British Journal of Pharmacology (2000) 129, 1021–1027

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Role of clot-associated (-derived) thrombin in cell proliferation induced by fibrin clots *in vitro*

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1 Thrombin is a potent mitogenic agent. Clot-associated thrombin retains its amidolytic and pro-aggregant activity. We therefore studied the ability of fibrin clots to induce proliferation in CCL39 cells (Chinese hamster lung fibroblasts), in the absence and presence of the thrombin inhibitors PPACK, recombinant hirudin (rHV2 Lys47) and heparin:antithrombin III.

2 Fibrin clots incubated for 48 h with CCL39 cells led to significant cell proliferation, which was dependent on the concentration of thrombin used to prepare the clots. Thus, clots prepared with 91 nmol l^{-1} thrombin produced a similar proliferation ($231 \pm 21\%$) to that obtained with 50 nmol l^{-1} thrombin in solution ($213 \pm 29\%$). Rabbit plasma clots led to a $499 \pm 41\%$ increase in cell number under identical conditions.

3 Fibrin clot-induced cell proliferation was inhibited by all three thrombin inhibitors with no difference in IC_{50} values compared to those obtained against thrombin in solution, suggesting that cell proliferation be due to thrombin leaching from the clots.

4 We found a time-dependent increase in thrombin release from the clots attaining a plateau at 24 h (~61% of the total thrombin used in clot formation). Clots separated from the cells using porous cell culture chamber inserts led to similar proliferation to that of clots in contact with the cells.

5 Thus fibrin-clot induced CCL39 proliferation is due to thrombin released from the clots.
British Journal of Pharmacology (2000) **129,** 1021–1027

Keywords: Fibrin clots; cell proliferation; mitogen; thrombin inhibitors

Abbreviations: CCL39, Chinese hamster lung fibroblast line; DMEM, Dulbecco's modified Eagle medium; PAR, protease activated receptors; PBS, Phosphate buffered saline; PPACK, (D)-Phe-Pro-Arg-chloromethylketone

Introduction

Thrombin (EC 3.4.21.5) is a multifunctional serine protease that is generated at sites of vascular injury. It is a critical component of the blood coagulation cascade, where it is a rate-limiting step in thrombus formation, and it promotes platelet aggregation (see Fenton, 1995 for review). It can activate signalling pathways in a variety of cells including fibroblasts (Chambard *et al*., 1987), vascular muscle cells (McNamara *et al*., 1993) and endothelial cells (Jaffe *et al*., 1987; Goligorsky *et al*., 1989), including increases in intracellular calcium, stimulation of protein synthesis and induction of c-*fos* expression (Berk *et al*., 1991). The cellular responses to thrombin are induced through the proteolytic activation of PAR-1, -3 and -4, (Vu *et al*., 1991; Ishihara *et al*., 1997; Xu *et al*., 1998). PAR-1 has been identified in several cell types such as CCL39 (Rasmussen *et al*., 1991), human endothelial cells (Bahou *et al*., 1993) and rat vascular muscle cells (Zhong *et al*., 1992). Thrombin can remain present at sites of injury due to its release from clots undergoing fibrinolysis, and, theoretically, high local concentrations could be available for interaction with cell types involved in tissue repair.

It is now well established that fibrin or plasma clot-associated thrombin retains its amidolytic activity (Francis *et al*., 1983; Weitz *et al*., 1990, 1998; Berry *et al*., 1994), is resistant to inhibition by heparin:antithrombin III complexes, (Mirshahi *et al*., 1989; Weitz *et al*., 1990; Berry *et al*., 1994) is neutralized by low molecular weight thrombin inhibitors such as PPACK (Weitz *et al*., 1990), napsagatran (Gast *et al*., 1994), argatroban (Berry *et al*., 1994) and to a lesser extent by hirudins (Weitz *et al*., 1990; Berry *et al*., 1994). In addition, fibrin clot-bound thrombin retroactivates the coagulation cascade (Kumar *et al*., 1994) and enhances platelet pro-coagulant activity (Kumar *et al*., 1995). Fibrin and plasma clot-bound thrombin can also induce platelet aggregation, which is inhibited by direct thrombin inhibitors such as argatroban, recombinant hirudin and Bothrojaracin, but is resistant to heparin:antithrombin III (Arocas *et al*., 1996; Lunven *et al*., 1996; Gandossi *et al*., 1998).

In the light of the above, we decided to explore whether or not fibrin clot-associated thrombin prepared by incubating human fibrinogen with human thrombin could induce the proliferation of a Chinese hamster lung fibroblast cell line (CCL39 cells), and to explore the role of clot-associated thrombin in such proliferation, using the irreversible thrombin inhibitor PPACK, (Kettner & Shaw, 1979), recombinant hirudin (rHV2 Lys47) and heparin in the presence of antithrombin III. We show that the proliferative effects are due to active thrombin released from clots and are thus neutralized by both antithrombin III-independent and -dependent thrombin inhibitors.

Methods

Drugs and reagents

Heparin (calcium salt), purified human thrombin, fibrinogen and antithrombin III were purchased from Sigma Chemical

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Co. (St. Louis, MO, U.S.A.). Reptilase was obtained from Diagnostica Stago (Asnières, France). The thrombin-specific synthetic chromogenic substrate S-2238 (H-D-Phe-Pip-Arg-pNA) was from Chromogenix (Mölndal, Sweden). (PPACK) was obtained from Calbiochem Corp. (San Diego, CA, U.S.A.). Recombinant hirudin (rHV2 Lys47) was a generous gift of Dr Carolyn Roitsch, Transgène (Strasbourg, France). CCL39 cells were purchased from the American Type Culture Collection (Rockville, MD, U.S.A.). Dulbecco's modified Eagle medium (DMEM)/Nut mix F-12 (HAM), foetal bovine serum (FBS), Phosphate Buffered-Saline (PBS), pH 7.4 trypsin-EDTA, L-glutamine, penicillin and streptomycin were from Life Technologies SARL (Cergy-Pontoise, France). Other reagents were obtained from standard suppliers.

Clot preparation

Fibrin clots were prepared by incubating 4.8 mg ml^{-1} fibrinogen (final concentration, dissolved in 10 mM Hepes buffer, pH 7.4 containing 100 mM NaCl) with varying concentrations of human thrombin (0.01 – 10 NIH Units ml^{-1} or 0.091 – 91 nM, based on the manufacturer's specific activity data) in the presence of 16 mM calcium chloride for 30 min at 37°C in a final volume of 250 μl. The clots were then washed five times with 2 ml PBS to remove thrombin present on the clot surface, and any drops of buffer solution adhering to the surface of the preparations were removed by very brief blotting on filter paper, where care was taken to avoid any clot dehydration. In the case of clots formed with reptilase, fibrinogen was incubated with 2.7 batroxobin Units ml^{-1} reptilase using the same conditions as for thrombin.

In studies using rabbit plasma clots, Male New Zealand White rabbits (3 – 3.5 kg E.S.D., France) were sedated with 8 mg kg^{-1} i.m. chlorpromazine (Largactil, Spécia). Thirty minutes later, animals were anaesthetised with 9 mg kg^{-1} i.v. ketamine (Imalgène, Rhône Mérieux) *via* a marginal ear vein. After anaesthesia was established (loss of corneal reflex), a carotid artery was dissected free from the surrounding tissue and cannulated. Blood (60 ml) was collected *via* the carotid catheter onto 3.8% tri-sodium citrate (9 vol blood : 1 vol citrate) as anticoagulant, and centrifuged at $2500 \times g$ for 10 min at 25°C to obtain platelet poor plasma (PPP). Clots were then prepared by incubating rabbit PPP 16 mM calcium chloride solution (final concentration). The rabbits were then killed by an overdose of anaesthetic.

CCL39 cell culture and proliferation

CCL39 cells derived from Chinese hamster lung fibroblasts were cultured in Dulbecco's minimum essential medium (DMEM/F-12 HAM), 10% foetal bovine serum (FBS), 2 mM L-glutamine, 100 units ml^{-1} penicillin, 100 μg ml^{-1} streptomycin and maintained at 37°C in a humidified atmosphere with 5% CO_2. Cells were centrifuged and seeded in culture medium (10^4 cells ml^{-1}) for 3 days in 75 ml vented flasks. For proliferation assays, cells were trypsinized with 0.05% trypsin/0.02% EDTA (pH 7.4) and were centrifuged at 1200 r.p.m. in growth medium. The pellet was then washed in PBS and cells were maintained in a quiescent state using DMEM/F-12 HAM + 0.2% FBS + antibiotics. Cells were plated (5.10^4 cells ml^{-1}, 1 ml per well) onto 24-well microtitre plates (ATGC), and incubated for 24 h to enable them to adhere to the wells prior to experimentation. Cells were washed once with PBS before stimulation for 48 h by either thrombin in solution or clots (prepared as above and placed directly onto the adherent cells) in a final volume of 1 ml. Thrombin inhibitors were added at the same time as thrombin or clots as appropriate. After 48 h in culture, cells were detached from the wells by trypsin treatment, and harvested cells were counted in a Coulter Z1 cell counter (Coultronics France S.A., Margency, France). The proliferation of CCL39 cells was calculated from the cell count after stimulation, compared to that obtained with cells incubated in quiescent medium alone, and expressed as a percentage.

In some experiments, fibrin clots or thrombin in solution were incubated with cells in quiescent medium for different times (0 – 48 h) during the 48 h incubation period in order to obtain a time course for proliferation. At the end of each designated time, the clots (or thrombin in solution) were removed, the medium changed and the proliferation allowed to continue to the end of the 48 h incubation period. In some cases, clots were separated from the cells during the incubation period using Costar Transwell cell culture chamber inserts. The pore size of the polycarbonate membrane (low protein binding) insert was 3 μm. In these experiments, the Transwells were added to each well containing cells and medium, and the clots were then placed in the Transwells.

Measurement of thrombin release from fibrin clots

Fibrin clots were incubated in 500 μl PBS at 37°C in 24-well microtitre plates for different incubation times (0 – 48 h), and the thrombin present in the incubation medium or remaining associated with the clots was measured using a chromogenic substrate assay with a thrombin calibration curve as follows. At the end of the incubation period, clots or 0.1 ml of supernatant were incubated for 30 min with 200 μM chromogenic synthetic substrate (S-2238) in a final volume of 0.5 ml assay buffer (100 mM tris-buffer, pH 8.5 containing 0.02% bovine serum albumin). Reactions were stopped with 0.1 ml of 50% acetic acid solution. The specific cleavage of S-2238 (H-D-Phe-Pip-Arg-pNa) by thrombin was detected at 405 nm by the release of paranitroaniline. The amidolytic activity was calculated from the ΔOD 405 nm and titrated against known concentrations of human thrombin. The chromogenic assays were performed on an IEMS instrument using Biolise software from Labsystems (Cergy-Pontoise, France). Amidolytic activity was expressed as picomoles of thrombin present on the clot surface or in the incubation medium. The percentage thrombin release from the clots into the incubation medium was evaluated from the initial thrombin concentration used to prepare the clots (91 nM in 250 μl being equivalent to 22.85 picomoles).

Statistical analysis

Values are expressed as the mean $\pm$ s.e.mean of n experiments for each experimental condition. Tests for statistical significance between clot-associated thrombin and thrombin in solution for each drug treatment were performed using Student's t-test for unpaired samples, where a probability value of 5% or less was regarded as being significant. The inhibition of proliferation by thrombin inhibitors is expressed as mean estimated IC_{50} values ($\pm$ s.e.mean) from each individual experiment.

Results

Proliferation of CCL39 cells induced by fibrin clot-associated thrombin

When fibrin clots prepared from human fibrinogen and human thrombin were incubated with CCL39 cells in quiescent medium for 48 h, a marked proliferative response was seen,

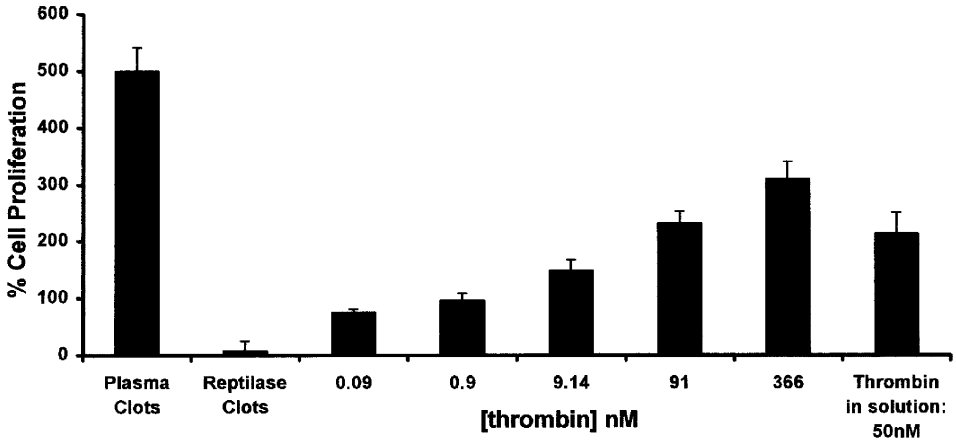

Figure 1 Effects of fibrin clot-associated thrombin on the proliferation of CCL39 cells. Fibrin clots were prepared using different thrombin concentrations, and were incubated with cells for 48 h. Cells incubated with 50 nM thrombin (IIa) in solution and clots prepared from rabbit plasma or reptilase are shown for comparison. The results show the mean ± s.e.mean per cent proliferation compared to cells incubated alone from three (reptilase clots) five (fibrin clots) or nine (plasma clots) separate experiments.

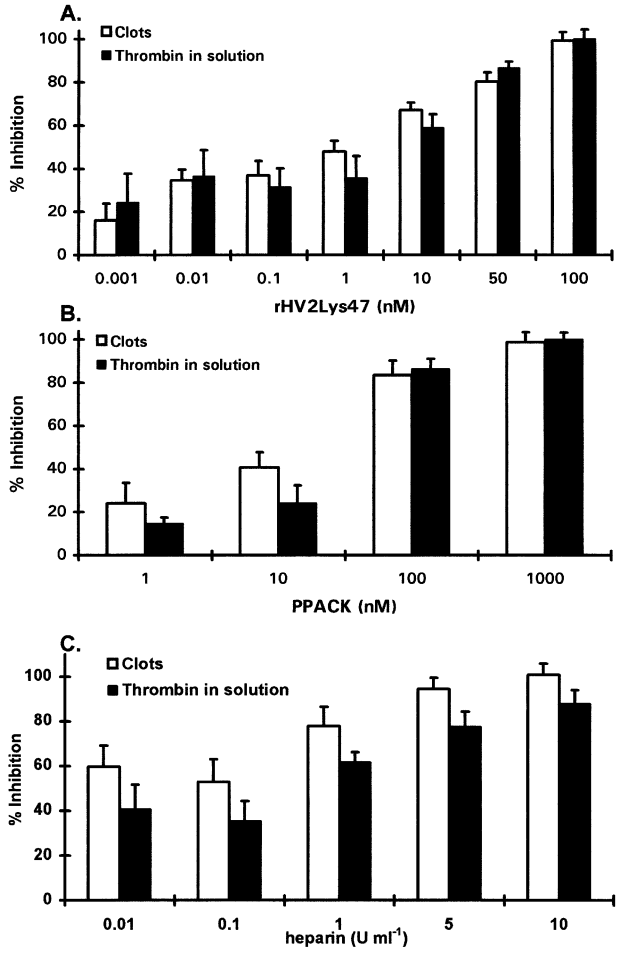

Figure 2 Effects of thrombin inhibitors on the proliferation of CCL39 cells induced by fibrin clots prepared using 91 nM thrombin and induced by 50 nM thrombin in solution. The results are expressed as the mean ± s.e.mean per cent inhibition by rHV2 Lys 47 (A, $n=8$–10), PPACK (B, $n=4$) and heparin in the presence of 20 nM antithrombin III (C, $n=5$).

which was dependent upon the concentration of thrombin used to form the clots (Figure 1). Moreover, clots prepared using 91 nM thrombin were as effective as 50 nM thrombin in solution tested under the same conditions in that the cell number increased from 78,390 ± 13,260 cells ml^{-1} in the absence of stimulation to 241,950 ± 36,030 cells ml^{-1} (mean ± s.e.m., $n=8$) in the presence of fibrin clots, and to 239,360 ± 42,230 cells ml^{-1} (mean ± s.e.m., $n=6$) in the presence of thrombin in solution. These thrombin concentrations were thus chosen for further studies. Figure 1 also shows that fibrin clots prepared from reptilase were without any proliferative effect, whereas rabbit PPP clots led to marked increases in cell counts ($n=9$ experiments).

Effects of thrombin inhibitors

Fibrin clots prepared with 91 nM thrombin or 50 nM thrombin in solution were added to CCL39 cells in culture at the same time as thrombin inhibitors. Cell numbers were determined after a 48 h incubation in quiescent medium as above. The results obtained are summarized in Figure 2. All three thrombin inhibitors were as effective in inhibiting fibrin clot-induced cell proliferation as they were against thrombin in solution. PPACK inhibited clot-induced proliferation with an IC_{50} value estimated to be 33.1 ± 11.7 nM compared to 44.0 ± 7.1 nM against thrombin in solution ($n=4$ experiments). rHV2Lys47 was more potent with estimated IC_{50} values of 3.7 ± 1.1 and 8.9 ± 2.8 nM against proliferation induced by clots ($n=10$) and thrombin in solution ($n=8$) respectively. Heparin in the presence of 20 nM antithrombin III had estimated IC_{50} values of 0.40 ± 0.17 U ml^{-1} vs fibrin clots, ($n=4$) and 0.67 ± 0.16 U ml^{-1} vs thrombin in solution ($n=4$). In addition, antithrombin III alone concentration-dependently inhibited the proliferation of CCL39 cells induced by thrombin in solution and by fibrin clots with IC_{50} values of 72.7 ± 8.2 ($n=3$) and 81.0 ± 7.3 ($n=3$) nM respectively.

In contrast to its ability to inhibit CCL39 cell proliferation induced by thrombin in solution and fibrin clots, PPACK even at 1 μM did not inhibit cell proliferation induced by rabbit plasma clots. In these experiments, control proliferation was 499 ± 41%, compared to 526 ± 53% ($n=9$ clots in each case) in the presence of PPACK.

Thrombin release from fibrin clots

The observation that CCL39 cell proliferation induced by fibrin clots was not resistant to inhibition by antithrombin III

(alone or with heparin) led us to explore the possibility that proliferation was induced by thrombin released from the clots during the incubation period.

In a first series of experiments, fibrin clots prepared from 91 nM thrombin were added to the quiescent medium, and were then incubated for different times (0–48 h) with CCL39 cells as described in the Methods. The cell number was determined at the end of the 48 h incubation period. The degree of proliferation of CCL39 cells was dependent on the incubation time of the clots with the cells as shown in Figure 3 ($n=5$ separate experiments). Maximal responses were observed when cells were maintained in the presence of clots throughout the 48 h incubation period, and was similar to that observed with 50 nM thrombin in solution as before. Nevertheless, a significant, albeit modest, proliferation was observed with clots incubated with cells for 30 min compared to that of cells incubated in the absence of clots or thrombin. This proliferation was also inhibited by 0.1 Units ml^{-1} heparin in the presence of 20 nM antithrombin III (data not shown). Figure 3 also shows that only a short contact time with thrombin in solution is necessary to trigger a significant proliferative response. Indeed, when cells were incubated with 50 nM thrombin for as little as 30 min the proliferative response was already 60% of the response seen when cells were incubated for 24 h in the presence of thrombin prior to changing the incubation medium.

To confirm that the proliferation induced by fibrin clots was due to thrombin release, clots were incubated for 48 h either directly in contact with the cells or separated from the cells by placing them in a Transwell polycarbonate membrane insert. The extent of cell proliferation ($205.2\pm21.2\%$) obtained for clots incubated in the Transwell inserts was not significantly different from that obtained when clots were placed directly in contact with the cells ($255.6\pm25.5\%$, $n=11$ experiments).

Finally, the time course of thrombin release from fibrin clots was measured using a chromogenic substrate. Clots prepared using 91 nM thrombin (i.e. 22.85 picomoles in 250 μl) were incubated for various times up to 48 h at 37°C in PBS prior to measurement of the amidolytic activity of the incubation medium and the clot. Amidolytic activity was

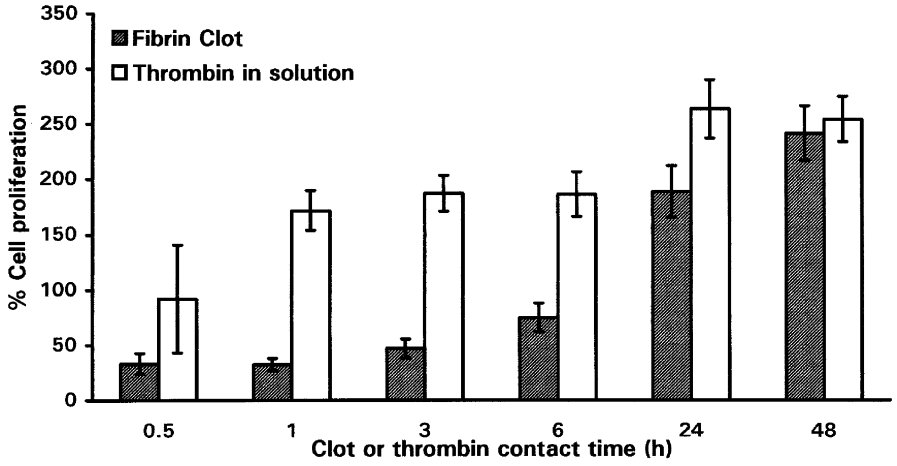

Figure 3 CCL39 cell proliferation induced by fibrin clots or thrombin in solution incubated with the cells for different times throughout the 48 h proliferation period, after which times the clots were removed and the medium changed (thrombin in solution) and cell proliferation was estimated at 48 h. The results are expressed as the mean±s.e.mean per cent proliferation from three (0.5 h) or five separate experiments.

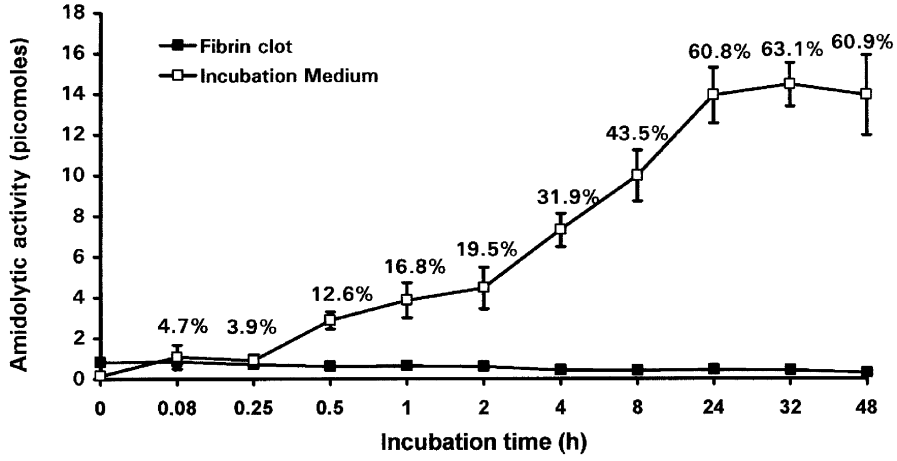

Figure 4 Time course of the amidolytic activity of thrombin on the clot surface and released into the supernatant. Fibrin clots were incubated for different periods and the amidolytic activity of the incubation media and the clots were determined using a synthetic chromogenic substrate (S-2238). The results are expressed as the mean±s.e.mean pmoles of thrombin determined using a thrombin standard curve in each experiment ($n=3$ (5 and 15 min) or five determinations). The figures in parentheses denote the per cent thrombin release into the medium based on the amount of thrombin used to prepare the clots (22.85 picomoles).

detected in the supernatant after only 5 min incubation as shown in Figure 4. By 30 min, the thrombin activity present in the incubation medium was higher than that observed on the fibrin clot surface: 2.9 ± 0.4 and 0.60 ± 0.06 pmoles respectively. Interestingly, the amount of thrombin released at 30 min represented 12.6% of the total thrombin used to prepare the clots, and, as mentioned above, clots incubated with CCL39 cells for the same time presented 13.5% of the maximum proliferative response. Thrombin release increased progressively with time before attaining a plateau between 24 and 48 h. After 32 h the equivalent of 14.4 ± 1.1 pmoles of thrombin were detected in the incubation medium which represented 63.1% (57 nM) of the total thrombin used to form the clots.

Discussion

Clots prepared by incubating human fibrinogen with human thrombin can induce proliferation of CCL39 cells. This effect is dependent on the concentration of thrombin used to prepare the clots, where clots prepared from 91 nM thrombin induced the same degree of proliferation as 50 nM thrombin in solution. The conclusion that this effect was due to thrombin and not simply due to fibrin is reinforced by the observation that fibrin clots prepared with reptilase were without effect, and that the mitogenic effect of the clots was inhibited by the thrombin inhibitors rHV2Lys47 and PPACK as effectively as thrombin in solution. The proliferation of CCL39 cells induced by fibrin clots was inhibited by both antithrombin III alone and by heparin in the presence of 20 nM antithrombin III. This latter result is in complete contrast to previous studies on the pharmacology of clot-associated thrombin, where its amidolytic and platelet activating activity *inter alia* are resistant to heparin/antithrombin III (Mirshahi *et al*., 1989; Weitz *et al*., 1990; Berry *et al*., 1994; Arocas *et al*., 1996; Lunven *et al*., 1996; Gandossi *et al*., 1998) and was at first sight surprising to us. However, in this study, the incubation period of the thrombin with the cells (48 h) was considerably longer than the incubation periods used to study the amidolytic and platelet activating activity of clot associated thrombin (3–20 min, *op. cit*.). Additionally, we have previously shown that when thrombin is used to prepare fibrin clots, less than 1% of the total clot thrombin is present on the clot surface (Berry *et al*., 1994; Lunven *et al*., 1996) which may be sufficient to cleave a chromogenic substrate or aggregate platelets (0.7–3 nM), but there would not be sufficient thrombin on the clot surface to induce proliferation under the experimental conditions used here.

We evaluated the minimum contact time necessary for fibrin clots to induce cell proliferation, and found that if cells were exposed to fibrin clots for 30 min there was a significant increase in cell number at 48 h. This effect was also inhibited by heparin/antithrombin III. The proliferation observed was dependent upon the time during which the fibrin clots were incubated with the cells with proliferation observed at 48 h being the same as that observed with thrombin in solution. It should be noted that the proliferation observed with thrombin in solution incubated with the cells for 1–6 h was markedly greater than that observed with fibrin clots in the same circumstances. The obvious explanation for the above observations would be that thrombin is leaching out of the fibrin clots. This was confirmed by the observation that clot-induced CCL39 cell proliferation was unchanged when the clots were separated from the cells by the use of the Transwell inserts, and that the time course of thrombin release from the clots measured using a chromogenic substrate assay was parallel to that of the CCL39 proliferation induced by the fibrin clots.

Human fibroblasts are able to adhere to fibrin(ogen) both by RGD-dependent and -independent binding sites (Farrell & al-Mondhiry, 1997). In addition, they can migrate into fibrin gel matrices, and their capacity to migrate is enhanced when the fibrin is cross-linked (Brown *et al*., 1993; Greiling & Clark, 1997). Furthermore, it has also been shown that fibrin can stimulate fibroblast proliferation (Ueyama & Kunimoto, 1978; Kasai *et al*., 1983; Sporn *et al*., 1995; Shats *et al*., 1997). However, none of these studies specifically addressed the question of the role of any residual thrombin on such proliferation. Only Sporn *et al*. (1995) appeared to be aware of the possibility that residual thrombin may induce a proliferative response, since they performed their study in the presence of an excess of PPACK, thus no attempt to quantify the effect of clot-associated thrombin on fibrin-induced fibroblast proliferation was made. Moreover, Shats *et al*. (1997) only washed their fibrin networks five times as we did. Thus, to our knowledge, this is the first study where a systematic attempt has been made to explore the mitogenic activity of clot-associated thrombin using CCL39 cells as an example. Indeed our results suggest that studies on the fibrin-induced proliferation of fibroblasts and other cell types expressing thrombin receptors which pre-date the findings that clots can be reservoirs of enzymatically active thrombin should perhaps be regarded in a different light, especially since we show that in our experimental system thrombin inhibitors are capable of totally inhibiting fibrin clot-induced proliferation.

High levels of thrombin may be generated at sites of injury, and thrombin molecules trapped in mural or extravascular thrombi could gradually be released during clot retraction and fibrinolysis. It should be noted that α-thrombin undergoes autocatalysis to β- and subsequently γ-thrombin with concomitant loss of fibrinogenolytic activity (Fenton *et al*., 1991), and our results imply therefore that the association of thrombin into a fibrin clot appears to stabilize the protease, although this would require experimental confirmation. Thus the fibrin clot could be considered to be an important reservoir of mitogenic thrombin, which may play role in the formation of granulation tissue and restenosis. Indeed, certain studies have shown that treatment with r-hirudin or PPACK reduces restenosis after balloon catheter angioplasty in rabbit models (Sarembock *et al*., 1991; Gimple *et al*., 1992; Walters *et al*., 1994). Prolonged treatment with r-hirudin (14 days) or with recombinant tick anticoagulant peptide (which inhibits thrombin generation by blocking Factor Xa, 5 days treatment) reduced luminal narrowing in a porcine model of balloon angioplasty (Gallo *et al*., 1998; Schwartz *et al*., 1996). However, care should be taken in extrapolating data obtained *in vitro* in a purified system to a complex *in vivo* setting as discussed in the next paragraph.

Clots prepared from plasma induced a marked proliferative response which was greater even than that observed by fibrin clots prepared with 366 nmol l^{-1} thrombin. However, this proliferation was not inhibited by PPACK at a final concentration of 1 μM, which was the threshold concentration capable of causing total inhibition of proliferation induced by fibrin clots ($IC_{50} = 33$ nM). This inability by PPACK to inhibit plasma clot induced CCL39 cell proliferation is almost certainly due to the presence of other mitogens present in the clots, since it has previously been shown that rHV2 Lys47 inhibited thrombin-induced, but not serum-induced mitogenesis (Bar-Shavit *et al*., 1992). It is perfectly plausible that

plasma clots undergoing minimal washing and blotting would contain mitogens also present in serum. Indeed, this result is also consistent with those of the HELVETICA study using hirudin in the prevention of restenosis, which were disappointing (Serruys *et al*., 1995), whereas platelet GPIIb/IIIa inhibitors have been shown to be effective (see Tcheng, 1997 for review). However, it could also be argued that the amount of thrombin present in the plasma clots exceeds that in fibrin clots to such an extent that much higher concentrations of inhibitor would be required. This merits further investigation.

In conclusion, we have shown that fibrin and plasma clots have mitogenic activity, and that these clots could act as a vector for thrombin or other mitogens (in the case of plasma clots), in addition to the mitogenic activities of the fibrin itself.

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(Received April 7, 1999
Revised August 16, 1999
Accepted December 7, 1999)