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C1q, a Subunit of the First Component of Complement, Enhances Binding of Plasma Fibronectin to Bacteria

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The interaction of plasma fibronectin with C1q of the complement system has been demonstrated in the past several years. In addition, the antibody-independent binding of C1q to bacteria, as well as the binding of plasma fibronectin to bacteria, is well documented. This study examines whether the binding of C1q to bacteria enhances the interaction of C1q and bacteria with plasma fibronectin. Highly purified ^{125}I-C1q bound to several species of bacteria in the absence of antibody. The binding of ^{125}I-C1q to bacteria was saturable and specific since the addition of unlabeled C1q inhibited binding while the presence of bovine serum albumin did not. Bacteria which had been pretreated with either buffer or unlabeled C1q were tested for their ability to bind ^{125}I-fibronectin. When bacteria were preincubated with buffer, *Staphylococcus aureus* bound fivefold more ^{125}I-fibronectin than did *Escherichia coli*. However, preincubation of *E. coli* with C1q increased the binding of ^{125}I-fibronectin by up to 20-fold, whereas pretreatment of *S. aureus* with C1q increased fibronectin binding by only twofold. These results were confirmed by immunoblotting studies which demonstrated the presence of C1q, as well as an increase in fibronectin antigens on the C1q-treated bacteria as compared with the level of fibronectin on buffer-treated bacteria. In addition, preincubation of ^{3}H-labeled bacteria with C1q enhanced their attachment to fibronectin-coated surfaces but not to albumin-coated surfaces. The biological consequences of these observations are discussed.

C1 is the first component of the classical pathway and consists of three subunits: C1q, C1r, and C1s (4, 17). The C1q subunit provides the recognition function for activation of the classical complement pathway (4, 17). The binding of C1q to immune complexes, as well as other activators of C1, induces activation of the zymogens C1r and C1s to form the enzymatic complex $C\bar{1}$ which initiates the classical complement pathway (reviewed in references 23 and 27). In the past several years, however, it has been demonstrated that a variety of other molecules and biological surfaces can readily bind C1 via the C1q subunit and result in activation of the classical pathway in the absence of antibody (7). Included among these substances are certain RNA tumor viruses (8), subcellular and cellular membranes (32), and bacteria (2, 5, 6, 16, 18, 36).

C1q is an assymetric molecule composed of two distinct domains. The C terminus of the molecule is composed of six globular "heads" which mediate C1q binding to immunoglobulin and other activators of C1. The N terminus of C1q is composed of a collagenlike domain which contains the binding sites for the C1r and C1s subunits (23, 27). During activation of the classical complement pathway, the C1 inhibitor, the plasma inhibitor of activated C1, binds to $C\bar{1}r$ and $C\bar{1}s$ to form an irreversible complex and dissociates them from the C1q molecule (41). Under these conditions, the collagenlike region of C1q becomes exposed and may interact with cell surface receptors (34) or plasma fibronectin (3, 12, 19, 22, 30, 31).

Fibronectin is a high-molecular-weight glycoprotein present in plasma, cell matrices, and basement membranes and on cell surfaces. Fibronectin binds to a number of macromolecules, including fibrin, DNA, heparin and heparan sulfate, native and denatured collagen, acetylcholinesterase, and C1q (reviewed in references 10, 11, and 21). Several functions have been suggested for this protein, including an opsonic role for fibronectin in promoting phagocytosis of immunoglobulin G- (IgG-) and C3b-bearing particles (40) and possibly bacteria (1, 9, 13, 14, 20, 24–26, 28, 29, 33, 38, 39).

The binding of fibronectin to bacteria is well documented, although its role in promoting their phagocytosis remains unclear (25, 38, 39). Studies have shown that gram-positive bacteria such as *Staphylococcus* spp. differ in their ability to bind fibronectin, whereas gram-negative bacteria such as *Escherichia coli* exhibit little affinity for fibronectin (1, 9, 13, 14, 20, 24–26, 28, 29, 33, 38, 39). Since C1q binds to bacteria in the absence of antibody (2, 5, 6, 16, 18, 36) and fibronectin interacts with C1q, it was of interest whether C1q could mediate binding of fibronectin to bacteria which exhibit little affinity for fibronectin.

In this report we demonstrate that the antibody-independent binding of C1q to bacteria can enhance fibronectin binding to the bacteria via C1q-fibronectin interaction. The presence of C1q on bacteria augmented the binding of either soluble or substrate-bound fibronectin to the bacteria.

MATERIALS AND METHODS

Bacteria. *E. coli* O75 and *S. aureus* 25923 were obtained from ATCC (American Type Culture Collection, Rockville, Md.). *E. coli* W12 and *Staphylococcus epidermidis* K 160 are part of the permanent clinical-isolate collection, Department of Microbiology, New York University Medical Center. Bacteria were inoculated into tryptic soy broth (Difco Laboratories, Detroit, Mich.), grown for 18 h at 37°C, harvested by centrifugation at 8,000 × *g* for 10 min, and washed and suspended in DVB^{2+}-BSA (Veronal-buffered saline [pH 7.5], diluted 1:2 with 5% dextrose [ionic strength, 0.075], containing 0.15 mM Ca^{2+}, 0.5 mM Mg^{2+}, and 0.2%

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bovine serum albumin [BSA]). Bacterial counts were performed in a Petroff-Hausser chamber or adjusted to the appropriate concentration from a standard curve at A_{610}. ^{3}H-labeled bacteria were prepared by inoculation of bacteria into 2 ml of tryptic soy broth containing [^{3}H]thymidine (10 μCi/ml; I.C.N. Chemicals, Inc., Irvine, Calif.), grown for 18 h at 37°C and harvested as described above.

Purification of proteins. C1q was isolated from human serum prepared from outdated plasma by the method of Tenner et al. with ion-exchange chromatography and gel filtration as previously described (35). The C1q was homogeneous, as assessed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (SDS-PAGE), and hemolytically active and contained no detectable immunoglobulins as measured by Ouchterlony analysis. The C1q was stored at −70°C. Fibronectin was purified from pooled, outdated, normal human plasma by gelatin affinity chromatography (22). The pool containing fibronectin from the gelatin affinity column was subsequently chromatographed over Sepharose 6B (Pharmacia Fine Chemicals, Uppsala, Sweden) in Tris-buffered saline–2 mM phenylmethylsulfonyl fluoride (pH 7.4) at 4°C. The fractions containing fibronectin were pooled, concentrated, dialyzed against phosphate-buffered saline (PBS), and stored at −70°C.

Iodination of proteins. Fibronectin was radiolabeled by solid-phase lactoperoxidase-catalyzed iodination as previously described (22, 30). The specific activity of labeled fibronectin preparations was usually 10^6 to 10^8 cpm/μg. C1q was iodinated with Enzymobeads (Bio-Rad Laboratories, Richmond, Calif.) as described by Tenner et al. (35). Specific activities usually ranged between 10^4 and 10^6 cpm/μg. The labeled C1q was stored in portions at −70°C.

Antisera. A rabbit antiserum to human plasma fibronectin was prepared as previously described, and the specificity was determined by immunoelectrophoresis of human plasma and immunoprecipitation of metabolically labeled fibronectin from conditioned medium of normal human fibroblasts (21). Rabbit anti-human C1q was purchased from Atlantic Antibodies, Westport, Maine. By Ouchterlony analysis, this antibody gave one precipitin line with C1q and failed to cross-react with fibronectin.

SDS-PAGE. SDS-PAGE of all proteins was performed in slab gels as described by Laemmli (15). Stacking gels were usually 5% acrylamide, and separating gels were 7.5, 10, or 12% acrylamide. Molecular weights were determined by comparison with known standards (Pharmacia Fine Chemicals).

Detection of proteins by immunoblotting. After SDS-PAGE, electrophoretic transfer of proteins was performed by the method of Towbin et al. (37). Briefly, electrophoretic transfer was carried out for 18 h at 150 mA in 25 mM Tris base–192 mM glycine–20% (vol/vol) methanol (pH 8.3). The filters were removed and incubated in PBS-Tween (PBS containing 0.05% Tween 20 [Sigma Chemical Co., St. Louis, Mo.]) for 2 h at 37°C. Filters were probed with a 1:500 dilution of either rabbit anti-human C1q or anti-human fibronectin for 2 h at 37°C. The filters were washed and incubated with radioiodinated protein A (0.2 μCi/50 ml of PBS-Tween 20) for 45 min at 37°C. The filters were washed, dried, and exposed to Kodak X-OMAT AR or RP film as required.

Antibody-independent binding of C1q to bacteria. ^{125}I-C1q (1.6 μg) was diluted into increasing concentrations (0 to 250 μg/ml) of unlabeled C1q in DVB^{2+}-BSA, and 0.5 ml of this mixture was added to 2.5×10^8 bacteria. After agitation for 30 min at 37°C, the mixtures were centrifuged at 8,000 × *g* for 15 min at 4°C and washed two times, and the bacterial pellets were counted for radioactivity. The total amount of C1q bound to the bacteria was calculated. Nonspecific binding was determined by dilution of ^{125}I-C1q in a 1,000-fold excess of unlabeled C1q, and experimental values were corrected.

Binding of ^{125}I-fibronectin to bacteria coated with C1q. Bacteria (10^9 cells) were preincubated in buffer alone or buffer containing either 96 or 19 nM unlabeled C1q and were washed as described above. ^{125}I-fibronectin (105,000 cpm; 1.6×10^6 cpm/μg) diluted in DVB^{2+}-BSA was added to the pelleted bacteria, and the mixtures were vortexed. After incubation at 30°C for 30 min, the mixtures were washed two times, and the bacterial pellets were counted for radioactivity. All samples were run in duplicate.

Immunoblotting experiments. Bacteria (10^9) were incubated with either DVB^{2+}-BSA or buffer containing 0.73 nM C1q as described above. All samples were washed and then incubated with 150 μg of fibronectin per ml diluted in PBS-BSA (2 mg/ml) for 30 min at 37°C. The bacteria were pelleted by centrifugation and washed two times, and the bound proteins were eluted by boiling in buffer containing 2% SDS and 0.1 M dithiothreitol. The mixtures were centrifuged and the supernatants were run on SDS-PAGE.

Attachment of ^{3}H-labeled bacteria to fibronectin-coated surfaces. To measure the binding of ^{3}H-labeled bacteria to fibronectin- or BSA-coated surfaces, plastic tubes (12 × 75 mm) were incubated with 1 ml of PBS containing 100 μg of fibronectin or BSA for 2 h at 37°C. The tubes were washed with PBS, and the nonspecific sites on the plastic were blocked with PBS-BSA (2 mg/ml) for 1 h at 37°C. ^{3}H-labeled bacteria were prepared and coated with C1q, as described above. Bacteria (1 ml; 10^9 cells) in PBS-BSA were added to the fibronectin-coated tubes and incubated for 45 min at 37°C. The bacteria were aspirated, and the tubes were washed three times with PBS-BSA. The attached bacteria were lysed with 1.0% SDS, and a portion of each sample was counted in a liquid scintillation counter. All samples were run in duplicate.

RESULTS

Purification and radiolabeling of C1q. SDS-PAGE of human C1q purified by the method of Tenner et al. (35) is shown in Fig. 1A. When this material was labeled with ^{125}I by using Enzymobeads, the labeled C1q was hemolytically active and retained the ability to bind to immune complexes. A representative gel of the ^{125}I-C1q is shown in Fig. 1B. In agreement with previous reports (35), the C dimers of C1q contained 90% of the total radioactivity, and minor bands detected in overloaded gels contained approximately 5% of the total radioactivity applied to the gel.

Antibody-independent binding of C1q to bacteria. The following four strains of bacteria were tested for their ability to bind C1q: *E. coli* W12, *E. coli* O75, *S. aureus* 25923, and *S. epidermidis* K 160. The total amount of C1q bound to the bacteria with ^{125}I-C1q diluted into increasing concentrations of unlabeled C1q in buffer containing 0.2% BSA is shown in Fig. 2. The binding of ^{125}I-C1q was saturable and specific since the addition of unlabeled C1q inhibited the binding of the radiolabeled ligand, whereas the presence of BSA did not affect C1q binding. *E. coli* W12 and *E. coli* O75 bound equal amounts of C1q; *S. aureus* 25923 bound slightly less C1q than did the gram-negative strains, whereas *S. epidermidis* K 160 bound the greatest amount of C1q.

Binding of soluble fibronectin to C1q-coated bacteria. *E. coli* W12 and *S. aureus* were preincubated with either buffer

or C1q and washed, and their ability to bind ^{125}I-fibronectin was tested as described above. When bacteria were preincubated in buffer, *S. aureus* bound fivefold more fibronectin when compared with *E. coli* (Fig. 3, closed columns). However, preincubation of *E. coli* with 19 nM C1q caused a fivefold enhancement in fibronectin binding (hatched columns), while incubation with 96 nM C1q increased fibronectin binding by more than 20-fold (open columns). In contrast, the amount of fibronectin bound by *S. aureus* preincubated with 96 nM C1q was only twofold greater than that of buffer-treated *S. aureus*. In both cases the amount of fibronectin bound to C1q-coated bacteria was dependent on the amount of C1q used in the preincubation.

To confirm these results, bacteria were preincubated with either C1q or buffer and washed, and all samples were then incubated with unlabeled fibronectin. The bacteria were then analyzed for C1q and fibronectin antigens by the immunoblotting technique described above. The results are shown in Fig. 4. In agreement with the data shown in Fig. 2, C1q was detected on all bacteria preincubated with C1q (indicated by the bracket in Fig. 4A); control filters incubated with ^{125}I-labeled protein A in the absence of anti-C1q did not show any detectable C1q antigens (data not shown). A nitrocellulose filter that was similar to control filters except that it was probed with antifibronectin instead of anti-C1q is shown in Fig. 4B. Both strains of *E. coli* bound little fibronectin when preincubated in buffer (lanes 2 and 4). Preincubation of the bacteria with C1q markedly increased the amount of fibronectin bound by *E. coli* (indicated by the arrow; cf. Fig. 4B, lanes 1 with 2; lanes 3 and 4). In contrast, *S. aureus* bound fibronectin when preincubated with buffer alone (cf. lanes 2 and 4 with lane 6), and the binding was augmented when bacteria were preincubated with C1q (cf. lanes 5 and 6). The strain of *S. epidermidis* tested bound fibronectin only when preincubated with C1q (cf. lanes 7 and 8).

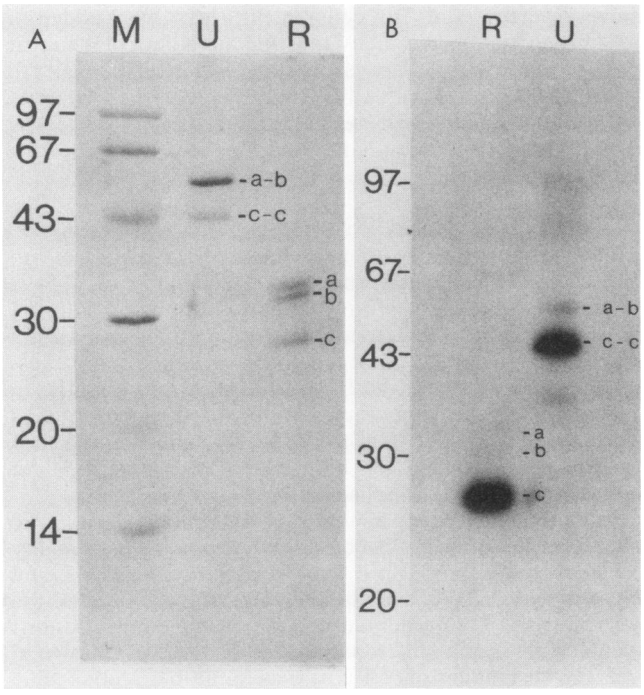

FIG. 1. SDS-PAGE analyses of purified C1q (A) and of ^{125}I-C1q (B). SDS-PAGE analysis of purified human C1q (panel A) was performed on a 12% acrylamide gel. The material shown in panel A was then labeled with Enzymobeads and analyzed by SDS-PAGE with a 10% acrylamide gel. After electrophoresis, the gel was dried and subjected to autoradiography (panel B). M, Marker proteins; U, unreduced C1q; R, reduced C1q; a, b, and c, subunit polypeptide chains of C1q.

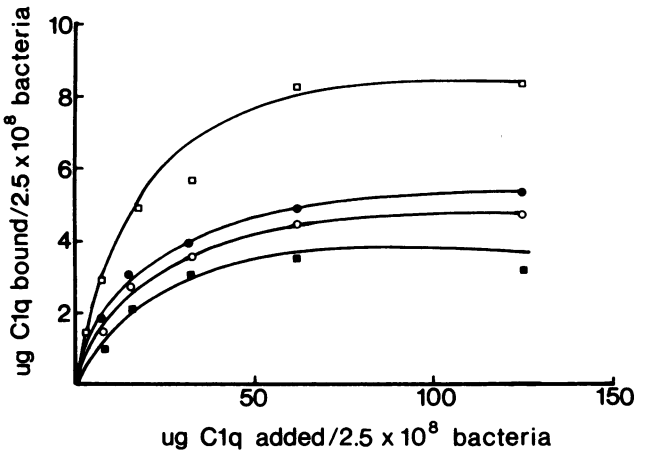

FIG. 2. Antibody-independent binding of C1q to bacteria. ^{125}I-C1q was diluted in increasingly concentrated unlabeled C1q and incubated with 2.5 × 10^8 bacteria for 30 min at 37°C. The bacteria were centrifuged, washed, and counted for radioactivity, and the total amount of C1q bound was calculated. Nonspecific binding was measured as described in the text. Symbols: ●, *E. coli* W12; ○, *E. coli* O75; ■, *S. aureus* 25923; □, *S. epidermidis* K 160.

Attachment of C1q-coated bacteria to fibronectin-coated surfaces. Bacteria which bind C1q in vivo may interact with either soluble fibronectin in plasma and other body fluids or extracellular fibronectin in the connective-tissue matrix. As the first step in studying the attachment of C1q-coated bacteria to tissue fibronectin, the binding of labeled C1q-coated bacteria to fibronectin-coated tubes was measured.

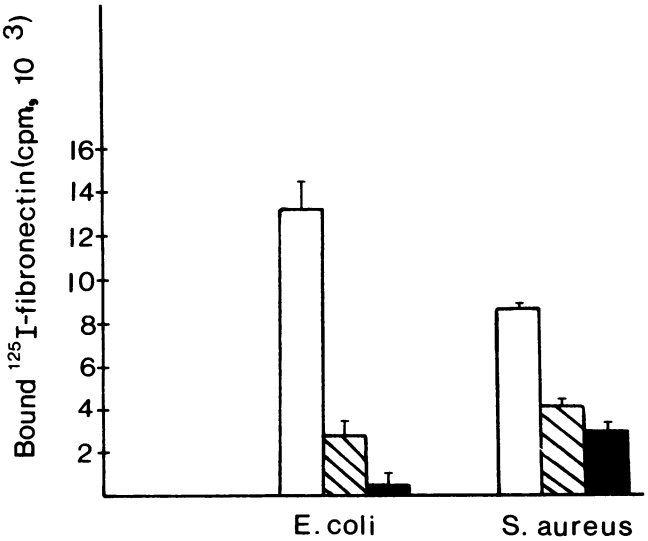

FIG. 3. Binding of ^{125}I-fibronectin to C1q-coated bacteria. *E. coli* W12 or *S. aureus* 25923 (10^9 cells per ml) was incubated in C1q (96 nM) (□), C1q diluted 1:5 (19 nM) (▨), or buffer (■) for 30 min at 37°C. The bacteria were washed and suspended in buffer containing ^{125}I-fibronectin. After incubation for 30 min at 37°C, the bacteria were washed twice and counted for radioactivity.

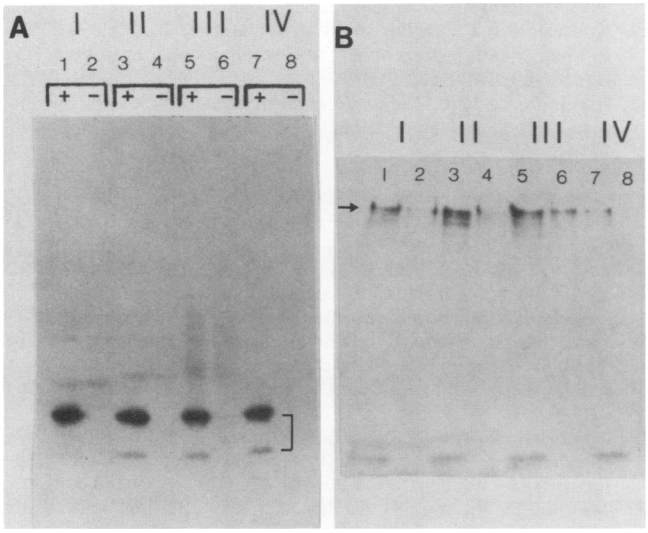

FIG. 4. Detection of C1q and fibronectin antigens on bacteria by the immunoblotting technique. Bacteria were preincubated with either 0.7 μM C1q (+) or buffer (−) and washed, and then all samples were incubated with 150 μg of fibronectin per ml. The bacteria were pelleted, washed, run on reduced SDS-PAGE, and analyzed for C1q and fibronectin antigens. I, *E. coli* O75; II, *E. coli* W12; III, *S. aureus* 25923; IV, *S. epidermidis* K 160. (A) Filter probed with anti-C1q. C1q a, b, and c chains are indicated by the bracket. (B) Duplicate filter probed with antifibronectin. Fibronectin is indicated by the arrow.

Fibronectin or BSA-coated tubes and C1q-coated, ^{3}H-labeled bacteria were prepared, and attachment was measured as described above. The results are shown in Fig. 5. Pretreatment of both strains of *E. coli* as well as *S. epidermidis* with C1q increased the attachment to fibronectin-coated surfaces by two- to threefold (open columns) as compared with their attachment when pretreated with buffer alone (closed columns). In contrast, *S. aureus* attached equally well to fibronectin-coated surfaces when preincubated with either buffer or C1q. Furthermore, relatively few C1q-coated bacteria bound to surfaces coated with BSA (hatched columns).

DISCUSSION

A direct interaction of human plasma fibronectin with the C1q subcomponent of the complement system has been demonstrated in the past several years (3, 12, 19, 22, 30, 31). The present investigation was undertaken to determine whether the antibody-independent binding of C1q to bacteria could mediate or enhance fibronectin binding to bacteria which exhibit little affinity for fibronectin.

In agreement with previous reports (2, 5, 6, 16, 18, 36), our results show that highly purified C1q binds to several strains of bacteria in the absence of specific antibody (Fig. 2). The binding of C1q to the four strains of bacteria tested was specific and saturable. We also demonstrated the binding of C1q to both gram-positive and gram-negative organisms, whereas previous studies have investigated the binding of C1q to only gram-negative bacteria. It should be pointed out that there is little information regarding the bacterial structure(s) involved in the direct binding of C1q by the strains used in the present as well as previous studies (2, 5, 6, 16, 18, 36).

Initial studies investigating the interaction of fibronectin with bacteria showed that fibronectin bound avidly to gram-positive bacteria but bound very poorly to gram-negative bacteria (1, 9, 13, 14, 20, 22–26, 28, 29, 33, 38, 39). Our results (Fig. 3 and 4) show that fibronectin bound well to *S. aureus* but poorly to both strains of *E. coli* used in this study. However, a recent study by Froman et al. (9) has shown that 4 out of 17 enterotoxigenic strains of *E. coli* isolated from infantile diarrhea expressed fibronectin receptors. Interestingly, it was found that bacteria grown at 40°C did not bind fibronectin. Therefore, it appears that the amount of fibronectin bound by bacteria may be influenced by factors such as the type of media, temperature, and pH during growth as well as the phase of growth of bacteria at harvest.

The strain of *S. epidermidis* used in this study bound little fibronectin (Fig. 4). This result was not surprising since Świtalski et al. (33) have shown that fibronectin-binding capacity varies greatly between different staphylococcal species, as well as from one strain to another within the same species.

We demonstrated in this study that preincubation of bacteria with C1q enhances fibronectin binding. Preincubation of *E. coli* and *S. epidermidis* with C1q caused a marked increase in the binding of fibronectin to the bacteria (Fig. 3 and 4). In the immunoblot shown in Fig. 4, the amounts of fibronectin bound by both untreated and C1q-treated bacteria were quantitated by cutting the lanes from the nitrocellulose filters and counting the radioactivity. There was a two- to threefold increase in radioactivity bound by bacteria pretreated with C1q as compared with bacteria pretreated with buffer (data not shown).

Precoating bacteria with C1q enhanced their attachment to surface-bound fibronectin (Fig. 5). However, it appears that *S. aureus* would attach to surface-bound fibronectin without C1q pretreatment. Although *E. coli* W12 would attach to surface-bound fibronectin in the presence of C1q, significant attachment would occur in the absence of C1q. These results may be due to the clustering or aggregation of fibronectin on the plastic, which could expose cryptic binding sites on the fibronectin molecule as has been suggested by Proctor et al. (25). In this regard, recent reports (9, 25) have demonstrated

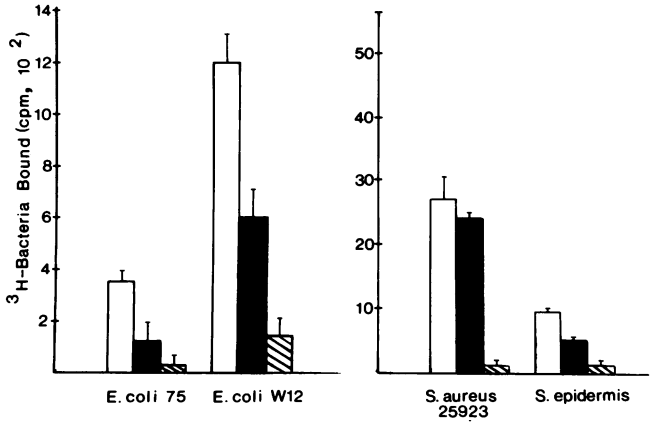

FIG. 5. Attachment of C1q-coated, ^{3}H-labeled bacteria to surface-bound fibronectin. ^{3}H-labeled bacteria (10^9 cells) were preincubated with C1q or buffer and washed, and their attachment to either fibronectin or BSA-coated tubes was measured. Symbols: □, C1q preincubation and fibronectin surface; ■, buffer preincubation and fibronectin surface; ▨, C1q preincubation and BSA-coated surface. The values shown have been corrected for the nonspecific binding of buffer-treated, ^{3}H-labeled bacteria to BSA-coated tubes.

that fibronectin has two binding sites for both gram-positive and -negative bacteria, one which binds bacteria reversibly and the other which binds bacteria irreversibly. Our results are in agreement with previous reports (1, 9, 24, 28) which suggest that the binding of bacteria to surface-bound fibronectin may represent a mechanism of tissue adherence. Further studies are needed to test this possibility.

In summary, we have shown that the antibody-independent binding of C1q to bacteria mediated or augmented the binding of soluble or substrate-bound fibronectin. The biological significance of this observation can only be speculated on at this time. It is known that the binding of fibronectin to bacteria causes their attachment to neutrophils and monocytes but does not promote phagocytosis by these cells (26, 29, 38, 39). However, recent studies by Wright et al. (40) have demonstrated that particles bearing both fibronectin and C3b are avidly ingested by monocytes. In this regard, several studies have shown that the antibody-independent binding of C1q, $C1r_2$, and $C1s_2$ can generate the classical C3 convertase and lead to C3b deposition onto the bacterial surface (2, 16). The binding of fibronectin to C1q could occur after $C\bar{1}$ inhibitor-mediated dissociation of $C\bar{1}r_2$ and $C\bar{1}s_2$. This mechanism would generate bacteria coated with both C3b and fibronectin, and these particles should be avidly ingested by monocytes (40).

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