Fibronectin as an opsonic regulator of monocyte phagocytosis

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SUMMARY Experiments using human monocytes and the yeast *Saccharomyces cerevisiae* have shown that fibronectin is a major plasma opsonin. Further studies have shown that fibronectin promotes the ingestion as well as the adherence of micro-organisms. These observations are independent of the non-physiological concentrations of heparin used in other assay systems.

Evidence from rat liver slice and macrophage monolayer assays suggests that plasma fibronectin is a major opsonic regulator of fixed macrophage function.¹⁻⁴ These assays, however, use target particles coated with gelatin and depend to a varying degree on the presence of heparin. We have recently developed an opsonin assay which utilises human peripheral blood monocytes and the micro-organism *Saccharomyces cerevisiae*. We have used this assay to examine the effect of plasma fibronectin on the phagocytosis of micro-organisms by human monocytes.

Material and methods

Human plasma was obtained from blood drawn into 3.13% wt/vol trisodium citrate, and plasma fibronectin was prepared by affinity chromatography on gelatin Sepharose and arginine Sepharose.⁵ Opsonic activity was assayed by electronic particle size analysis of human monocytes and the yeast *S. cerevisiae* as previously described.⁶ In addition, ingested and adherent yeasts were visually distinguished by staining with toluidine blue and Procion rubine MX dye. With this technique, ingested and adherent yeasts are coloured red and blue respectively.⁷ Heparin was not added to either of these two assays.

Fibronectin free plasma was obtained by applying 10 ml citrated plasma to a 10 ml column of gelatin Sepharose 4B, equilibrated with 50 mM Tris HCl pH 7.5. The eluate was concentrated by ultrafiltration to its original volume using fibrinogen concentration as a guide.⁸ Fibronectin antigen concentrations were assayed by immunoelectrophoresis using a monospecific polyclonal sheep antihuman

fibronectin antibody.⁹ In order to inhibit activation of the C₃ component of complement, dipotassium edetic acid was added to plasma to a final concentration of 20 mM. Purified human IgG (14g/l) was obtained from the Immuno Diagnostic Research Laboratory at Birmingham University.

The statistical significance of differences between mean values was assessed by Student's *t* test.

Results

Fibronectin concentrations in citrated plasma from 30 normal healthy subjects gave a range (mean $\pm$ 2 SD) of 254-501 mg/l. In all of the following experiments fibronectin was used in concentrations which did not exceed the range in normal plasma.

Fig. 1 shows the stimulation of phagocytosis by increasing concentrations of purified fibronectin and

Fig. 1 Monocyte uptake of yeasts after opsonisation with increasing concentrations of fibronectin or whole plasma.

Fig 2 Percentage of yeasts ingested or adhering to monocytes after opsonisation with increasing concentrations of purified fibronectin. Mean value $\pm$ 1 SEM for each concentration is shown. Hatched areas represent the adherent yeasts which have not yet been ingested.

whole plasma. A dose response was seen in both cases and the mean values for opsonisation at each fibronectin concentration were not significantly different ($p > 0.1$).

Fig. 2 shows a visual assessment of yeast adherence and ingestion in response to increasing fibronectin concentrations. In the absence of fibronectin no significant adhesion or ingestion of yeasts was observed, but these were considerably stimulated by increasing concentrations of fibronectin. These results show clearly that higher fibronectin concentrations stimulate the ingestion of a higher proportion of adherent organisms.

The relative importance of fibronectin as an opsonin is shown in Fig. 3. Fibronectin accounted for 60% of the opsonic activity of citrated plasma, and most of the activity in fibronectin free plasma was due to IgG. When purified IgG was used with fibronectin as an opsonin the combined effect appeared additive and no synergy was observed. Inhibition of complement with 20 mM edetic acid reduced opsonic activity by only 10%. A fibronectin free preparation of human albumin (35 g/l) was used to examine non-specific protein effects and this showed no opsonic activity.

Discussion

The uptake of *S cerevisiae* opsonised with purified fibronectin or whole plasma showed a clear dose response. The experiments with dyed yeast showed that purified fibronectin promoted the ingestion of

Fig. 3 Opsonic effect of purified fibronectin (FN), whole citrated plasma, fibronectin free plasma, purified IgG, and EDTA plasma. Plasma samples from the same donor pool and purified fibronectin were used at a concentration of 400 mg/l. No fibronectin could be detected in fibronectin free plasma samples or in the purified IgG. Opsonisation is shown as a percentage of the activity shown by citrated plasma, and values are indicated as a mean $\pm$ 1 SEM.

micro-organisms by monocytes in addition to its effect as an adhesion promoter. These findings contrast with those obtained with gelatinised target particles and monocyte monolayers, in which adhesion but not the subsequent ingestion of particles could be demonstrated.¹⁰

Since fibronectin does not bind to certain Gram negative organisms and in some assay systems has not promoted the ingestion of particles by human monocytes, it has been suggested that fibronectin may not be an important opsonin.¹¹ Our own studies suggest, however, that it accounts for most of the non-specific opsonic activity of plasma. The absence of opsonic activity in fibronectin free albumin excluded the possibility of non-specific protein effects.

Heparin is essential for the uptake of gelatinised target particles in the rat liver slice assay.³ A rat peritoneal macrophage assay is also, to some extent,

heparin dependent.⁴ Our own experiments with human monocytes were conducted without heparin present, but we have found that the opsonic activity of plasma was enhanced by heparin. The activity of purified fibronectin was greatly reduced, however, and this is the subject of further investigations.

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