A Matrix Form of Fibronectin Mediates Enhanced Binding of Streptococcus pyogenes to Host Tissue*

Nobuhiko Okada‡§, Masahisa Watarai‡, Vered Ozeri§, Emanuel Hanski‡, Michael Caparon†, and Chihiro Sasakawa‡

From the ‡Department of Bacteriology, Institute of Medical Science, University of Tokyo, Tokyo 108, Japan, the §Department of Clinical Microbiology, The Hebrew University Hadassah Medical School, Jerusalem 91010, Israel, and the †Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri 63110

The pathogenic Gram-positive bacterium Streptococcus pyogenes (group A streptococcus) binds to fibronectin via protein F. In this study, we have investigated the binding properties of protein F to various multimeric tissue forms of fibronectin that appear on cell surfaces and in the extracellular matrix. We show that binding of S. pyogenes through protein F is more efficient to an in vitro-derived polymerized form of fibronectin (superfibronecin) than to soluble fibronectin immobilized in a solid phase. In addition, Chinese hamster ovary cells overexpressing the α5β1 integrin produced an increased amount of a fibronectin matrix and consequently bound a higher number of S. pyogenes cells. Inhibition and direct binding assays using purified proteins demonstrated that binding to a fibronectin matrix involved both domains of protein F (UR and RD2) that have previously been implicated in interactions with fibronectin. Using intact S. pyogenes bacteria in which various domains of protein F were expressed as hybrids with the surface-exposed region of an unrelated protein, we revealed that, in contrast to the predominantly UR-mediated binding to soluble fibronectin, the maximal binding to the fibronectin matrix required RD2 in addition to UR. Since in some infections S. pyogenes may initially encounter a matrix form of fibronectin, these results suggest that UR and RD2 may be important for the initiation of streptococcal infectious processes.

The adherence of pathogenic bacteria to host tissues is an important prerequisite for bacterial colonization and subsequent development of disease (1). This interaction is mediated by structures on the bacterial surface (adhesins) and specific structures associated with host cells (receptors) (2). A host protein utilized as a receptor by numerous pathogenic bacteria is fibronectin (3), a glycoprotein found either as a soluble dimer in most body fluids including plasma, cerebrospinal fluid, and amniotic fluid and as an insoluble multimer in association with cell surfaces, the extracellular matrix, and basement membrane (4, 5). An interesting feature of fibronectin is that it is composed of distinct domains that bind to a number of proteins that include integrins, collagens, fibrin, gelatin, and heparin, as well as other proteins and nonprotein compounds (4–6). For bacteria that bind fibronectin, its multiple binding properties can contribute to the ability to colonize many different sites in the host.

The interaction between fibronectin and the Gram-positive bacterium Streptococcus pyogenes (group A streptococcus) has been extensively studied. S. pyogenes is one of the most versatile human pathogens with regards to the number of different tissues it can infect and the wide range of different diseases it can cause, including supplicative infections of the throat (pharyngitis) and of the skin and soft tissues (impetigo, erysipelas, cellulitis, necrotizing fasciitis, and myositis). Several systemic infections can result from the production of different toxins (scarlet fever and toxic shock-like syndrome), and a host immune response to streptococcal antigens has been implicated to play a role in rheumatic fever. Each streptococcal infection is initiated by attachment and colonization of bacteria to epithelial cells of the pharyngeal mucosa or the skin (6). Considerable evidence has accumulated to suggest that binding of S. pyogenes to fibronectin promotes adherence to epithelial cells during infection (1, 7, 8).

In S. pyogenes, several different fibronectin-binding proteins have been identified, including the 28-kDa antigen (9), FBP54 (10), glyceraldehyde-3-phosphate dehydrogenase (11), a serotype 3 M protein (12), serum opacity factor/SfbII (13, 14), and protein H (15). Perhaps the best characterized streptococcal fibronectin-binding molecules are the closely related proteins SfbI (16, 17) and protein F (18). Previous studies have shown that protein F contains two distinct fibronectin binding domains, a tandem repetitive domain (RD2), and a domain immediately N-terminal to the repetitive domain (UR, Fig. 1) (19, 20). The minimal functional binding unit of the RD2 repeat domain consists of 44 amino acids located at the junction of two adjacent sequence repeats and is flanked by an MGGQSES motif. This functional unit recognizes the N-terminal fibrin binding domain of fibronectin (20). UR contains 49 amino acids and consists of the 43 amino acids located immediately N-terminal to RD2 and the first six amino acids from the first repeat of RD2. This domain binds to a large region of fibronectin that includes both the N-terminal fibrin and adjacent collagen binding domains (20). UR binds fibronectin with high affinity and is the dominant domain for the binding of soluble fibronectin to protein F (20).

In tissues, fibronectin forms disulfide cross-linked fibrils (5). Several lines of evidence suggest that this tissue form of fibronectin is functionally distinct from that of the soluble form. Chinese hamster ovary (CHO)1 cells with an increased capacity...
Enhanced Streptococcal Binding to a Fibronectin Matrix

of the fibronectin binding domains of protein F as fusions to an N-terminal affinity tag consisting of six histidine residues (His6) has been described elsewhere (19, 20, 28). These included pUR-4 (UR), pRD-2 (RD2), and pPTF54 (UR plus 5 repeats of RD2). The fusion proteins were purified from SG13009(pREP4), containing the appropriate plasmid, by affinity chromatography using a nickel-nitrilotriacetic acid resin and native conditions according to the recommendations of the manufacturer. The purified proteins were dialyzed against phosphate-buffered saline (PBS, pH 7.2). Each preparation was 95% pure as judged by SDS-polyacrylamide gel electrophoresis and staining of gels with Coomassie Blue.

Superfibronectin Production—Superfibronectin was produced as described previously (22). Briefly, 1 μg/ml of purified human plasma fibronectin (Upstate Biotechnology Inc.) was incubated at 37 °C for 24 h with 1 μg of a purified fusion protein that consists of the C-terminal two-thirds of the third type III repeat of fibronectin (III,-C) and corresponds to amino acids 600 to 674 of the whole molecule (32). The typical yield was 1 μg/ml superfibronectin as determined by nonreducing SDS-polyacrylamide gel electrophoresis (22). Since a region from the eleventh type III repeat corresponding to amino acids 1532 to 1599 of the fibronectin molecule (III,-C) is similar in composition to III,-C, but does not promote the formation of superfibronectin (22), the fusion protein containing III,-C was incubated with fibronectin in a similar manner to produce a nonpolymerized control. The construction of the His3-III,-C and III,-C fused proteins is described elsewhere (22). In all of these experiments, the fusion proteins were purified from E. coli MV1184 as outlined above.

Superfibronectin Binding Assay—Superfibronectin, fibronectin, or the III,-C fragment at various concentrations was coated onto 96-well ELISA plates (Costar). Following a 24-h incubation at 37 °C, the wells were then blocked with 5% bovine serum albumin in PBS (pH 7.2). Streptococci from overnight cultures were resuspended in PBS (pH 7.2) to a density of 1 × 10⁶ cells/ml, and a 100-μl aliquot of the bacterial suspension was added to each well. After incubation for 2 h at room temperature, unbound bacteria were removed by washing the wells five times with PBS containing 0.05% Tween 20 (PBS/T). The amount of streptococci bound to each protein was determined by ELISA using a rabbit antiserum specific for the S. pyogenes cell wall carbohydrate (Difco) and an alkaline phosphate-conjugated anti-rabbit IgG anti-serum (Sigma). In control experiments, the monoclonal antibody 3E1 (Life Technologies, Inc.) that recognizes C-terminal heparin binding domain of fibronectin (33) was used at similar molar concentrations as the III,-C fragment.

For inhibition assays, superfibronectin at a concentration of 5 μg/ml was used to coat wells of 96-well ELISA plates. Purified protein containing two-thirds of either UR, RD2, or RD2 plus RD2 repeats was then added to superfibronectin-coated wells for 1 h at room temperature. In some experiments, a partially purified full-length protein F prepared as the periplasmic fraction of an E. coli strain carrying the chimeric plasmid pPTF5 (18) was used as an inhibitor at a concentration of 1.22 mg/ml in place of the purified fusion proteins. Streptococcal adherence to the treated wells was then determined as described above. For direct binding assays, 100 μl of the purified fusion protein containing either UR, RD2, or RD2 plus five repeats of RD2 at 10 μg/ml was added to superfibronectin- or the III,-C-coated well at various concentrations and incubated for 2 h at room temperature. After washing the wells three times with PBS/T, the amount of each purified protein bound to superfibronectin or to the III,-C fragment was measured by ELISA using a monoclonal 16305-His antibody (Qiagen Inc.) and an alkaline phosphate-conjugated anti-mouse IgG antisera (Sigma). Streptococcal Adherence to CHO-HFR4 and CHO-HFR5 Cells—The α1β1 integrin overexpressing CHO cell lines, CHO-HFR4 and CHO-HFR5, produce an abundant fibronectin matrix composed of fibrils longer and thicker than CHO cells alone (21, 25). Cells were cultured for 4 days before use in 24-well plates on glass coverslips in minimal essential medium supplemented with 10% fetal calf serum and 1 mg/ml Geneticin (Wako Pure Chemical Industries, Ltd) at 37 °C in a humidified atmosphere of 5% CO₂ and 95% air. Adherence of S. pyogenes to these cells was evaluated by staining with crystal violet and light microscopy and was quantified by determination of the number of bacteria bound per cell. For each experiment, at least 50 cells from each of five randomly chosen microscope fields were evaluated, and the data reported are the mean number of bacteria bound per cell obtained from three independent experiments. In some experiments, percentage of cultured cells that bound bacteria was determined as described elsewhere (34). Inhibition assays with a purified rabbit polyclonal antibody specific for human fibronectin (COSMO BIO Co. Ltd.) were performed by incubation of CHO-HFR5 cells with various concentrations of antibody for 1 h at 37 °C prior to addition of S. pyogenes cells. Purified
Enhanced Streptococcal Binding to a Fibronectin Matrix

RESULTS

Enhanced Binding of \textit{S. pyogenes} to Superfibronectin—In some infections, such as infection of a wound, many bacterial species including \textit{S. pyogenes} may initially encounter a matrix form of fibronectin. To investigate the binding of \textit{S. pyogenes} to a fibronectin matrix, we employed a form of fibronectin (superfibronectin) that resembles the tissue form of fibronectin (22–24). Superfibronectin was produced by the incubation of fibronectin with a fragment corresponding to the C-terminal two-thirds of the fibronectin repeat, which binds to fibronectin with high affinity and induces spontaneous disulfide cross-linking of the fibronectin dimers into matrix fibrils (22, 23, 24) (Fig. 2A). Analysis of the binding of protein F-expressing \textit{S. pyogenes} JRS145 revealed that binding to superfibronectin was much more efficient than to fibronectin (Fig. 2B). Binding to 2.5 \( \mu \text{g/ml} \) superfibronectin was increased 7-fold relative to fibronectin at this concentration and was comparable with the degree of binding obtained with 10 \( \mu \text{g/ml} \) fibronectin. To circumvent a possibility that the increased binding of JRS145 to the superfibronectin might result from the fibronectin bound to immobilized \( \text{III}_1\)-C, thereby protecting the fibronectin from denaturation on a plastic dish, fibronectin was coated onto 96-well plates with the monoclonal antibody 3E1, which binds to the C-terminal heparin binding domain of fibronectin (33), and then binding of JRS145 was examined. As shown in Fig. 2C, treatment of fibronectin with the monoclonal antibody 3E1 in place of the \( \text{III}_1\)-C did not result in increased binding of JRS145 strain under the same conditions as that of Fig. 2B. Binding to superfibronectin required protein F as demonstrated by the dramatically reduced binding of a protein F-deficient mutant SAM2 to superfibronectin under these conditions (Fig. 2D). Minimal binding was observed to the immobilized \( \text{III}_1\)-C fragment alone (Fig. 2B), and control experiments indicated that total amount of fibronectin bound to wells did not differ between superfibronectin- and fibronectin-coated wells. In addition, the levels of binding observed were not influenced by any M protein-induced streptococcal aggregation since the gene that encodes M protein has been deleted from both JRS145 and SAM2. These data suggest that \textit{S. pyogenes} binds much more efficiently to the fibrillar form of fibronectin via protein F than to immobilized plasma fibronectin.

Two Distinct Fibronectin Binding Domains of Protein F Bind Superfibronectin—Previous studies have demonstrated that two domains of protein F, UR and RD2, are involved in the binding of \textit{S. pyogenes} to extracellular matrix (20). To examine the binding of protein F to superfibronectin, purified proteins containing either UR, RD2, or UR plus five RD2 repeats were evaluated for their ability to compete with protein F-expressing \textit{S. pyogenes} for binding to superfibronectin. A purified protein using a rabbit antiserum specific for the \textit{S. pyogenes} cell wall carbohydrate and an alkaline phosphatase-conjugated anti-rabbit IgG antiserum. Data represents the mean of duplicate samples, which differed by less that 5\% from the mean value for each pair at all concentrations tested.
containing both fibronectin binding domains of protein F (UR plus five RD2 repeats) blocked over 90% of the streptococcal binding to superfibronectin at a final concentration of 1000 nM, whereas purified proteins containing either UR alone or RD2 alone blocked binding by only 70% and 50%, respectively, under identical conditions (Fig. 3). The level of inhibition obtained with the complete protein F molecule was essentially the same as that by the purified protein containing both UR plus the five repeats of RD2 (data not shown). To examine the relative contributions of the UR and RD2 domains to the whole fibronectin binding domains of protein F, the binding ability of each of the purified proteins to superfibronectin was determined by the direct binding assay. The specific binding of the purified protein containing UR and five repeats of RD2 to 10 μg/ml superfibronectin was approximately 1.6- and 2.0-fold higher than that by the purified proteins containing UR and RD2, respectively (Fig. 4). In contrast, the binding to the same concentration of immobilized fibronectin was mainly required for the purified protein containing UR domain (Fig. 4). Only a minimal binding of these purified derivatives of protein F to the immobilized III1-C fragment was detected (Fig. 4). Thus, both UR and RD2 are required for efficient binding of protein F to superfibronectin.

**Binding of *S. pyogenes* to a Fibronectin Matrix in Vivo**—Further characterization of the efficient binding to the matrix form of fibronectin utilized CHO cell lines that demonstrate a 3.1- (CHO-HFR4) and 7.4-fold (CHO-HFR5) overexpression of the human α5β1 integrin (25). These integrin-overexpressing cells accumulate a more abundant fibronectin matrix than the parental CHO cells, which results in the organization of fibrillar network (21). As shown in Fig. 5, the levels of fibronectin fibrils produced from CHO-HFR4 and CHO-HFR5 cells correlate with the increased levels of α5β1 integrin expressed. JRS145 adhered efficiently to CHO-HFR5 cells, with bacteria being largely clustered on defined areas of the cell surface (Fig. 5). For CHO-HFR4 cells, an intermediate amount of binding was seen, which appeared as small clusters of streptococci covering the surface of the cells (Fig. 5). The numbers of bacteria bound to CHO-HFR4 and CHO-HFR5 cells were 6.2- and 9.8-fold greater than to CHO cells. To see if *S. pyogenes* cells bound specifically to a fibronectin matrix produced from α5β1 integrin-overexpressing CHO cells, a polyclonal antibody specific for human fibronectin was tested for its ability to inhibit bacterial binding to CHO-HFR5 cells. Following preincubation of CHO-HFR5 cells with either 0.1, 1, or 10 μg/ml of anti-human fibronectin antibody, a concentration-dependent inhibition of streptococcal adherence to CHO-HFR5 cells was observed (Table I). Approximately 70% of bacterial adherence was blocked by incubation of CHO-HFR5 cells with 10 μg/ml of antibody (Table I). In addition, protein F-deficient mutant SAM2 did not adhere to any CHO cell line at a detectable level.

These findings demonstrate that an elevated deposition of a fibronectin and subsequent formation of a matrix on the surface of host cells promoted increased binding of *S. pyogenes* via protein F.

**Efficient Binding of Protein F to a Fibronectin Matrix in Vivo Requires Both Fibronectin Binding Domains of Protein F**—To determine which fibronectin binding domain of protein F was responsible for binding to a fibronectin matrix produced from
CHO-HFR5 cells, purified proteins of either UR, RD2, or both UR and five repeats of RD2 were tested for their ability to block bacterial binding to CHO-HFR5 cells. A purified protein containing both domains (UR plus five repeats of RD2) at a final concentration of 100 nM blocked adherence of JRS145 to CHO-HFR5 cells by 88%, whereas a purified protein containing either UR alone or RD2 alone blocked the binding by 66% and 34% of the control level, respectively (Table II), suggesting that both domains participate in the bacterial binding to a fibronectin matrix in vivo.

Further examination included analysis of a series of S. pyogenes mutants that express various specific domains of protein F as hybrids, which replace the surface-exposed region of M protein, a surface protein unrelated to protein F (20, 28). The structure of the chimeric proteins expressed by various strains is shown in Fig. 6A. Microscopic examination revealed that S. pyogenes strain SAM19 (UR plus five RD2 repeats) bound to the cells at a similar level to protein F-expressing JRS145, whereas SAM17 (five repeats of RD2) or SAM25 (UR plus a single RD2 repeat) demonstrated a reduced level of binding (Fig. 6B). SAM2, the protein F-deficient parent of these hybrid-expressing strains, did not bind at any significant level to CHO-HFR5 cells (Fig. 6B). These results were confirmed by measurement of the number of CHO-HFR5 cells-bound streptococci, in which the number of bacteria bound per cell were counted. The average number of SAM17 and SAM25 bound per CHO-HFR5 cell was approximately 30 and 60% fewer, respectively, than that by SAM19 (Table III). These data provide

**TABLE I**

| Treatment                          | Concentration | % Inhibition | % Inhibition* |
|-----------------------------------|---------------|--------------|---------------|
| **Treatment**                     | **Concentration** | **% Inhibition** | **% Inhibition** |
|                                   | µg/ml         |              |              |
| Purified rabbit IgG               | 10            | 0 ± 2        |              |
| Anti-human fibronectin antibody   | 0.1           | 6 ± 2        |              |
|                                   | 1             | 49 ± 4       |              |
|                                   | 10            | 71 ± 7       |              |

*Data represent the mean value and standard error of the mean of triplicate determinants.

**TABLE II**

| Treatment                          | Protein concentration | % Inhibition | % Inhibition* |
|-----------------------------------|------------------------|--------------|---------------|
|                                   | nM                     |              |              |
| Bovine serum albumin              | 100                     | 0            |              |
| UR                                | 1                      | 14 ± 4       |              |
|                                   | 10                      | 46 ± 8       |              |
|                                   | 100                     | 65 ± 7       |              |
| RD2                               | 1                      | 10 ± 7       |              |
|                                   | 10                      | 20 ± 4       |              |
|                                   | 100                     | 34 ± 5       |              |
| UR + five RD2                     | 1                      | 38 ± 6       |              |
|                                   | 10                      | 66 ± 5       |              |
|                                   | 100                     | 88 ± 3       |              |

*Data represent the mean value and standard error of the mean of triplicate determinants.

* These proteins were purified as a fusion protein with N-terminal affinity tag consisting of His6 (see “Experimental Procedures”). The localization of these domains in the protein F molecule is shown in Fig. 1.
Enhanced streptococcal binding to a fibrinectin matrix

**TABLE III**

| Strain   | % of adherencea | No. of bacteria bound per cellb |
|----------|-----------------|---------------------------------|
| JRS145   | 93 ± 5           | 137 ± 8                         |
| SAM17    | 56 ± 4           | 44 ± 2                          |
| SAM19    | 90 ± 5           | 125 ± 10                        |
| SAM25    | 82 ± 5           | 80 ± 8                          |
| SAM2     | 1 ± 3            | 7 ± 3                           |

a The number of CHO-HFR5 cells with adherent streptococci of the indicated strain was determined by light microscopy.

b The number of adherent bacteria of the indicated strain per CHO-HFR5 cell was shown (see "Experimental Procedures" for details).

Data represent the mean and standard error of the mean of triplicate determinants.

Additional evidence that efficient binding to matrix fibrinectin requires both the UR and RD2 domains of protein F.

**DISCUSSION**

Fibronectin exists in several forms, which can arise through alternative splicing of the fibrinectin mRNA by posttranslational modifications including glycosylation and phosphorylation or by multimer formation produced by disulfide bond cross-linking (5). These variations have been shown to affect the binding properties of many different host proteins to fibrinectin (5, 22), and several lines of evidence from the present study suggest that the form of fibrinectin can effect its interaction with protein F. These include: 1) that protein F-mediated binding of *S. pyogenes* to superfibronectin, an *in vitro* derived form of fibrinectin similar to matrix fibrinectin, is more efficient than to immobilized soluble fibrinectin; 2) that increased protein F-mediated binding to host cells can be enhanced by increasing the ability of the cells to induce the formation of a fibrinectin matrix; and 3) that while UR is the dominant protein F domain for binding to soluble fibrinectin (20), efficient binding to a fibrinectin matrix requires RD2 in addition to UR.

Because of its heterogeneity, it is not surprising that a variety of microorganisms, including both Gram-positive and Gram-negative bacterial species, have evolved numerous unrelated adhesins that bind to different specific domains of the fibrinectin molecule. For example, protein F recognizes the N-terminal fibrin binding domain of fibrinectin via RD2, while UR binds to a larger N-terminal domain consisting of the fibrin binding domain and adjacent collagen binding domain (20). The N-terminal fibrin binding domain and the C-terminal heparin binding domain of fibrinectin interacts with both staphylococci and streptococci (3, 20). The collagen binding domain of fibronectin contains a region recognized by mycobacteria and *Streptococcus agalactiae* (35, 36), whereas the fibrinectin type III repeat domain is recognized by protein H of *S. pyogenes* (15). The numerous mechanisms that have evolved for promoting bacterial binding to fibrinectin enable many organisms to adhere and colonize different niches in the host.

In many instances, an infecting bacterium could encounter both a matrix form of fibrinectin in addition to a soluble form of fibrinectin. Although the molecular basis of binding is poorly understood, some bacterial fibrinectin-binding proteins possess preferential recognition of a specific state of the fibrinectin molecule. For example, the P-pilus of urapathogenic *E. coli*, an adhesin that binds to Galα1-3Gal-containing glycosphingolipids on epithelial cells, can bind efficiently to immobilized fibrinectin but not to the soluble form (37). Similarly, the uncharacterized fibrinectin-binding proteins of *Streptococcus pneumoniae* and *Streptococcus sanguis* also seem to preferentially recognize immobilized versus soluble fibrinectin (38, 39). The YadA outer membrane protein found in several *Yersina* species can mediate binding to cartilage-derived human cellular fibrinectin but fails to bind to human plasma fibrinectin in a solid phase (40). Discrimination between different forms of fibrinectin may be a consequence of the sensitivity of a bacterial adhesion to detect conformational changes in fibrinectin induced by the constraints imposed by any particular state, like soluble, immobilized, multimeric, or dimeric structures. The enhanced binding of *S. pyogenes* to superfibrinectin versus fibrinectin may suggest that protein F is sensitive to a conformational change between these structures. However, since both fibrinectin binding domains of protein F are required for optimal binding, matrix formation may either allow protein F greater access to the receptor domains of fibrinectin or may create a high receptor density. Further analysis of streptococcal binding to superfibrinectin and to CHO cells overproducing the α5β1 integrin will be useful for elucidation of the molecular mechanism of enhanced binding to matrix fibrinectin.

It is unknown why protein F possess two distinct domains for binding to fibrinectin. The genes that encode proteins of the protein F/SfbI family are widely distributed among diverse isolates of *S. pyogenes* (41, 42), and the UR and RD2 domains are highly conserved (41, 42), suggesting that these two different domains are critical for protein F function. In this study, we have shown in several assays that both UR and RD2 are required to obtain a level of binding to matrix fibrinectin that is as efficient as that of the whole protein F molecule. Thus, the conservation of both UR and RD2 may result from a requirement for both domains to promote efficient binding to tissue forms of fibrinectin. This suggests that an interaction between two distinct fibrinectin binding domains and a fibrinectin matrix plays an important role in the pathogenesis of streptococcal infections.

Acknowledgments—We thank Erkki Ruusalahti for generous gifts of α5β1 integrin cDNAs and expression plasmids for purification of the III1-C and III11 fragments.

REFERENCES

1. Beachey, E. H. (1981) *J. Infect. Dis.* **143**, 325–345
2. Halgren, S. J., Abraham, S., Caparon, M., Falk, P., St. Gene J., III, and Normark, S. (1993) *Cell* **73**, 887–901
3. Patti, J. M., and Hook, M. (1994) *Curr. Opin. Cell Biol.* **6**, 752–758
4. Hynes, R. O., and Yamada, K. M. (1982) *J. Cell Biol.* **95**, 369–377
5. Ruusalahti, E. (1988) *Annu. Rev. Biochem.* **57**, 375–413
6. Wannamaker, L. W. (1970) *N. Engl. J. Med.* **282**, 23–30
7. Hasty, D. L., Oefc, I., Courtney, H. S., and Doyle, R. (1992) *Infect. Immun.* **60**, 2147–2152
8. Simpson, W. A., Courtney, H. S., and Oefc, I. (1987) *Rev. Infect. Dis.* **9**, 8251–8258
9. Courtney, H. S., Hasty, D. L., Dale, J. B., and Poirier, T. P. (1992) *Curr. Microbiol.* **25**, 245–250
10. Courtney, H. S., Li, Y., Dale, J. B., and Hasty, D. L. (1994) *Infect. Immun.* **62**, 3937–3946
11. Pancholi, V., and Fischetti, V. A. (1992) *J. Exp. Med.* **176**, 415–426
12. Schmidt, K. H., Mann, K., Conney, J., and Kohler, W. (1993) *FEMS Immunol. Med. Microbiol.* **7**, 135–144
13. Rakonjac, J. V., Robbins, J. C., and Fischetti, V. A. (1995) *Infect. Immun.* **63**, 622–631
14. Kreikmeyer, B., Talay, S. R., and Chhatwal, G. S. (1995) *Microb. Res.* **150**, 137–145
15. Fick, I.-M., Crossin, K. L., Edelman, G. M., and Björk, L. (1995) *EMBO J.* **14**, 1674–1679
16. Talay, S. R., Ehrenfeld, E., Chhatwal, G. S., and Timmis, K. N. (1991) *Mol. Microbiol.* **5**, 1727–1734
17. Talay, S. R., Valentin-Weigand, P. G. J., Timmis, K. N., and Chhatwal, G. S. (1992) *Infect. Immun.* **60**, 2387–2394
18. Hanski, E., and Caparon, M. G. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 6172–6176
19. Sela, S., Aviv, A., Tovi, A., Burstein, I., Caparon, M. G., and Hanski, E. (1993) *Mol. Microbiol.* **10**, 1049–1055
20. Ozeri, V., Tovi, A., Burstein, I., Natanson-Yaron, S., Caparon, M. G., Yamada, K. M., Akiyama, S. K., Vlodavski, I., and Hanski, E. (1996) *EMBO J.* **15**, 989–996
21. Giancotti, F. G., and Ruoslahti, E. (1990) *Cell* **60**, 849–859
22. Moria, A., Zhang, Z., and Ruoslahti, E. (1994) *Nature* **367**, 193–196
23. Hocking, D. C., Sottile, J., and McKeown-Longo, P. J. (1994) *J. Biol. Chem.* **269**, 19183–19187
24. Hocking, D. C., Smith, B. K., and McKeown-Longo, P. J. (1996) *J. Cell Biol.* **133**, 431–444
25. Watara, M., Funato, S., and Sasakiwa, C. (1996) *Exp. Med.* **183**, 991–999
Enhanced Streptococcal Binding to a Fibronectin Matrix

26. Scott, J. R., Guenther, P. C., Malone, L. M., and Fischetti, V. A. (1986) J. Exp. Med. 164, 1641–1651
27. Caparon, M. G., Geist, R. T., Perez-Casal, J., and Scott, R. S. (1992) J. Bacteriol. 174, 5693–5701
28. Hanski, E., Fogg, G., Tovi, A., Okada, N., Burnsein, I., and Caparon, M. (1995) Methods Enzymol. 253, 269–305
29. Scott, J. R. (1972) Virology 52, 344–349
30. Fogg, G. C., Gibson, C. M., and Caparon, M. G. (1993) Mol. Microbiol. 11, 671–684
31. Lee, S.-Y., and Caparon, M. (1996) Infect. Immun. 64, 413–421
32. Kornblith, A. R., Umezawa, K., Vibe-Pederson, K., and Baralle, F. E. (1985) EMBO J. 4, 1755–1759
33. Pierschbacher, M. D., Hayman, E. G., and Ruoslahti, E. (1981) Cell 26, 259–267
34. Okada, N., Pentland, A. P., Falk, P., and Caparon, M. G. (1994) J. Clin. Invest. 94, 965–977
35. Peake, P., Gooley, A., and Britton, W. J. (1993) Infect. Immun. 61, 4828–4834
36. Tamura, G. S., and Rubens, C. E. (1995) Mol. Microbiol. 15, 581–589
37. Westerlund, B., Kunnasla, P., Vartio, T., Van Die, I., and Korhonen, T. K. (1989) FEBS Lett. 243, 199–204
38. von der Flier, M., Chhun, N., Wizemann, T. M., Min, J., McCarthy, J. B., and Tuomanen, E. I. (1995) Infect. Immun. 63, 4317–4322
39. Lowrance, J. H., Hasty, D. L., and Simpson, W. A. (1988) Infect. Immun. 56, 2279–2285
40. Schulze-Koops, H., Burkhardt, H., Heesemann, J., Kirch, T., Sweboda, B., Bull, C., Goodman, S., and Emmrich, F. (1993) Infect. Immun. 61, 2513–2519
41. Talay, S. R., Valentin-Weigand, P., Timmis, K. N., and Chhatwal, G. S. (1994) Mol. Microbiol. 13, 531–539
42. Natanson, S., Sela, S., Moses, A. E., Musser, J. M., Caparon, M. G., and Hanski, E. (1985) J. Infect. Dis. 151, 871–878