as described by Ploplis et al. (31).

Animal Studies

The effect of the purified monoclonal antibodies on thrombolysis was tested in a simple model animal system. Male Lobund-Wistar rats were anaesthetised and injected intravenously (i.v.) via the saphenous with a 15 μCi dose of human ^{125}I-labelled fibrinogen. A saline solution of monoclonal antibody was adminstered i.v., prior to the induction of intravascular coagulation by infusion with thrombin (700 NIH units kg^{-1} h^{-1}). The accumulation of microthrombi in the pulmonary capillary bed was externally monitored using a NaI detector, connected to a single channel analyser (Nucleus).

Results

Preparation of Monoclonal Antibodies

The spleens of Balb/c mice which showed a positive immune response against the fibrin antigen were fused with the NS-1 myeloma cell line. In the primary cloning phase, 121 wells showed vigorous growth and 18 of these hybrid colonies produced antibody to the antigen. The 18 hybridomas were recloned three times by limiting dilution with the tertiary cloning phase performed at 1 cell per well. Two stable antibody producing cell lines were eventually established.

Monoclonal antibodies were purified from tissue culture supernatant by affinity chromatography, on a Sepharose-anti-mouse IgG column, and from ascites fluid by HPLC. The purified antibodies showed only one band after isoelectric focusing and after $NaDodSO_4$/PAGE under non-reducing conditions. Subtyping by ELISA, using a standard antimouse reference kit, showed that the two antibodies belonged to the IgG_1 class. Both antibodies gave positive ELISA results with antimouse κ chains and a negative with antimouse λ chains.

Immunospecificity

To determine the immunospecificity of the two antibodies isolated, immunoblotting analysis was performed.

Fibrin and fibrinogen degradation products were transferred from 5% acrylamide gels to nitrocellulose sheets in all blotting experiments. Visualization of antigen-antibody complex revealed that the hybridoma cell line, DG2, produces an antibody that binds to fibrinogen and it's X, Y and D fragments. While DG2 immunoreacts strongly with soluble fibrin only limited activity

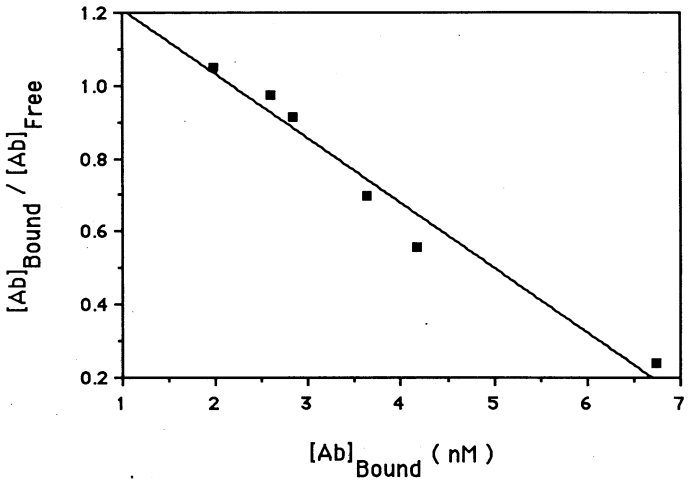

Fig. 1 Determination of binding constant of monoclonal antibody, DG1, to fibrin, using a modified Scatchard plot. The ratio of the concentration of bound antibody ($[Ab]_{Bound}$) to unbound ($[Ab]_{Free}$), is plotted against $[Ab]_{Bound}$

was observed with D-dimer from crosslinked fibrin. Further analysis indicated that the epitope is in the Aα chain of fibrinogen. The other established hybrid clone, DG1, crossreacts strongly with soluble fibrin and D-dimer. Only slight immunoreactivity, utilizing Western Transfer analysis, was observed with the fibrinogen degradation products D, X and Y. When immunoblotting was performed on a reduced fibrinogen sample no reactivity was observed with the Bβ or γ chains but slight reactivity was detected with the Aα chain.

Determination of Binding Constants

The avidity of the monoclonal antibody, DG1, for fibrinogen and fibrin was determined by solid phase RIA and the data was analyzed using a modified form of the Scatchard relationship as described by Frankel and Gerhard (30). Prior to the determination of the avidity constants, the effect of iodination on the binding efficiency of the antibody was tested using the standard ELISA technique. A decreased binding efficiency of approximately 10% was determined. A modified Scatchard plot of the binding data for DG1 to fibrin and fibrinogen is shown in Fig. 1. Approximate dissociation constants (K_D) were determined from the slope of the lines and calculated to be 1.2×10^{-9} M and 3.2×10^{-8} M for fibrin and fibrinogen, respectively. Similar experiments were performed to determine the binding constants of DG2 to fibrinogen and fibrin. The results are listed in Table 1.

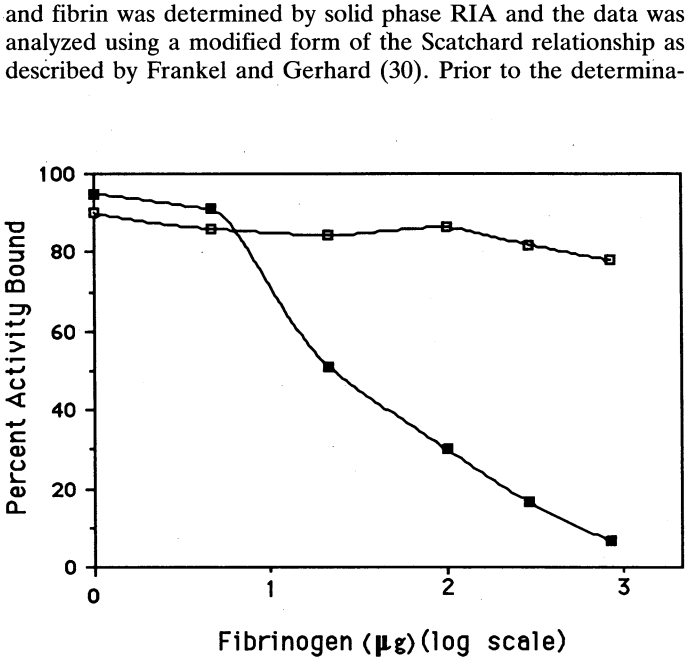

Fig. 2 Competitive binding of fibrinogen and fibrin for antibodies, DG1 (□) and DG2 (■). A subsaturating level of ^{125}I-labelled antibody was added to fibrin coated wells, containing various levels of fibrinogen. The 96-well plates were incubated at 37 °C for 90 min. After extensive washing with PBS-Tween, the amount of antibody bound to fibrin in the individual wells was measured in a gamma counter. The results are the means of triplicate experiments

Table 1 The binding constants, K_D, for antibodies, DG1 and DG2 for various antigens. K_D values were determined using a solid phase RIA and a modified form of the Scatchard analysis method

Antigen	K_D ($\times 10^8$ M)	
	DG1	DG2
Fibrin[a]	0.12	1.07
Fibrinogen	3.20	0.7
Soluble fibrin[b]	3.00	0.65

[a] Fibrin coated wells were prepared by thrombin treatment of fibrinogen-coated wells, as described in methods section.
[b] Soluble fibrin refers to that portion of a fibrin clot solubilised by 4 M urea.

Determination of the Fibrin Specificity Using a Competitive Assay

A competitive assay system was developed to further investigate the fibrin specificity of DG1. Solutions containing various levels of fibrinogen and a constant subsaturating level of ^{125}I-labelled antibody were added to fibrin coated wells. The experiment was essentially as described for determining avidity constants. A control experiment was performed in which the antibody DG1 was replaced with DG2. As shown in Fig. 2, competing fibrinogen levels as high as 2.5 mg/ml (500 fold excess of the fibrin antigen) did not cause any significant decrease in the amount of antibody, DG1, bound to fibrin. In contrast, antibody DG2 was displaced by approximately 50% at competing fibrinogen concentrations equivalent to fibrin and almost completely displaced at higher levels. An additional competition assay was performed to test fibrin specifity of the antibody DG1. In this assay, fibrinogen solutions were added to tubes containing a suspension of fibrin, formed by sonication of a crosslinked fibrin clot. The level of ^{125}I-labelled antibody which binds to fibrin was determined after extensive washing and centrifugation of the fibrin clots. Fig. 3 shows that increasing fibrinogen levels do not inhibit binding of DG1 to the fibrin clot whereas the same fibrinogen concentrations cause a significant decrease in the level of DG2 which bound to fibrin. The difference in the amount of DG1 and DG2 bound to fibrin clots in the absence of soluble fibrinogen is reflective of the diminished affinity of DG2 for fibrin as noted in Table 1.

Investigation of the Effect of Fibrin Bound Monoclonal Antibodies, DG1 and DG2, on the Activity of t-PA

The effect of fibrin bound antibodies, DG1 and DG2, on t-PA activity was determined using an adaption of the spectrophotometric assay described by Drapier et al., as outlined in the experimental section.

The assay was performed after preincubation of the fibrin coated wells with levels of antibody ranging from zero to concentrations well in excess of saturating levels (Fig. 4). A replot of this data (Fig. 5) shows a steady decline in t-PA activity with increasing levels of fibrin bound antibody, DG1. No additional

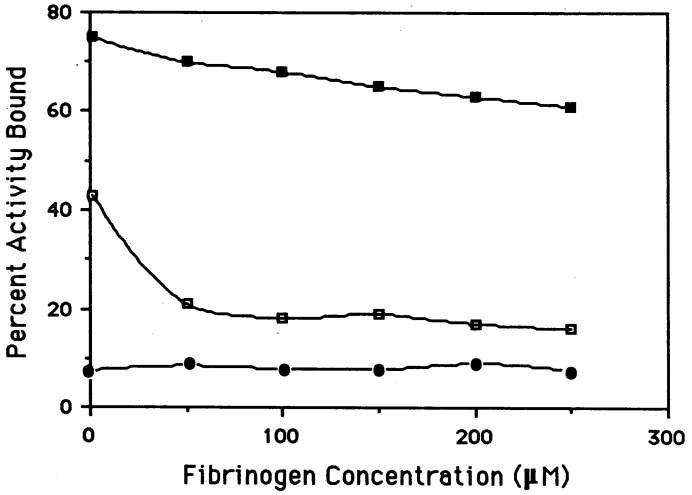

Fig. 3 Solution phase competition assay between fibrin and fibrinogen for monoclonal antibodies DG1 (■) and DG2 (□). A crosslinked fibrin clot, sonicated to produce a homogeneous fibrin suspension was mixed with a range of fibrinogen solutions. A constant subsaturating level of ^{125}I-labelled monoclonal antibody was added to the mixture and incubated at 37 °C for 1 h. The fibrin clot was recovered by centrifugation and washed extensively with PBS-Tween. The amount of antibody bound to the fibrin clot was determined by measuring the ^{125}I activity. ^{125}I-labelled BSA (●) was used in a control experiment to determine the extent of protein entrapment during centrifugation of the fibrin clot

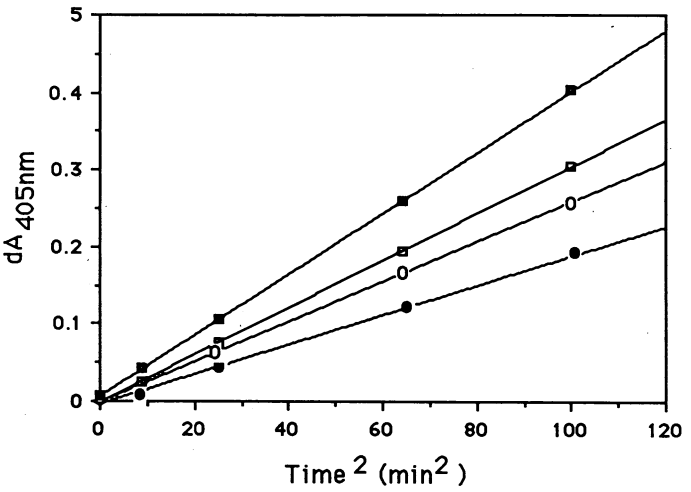

Fig. 4 Determination of the effect of monoclonal antibody, DG1, bound to fibrin, on the activity of t-PA. Aliquots of monoclonal antibody solution (0.2 ml) were incubated in fibrin coated wells of a 96-well microtiter plate for 90 min at 37 °C. The wells were extensively washed with PBS-Tween and a chromogenic assay was performed to determine the t-PA activity, as described in the experimental section. A rate assay was performed by measuring the change in absorbance at 405 nM (dA_{405nM}) at different time points. The rate of production of para-nitroanilide, in the presence of various antibody solutions [20 μM (●), 10 μM (○), 5 μM (□), 0 (■)] was determined from the slope of the line obtained by plotting dA_{405nM} against t_2

inhibition of the t-PA activity could be induced by increasing antibody levels in excess of those shown. In contrast, experiments utilizing the antibody DG2 indicated no effect on t-PA activity, even at concentrations of DG2, 100 fold higher than that used for DG1.

In another series of experiments, using an ^{125}I-fibrin plate assay, the effect of the antibodies DG1 and DG2 on the fibrinolytic activity of t-PA was determined. The fibrin coated wells were preincubated with solutions containing saturating levels of antibody. After removal of unbound antibody the fibrinolytic activity was measured as described in the experimental section. In control experiments the amount of bound activity was determined by trypsin digestion of the fibrin film. In those wells preincubated with antibody, DG1, only 60% of the bound activity was released after a 3 h incubation at 37 °C with t-PA and plasminogen (Table 2). Wells not preincubated with antibody released 90% of the bound activity over the same period. Wells, preincubated with antibody DG2, did not show any fibrinolytic inhibition. The t-PA activity measured was almost identical to that measured in the absence of antibody (Table 2).

The Effect of Fibrin Specific Antibody DG1 on Thrombolysis

Intravascular coagulation was induced in rats by infusion with thrombin and the fate of the resultant microclots was monitored in the capillary bed of the lungs by measuring the ^{125}I-activity as described in the experimental section. Fig. 6 illustrates the typical activity profile obtained in control animals. The sharp rise in activity, a short time after the commencement of thrombin infusion indicates the accumulation of thrombi in the capillary bed. After reaching a peak, which is sustained for less than 15 min, the activity decreases rapidly (Fig. 6). This decrease in activity is attributable to the animals fibrinolytic response resulting in the dissolution of the fibrin clots to soluble degradation products.

In the presence of the fibrin specific monclonal antibody, DG1, the onset of thrombolysis is significantly delayed (Fig. 6). This interference with the onset of fibrinolysis depended on the dose of antibody administered but the effect was not enhanced at antibody levels greater than one nanomole (Fig. 6). These experiments were repeated using the antibody DG2 and no deviation from the control activity profile was observed.

Table 2 ^{125}I-Fibrin plate competition assay. The effect of monoclonal antibodies DG1 and DG2 on fibrinolysis

Antibody	Activity (cpm)
DG1	70,810
DG2	100,900
No antibody	102,904

Values represent the ^{125}I-activity released after 3 h of incubation with t-PA and plasminogen.

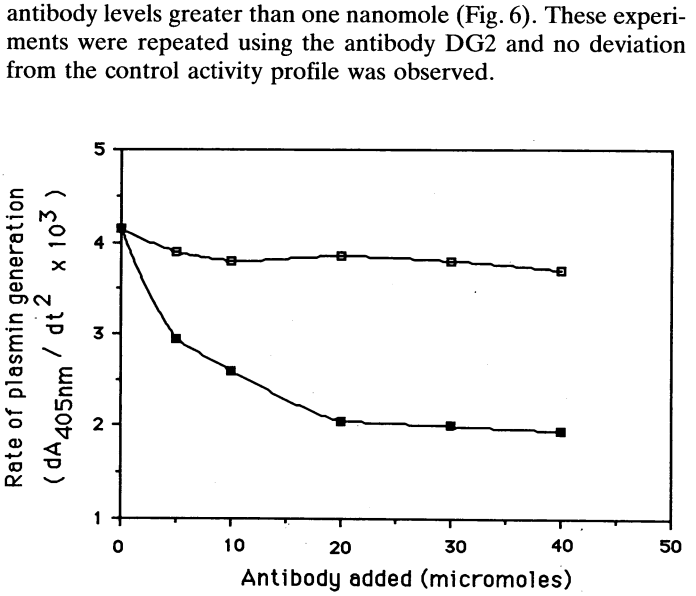

Fig. 5 The rate of t-PA mediated plasmin generation using a chromogenic substrate, S-2251, in the presence of monoclonal antibodies DG1 (□) and DG2 (■)

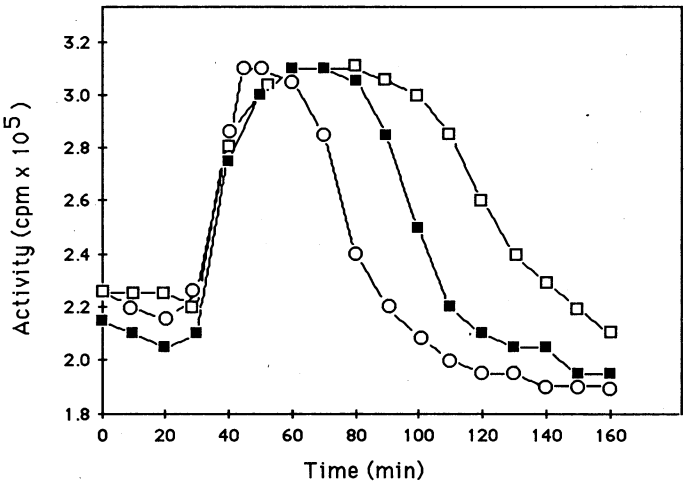

Fig. 6 The effect of monoclonal antibody, DG1, on thrombolysis, using an animal model system. ^{125}I-labelled fibrinogen (10 μg, 15 μCi in 0.5 ml saline) was injected (i.v.) at t = 0. Thrombosis was induced by i.v. infusion of thrombin starting at t = 30 min. The ^{125}I activity was measured over the lung field, for 1 min periods at 5 min intervals. Monoclonal antibody, DG1 (0.2 nanomoles [■] and 1 nanomole [□]) was injected, i.v., at t = 15 min. In the control experiment (○) no antibody was injected

Discussion

The conversion of fibrinogen to fibrin by the proteolytic effect of thrombin results in retention of 98% of the primary structure of the precursor molecule. As a result, efforts to obtain monoclonal antibody, specific for fibrin have proven to be difficult. One approach has taken advantage of the expression of new N-termini of the Aα and Bβ chains of fibrinogen after thrombin proteolysis. Peptides that mimic these regions have proven to be suitable antigens in developing antibodies that differentiate fibrin from fibrinogen *in vitro* (16, 17). It remains to be determined whether these antibodies can serve as diagnostic reagents in detecting thrombi *in vivo* where ongoing fibrinolysis results in the plasmin mediated degradation of these neoantigenic regions (5). Another approach has utilized crosslinked fibrin degradation products as immunogens (18, 19). It was reasoned that during the conversion of fibrinogen to fibrin conformational changes occur in the tertiary structure resulting in the expression of neoantigenic sites. Monoclonal antibodies have been developed that distinguish between fibrinogen and fibrin derivatives in serum immunoassay (18). It was further determined that the epitope is expressed in the D-dimer fibrin degradation product.

We have been successful in developing a fibrin specific antibody utilizing intact human fibrin as the immunogen. Evidence for the fibrin specificity of monoclonal antibody, DG1, was obtained using both solid and solution phase radioimmunoassays. The avidity constants determined by solid phase radioimmunoassays for DG1 and the antigens fibrin and fibrinogen indicated a 30 fold higher affinity for fibrin. Radioimmunoassays utilizing fibrinogen in solution as a competing antigen indicated much higher preference for the fibrin antigen. This was further confirmed in solution phase assays utilizing soluble fibrinogen and fibrin clots in suspension (Fig. 3). The apparent diminished albeit maintained affinity of DG1 for fibrinogen in assays in which this protein serves as a solid phase matrix may be indicative of a conformational alteration induced by its interaction with the plastic surface. This change in conformation may expose or induce the assembly of an epitope similar to the neoantigenic site recognised by DG1 when fibrinogen is converted to fibrin. Recently, Soria et al. (32) reported a similar effect when fibrinogen is bound to plastic surfaces. Using monoclonal antibodies, they demonstrated that an epitope, inaccessible in soluble fibrinogen, was expressed when the antigen was degraded by plasmin or bound to a polystyrene surface.

One of the unique characteristics of this antibody is its ability to interfere with the t-PA mediated generation of plasmin. Utilizing a chromogenic assay (Fig. 3), it was demonstrated that the antibody inhibits the rate of plasmin formation. However, even at saturating levels of antibody DG1, considerable plasmin generation was still observed. The mechanism of this inhibition is unclear but a possible explanation is that the antibody competes with t-PA or plasminogen for its fibrin binding site. To test this hypothesis ELISAs were performed on fibrin coated wells, preincubated with saturating levels of plasminogen or t-PA in the presence of a protease inhibitor. The results (unpublished) clearly indicated that neither the activator nor plasminogen interfered with the antibody-fibrin interaction. The most likely explanation for the mechanism of the inhibition, also observed in an ^{125}I-fibrin plate assay, is the close proximity of the antibody epitope and the site involved in the t-PA-plasminogen interaction.

Further evidence of this interference of plasminogen activator by the antibody was obtained from a model animal system. The thrombolytic capacity of the endogenous t-PA on microclots, labeled with human ^{125}I-fibrin, was somewhat decreased in the presence of the antibody, DG1. The species specificity of the antibody was determined using a range of fibrin(ogen) preparations and no crossreactivity with rat or with any other species was found (results not shown). This would indicate that the delayed fibrinolytic effect observed in this animal model is due to the interaction between the antibody and the human fibrin component of the labeled microclots. The antibody did not show any capacity for inhibition of fibrin polymerisation in the model system (Fig. 6). This observation discounts the possibility that the antibody is inhibiting the rate of formation of fibrin and therefore interfering with the potentiating effect of fibrin polymerisation on t-PA catalysed plasminogen activation.

Fibrin specific monoclonal antibodies, such as DG1, offer a new approach for detection and localisation of deep vein and coronary artery thrombi. With appropriate labelling, such antibodies could replace current angiographic methods with a much more specific and sensitive method of thrombus detection. In addition, applications of fibrin specific antibodies include, a vehicle or targeting reagent for carrying thrombolytic agents to the site of fibrin clots or cytotoxic reagents to fibrin encapsulated tumors.

Acknowledgments

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