Fibronectin binding to complement subcomponent C1q

Localization of their respective binding sites

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(Received 24 August 1984/Accepted 16 October 1984)

The interaction of purified human plasma fibronectin with the C1q subcomponent of complement was investigated by using a solid-phase radiobinding assay. ^{125}I -fibronectin binding to native C1q, purified collagen domain (C1q-c) or globular domain (C1q-g) was compared. When the purified domains were insolubilized by binding to plastic, the C1q-c exhibited 59% of the binding demonstrated with intact C1q, whereas the C1q-g exhibited 35% of the binding. *N*-Terminal sequencing of the globular domain showed that a sequence of seven collagen-like amino acids was retained on each chain of the C1q-g fragment. ^{125}I -fibronectin binding to C1q could be inhibited equally well by fluid-phase C1q and C1q-c, but not by fluid-phase C1q-g, implying that the collagen-like region retained on the C1q-g is masked in the fluid phase. In addition, studies were performed to determine which subunit(s) of C1q bind(s) fibronectin. The percentages of fibronectin bound by the A, B, and C chain of C1q were found to be 38, 21 and 41% respectively. Inhibition studies with purified 200–180 kDa, 50 kDa or 29 kDa fragments of fibronectin show that the binding site on fibronectin for C1q is the 50 kDa gelatin-binding domain.

Fibronectin is a high-*M*_r glycoprotein that exists *in vivo* in two different forms which are structurally and immunologically similar (Ruoslahti & Vaheri, 1975). Plasma fibronectin circulates as a dimer composed of two 220 kDa disulphide-linked monomers, whereas cell-surface fibronectin exists as large aggregates or multimers formed by non-covalent and covalent interactions. Fibronectin binds to a number of different ligands, including gelatin, collagen and collagen-derived fragments, fibrin and fibrinogen, heparin and proteoglycans, as well as cells and bacteria (reviewed by Pearlstein *et al.*, 1980; Mosher, 1980; Hynes & Yamada, 1982). Recently, fibronectin has been shown to interact with two other proteins that contain a collagen-like sequence, acetylcholinesterase (Emmerling *et al.*, 1981) and C1q (Menzel *et al.*, 1981;

Harris *et al.*, 1981; Isliker *et al.* 1981, 1982; Pearlstein *et al.*, 1982; Bing *et al.*, 1982; Sorvillo *et al.*, 1983; Ingham *et al.*, 1983; Kono *et al.*, 1983).

C1 is the first component of the classical pathway of complement and circulates in normal plasma as a complex composed of three subunits, C1q, C1r₂ and C1s₂, which are linked by Ca^{2+} (Porter & Reid, 1978). Subcomponent C1q is composed of three different polypeptide chains (A, B, C chains), which associate through covalent and non-covalent interactions. C1q is an asymmetric molecule composed of two distinct domains. The collagen-like domain is located at the *N*-terminus of the molecule and binds the C1r and C1s subunits. The C-terminus of C1q is composed of six compact globular 'heads' which contain the binding sites for immunoglobulins and other activators of the classical complement pathway. On binding and activation of C1, C1r and C1s are dissociated from C1q by the regulatory protein C1 inhibitor, ultimately unmasking the collagen-like region of C1q (Porter & Reid, 1979).

We, as well as others, have previously demonstrated a direct interaction of fibronectin with C1q (Menzel *et al.*, 1981; Harris *et al.*, 1981; Pearlstein

Abbreviations used: C1q, subcomponent of the first component of complement; C1q-c, collagen-like domain of C1q; C1q-g, globular domain of C1q; SDS, sodium dodecyl sulphate; HRP-Fn and HRP-50 kDa, horseradish peroxidase conjugated to intact fibronectin or 50 kDa gelatin-binding domain respectively.

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et al., 1982; Isliker *et al.*, 1982; Bing *et al.*, 1982; Sorvillo *et al.*, 1983; Ingham *et al.*, 1983; Kono *et al.*, 1983). Although the collagen-like region has been shown to be the binding site on C1q for fibronectin (Menzel *et al.*, 1981; Isliker *et al.*, 1982; Bing *et al.*, 1982), other evidence suggests that fibronectin binding may also occur elsewhere on the C1q molecule (Harris *et al.*, 1981; Bing *et al.*, 1981; Pearlstein *et al.*, 1982). In the present paper the binding of fibronectin to intact C1q was compared with its binding to purified collagen or globular domain of C1q. These studies suggest that the binding site for fibronectin on the C1q molecule is near the collagen/globular junction. In addition, we show that fibronectin interacts with all three subunits of C1q (A, B and C chains) with different efficiency. We also show that the C1q-binding site on fibronectin is the 50kDa gelatin-binding domain.

Materials and methods

Purification of Fibronectin and C1q

Fibronectin was purified from pooled human plasma by affinity chromatography on gelatin-Sepharose 4B columns (Engvall & Ruoslahti, 1977). Purified fibronectin contained no detectable C1q as determined by SDS/polyacrylamide-gel electrophoresis and Ouchterlony analysis.

C1q was isolated from normal human serum by ion-exchange chromatography using Bio-Rex-70 (Bio-Rad, Richmond, CA, U.S.A.) and gel filtration on Bio-Gel A-5M (Bio-Rad) as described by Tenner *et al.* (1981). The C1q was adsorbed with gelatin-Sepharose (1ml of resin/5mg of protein) before use and contained no detectable fibronectin as determined by SDS/polyacrylamide-gel electrophoresis and Ouchterlony analysis. Gelatin (type 1 from swine skin) was purchased from Sigma Chemical Co.

Purification of C1q and fibronectin domains

The globular portion of C1q (C1q-g) was prepared by digestion with bacterial collagenase (Worthington) and isolated by the method of Hughes-Jones & Gardner (1979). The collagen portion of C1q (C1q-c) was obtained by pepsin digestion (Worthington) and purified by gel filtration as described by Reid (1976). The purity of both C1q-g and C1q-c was assessed by SDS/polyacrylamide-gel electrophoresis (Fig. 1a) and amino acid analysis.

The 50kDa gelatin-binding domain of fibronectin was generously given by Dr. L. Gold (Gold *et al.*, 1979). The 29kDa N-terminal domain of fibronectin and the 200–180kDa fragment comprising the remaining portion of the fibronectin molecule minus the extreme C-terminal region

were kindly provided by Dr. A. Garcia-Pardo (Garcia-Pardo *et al.*, 1983). Fig. 1(b) shows SDS/polyacrylamide-gel electrophoresis of fibronectin and its fragments.

Iodination of fibronectin

Fibronectin was ^{125}I -labelled by solid-phase lactoperoxidase-catalysed iodination as previously described (Pearlstein & Baez, 1981). The specific radioactivities after different iodinations were usually in the range 10^6 – 10^7 c.p.m./ μg of protein.

Iodination of C1q, C1q-g and C1q-c

C1q, C1q-g and C1q-c were labelled with Bolton-Hunter reagent as described by Tenner *et al.* (1981) and used to determine the amount of protein adsorbed to the plastic.

Binding assay

Microtitre wells (Dynatech Corp.) were incubated with 50 μl of C1q, gelatin, C1q-c or C1q-g (50 $\mu\text{g}/\text{ml}$) in 0.05M-Tris/acetate, pH 7.2, for 2 h at 37°C. The wells were washed three times in 0.05M-Tris/acetate, pH 7.2, containing 2.0mg of bovine serum albumin/ml and incubated with bovine serum albumin/Tris/acetate buffer for 30 min at 37°C to block non-specific sites on the plastic. The fluid was decanted and 50 μl of ^{125}I -fibronectin (3.7×10^4 c.p.m.) in bovine serum albumin/Tris/acetate was added to each well. The plate was incubated for 1 h at 37°C, washed three times, and the wells were cut from the plastic plates and placed in 12mm $\times$ 75mm plastic tubes for determination of radioactivity in a well-type scintillation counter (TM Analytic). In the inhibition studies, a constant amount of ^{125}I -fibronectin was diluted in unlabelled inhibitor before addition to the solid-phase C1q, C1q-c or C1q-g.

Amino acid analysis

The proteins to be analysed were dissolved in 0.2ml of 6M-HCl and hydrolysed at 110°C for 24 h. Phenol was added to the HCl [40 μl of an aq. 10% (v/v) solution] to prevent degradation of tyrosine. Amino acid compositions of hydrolysed peptides were determined in a Durrum D-500 amino acid analyser.

Automated sequence determination

C1q-g was reduced and alkylated as described by Reid (1976) and the N-terminal sequence was obtained by use of a Beckman 890C sequence analyser equipped with a P-6 Sequemat Auto-converter. Phenylthiohydantoin derivatives were identified directly by h.p.l.c. (Waters Associates, model 440) and amino acid analysis after hydro-

Fig. 1. SDS/polyacrylamide-gel electrophoresis of C1q, C1q-c and C1q-g and of fibronectin and its fragments (a) SDS/12.5%-(w/v)-polyacrylamide-gel electrophoresis: lane 1, C1q; lane 2, C1q-g; lane 3, C1q-c. Samples were unreduced. (b) SDS/5-15%-(w/v)-polyacrylamide-gradient-gel electrophoresis of fibronectin and purified fragments: lane 1, intact fibronectin; lane 2, 200-180kDa fragment; lane 3, 50kDa gelatin-binding fragment; lane 4, N-terminal 29kDa fragment. Samples were reduced with 100mM-dithiothreitol.

lysis *in vacuo* with 6M-HCl and 1% stannous chloride for 24h at 110°C.

SDS/polyacrylamide-gel electrophoresis

SDS/polyacrylamide-gel electrophoresis of all proteins was performed in slab gels as described by Laemmli (1970). M_r values were determined by comparison with known standards (Pharmacia).

Detection of proteins by immunoblotting

Electrophoretic transfer of proteins was performed, by the method of Towbin *et al.* (1979), with a Trans Blot cell (Bio-Rad Laboratories). After electrophoretic transfer, the nitrocellulose filters were incubated in phosphate-buffered saline (4mM-KH₂PO₄/16mM-Na₂HPO₄/115mM-NaCl, pH7.3) containing 0.05% Tween 20 (Sigma) for 2h at 37°C. The filters were subsequently incubated with ¹²⁵I-fibronectin, HRP-Fn or HRP-50kDa for 2h at 37°C. Proteins were conjugated to horseradish peroxidase by the method of Bell & Engvall (1982). After the filters had been washed with phosphate-buffered saline/Tween, the filters probed with ¹²⁵I-fibronectin were dried and exposed to Kodak X-Omat AR film for 2-6 days.

Filters probed with horseradish peroxidase-conjugated proteins were developed with horseradish peroxidase developing reagent (Bio-Rad) according to the manufacturer's instructions.

Protein determination

Protein concentrations were determined with Bio-Rad protein dye as reagent and bovine serum albumin as a standard.

Results

Fluid-phase inhibition of ¹²⁵I-fibronectin binding to C1q by C1q-c and C1q-g

It has fairly recently been demonstrated that the collagen-like region of C1q can competitively inhibit the binding of ¹²⁵I-C1q to solid-phase fibronectin, whereas the globular region of C1q had no inhibitory effect (Bing *et al.*, 1982). To determine if purified fluid-phase C1q-c or C1q-g could inhibit fibronectin binding to solid-phase C1q, ¹²⁵I-fibronectin was preincubated with increasing concentrations of unlabelled C1q-c or C1q-g for 30min at 37°C before addition to solid-phase C1q. The results in Fig. 2 show that fluid-

Fig. 2. Fluid-phase inhibition of ^{125}I -fibronectin binding to C1q by collagen and globular domain of C1q

The binding assay was performed as described in the Materials and methods section. ^{125}I -fibronectin was preincubated with C1q (●), C1q-c (▲) or C1q-g (■) and then incubated with solid-phase C1q for 1 h at 37°C . The results are expressed as the percentage of fibronectin bound to solid-phase C1q in the presence of increasing concentrations of inhibitor.

phase C1q-g did not inhibit fibronectin binding to C1q. In contrast, both fluid-phase C1q-c as well as intact C1q inhibited fibronectin binding equally well.

Binding of ^{125}I -fibronectin to C1q, purified C1q-c and purified C1q-g

The direct binding of fibronectin to intact C1q, purified C1q-c or purified C1q-g can be demonstrated. Microtitre wells were incubated with $50\mu\text{l}$ of C1q, C1q-c or C1q-g at $50\mu\text{g/ml}$ and tested for ^{125}I -fibronectin binding as described in the Materials and methods section. A representative experiment is shown in Table 1. Under the assay conditions used, fibronectin bound to intact C1q, the C1q-c and C1q-g domains (Table 1). The domains contained partial binding activity when compared with the intact C1q molecule; the C1q-c and C1q-g domains exhibited 59 and 35% binding activity respectively of the total binding of fibronectin to intact C1q. This result may be attributed to one of two possibilities: either the globular domain contains a distinct binding site for fibronectin, or collagen-like sequences are still present on the C1q-g preparation. The latter explanation may be more likely, since Reid (1974) has shown that, after limited digestion of the A chain of C1q with collagenase for 8 h, a residual collagen-like sequence of 16 amino acids is still retained on the globular moiety of the molecule. Therefore *N*-terminal-sequence analysis was performed on our preparation of the C1q-g domain as described in the Materials and methods section. The results shown in Fig. 3 demonstrate that a stretch of seven collagen-like amino acid residues on each chain of

Table 1. Direct binding of ^{125}I -fibronectin to purified C1q, C1q-c and C1q-g

Microtitre well coated with*	Bound radioactivity (c.p.m.)†
C1q	5720 ± 35
C1q-c	3385 ± 212
C1q-g	2009 ± 158
BSA	352 ± 17

* By using ^{125}I -labelled C1q, C1q-c or C1q-g it was determined that there were no differences between the amount of protein adsorbed to the plastic for these ligands.

† Results are averages ($\pm$ S.E.M.) of triplicate determinations in a representative experiment.

	81		88
A chain	Gly-	-*	Pro-Gly-Ser-Pro-Gly-Asn
B chain	Gly-Pro-	*Lys-Gly-Glu-Ser-Gly-Asp	
C chain	Gly-Glu-	Pro-Gly-	-Glu-Gly-Arg

Fig. 3. *N*-Terminal amino acid sequence of C1q-g domain. Numbering and alignment of residues is in accord with that given by Reid (1974). * indicates an hydroxylated residue. '-' indicates an unidentified residue.

C1q was still retained on the C1q-g domain. This result, along with the ineffectiveness of the fluid-phase globular domain to inhibit fibronectin binding to intact C1q, suggests that the collagen sequences are not available for fibronectin binding in the fluid phase, but become exposed on attachment of the C1q-g to a surface (Table 1, Fig. 2). Attempts to remove the collagen residues by increasing enzyme/substrate ratios or incubation time failed to remove the sequences.

Detection of the collagen-like sequences on C1q-g with the blotting technique

In order to verify that the collagen-like sequences described above were present on our C1q-g domains, we employed a method to detect collagenous proteins described by Bell & Engvall (1982). This method has been shown to detect collagenous proteins in a mixture of heterogeneous proteins. The proteins to be tested are subjected to SDS/polyacrylamide-gel electrophoresis, transferred to nitrocellulose, and the collagenous proteins are detected by staining the filters with the purified gelatin-binding domain of HRP-50kDa. It is specific for all known types of collagens and does not react with non-collagenous proteins, with one exception: it reacts slightly with carbonic anhydrase, one of the M_r marker proteins (Bell & Engvall, 1982).

C1q, C1q-g, C1q-c or markers were subjected to SDS/polyacrylamide-gel electrophoresis, transferred to nitrocellulose and the filters were probed with HRP-50kDa. The results in Fig. 4 demonstrate that the HRP-50kDa probe reacted with the collagen-like sequences of intact C1q, as well as those in the C1q-g and C1q-c.

Determination of the C1q subunit(s) that bind(s) fibronectin

Isliker *et al.* (1982) previously suggested that the binding of C1q by fibronectin is mediated by the A chain of C1q. The assay compared the amount of A chain or mixture of A, B and C chains that was

bound by solid-phase fibronectin when an anti-C1q antibody enzyme-linked immunosorbent assay was used. It was found that the A chain displayed the same fibronectin-binding capacity as an equimolar mixture of the A, B and C chains. Because this result could have measured differences in binding of the anti-C1q antibody to the individual chains of C1q rather than which chain of C1q actually binds fibronectin, we also investigated which chain mediates fibronectin binding by using a more direct approach.

C1q was subjected to SDS/polyacrylamide-gel electrophoresis; the proteins were transferred to nitrocellulose filters and processed as described in the Materials and methods section. The filters were then probed with HRP-Fn, HRP-50kDa or 125 I-fibronectin. The results are shown in Fig. 5. Lane 1 shows the filter stained with Amido Black; lanes 2, 3, 4 are filters probed with HRP-Fn, HRP-50kDa and 125 I-fibronectin respectively.

Identical results were obtained with three different probes, i.e., all three chains of C1q bound fibronectin with apparently different efficiencies. Both the C and A chains of C1q bound fibronectin equally well, whereas the B chain bound much less than either the C or A chain. In order to quantify

Fig. 4. Detection of collagen-like sequences on C1q, C1q-g and C1q-c

Lane 1, M_r markers; lane 2, intact C1q; lane 3, C1q-g; lane 4, C1q-c. Equal amounts of C1q-g and C1q-c were applied to the gel. The filter was probed with HRP-50 kDa.

Fig. 5. Binding of fibronectin to the A, B and C chains of C1q

Reduced C1q was subjected to SDS/polyacrylamide-gel electrophoresis, transferred to nitrocellulose and filters were stained with Amido Black (lane 1), or probed with HRP-Fn (lane 2), HRP-50kDa (lane 3), or 125 I-fibronectin (lane 4). a, b, and c designate the three polypeptide subunits of C1q.

the amount of fibronectin bound by each chain, the filter which had been probed with ^{125}I -fibronectin was cut into 2 mm slices and counted for radioactivity. Fig. 6 shows that the percentage of fibronectin bound by the A, B and C chains was 38, 21 and 41% respectively.

Localization of the domain on fibronectin that binds to C1q

In order to determine which domain on fibronectin is responsible for binding to C1q, we

Fig. 6. Quantification of fibronectin binding to the A, B and C chains of C1q

Reduced C1q was subjected to SDS/polyacrylamide-gel electrophoresis, transferred to nitrocellulose and the filter was probed with ^{125}I -fibronectin (lane 4, Fig. 5). The filter was then cut into 2 mm strips and counted for radioactivity.

measured ^{125}I -fibronectin binding to C1q in the presence of unlabelled intact fibronectin or purified fragments of fibronectin. ^{125}I -fibronectin was diluted in unlabelled intact fibronectin, 200–180 kDa, 50 kDa or 29 kDa fragment before the addition to solid-phase C1q or gelatin. The results in Table 2 demonstrate that the 50 kDa gelatin-binding fragment, although somewhat less effective than the intact fibronectin as an inhibitor, is still more effective than the 29 kDa fragment in inhibiting ^{125}I -fibronectin binding to either C1q or gelatin.

We have confirmed that C1q-c interacts with the gelatin-binding domain of fibronectin by comparing the binding of fibronectin to C1q or C1q-c in the presence of intact fibronectin, the 50 kDa or the 29 kDa fragment. The results in Fig. 7(a) show that the binding of fibronectin to intact C1q is inhibited by intact fibronectin and 50 kDa fragment; minimal inhibition of fibronectin binding to C1q by 29 kDa fragment is reached only at much higher concentrations of the 29 kDa fragment. The same experiment using C1q-c shows that the 50 kDa fragment inhibits fibronectin binding equally as well as intact fibronectin, whereas the 29 kDa fragment shows essentially no inhibition of fibronectin binding to the collagen-like region of C1q (Fig. 7b). We have also performed similar inhibition experiments using C1q-g domain bound to plastic and have found that the binding of ^{125}I -fibronectin can be inhibited by intact fibronectin and 50 kDa fragment but not by the 29 kDa fragment (results not shown). We conclude that fibronectin binds to the collagen-like region of C1q via the 50 kDa gelatin-binding domain.

Fig. 7. Binding of ^{125}I -fibronectin to C1q in the presence of purified proteolytic fragments of fibronectin (a) ^{125}I -fibronectin was diluted in increasing concentrations of unlabelled intact fibronectin (●), 50 kDa gelatin-binding domain (■) or 29 kDa fragment (▲) and added to solid-phase C1q. (b) ^{125}I -fibronectin was diluted in unlabelled fibronectin (●), 50 kDa (■), or 29 kDa (▲) fragment and then added to solid-phase C1q-c.

Table 2. *Inhibition of ^{125}I -fibronectin binding to C1q or gelatin by proteolytic cleavage products of fibronectin*
For full experimental details, see the text. Inhibition was achieved by dilution of the ^{125}I -fibronectin in increasing concentrations of unlabelled inhibitor before addition to the C1q-coated microtitre wells.

Inhibitor	Protein (μg) required to inhibit 50% of the binding to	
	C1q	Gelatin
Intact fibronectin	10	15
200–180kDa fragment	22	35
50kDa fragment	20	15
29kDa fragment	> 250	> 250

Discussion

Within the past several years, a direct interaction of human plasma fibronectin with subcomponent C1q of the complement system has been demonstrated by several laboratories (Menzel *et al.*, 1981; Harris *et al.*, 1981; Isliker *et al.*, 1982; Pearlstein *et al.*, 1982; Bing *et al.*, 1982; Sorvillo *et al.*, 1983; Ingham *et al.*, 1983; Kono *et al.*, 1983). By using various assay systems it has been established that fibronectin binds to C1q, but interacts poorly with macromolecular C1 (Pearlstein *et al.*, 1982; Isliker *et al.*, 1982). Removal of the C1r and C1s subunits from the C1 macromolecular complex with either EDTA or the C1-inhibitor *in vitro* allows fibronectin binding to C1q (Pearlstein *et al.*, 1982). The functional activity of C1 is not affected by fibronectin; however, adsorption of purified C1q with gelatin-Sepharose increases the haemolytic activity of C1q (Isliker *et al.*, 1981), suggesting that the binding of fibronectin impairs the reconstitution of the C1 macromolecule. Lowering the ionic strength or immobilization of C1q on Sepharose beads facilitates fibronectin binding to C1q (Bing *et al.*, 1982; Sorvillo *et al.*, 1983; Kono *et al.*, 1983). Despite the general agreement with the above data, there have been apparent discrepancies as to which domains on both C1q and fibronectin are responsible for the interaction between these two proteins (Bing *et al.*, 1981, 1982; Harris *et al.*, 1981; Menzel *et al.*, 1981; Pearlstein *et al.*, 1982).

In the present study we compared the binding of ^{125}I -fibronectin to intact C1q with that of purified C1q-c or C1q-g. Our studies show that the C1q-c preparations exhibited approx. 59% of the binding demonstrated with intact C1q, whereas the C1q-g exhibited 35% of the binding (Table 1). When calculated on a molar basis, these numbers are decreased to 24 and 3.3% respectively for C1q-c and C1q-g compared with intact C1q. However,

when the binding site is present on the smaller fragments, enhanced steric inhibition of fibronectin binding by adjacent fibronectin bound to the more closely spaced proteins (due to their smaller size), would tend to underestimate their binding capability. Thus these experiments are qualitative and cannot be used to calculate binding constants for these fragments.

Although these results initially suggested that the C1q molecule may contain two binding sites for fibronectin, this appears not to be the case. First, previous studies have demonstrated that Scatchard plots of the fibronectin–C1q reaction are linear, with a single class of high-affinity binding sites (Bing *et al.*, 1982; Sorvillo *et al.*, 1983). Second, *N*-terminal sequencing of the C1q-g domain demonstrated that a stretch of eight collagen-like amino acids remained attached to the C1q molecules even after extensive digestion with collagenase (Fig. 3). The presence of the collagen-like amino acids was confirmed by using the immunoblotting technique when purified HRP-50kDa was used as a probe (Fig. 4). Although it appears that the C1q-g domain bound more than the C1q-c domain, this difference is probably attributed to the greater number of molecules of C1q-g than C1q-c present on the filter. Because equal amounts of protein, rather than equimolar amounts, were initially applied to the gel, and since the M_r values for C1q-g and C1q-c are 56000 and 170000 respectively, there are 3-fold more binding sites in C1q-g (lane 3) compared with C1q-c (lane 4). Examination of sequences and possible cleavage sites of C1q by pepsin and collagenase taken from Reid (1974, 1976) show that this residual stretch of collagen-like amino acids is retained on both the collagen and globular domains of the purified fragments. This would suggest that fibronectin may bind to C1q at or near the collagen/globular junction of the molecule.

It has fairly recently been demonstrated that, under physiological conditions, the fluid-phase interaction of fibronectin with C1q is very weak; however, by lowering the ionic strength or immobilizing C1q to Sepharose beads, fibronectin binding to C1q can be enhanced (Ingham *et al.*, 1983). Our studies involving inhibition of ^{125}I -fibronectin binding to C1q by fluid-phase C1q, C1q-c or C1q-g support the above observations. Since our binding assay was performed at low ionic strength, fluid-phase intact C1q and C1q-c inhibited the binding of fibronectin to C1q, whereas the C1q-g showed no inhibition (Fig. 2). This lack of inhibition by the C1q-g, which contained the residual collagen sequences discussed above, was surprising, since the preparations used in these studies bound fibronectin when they were insolubilized by binding to the plastic or the nitrocellulose

(Table 1 and Fig. 4). Perhaps owing to compactness of the globular heads, these residual sequences are masked in the fluid phase, whereas on binding of C1q-g to the plastic they become exposed. Two lines of evidence support this conclusion. First, on binding of C1q to immunoglobulin as well as to other activators, there is a conformational change in the C1q molecule that has been demonstrated with monoclonal antibodies (Golan *et al.*, 1982). Secondly, Isliker *et al.* (1982) have shown that binding of fibronectin to gelatin in the presence of soluble C1q-g showed little inhibition, whereas the C1q-c inhibited binding of fibronectin to gelatin. Similarly, Bing *et al.* (1982) have demonstrated that fluid-phase C1q-c competitively inhibited the binding of ^{125}I -C1q to fibronectin, whereas the C1q-g domain showed no inhibition.

We had previously suggested that the globular domain of C1q may interact with fibronectin, since treatment of C1q with pepsin before adsorption on to plastic reduced the binding of ^{125}I -fibronectin (Pearlstein *et al.*, 1982). Our present results demonstrate that the C1q-c domain contains the binding site for fibronectin, and our previous observation, that C1q-g binds fibronectin, is explained by the residual collagen sequences remaining on the C1q-g as described above.

We have also demonstrated that fibronectin appears to bind to all three polypeptide chains of C1q. By using the blotting technique with several different probes, we showed that both intact fibronectin or the 50 kDa gelatin-binding domain of fibronectin interacts with the individual chains of C1q (Fig. 5). Comparison of the amount of fibronectin bound by each chain shown in Fig. 5 suggests that the C and A chains bound much more fibronectin than the B chain. This was confirmed by cutting the filter in Fig. 5 that had been probed with ^{125}I -fibronectin and counting the slices for radioactivity. Fig. 6 shows that the C chain quantitatively bound slightly more fibronectin than the A; the B chain bound the least amount of fibronectin.

Although many workers have studied the interaction between the collagen and/or globular domains of C1q and fibronectin (Menzel *et al.*, 1981; Isliker *et al.*, 1981; Pearlstein *et al.*, 1982; Bing *et al.*, 1982), no studies have addressed the converse question of which fragment of fibronectin is responsible for C1q binding. We now report that the binding site on fibronectin for C1q is the 50 kDa gelatin-binding domain. Table 2 demonstrates that the 50 kDa gelatin-binding domain of fibronectin is as effective in inhibiting fibronectin binding to C1q as is intact fibronectin or the 200–180 kDa fragment (which contains the gelatin-binding site). When C1q or C1q-c (Fig. 7) were immobilized on to the plastic, only the 50 kDa

fragment or intact fibronectin inhibited fibronectin binding; the 29 kDa fragment showed minimal inhibition.

Since fibronectin is known to play a role in clearance of particulate matter (Saba & Jaffe, 1980), the interaction of fibronectin with C1q may assist in clearance of C1q containing immune complexes (Porter & Reid, 1979), Gram-negative bacteria coated with C1q (Loos *et al.*, 1978; Betz & Isliker, 1981) and other particles that bind C1q independent of specific antibody (Giclas *et al.*, 1979).

After completion of this work a report appeared (Reid & Edmundson, 1984) demonstrating the binding of fibronectin to both C1q-g and C1q-c fragments.

We thank B. Grofe for typing this manuscript, and C. Longobardi for technical assistance. This work was supported by grants AM01431, AI20067 and CA09161 from the National Institutes of Health.

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