HUMAN PLASMA FIBRONECTIN PROMOTES THE ADHESION AND SPREADING OF PLATELETS ON SURFACES COATED WITH FIBRILLAR COLLAGEN

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1. Introduction

Fibronectin is a high molecular weight glycoprotein present on cell surfaces, in tissues, blood, and other physiological fluids (reviews [1–4]). The cellular form of fibronectin plays an important role in cell–cell and cell–substrate interactions [1–4]. Fibronectin forms complexes with collagen, fibrin, heparin and glucosaminoglycans [1–4]. Plasma fibronectin is of special interest. Although the concentration of this protein is high in plasma (0.3 mg/ml), its biological significance is not yet clear. It has been suggested that fibronectin from plasma probably mediates the opsonin-like function of the reticuloendothelial system [5,6].

Attempts have been made to elucidate the role of fibronectin in the interaction of platelets with fibrillar collagen, the adhesive component of damaged vessel walls [7–10]. In [7], platelet fibronectin was proposed as the collagen receptor on the platelet cell membrane. In [9] it was concluded that fibronectin has only a limited role in the adhesion of platelets to collagen. However, the effect of plasma fibronectin on the adhesion of platelets to fibrillar collagen has not yet been the object of any direct investigation.

We present here a study of the role of plasma fibronectin in the adhesion of platelets to a surface coated with fibrillar collagen. We show that fibronectin at physiological concentrations stimulates the level of total platelet adhesion and their spreading on the collagen-coated surface.

2. Materials and methods

Fibronectin from human plasma was prepared by gelatin–Sepharose affinity chromatography with subsequent chromatography on Whatman DE-52 DEAE-cellulose [11,12]. The homogeneity of the fibronectin preparation was assessed electrophoretically (see fig.1). The purity of the protein was ⩾96–98%. The fibronectin was also completely readsorbed on gelatin–Sepharose.

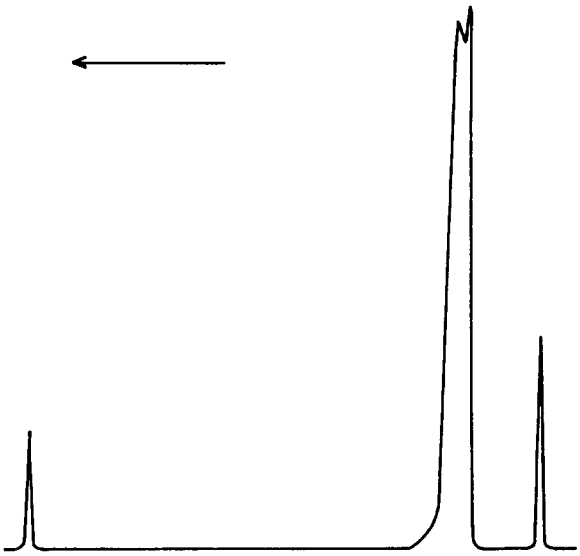

Fig.1. SDS–polyacrylamide gel electrophoresis of the fibronectin preparation. Densitogram of the 5–15% polyacrylamide gel gradient. Fibronectin migrates as a characteristic doublet with M_r 220 000–240 000.

Platelets from human plasma were isolated by gel-filtration on Sepharose 2B [13]. The labeling of platelets with $Na_2{}^{51}CrO_4$ (100–400 μCi/mg chromium, Amersham) and their interaction with the collagen-coated surface was performed as in [14]. The bottom of 16.4 mm wells of the multiwell tissue culture plates (Falcon) were coated with fibrillar collagen (type 1 from calf skin; Sigma C-3511) [14]. A 0.15 ml sample of the gel-filtered platelet suspension ($1.5–2.5 \times 10^7$

cells) was added to the wells in Tyrode solution, containing 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.2 mg apyrase/ml (Sigma, A-6132) and 3.5 mg/bovine albumin/ml (Sigma, A-4503). The incubation was at 37°C for 40 min by rotating the multiwell plate in a horizontal shaker at 36 rev./min. The non-adherent platelets were removed and the wells were washed with 0.2 ml Tyrode solution. The number of adherent platelets was determined by the radioactivity adsorbed at the bottom or by scanning electron microscopy [14].

In the experiments with fibronectin, 0.15 ml of the protein at the required concentration in Tyrode solution was added to the wells, rotated for 90 min at 36 rev./min at 37°C, then 0.15 ml of the platelet suspension added. When necessary the excess of fibronectin was removed and the wells were washed 3 times with a 1.5 ml Tyrode solution. Then 0.15 ml Tyrode solution and 0.15 ml platelet suspension were added.

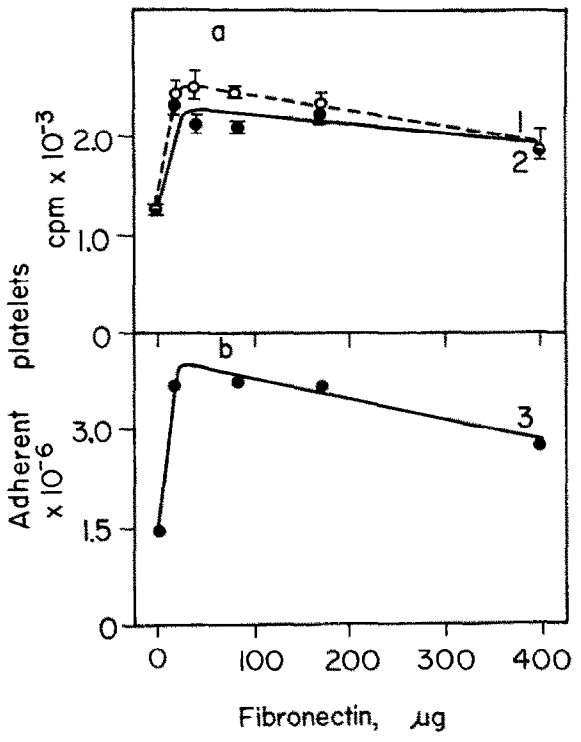

Fig.2. Fibronectin dose–response of platelet adhesion to the surface coated with fibrillar collagen. ^{51}Cr-labeled platelets (1.8×10^7 cells) were added to the collagen-coated wells and platelet adhesion was determined by radioactivity (a) and by scanning electron microscopy (b): (1) free fibronectin was present in the incubation mixture; (2) fibronectin was removed from the incubation mixture; (3) as in (2).

Table 1
Effect of plasma fibronectin on the total adhesion and shape change of adherent platelets on a surface coated with fibrillar collagen

Surface	n	Adherent platelets		
		$\times 10^{-3}$, mm^{-2}	Spread (%)	Nonspread (%)
Collagen	6	4.65 ± 0.94	12.9 ± 2.4	87.1 ± 2.4
Collagen treated with fibronection	7	11.47 ± 1.83[a]	42.2 ± 3.1[b]	57.8 ± 3.1[b]

[a] $p < 0.01$; [b] $p < 0.001$

Collagen-coated wells were treated with Tyrode solution or fibronectin (50–400 μg/well) in Tyrode solution. Non-bound fibronectin was removed and a platelet suspension ($1.8–2.2 \times 10^7$ cells) was added to the wells. Adhesion was measured by scanning electron microscopy. From 200–500 platelets were counted in each experiment. Mean values ± standard errors are shown. The statistical significance of the differences between means was determined using Student's t-test

3. Results and discussion

Fig.2 represents the fibronectin dose–response curve for platelet adhesion to a surface coated with fibrillar collagen. Two versions of the experiments were done: the collagen-coated surface was pretreated with fibronectin and, before addition of platelets, the protein was either removed or remained present in the incubation mixture. It is seen (fig.2a) that independent of the procedure used, plasma fibronectin stimulated the adhesion of the ^{51}Cr-labeled platelets to collagen by 2 or 3 times. A similar effect is observed when the platelet adhesion was determined by scanning electron microscopy (fig.2b, table 1). The results obtained indicate that the effect of fibronectin consists in the promotion of platelet adhesion to the collagen-coated surface (conditioning of the collagen substratum) and does not depend on the presence of exogeneous fibronectin in the incubation mixture. It is known that in the process of adhesion the shape of adherent platelets is drastically changed [15]. After contact with collagen, discoid (native) platelets are transformed into discs and spheres with pseudopods which are then spread over the collagen-coated surface (fig.3, upper row) [14]. That is why the morphological study was made on the effect of plasma fibronec-

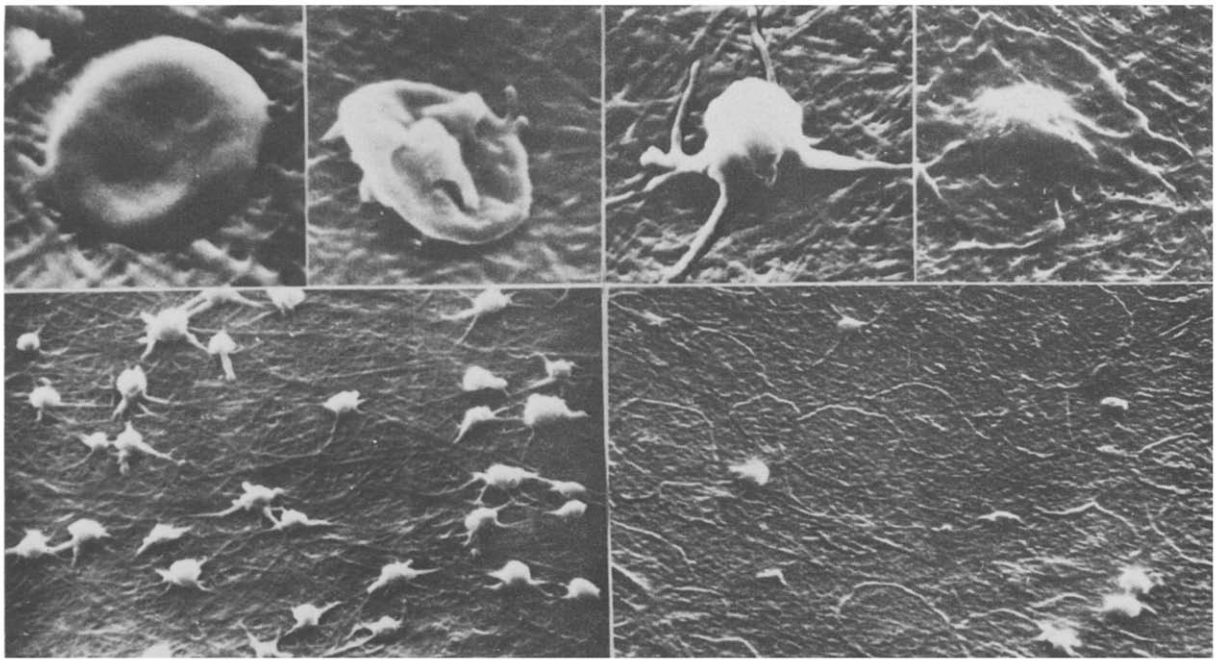

Fig.3. Scanning electron micrographs of platelets adherent to the fibrillar collagen. Upper row: main form of adherent platelets (left–right): disc (×11 789); disc with ruffles and pseudopods (×91 636); sphere with pseudopods (×5894); spread platelet (×4582). Bottom row: platelets adherent to collagen (left, ×1768) and to collagen treated with 100 μg fibronectin (right, ×1374); free fibronectin was removed.

tin on the shape of the platelets adherent to the collagen-coated surface (fig.3 bottom row, fig.4 and table 1). It is seen that plasma fibronectin promotes significantly the spreading of adherent platelets.

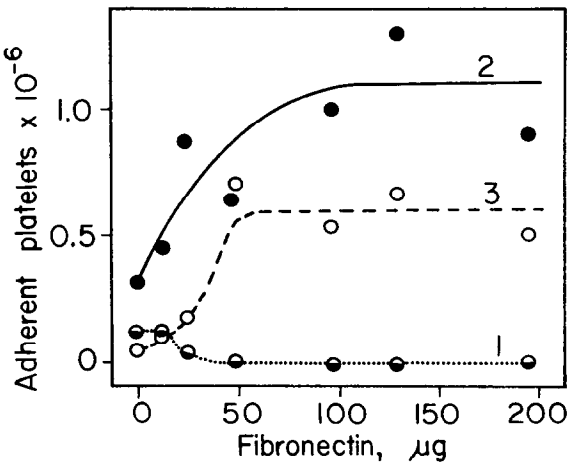

Fig.4. Effect of fibronectin on the shape of adherent platelets: (1) discs; (2) discs and spheres with pseudopods; (3) spread platelets. The other conditions were as in fig.2b.

4. Conclusion

This letter reports that human plasma fibronectin stimulates the interaction of platelets with fibrillar collagen (cell adhesion) and also dramatically changes the shape of adherent platelets, promoting their spreading.

The stimulating effect of fibronectin is due to its interaction with collagen fibrils since free fibronectin does not influence the adhesion or spreading of platelets.

The results obtained suggest a new physiological function for plasma fibronectin in the control of platelets interaction with damaged vessel walls.

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