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Interaction of Viral Envelope Glycoproteins with Fibronectin

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An interaction between fibronectin and viral envelope glycoprotein micelles isolated from influenza A, parainfluenza 1, and mumps viruses was found by enzyme immunoassay. All three different glycoprotein micelles bound efficiently to solid-phase fibronectin. When fibronectin was permitted to bind to solid-phase viral glycoproteins, dose-dependent binding was observed. Soluble glycoprotein micelles inhibited the binding of fibronectin to immobilized glycoprotein preparations. The binding was not observed when fibronectin was pretreated with neuraminidase, suggesting that the sugar moieties of fibronectin are responsible for the affinity. This affinity may play a role in virus-cell interactions or in the opsonization of certain viruses during infection.

A number of biological interactions between fibronectin and different macromolecules have previously been described (19, 23, 31). Fibronectin can bind to collagens, heparin, fibrinogen and fibrin, actin, and DNA. The strong affinity of fibronectin for gelatin has been of special importance in studies on the structure of fibronectin (6, 8). Fibronectin is considered to be one of the nonspecific opsonins of blood and is also known as an opsonic α2-surface-binding glycoprotein (2, 24). This concept accords with the observation that it binds to *Staphylococcus aureus* (11).

Viral envelope glycoproteins play a central role in the infectivity of viruses. The hemagglutinin molecules of myxo- and paramyxoviruses mediate the binding of the virus onto sialic acid containing glycoproteins on the cell surface (4, 7, 15, 27), and antibodies against virus envelope glycoproteins can protect the host from infection (4, 18). Hemagglutinin can attach to glycophorin on the erythrocyte membrane. The receptor structure in glycophorin is also sialic acid (3). Envelope glycoproteins of certain viruses can easily be purified, and they form protein micelles. Viral glycoprotein micelles are composed of several viral envelope spike proteins attached together with their hydrophobic membrane-bound segments in the middle of the micelle and hydrophilic parts at the periphery (29). These glycoprotein micelles are stable and can be used as models for viral envelope glycoproteins.

The role of fibronectin in the pathogenesis of bacterial infections has been studied widely (11, 22, 24, 33, 35, 36), whereas the interactions of fibronectin with viruses and viral proteins are poorly understood. We therefore studied in the present investigation the interactions between viral surface glycoproteins and human plasma fibronectin, using isolated glycoprotein micelles from influenza A, parainfluenza 1, and mumps viruses. The binding was detected either by enzyme immunoassay (EIA) or by measuring the binding of radiolabeled viral glycoprotein micelles to fibronectin. All viral glycoprotein micelles bound efficiently to fibronectin; the binding was inhibited by soluble fibronectin, but not by neuraminidase-treated fibronectin. The binding of fibronectin to viral glycoproteins was inhibited by all viral glycoprotein preparations tested, suggesting that the binding site for viral envelope glycoproteins can be localized to a specific domain of the fibronectin molecule.

MATERIALS AND METHODS

Viruses and viral glycoprotein micelles. Influenza A (H1N1, Brazil strain and H3N2, Bangkok strain), parainfluenza 1 (Sendai strain), and mumps viruses (Enders strain) were grown in embryonated chicken eggs. At 3 to 5 days postinfection, allantoic fluid was collected and clarified by low-speed centrifugation (14,600 × *g*, 40 min). The allantoic fluid was concentrated with a liquid concentrator, and after another low-speed centrifugation, the virus was pelleted between 30 and 50% sucrose cushions in phosphate-buffered saline (PBS) in a Spinco L4 Ti-15 rotor at 32,000 rpm for 2 h. The virus was concentrated on a 50% sucrose cushion in a SV-27 rotor for 1.25 h at 25,000 rpm. The virus was diluted and centrifuged in a 15 to 30% sucrose gradient for 20 min at 25,000 rpm at 4°C. A visually detected virus band was collected from the middle of the gradient, pelleted (25,000 rpm, 1 h), and after suspension in PBS in an ice bath, stored in aliquots at −70°C.

Viral glycoprotein micelles were prepared as described elsewhere (29) with the following modification. After disruption of the viruses with Triton X-100 for 30 min at 37°C (virus protein/detergent ratio of 1:4), the nucleocapsids were pelleted at 100,000 × *g* for 30 min at 20°C (28). Viral envelope glycoprotein micelles were

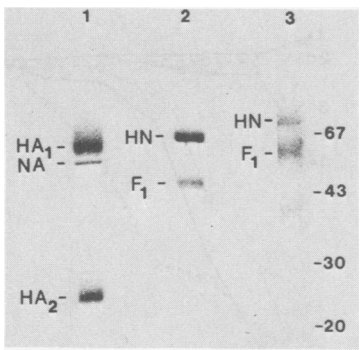

FIG. 1. Polypeptide analysis of viral glycoprotein micelles. Purified glycoprotein micelles (5 to 8 μg/slot) were run under reducing conditions on a 10 to 15% polyacrylmide gel and stained. Lane 1, Influenza A, H1N1 strain; lane 2, parainfluenza 1; and lane 3, mumps glycoprotein micelles. HA_1 and HA_2, Hemagglutinin; NA, neuraminidase; HN, hemagglutinin-neuraminidase; F1, fusion protein; F2 stained poorly, and its migration is not indicated.

formed in a 20 to 50% sucrose gradient as described previously (29). Analysis by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was carried out to demonstrate the purity of the viral glycoprotein micelle preparations (Fig. 1). The polypeptides of the micelles were as described elsewhere (5, 17). Hemagglutinin and neuraminidase activities were well preserved in glycoprotein micelles as detected by hemagglutinin titration with 0.5% 1-day-old chicken erythrocytes in PBS plus 0.2% bovine serum albumin and by neuraminidase assay (26).

Radioactive labeling of viral glycoprotein micelles. Influenza A (H3N2) glycoprotein micelles were labeled for galactose residues by a modification of the method of Luukkonen et al. (14). The glycoprotein micelles (500 μg/200 μl in PBS) were incubated with 20 IU (20 μl) of galactose oxidase (Kabi AB, Stockholm, Sweden) at 37°C for 30 min. The protein mixture was moved onto frozen tritiated sodium borohydride (NaB^3H_4, 2 mCi in 200 μl of 0.01 N NaOH); after 30 min of incubation at 20°C, the reaction mixture was passed through a Sepharose 4B column (Pharmacia, Uppsala, Sweden; 0.7 by 8 cm), and the labeled glycoprotein micelles were separated from free NaB^3H_4. The specific activity was 140,000 cpm/μg of protein.

Fibronectin, macromolecules, and other reagents. Fibronectin was purified from human plasma by affinity chromatography over gelatin-agarose columns (8) modified as described elsewhere (32). The purity of the fibronectin was >97% as judged by analysis in polyacrylamide gels. The following were obtained from commercial sources: bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.), gelatin (type I; Sigma), fetuin (type III; Sigma), heparin (Medica Oy, Helsinki, Finland), *N*-acetylneuraminic acid (NeuAc, sialic acid; Sigma), 2-(3-methoxyphenyl)-*N*-acetyl-D-neuraminic acid (Boehringer, Mannheim, West Germany), and neuraminidase (*Vibrio cholerae*, 1 U/ml; Behringwerke AG, Marburg, West Germany).

Antisera and enzyme conjugates. Antisera against virus envelope glycoproteins were obtained by immunizing rabbits with glycoprotein micelles. Twenty micrograms of protein in Freund complete adjuvant was injected three times directly into popliteal lymph nodes at 2-week intervals, and the rabbits were bled 2 weeks after the last injection. Serum antibody titers varied from 300,000 to 1,000,000 as measured by the EIA described below, and the antisera did not react with fibronectin in the EIA. Antifibronectin antiserum was produced in rabbits, and the specificity of the antiserum was determined as described earlier (32). Antifibronectin antiserum did not react with viral glycoprotein micelles in the EIA. Horseradish peroxidase-labeled swine antibodies against rabbit immunoglobulins were used as an enzyme conjugate (Dako, Copenhagen, Denmark). A background absorbance of the conjugate was less than 0.200 for all of the glycoprotein micelle preparations, as well as for fibronectin.

EIA. Polystyrene microtiter plates (Linbro, Titertek; Flow Laboratories, Inc., Hamden, Conn.) were coated with 50 μl (5 μg/ml) of fibronectin, glycoprotein micelles, bovine serum albumin, or *V. cholerae* neuraminidase all in PBS at 37°C for 2 h. Plates incubated with PBS served as controls. The plates were washed twice with 150 μl of PBS containing 0.05% Tween 20 (PBS-Tween) and once with distilled water and kept dry at 4°C until used (25).

Different dilutions of fibronectin (50 μl in PBS-Tween) were incubated in viral glycoprotein-coated and noncoated wells for 2 h at 37°C. After two washings with PBS-Tween, 50 μl of antifibronectin serum in PBS-Tween at a 1:30,000 dilution (30 times the endpoint titer) was added, and the wells were incubated at 37°C for 2 h. They were then washed twice with PBS-Tween, and 50 μl of horseradish

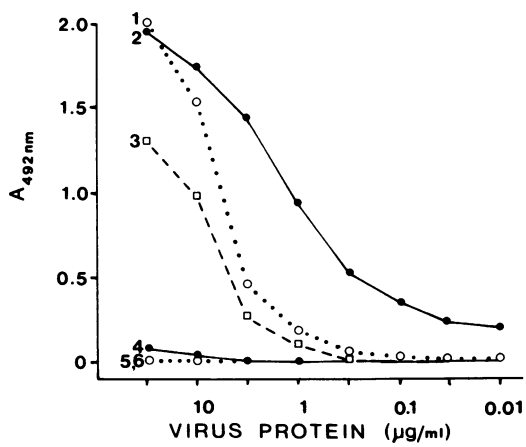

FIG. 2. Binding of virus glycoprotein to solid-phase fibronectin. Virus glycoprotein micelles were allowed to react with solid-phase fibronectin and noncoated microtiter wells, and the bound virus proteins were quantified by EIA. The binding of parainfluenza 1 (1), influenza A, H1N1 strain (2), and mumps virus (3) glycoprotein micelles to fibronectin was detected with the respective antisera. The binding of influenza A (4), parainfluenza 1 (5), and mumps (6) virus glycoprotein micelles to noncoated microtiter wells is also shown. A_{492}, Absorbance at 492 nm.

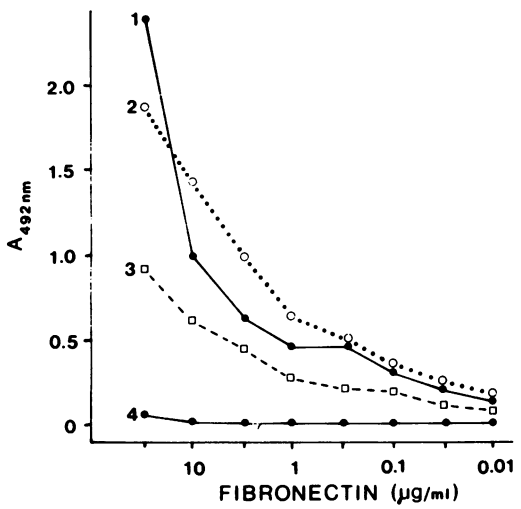

FIG. 3. Binding of fibronectin to solid-phase viral glycoprotein micelles. Fibronectin was allowed to react with solid-phase parainfluenza 1 (1), influenza A, H1N1 strain (2), and mumps (3) glycoprotein micelle-coated wells and noncoated wells (4). The binding was detected with antifibronectin sera by EIA.

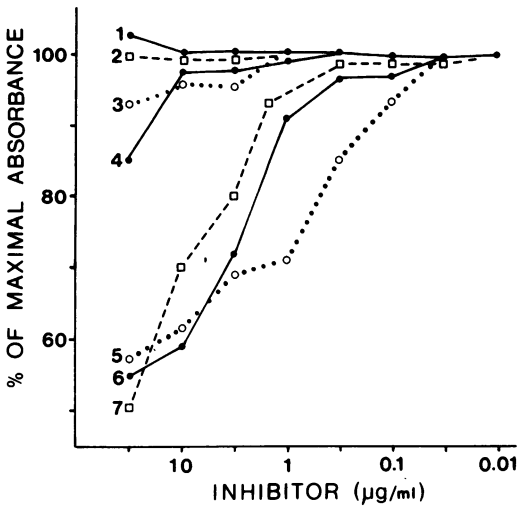

FIG. 4. Inhibition of fibronectin binding to solid-phase mumps glycoproteins. Fibronectin (3 μg/ml) was added simultaneously to mumps glycoprotein micelle-coated wells with different concentrations of bovine serum albumin (1); heparin (2); gelatin (3); fetuin (4); and mumps (5), parainfluenza 1 (6), and influenza A (7) glycoprotein micelles, and the bound fibronectin was determined by EIA. The results are expressed as a percentage of the absorbance ratio of uninhibited fibronectin binding.

peroxidase-labeled swine antibodies against rabbit immunoglobulins was added at a 1:800 dilution in PBS-Tween to each well followed by incubation at 37°C for 2 h or at 20°C for 16 h. After two washings with PBS-Tween and one washing with distilled water, 100 μl of *o*-phenylenediamine (0.4 mg/ml; Koch-Light Laboratories, Colnbrook, Bucks, England) together with H_2O_2 (0.2 mg/ml) in phosphate-citrate buffer (pH 5.5) was added, and after 30 min of incubation at 20°C, the enzyme reaction was stopped with 50 μl of 4 N sulfuric acid (34). The intensity of the color reaction was read at 492 nm with a multichannel photometer (Titertek, Multiskan; Eflab OY, Helsinki, Finland).

We studied the binding of viral glycoprotein micelles to fibronectin-coated cuvettes as described above, using the appropriate antisera (30 times the endpoint dilution). To study the inhibition of fibronectin binding to solid-phase glycoprotein micelles, we added 25 μl of a fibronectin dilution in PBS-Tween simultaneously with 25 μl of the inhibitor studied. The final concentration of fibronectin was 3 μg/ml. We performed the inhibition studies for viral glycoprotein binding to solid-phase fibronectin similarly, using fibronectin as an inhibitor.

The titers of rabbit antisera against fibronectin and glycoprotein micelles were determined as the reciprocal of the last serum dilution giving twice the absorbance of preimmune sera. The EIA was carried out as described above. Triplicate determinations were used throughout the study.

Radioassay. Radiolabeled influenza A (H3N2) glycoprotein micelles were diluted with unlabeled micelles at a ratio of 1:3 and allowed to bind onto solid-phase fibronectin, neuraminidase-treated fibronectin, bovine serum albumin, or noncoated microtiter wells at protein concentrations of 0.1 to 32 μg/ml (50 μl/well in PBS-Tween). After 2 h of incubation at 37°C, the wells were washed twice with PBS-Tween and once with distilled water, and 150 μl of 2% sodium dodecyl sulfate in H_2O was added for 30 min. The liquid was moved into scintillation liquid, and the radioactivity was determined in a liquid scintillation counter. Triplicate determinations were used.

Other techniques. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was carried out as described by Laemmli (12) with gradient gels (10 to 15% acrylamide monomer concentration). Protein concentrations were determined by the method of Lowry et al. (13). Liquid-phase fibronectin was treated with *V. cholerae* neuraminidase (substrate/enzyme ratio in PBS, 15:1; final pH, 6.2) at 37°C for 16 h.

RESULTS

Binding of viral glycoprotein micelles to fibronectin. The binding of three different preparations of viral glycoprotein micelles to fibronectin-coated microtiter wells was studied by EIA with different concentrations of the micelles. The binding was detected by antibodies specific for each preparation used. Efficient, dose-dependent binding to solid-phase fibronectin was observed with influenza A (H1N1), parainfluenza 1, and mumps glycoprotein micelles (Fig. 2). Influenza A glycoprotein micelles bound slightly better than did the other two viral glycoprotein preparations. No EIA reaction was observed when noncoated microtiter wells were used.

The binding could be inhibited by soluble fibronectin. A decrease of 25 to 40% in absor-

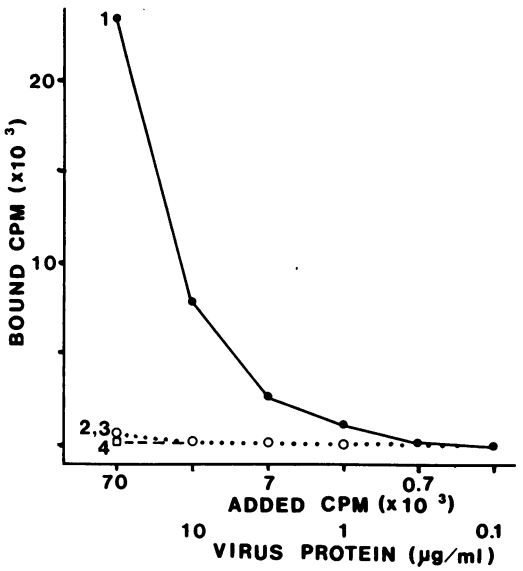

FIG. 5. Binding of radiolabeled influenza A glycoprotein micelles to fibronectin. Radiolabeled influenza A (H3N2) glycoproteins were permitted to bind to fibronectin- (1) and neuraminidase-treated fibronectin- (2) coated wells, noncoated microtiter wells (3), and bovine serum albumin-coated wells (4), and the bound radioactivity was determined.

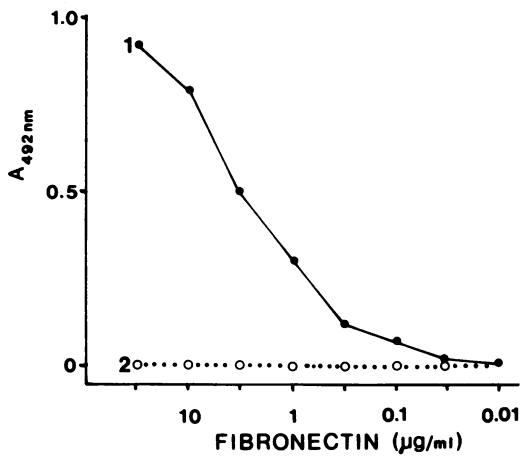

FIG. 6. Binding of fibronectin to *V. cholerae* neuraminidase. Fibronectin was allowed to react with neuraminidase-coated wells (1) and noncoated wells (2), and the binding was detected by EIA.

bance was achieved, depending on the glycoprotein micelle preparation used, by a 10-fold (30 μg/ml) excess of soluble fibronectin (data not shown).

Binding of fibronectin to solid-phase viral glycoproteins. Soluble human plasma fibronectin was permitted to bind to microtiter wells coated with glycoprotein micelles. The dose-dependent binding of fibronectin to all glycoprotein micelle preparations was observed, but some differences in the binding efficiency were seen (Fig. 3). No binding of fibronectin to noncoated wells was found.

Inhibition of fibronectin binding to mumps virus glycoprotein micelles. Fibronectin was incubated in wells coated with glycoprotein micelles of mumps virus, together with some other macromolecules. Bovine serum albumin, heparin, and gelatin did not inhibit the binding of fibronectin to mumps proteins. High concentrations (32 μg/ml) of fetuin had some inhibitory effect. In contrast, all three preparations of virus glycoprotein micelles were effective inhibitors, and mumps glycoprotein micelles had possibly the best inhibitory effect (Fig. 4).

Inhibition experiments were also carried out on fibronectin binding to wells coated with influenza A glycoprotein micelles. An inhibition similar to that of fibronectin binding to mumps glycoprotein micelles was observed.

Sialic acid and 2-(3-methoxyphenyl)-*N*-acetyl-D-neuraminic acid, a synthetic substrate for neuraminidase (0.01 to 100 μg/ml), were also tested for their ability to inhibit the binding. No inhibitory effect on fibronectin binding to solid-phase influenza A, parainfluenza 1, and mumps virus glycoproteins could be observed.

Interaction of influenza A glycoprotein micelles with *V. cholerae* neuraminidase-treated fibronectin. Since the hemagglutinins of myxo- and paramyxoviruses can bind to sialic acids, we treated liquid-phase fibronectin with neuraminidase to find out whether the removal of sialic acids from fibronectin affected its ability to bind viral glycoprotein micelles. A significant decrease in the binding of radioactive influenza A (H3N2) glycoprotein micelles to neuraminidase-treated solid-phase fibronectin was observed (Fig. 5). Unlike untreated fibronectin, neuraminidase-treated fibronectin did not inhibit radiolabeled influenza A glycoprotein binding to solid-phase fibronectin.

To study whether purified neuraminidase could interact with fibronectin, we tested the ability of fibronectin to bind to neuraminidase purified from *V. cholerae*, which is commercially available in pure form. *V. cholerae* neuraminidase can remove any sialic acid irrespective of the attaching linkage. *V. cholerae* neuraminidase (5 μg/ml in PBS) was used to coat the wells, and different concentrations of fibronectin were permitted to bind to the coated wells. Dose-dependent binding of fibronectin to solid-phase neuraminidase was observed (Fig. 6). The binding of fibronectin to solid-phase *V. cholerae* neuraminidase was inhibited by all three glycoprotein micelles. A decrease in absorbance of 25 to 35% was achieved by a 10-fold excess of viral

glycoprotein (30 μg/ml) (data not shown). This affinity was not characterized further.

DISCUSSION

In the present work, we have demonstrated that fibronectin can interact with viral glycoprotein micelles in a dose-dependent manner. Binding was observed when either fibronectin or viral glycoproteins were in the solid phase, whereas it was inhibited by fluid-phase proteins.

Sialic acid-containing glycoproteins may serve as receptors when myxo- and paramyxoviruses, as well as many other virus groups, bind onto the cell surface (15, 27). We tested the effects of free sialic acid and methoxyphenyl neuraminic acid on the binding, but they did not show any inhibition. Sialic acids alone are ineffective inhibitors of viral hemagglutination, whereas macromolecules with large quantities of sialic acids are effective (3, 21). Fetuin, rich in sialic acids with NeuAcα2-3Gal linkages (1), slightly inhibited the binding of fibronectin to viral glycoprotein micelles. The inhibition, however, may result from fetuin-viral glycoprotein interaction, which may sterically inhibit the fibronectin binding.

The capacity of the virus to attach to the cell surface can be abolished, and even cell surface-bound virus can be removed by treatment with neuraminidase (7, 15, 16). Plasma fibronectin contains sialic acids linked mainly by NeuAcα2-4Gal linkages (30, 37). Myxo- and paramyxovirus hemagglutinin predominantly attaches to α2-3-positioned sialic acids, suggesting this linkage to be important as a receptor (3, 15). We treated fibronectin with neuraminidase and observed a marked decrease in its ability to bind viral glycoprotein micelles. Our data suggest that sialic acids with suitable linkages to galactose on the fibronectin molecule can serve as binding sites for viral hemagglutinin. The binding of viral glycoprotein micelles to fibronectin may thus be mediated via the sugar moieties of fibronectin.

The gelatin-binding site of fibronectin contains the carbohydrate-rich part of the molecule (19, 23, 31). The affinity of fibronectin for collagen, especially gelatin, is high. The binding of fibronectin to fibrinogen and actin is efficiently inhibited by gelatin (9, 10). Both fibronectin and actin have binding sites that are close to the gelatin-binding site but are distinct (23, 31). Gelatin was, however, ineffective in the present inhibition assays, suggesting that the viral envelope glycoprotein-binding site is distinct from the gelatin-binding site. On the other hand, the binding sites for the different viral glycoproteins may be identical or close to one another. Fibronectin also bound to *V. cholerae* neuraminidase, suggesting a more wide-ranging binding capacity of fibronectin. The binding of fibronectin to solid-phase *V. cholerae* neuraminidase was inhibited by liquid-phase viral glycoprotein micelles. The binding site of the glycoprotein micelles on the fibronectin molecule might thus be close to the binding site of *V. cholerae* neuraminidase. Steric aspects may play a role in the binding because viral glycoprotein micelles in the liquid phase bound more efficiently to solid-phase fibronectin than vice versa. Differences in the binding efficiency of glycoprotein micelles onto plastic, as well as differences between the sizes of different glycoprotein micelles, may affect the quantity of bound protein.

The interaction of fibronectin with staphylococci (11) involves a surface protein common to a number of different strains of *Staphylococcus aureus* and *S. epidermidis* (33). Mosher and Proctor (20) have localized the binding site for staphylococci in fibronectin to a 27,000-dalton fragment at the amino-terminal end of the molecule. It has been proposed that fibronectin acts as a staphylococcal opsonin (22), but its opsonic activity is probably relatively weak as compared with C3b and immunoglobulin G (33). However, the adherence of certain bacteria to epithelial cell surfaces is affected by fibronectin (35). Further, the decrease in cell surface fibronectin correlates with increased susceptibility to adherence by *Pseudomonas aeruginosa* (36). The affinity of fibronectin for viral envelope glycoproteins may play a role in the opsonization of viruses in vivo or in virus-cell interactions.

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LITERATURE CITED

1. **Baenziger, J. U., and D. Fiete.** 1979. Structure of the complex oligosaccharides of fetuin. J. Biol. Chem. **254**:789–795.
2. **Blumenstock, F. A., T. M. Saba, P. Weber, and R. Laffin.** 1978. Biochemical and immunological characterization of human α2-SB glycoprotein: its identity with cold-insoluble globulin. J. Biol. Chem. **253**:4287–4291.
3. **Burness, A. T. H.** 1981. Glycophorin and sialylated components as receptors for viruses, p. 63–84. *In* K. Lönberg-Holm and L. Philipson (ed.), Virus receptors, part 2, animal viruses. Chapman and Hall, London.
4. **Choppin, P. W., and A. Scheid.** 1980. The role of viral glycoproteins in adsorption, penetration and pathogenicity of viruses. Rev. Infect. Dis. **2**:40–61.
5. **Compans, R. W., and H. D. Klenk.** 1979. Viral membranes, p. 293–407. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology, vol. 13. Plenum Publishing Corp., New York.
6. **Dessau, W., F. Jilek, B. C. Adelmann, and H. Hörmann.** 1978. Similarity of antigelatin factor and cold-insoluble globulin. Biochim. Biophys. Acta **533**:227–237.
7. **Enegren, B. J., and A. T. H. Burness.** 1977. Chemical

structure of attachment sites for viruses on human erythrocytes. Nature (London) **268**:536–537.
8. **Engvall, E., and E. Ruoslahti.** 1977. Binding of soluble form of fibroblast surface protein, fibronectin, to collagen. Int. J. Cancer **20**:1–5.
9. **Engvall, E., E. Ruoslahti, and E. Miller.** 1978. Affinity of fibronectin to collagens of different genetic types and to fibrinogen. J. Exp. Med. **147**:1584–1595.
10. **Keski-Oja, J., E. Ruoslahti, and E. Engvall.** 1981. Binding of fibronectin to actin is inhibited by gelatin. Biochem. Biophys. Res. Commun. **100**:1515–1522.
11. **Kuusela, P.** 1979. Fibronectin binds to *Staphylococcus aureus*. Nature (London) **276**:718–720.
12. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
13. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
14. **Luukkonen, A., C. G. Gahmberg, and O. Renkonen.** Surface labeling of Semliki Forest virus glycoproteins using galactose oxidase. Exposure of E3-glycoprotein. Virology **76**:55–59.
15. **Markwell, M. A. K., and J. Paulson.** 1980. Sendai virus utilizes specific sialyloligosaccharides as host cell receptor determinants. Proc. Natl. Acad. Sci. U.S.A. **77**:5693–5697.
16. **Matlin, K. S., H. Reggio, A. Helenius, and K. Simons.** 1981. Infectious entry pathway of influenza virus in a canine kidney cell line. J. Cell Biol. **91**:601–613.
17. **McCarthy, M., and R. T. Johnson.** 1980. A comparison of the structural polypeptides of five strains of mumps virus. J. Gen. Virol. **46**:15–27.
18. **Morein, B., A. Helenius, K. Simons, R. Pettersson, L. Kääriäinen, and R. Schirrmacher.** 1978. Effective subunit vaccines against an enveloped animal virus. Nature (London) **276**:715–718.
19. **Mosher, D. F.** 1980. Fibronectin. Prog. Hemostasis Thromb. **5**:111–151.
20. **Mosher, D. F., and R. A. Proctor.** 1980. Binding and factor XIIIa-mediated cross-linking of a 27-kilodalton fragment of fibronectin to *Staphylococcus aureus*. Science **209**:927–929.
21. **O'Callaghan, R. J., S. M. Loughlin, D. D. Labat, and C. Howe.** 1977. Properties of influenza C virus grown in cell culture. J. Virol. **24**:875–882.
22. **Proctor, R. A., E. Prendengast, and D. F. Mosher.** 1982. Fibronectin mediates attachment of *Staphylococcus aureus* to human neutrophils. Blood **59**:681–687.
23. **Ruoslahti, E., E. Engvall, and E. G. Hayman.** 1981. Fibronectin: current concepts of its structure and functions. Collagen Rel. Res. **1**:95–128.
24. **Saba, T. M., F. A. Blumenstock, P. Weber, and J. E. Kaplan.** 1978. Physiologic role of cold-insoluble globulin in systemic host defence: implications of its characterization as the opsonic a2-surface-binding glycoprotein. Ann. N.Y. Acad. Sci. **312**:43–55.
25. **Salonen, E.-M., and A. Vaheri.** 1979. Immobilization of viral and mycoplasma antigens and of immunoglobulins on polystyrene surface for immunoassays. J. Immunol. Methods **30**:209–218.
26. **Santer, U. V., J. Yee-Foon, and M. C. Glick.** 1978. A rapid assay for neuraminidase. The detection of two differences in activity associated with virus transformation. Biochim. Biophys. Acta **523**:435–442.
27. **Scheid, A.** 1981. Subviral components of myxo- and paramyxo-viruses which recognize receptors, p. 49–62. *In* K. Lönberg-Holm and L. Philipson (ed.), Virus receptors, part 2, animal viruses. Chapman and Hall, London.
28. **Scheid, A., and P. W. Choppin.** 1973. Isolation and purification of the envelope proteins of Newcastle disease virus. J. Virol. **11**:263–271.
29. **Simons, K., A. Helenius, K. Leonard, M. Sarvas, and M. J. Gething.** 1978. Formation of protein micelles from amphilic membrane proteins. Proc. Natl. Acad. Sci. U.S.A. **75**:5306–5310.
30. **Takasaki, S., K. Yamashita, K. Suzuki, S. Iwanaga, and A. Kabata.** 1979. The sugar chains of cold-insoluble globulin. J. Biol. Chem. **254**:8548–8553.
31. **Vaheri, A., J. Keski-Oja, T. Vartio, K. Alitalo, K. Hedman, and M. Kurkinen.** 1980. Structure and functions of fibronectin. Dev. Biochem. **15**:161–178.
32. **Vaheri, A., M. Kurkinen, V.-P. Lehto, E. Linder, and R. Timpl.** 1978. Codistribution of pericellular matrix proteins in cultured fibroblasts and loss in transformation: fibronectin and procollagen. Proc. Natl. Acad. Sci. U.S.A. **75**:4944–4948.
33. **Verbrugh, H. A., P. K. Peterson, D. E. Smith, B.-Y. T. Nguyen, J. R. Hoidal, B. J. Wilkinson, J. Verhoef, and L. T. Furcht.** 1981. Human fibronectin binding to staphylococcal surface protein and its relative inefficiency in promoting phagocytosis by human polymorphonuclear leukocytes, monocytes and alveolar macrophages. Infect. Immun. **33**:811–879.
34. **Wolters, G., L. Kuijpers, J. Kacaki, and A. Schuurs.** 1976. Solid-phase enzyme-immunoassay for detection of hepatitis B surface antigen. J. Clin. Pathol. **29**:873–879.
35. **Woods, D. E., J. A. Bass, W. G. Johanson, Jr., and D. C. Straus.** 1980. Role of adherence in the pathogenesis of *Pseudomonas aeruginosa* lung infection in cystic fibrosis patients. Infect. Immun. **30**:694–699.
36. **Woods, D. E., D. C. Straus, W. G. Johanson, Jr., and J. A. Bass.** 1981. Role of fibronectin in the prevention of adherence of *Pseudomonas aeruginosa* to buccal cells. J. Infect. Dis. **143**:784–790.
37. **Wrann, M.** 1978. Methylation analysis of the carbohydrate portion of fibronectin isolated from human plasma. Biochem. Biophys. Res. Commun. **84**:269–274.