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Acidic Fibroblast Growth Factor Modulates *Staphylococcus aureus* Adherence to Human Endothelial Cells

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Alteration of human endothelial cells may increase their susceptibility to staphylococcal invasion and thus may contribute to the development of intravascular staphylococcal disease. Acidic fibroblast growth factor, a potent regulator of endothelial cell function, had a significant effect on *Staphylococcus aureus* infection of cultured human endothelial cells. Three of four *S. aureus* strains had diminished adherence to endothelial cells when the latter were grown in the presence of acidic fibroblast growth factor ($P < 0.05$). The diminished adherence was time dependent, maximal at 72 h, and independent of the initial bacterial inoculum. A twofold enhancement of *S. aureus* adherence was observed when endothelial cells were pretreated with heparitinase. Adherence was unaffected by endothelial cell activation by interleukin-1 or endotoxin. Thus, acidic fibroblast growth factor exerted a protective effect, deterring *S. aureus* adherence to cultured endothelial cells. Endothelial cell heparan sulfate was also directly involved in the adherence process. Subtle modulations of endothelial cells can significantly affect the ability of *S. aureus* to adhere to and then infect these cells. Similar alterations may contribute to the ability of *S. aureus* to infect endovascular tissue in vivo.

The pathogen *Staphylococcus aureus* is notable for its ability to cause fulminant disease. In acute endocarditis, *S. aureus* is often associated with metastatic dissemination, abscess formation, high mortality, and a propensity for involving apparently normal valvular tissue (6, 11, 29). The basis for this affinity for endovascular tissue is unexplained. Previous studies have demonstrated that *S. aureus* has a remarkable ability to adhere to valvular tissue and to cultured human endothelial cells (17, 26, 35). Thus, the intrinsic properties of the bacterium appear to be important in the characteristic development of *S. aureus* endocarditis. The role of the endothelium in this process is less clearly understood.

Many investigators have demonstrated that endothelial cell activation by immunomodulators such as interleukin-1 or endotoxin leads to increased leukocyte adhesion (1–3, 22, 23, 32). This activation appears to be critical in the initiation of both the inflammatory response and coagulation (8, 9). Thus, the endothelial cell can respond to environmental stimuli with significant alterations of surface characteristics.

We hypothesized that the vascular endothelium was actively involved in the initial bacterial adhesion interaction (26) and therefore contributed to the pathogenesis of staphylococcal endovascular disease. Modulation of the endothelial cell might contribute significantly to this process. To investigate this question, we studied whether the endothelial cell modulators acidic fibroblast growth factor (aFGF), interleukin-1, and endotoxin induced changes which rendered the endothelial cells more susceptible to invasion by *S. aureus* in tissue culture.

MATERIALS AND METHODS

Endothelial cell preparation. Endothelial cells were harvested from human umbilical veins by using 0.1% collagenase (Sigma Chemical Co., St. Louis, Mo.) and maintained in tissue culture at 37°C in 5.5% CO_2 as previously described (14). Cells between the second and fifth passages (inclusive) were grown to confluence in 100-mm Falcon tissue culture plates (Becton Dickinson Labware, Oxnard, Calif.) and passaged every 7 days in medium consisting of medium 199 (M199) (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 20% newborn calf serum (GIBCO), 1.6 mM glutamine (GIBCO), 15 mM HEPES (*N*-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (Calbiochem-Behring, La Jolla, Calif.), 25 μg of porcine heparin (Sigma) per ml, 50 μg of ascorbate (Fisher Scientific Co., Pittsburgh, Pa.) per ml, and partially purified aFGF (14). Infection assays were performed with these cells passaged into 24-well (16-mm) plastic trays (Corning Glass Works, Rochester, N.Y.). The maximal cell passage number for these studies was 4.

Preparation of bacteria. A blood culture isolate from a patient with *S. aureus* endocarditis was the primary strain (Webb) used for all of the experiments. Additional isolates including *S. aureus* (three isolates), *Staphylococcus epidermidis* (two isolates), and *Streptococcus sanguis* (one isolate) were used in some studies (Table 1). The strains were stored on glass beads in nutrient broth (Difco Laboratories, Detroit, Mich.) at −70°C and subcultured onto blood agar before each experiment. Fresh colonies were inoculated into Todd-Hewitt broth (BBL Microbiology Systems, Cockeysville, Md.) and grown up overnight at 37°C. Stationary-phase bacteria were collected by centrifugation and suspended in M199. The suspension was repeatedly aspirated through a 25-gauge needle and then filtered through a 5.0-μm-pore-size membrane (Nuclepore Corp., Pleasanton, Calif.) to disperse bacterial aggregates. The suspension was then adjusted to an optical density known to provide an average inoculum of 10^7 CFU/ml.

Infection assay. Samples of 0.25 ml of bacteria were added to well sets containing confluent monolayers of endothelial cells (4×10^4 to 8×10^4 cells per well) and incubated at 37°C in 5.5% CO_2 for 30 min (26). Supernatants containing nonadherent bacteria were aspirated, and the cell surfaces were washed with phosphate-buffered saline (PBS). Endothelial cells and adherent bacteria were removed with trypsin

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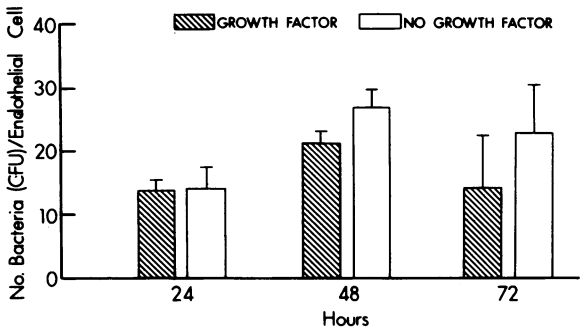

FIG. 1. Effect of aFGF on *S. aureus* adherence to endothelial cells. Endothelial cells were grown to confluence in the presence or absence of aFGF (3 mg%). The endothelial cell monolayer was washed, and the infection assay was performed (30-min bacterial incubation). Results represent the means ± SD.

(GIBCO), diluted into distilled water to lyse the endothelial cells, and then serially diluted and plated into heart infusion agar (Difco). Results were expressed as the number of bacteria per endothelial cell (BA/EC).

Effect of aFGF, interleukin-1, and endotoxin on adherence. All studies were performed by preincubating the endothelial cell monolayer with one of the immunomodulators as described below. After preincubation, monolayers were washed free of the immunomodulator and the infection assay was performed.

(i) aFGF. Endothelial cells were grown to confluence over periods ranging from 24 to 96 h either with or without partially purified bovine brain aFGF or heparin affinity-purified human brain aFGF (concentrations of 3 mg% and 50 ng/ml, respectively). The affinity-purified human aFGF was a gift of Gregory Conn, Albert Einstein College of Medicine, Bronx, N.Y. (7). The infection assay was performed simultaneously on washed endothelial cells of the same cell line grown with and without aFGF. Endothelial cell counts were similar in the wells grown with and without aFGF. In dose-response studies, the infection assay was performed at 4°C (to prevent bacterial endocytosis) with concentrations of bacteria ranging from 10^5 to 10^7 CFU/ml and monolayers grown to confluence over 72 h with and without aFGF.

(ii) Interleukin-1 and endotoxin. Endothelial cell monolayers grown to confluence in the presence and absence of aFGF were preincubated with interleukin-1 (5 to 10 U/ml) for 15 min to 96 h or *Escherichia coli* lipopolysaccharide 026B6 (gift of Julius Nemins, Bronx Veterans Administration) (0.5 to 1.0 μg/ml) for 15 min to 48 h. After preincubation, the immunomodulator was removed, the surface was washed, and the infection assay was performed.

(iii) Control study. To demonstrate that the interleukin-1 was active in this system, the study was performed with neutrophils in lieu of *S. aureus* (33). Neutrophils were obtained by the method of Lalezari (20) with a 6% dextran (Pharmacia Fine Chemicals, Piscataway, N.J.) sedimentation and lymphocyte separation medium (density, 1.077 g/ml) (Bionetics Lab Products, Kingston, Md.). Endothelial cell monolayers were grown to confluence on cover slips in the presence of aFGF. Washed monolayers were then incubated with interleukin-1 (10 U/ml) for 4 h and washed; each cover slip was then incubated with approximately 10^6 neutrophils in PIPES buffer [25 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (Sigma), 110 mM NaCl, 5 mM KCl (pH 7.3)] for 10 min at 37°C. Nonadherent neutrophils were washed off, and adherent cells were stained with Wright Giemsa to allow for visual identification of neutrophils and endothelial cells under light microscopy. Ten high-power fields (×40) were screened on each cover slip, and three separate cover slips per treatment group were examined for each experiment.

Endothelial cell morphologic studies. Endothelial cells grown to confluence over 24 to 96 h, both in the presence and absence of aFGF, were compared morphologically by using phase-contrast microscopy at multiple points before and during the infection assays (4 and 37°C) (18). Endothelial cells were also examined ultrastructurally. Monolayers were grown to confluence over 72 h with and without aFGF on 35-mm plastic petri plates (Corning). Cells were fixed with 2% glutaraldehyde and 0.1% ruthenium red in 0.1 M cacodylate buffer (pH 7.4) for 20 min, postfixed in 1% OsO_4 in 0.1 M cacodylate buffer, stained en bloc with 1% aqueous uranyl acetate, dehydrated in a graded ethanol series, and embedded in LX 112 (Ladd Research Industries, Inc., Burlington, Vt.). Thin sections taken perpendicular to the growth plane were stained with uranyl acetate and lead citrate and then compared ultrastructurally.

Enzymatic treatment of endothelial cell monolayers. Endothelial cells grown to confluence in the presence of aFGF were washed and incubated at 37°C in 5.5% CO_2 with M199, chondroitinase ABC (Seikagaku Kogyu, Tokyo, Japan), Hanks balanced salt solution (HBSS; GIBCO), heparitinase (Seikagaku Kogyu), or PBS. The heparitinase degraded isolated endothelial cell heparin sulfate proteoglycans (unpublished data). After incubations of 1 1/2 or 3 h, the wells were washed with M199 plus 1% bovine serum albumin (Miles Laboratories, Naperville, Ill.), and the infection assay was performed. Heparitinase was diluted into PBS to provide final concentrations of 2.5 or 1.25 U/ml, and chondroitinase ABC was diluted into HBSS to give final concentrations of 1.25 or 0.5 U/ml. The PBS and HBSS wells served as controls for wells preincubated with heparitinase and chondroitinase ABC, respectively.

Statistics. All studies were performed on at least two separate occasions. Each point, unless otherwise stated, was performed in triplicate. The results represent the means ± standard deviations (SD) of all studies. Results were analyzed by the Student two-tailed *t* test as described by Zar (36).

RESULTS

Effect of aFGF on bacterial adhesion. Endothelial cells grown to confluence in the presence and absence of aFGF demonstrated similar patterns of *S. aureus* adhesion at 24 and 48 h. By 72 h there was a significant decrease ($P < 0.05$) in *S. aureus* adherence to endothelial cells grown with aFGF (BA/EC ± SD, 14.4 ± 8.3 for cells grown with aFGF versus 22.9 ± 8.6 for controls [Fig. 1]). A similar diminution in adhesion to endothelial cells grown to confluence in the presence of heparin affinity-purified human aFGF was observed (BA/EC ± SD, 21.9+5.8 for cells grown with aFGF versus 29.1 + 4.4 for controls; $P < 0.05$). At 72 h the effect of aFGF on *S. aureus* was noted consistently with different inocula of *S. aureus*, ranging from 10^5 to 10^7 CFU per well (Fig. 2). The diminished adhesion in the presence of aFGF at 72 h was also noted with two of three additional *S. aureus* strains tested but was not observed with less-adherent clinical isolates of *S. epidermidis* or *S. sanguis* (Table 1). Scatchard analysis of this data (not shown) revealed a nonlinear representation, suggesting the presence of multiple binding sites with evidence for cooperative binding (10). The

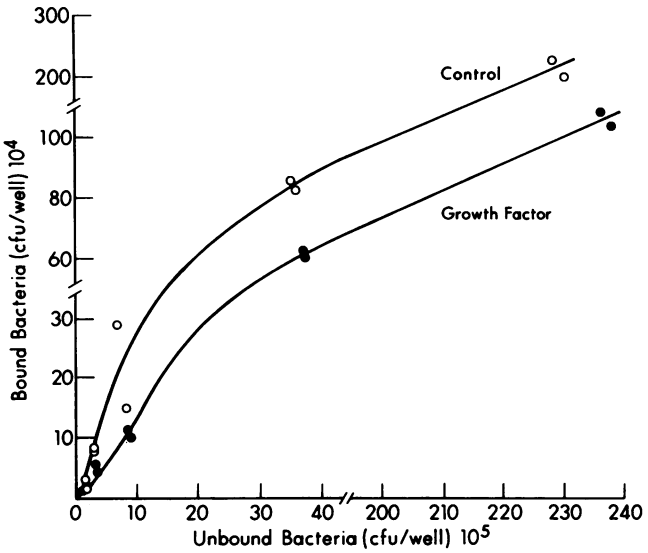

FIG. 2. Representative binding curves for the adherence of *S. aureus* to endothelial cells grown with and without aFGF. Monolayers were grown to confluence with or without aFGF for 72 h. aFGF was removed, and the infection assay was performed at 4°C with different bacterial inocula (30-min bacterial incubation). Each point represents the mean of two determinations.

diminished adhesion was not demonstrable on endothelial cells beyond the fourth passage.

Effect of interleukin-1 and endotoxin on *S. aureus* adhesion. Endothelial cells were preincubated with interleukin-1 (5 to 10 U/ml) for time periods spanning 15 min to 96 h or with endotoxin (0.5 to 1.0 μg/ml) for periods ranging from 15 min to 48 h. Regardless of the dose of immunomodulator used or the duration of the preincubation, neither interleukin-1 nor endotoxin had any effect on *S. aureus* adherence to endothelial cells. This was true whether the endothelial cells were grown to confluence in the presence or absence of aFGF (Fig. 3). All bacterial adherence to monolayers grown with aFGF was diminished when compared with adherence to monolayers grown without aFGF (BA/EC ± SD, 13.6 ± 4.5 versus 28.6 ± 4.4; $P < 0.05$). Similar results were noted with bacterial incubations of 15, 45, and 60 min.

TABLE 1. Adherence of bacteria to human endothelial cells in the presence or absence of aFGF[a]

Species and strain	Mean BA/EC ± SD	
	+aFGF	−aFGF
S. aureus		
Webb[b]	26.4 ± 4.1	37.2 ± 8.5
Fig[b]	17.5 ± 4.2	27.5 ± 4.8
Gold	23.6 ± 8.6	36.0 ± 16.3
Wood	43.5 ± 10.0	43.4 ± 10.7
S. epidermidis		
But	2.5 ± 0.5	2.1 ± 0.6
Sut	3.5 ± 0.8	3.5 ± 1.4
S. sanguis Wicky	14.4 ± 1.5	15.9 ± 3.7

[a] Comparison of bacterial adherence to human umbilical vein endothelial cells grown in the presence or absence of aFGF for 72 h. Bacteria were incubated with confluent endothelial cell monolayers for 30 min.

[b] Differences between the +aFGF and −aFGF groups are significant ($P < 0.05$).

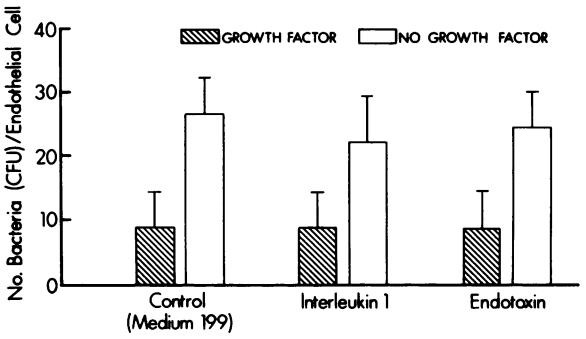

FIG. 3. Effect of interleukin-1 and endotoxin on *S. aureus* adherence to endothelial cells. Endothelial cells were grown to confluence in the presence or absence of aFGF. Confluent monolayers were preincubated with interleukin-1 (10 U/ml), endotoxin (1 μg/ml), or M199 for 30 min before the infection assay on washed monolayers. Results represent the means ± SD.

Interleukin-1 activation of the endothelial cells under our experimental conditions was demonstrated by measuring leukocyte adhesion to endothelial cells pretreated with interleukin-1 for 4 h. A sixfold increase in leukocyte adhesion to the endothelial cells was observed, suggesting activation (no. adherent leukocytes per high-power field [mean ± SD], 6.5 ± 3.0 for interleukin-1-treated cells versus 0.9 ± 1.1 for untreated cells; $P < 0.05$).

Morphologic comparisons of endothelial cells grown with and without aFGF. Endothelial cells grown with and without aFGF exhibited similar morphology upon examination by phase-contrast microscopy. Additionally, ruthenium-red-stained ultrastructural studies of the luminal surface of the endothelial cell revealed no significant morphologic differences to account for the disparity in *S. aureus* adherence. Cells grown with aFGF were able to withstand multiple passages, and they rapidly became confluent, even in the presence of low endothelial cell inocula and late-passaged cell lines. The cells could be detached from the well set with trypsin incubation at 37°C but not at 4°C. Cells grown without aFGF could not be passaged more than one time. In the absence of aFGF, endothelial cells became confluent only in the presence of higher inocula and were readily detached with trypsin incubation at both 4 and 37°C.

Enzymatic pretreatment of endothelial cell monolayers. Endothelial cells grown to confluence with aFGF were pretreated with chondroitinase ABC or heparitinase to assess the role of extracellular proteoglycans in *S. aureus* adherence to endothelial cells (Table 2). Chondroitinase ABC, an enzyme which disrupts polysaccharide bonds, thereby destroying chondroitin and dermatan sulfates, had no effect on adherence. Pretreatment with heparitinase, an enzyme which acts specifically on heparan sulfate, resulted in a twofold enhancement of *S. aureus* adherence to endothelial cells ($P < 0.05$). The morphology of the cells as determined by phase-contrast microscopy was not affected by heparitinase pretreatment. Chondroitinase ABC in concentrations greater than 0.5 U/ml promoted detachment of the cells from the substrate. Heparitinase treatment (1.25 U/ml) of endothelial cells grown in the absence of aFGF also promoted detachment of the cells.

DISCUSSION

Previous studies have demonstrated that activated endothelial cells have altered surface characteristics which pro-

TABLE 2. Effect of chondroitinase ABC and heparitinase on *S. aureus* adherence to human endothelial cells[a]

Prepn[b]	Mean BA/EC ± SD[c]
M199	4.7 ± 1.0
Chondroitinase ABC	6.2 ± 1.8
HBSS	5.4 ± 0.9
Heparitinase (1.25 U/ml)	9.7 ± 3.4
PBS	4.2 ± 0.6

[a] The endothelial cell monolayer was pretreated with the enzyme for 3 h at 37°C. The monolayers were then washed, and the infection assay was performed. Bacteria were incubated with the monolayer for 30 min.

[b] HBSS and PBS served as controls for chondroitinase ABC and heparitinase, respectively.

[c] Results are from three separate studies; each point represents an experiment performed in duplicate.

mote leukocyte adhesion (1–3, 22, 23, 32) and that this process may contribute to the inflammatory response and initiation of coagulation (8, 9). In this study, we investigated whether endothelial cell modulation would alter susceptibility to infection.

We have shown that endothelial cells grown in the absence of aFGF are more susceptible to *S. aureus* adherence. This process is time dependent but independent of the initial bacterial inoculum. We speculate that aFGF produces subtle biochemical or ultrastructural changes which affect the susceptibility of these cells to infection. Similar types of changes induced by other modulators may contribute to the development of infection by *S. aureus*.

aFGF, a peptide originally purified from bovine brain, has a crucial, multifunctional role in cell function. aFGF plays a major role in angiogenesis, cell growth, migration, and adhesion to the substrate (13–16, 21, 33). It affects the synthesis of glycosaminoglycans, including heparan sulfate (13). In our in vitro system, aFGF is crucial for the long-term maintenance of endothelial cell lines in culture. Maciag et al. (21) and Gordon et al. (14) have demonstrated that cells grown without growth factor cannot be serially propagated. Moreover, Gordon et al. (13) have reported that cells grown without aFGF are more readily detached from their matrix. We have confirmed these findings. Thus, despite their similar morphologic appearance, endothelial cells grown in tissue culture with aFGF behave differently from those grown without aFGF in tissue culture. Although fibroblast growth factors have primarily been studied in in vitro systems, they have been isolated from both normal and cancerous human tissues (12, 24, 25). In vivo effects of these growth factors include cell proliferation, limb regeneration, and the formation of new blood vessels (16).

In our series of experiments, heparitinase pretreatment greatly enhanced *S. aureus* adhesion to endothelial cells, suggesting that heparan sulfate played a role in *S. aureus* adhesion. The process of binding to cell surface receptors is complex, often involving multiple receptors (4, 19). In the atherosclerotic model, heparan sulfate has been implicated as a protector of endothelial cell integrity by inhibiting smooth muscle cell migration (5, 33). Parsons and colleagues (27, 28, 34) have demonstrated that glycosaminoglycans exert a protective effect, preventing the development of bladder infection, and that bladders with intact mucin are more resistant to bacterial adherence and subsequent infection. In our system, the removal of heparan sulfate promoted enhanced *S. aureus* adhesion. Heparan sulfate may provide steric hindrance, blocking *S. aureus* attachment to a binding site on the endothelial cells. Whether heparan sulfate is important in the aFGF-induced diminution of *S. aureus* adherence to endothelial cells is unknown and is a focus of current study.

Endothelial cell modulation appears to contribute to the pathogenesis of intravascular disease. Ryan and Ryan (30, 31) have documented the importance of subtle ultrastructural change in endothelial cell function. Endothelial cell exposure to granulocytes or viruses resulted in an alteration of surface glycocalyx, permitting the expression of previously unexposed Fc receptors. This favored the binding of immune complexes with subsequent activation of complement. The effects of modulation may be variable, based on the modulator and the system with which it is interacting. Thus, despite the ability of both interleukin-1 and endotoxin to enhance leukocyte adhesion to endothelial cells, neither immunomodulator affects *S. aureus* adherence under the conditions of our system.

In summary, we have utilized an in vitro infection assay to demonstrate that endothelial cell modulation significantly affects staphylococcal adhesion. Both aFGF and heparitinase can mediate these changes in vitro. The ability of aFGF to exert a protective role in bacterium-endothelial cell interactions is a novel function for this critical cellular peptide. Similar types of modulation in vivo caused by aFGF or other agents may contribute to the pathogenesis of staphylococcal endovascular infections such as acute endocarditis or infected vascular grafts. In both clinical settings, a morphologically normal endovascular surface may be seeded. Alteration of these surfaces by circulating modulators or underlying material (e.g., prosthetic grafts) may alter the susceptibility of these cells to infection.

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LITERATURE CITED

1. **Bevilacqua, M. P., J. S. Pober, G. R. Majeau, R. S. Cotran, and M. A. Gimbrone.** 1984. Interleukin 1 induces biosynthesis and cell surface expression of procoagulant activity in human vascular endothelial cells. J. Exp. Med. **160**:618–623.
2. **Bevilacqua, M. P., J. S. Pober, M. E. Wheeler, R. S. Cotran, and M. A. Gimbrone.** 1985. Interleukin 1 activation of vascular endothelium, effects on procoagulant activity and leukocyte adhesion. Am. J. Pathol. **121**:393–403.
3. **Bevilacqua, M. P., J. S. Pober, M. E. Wheeler, R. S. Cotran, and M. A. Gimbrone.** 1985. Interleukin 1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes, and related leukocyte cell lines. J. Clin. Invest. **76**:2003–2011.
4. **Bornens, M., E. Karsenti, and S. Avrameas.** 1976. Receptor mobility and its cooperative restriction by ligands. Immunol. Commun. **5**:569–589.
5. **Busch, P. C.** 1984. Sulfated glycosaminoglycans and vascular endothelial cells, p. 178–188. *In* E. A. Jaffe (ed.), Biology of endothelial cells. Martinus Nijhoff, Publisher, Boston.
6. **Chambers, H. F., O. M. Korzeniowski, and M. A. Sande.** 1983. *Staphylococcus aureus* endocarditis: clinical manifestations in addicts and nonaddicts. Medicine **62**:170–177.

7. **Conn, G., and V. B. Hatcher.** 1984. The isolation and purification of two anionic endothelial cell growth factors from human brain. Biochem. Biophys. Res. Commun. **124**:262–268.
8. **Dinarello, C. A.** 1984. Interleukin 1. Rev. Infect. Dis. **6**:51–95.
9. **Dinarello, C. A.** 1984. Interleukin 1 and the pathogenesis of the acute phase response. N. Engl. J. Med. **311**:1413–1418.
10. **Doyle, R. J., J. D. Oakley, K. R. Murphy, D. McAlister, and K. G. Taylor.** 1985. Graphical analysis of adherence data, p. 109–113. *In* S. E. Mergenhagen and B. Rosan (ed.), Molecular basis of oral microbial adhesion. American Society for Microbiology, Washington, D.C.
11. **Dreyer, N. P., and B. N. Fields.** 1973. Heroin-associated infective endocarditis, a report of 28 cases. Ann. Intern. Med. **78**:699-702.
12. **Folkman, J., and M. Klagsbrun.** 1987. Angiogenic factors. Science **235**:442–447.
13. **Gordon, P. B., G. Conn, and V. B. Hatcher.** 1985. Glycosaminoglycan production in cultures of early and late passage human endothelial cells. The influence of anionic endothelial cell growth factor and the extracellular matrix. J. Cell. Physiol. **125**:596–607.
14. **Gordon, P. B., I. I. Sussman, and V. B. Hatcher.** 1983. Long term growth of human endothelial cells. In Vitro **19**:661–671.
15. **Gospodarowicz, D.** 1983. Growth factors and their action in vivo and in vitro. J. Pathol. **141**:201–233.
16. **Gospodarowicz, D., N. Ferrara, L. Schweigerer, and G. Neufeld.** 1987. Structural characterization and biological functions of fibroblast growth factor. Endocrinol. Rev. **8**:95–114.
17. **Gould, D., C. H. Ramirez-Ronda, R. K. Holmes, and J. P. Sanford.** 1975. Adherence of bacteria to heart valves in vitro. J. Clin. Invest. **56**:1364–1370.
18. **Haudenschild, C. C.** 1984. Morphology of vascular endothelial cells in culture, p. 129–140. *In* E. A. Jaffe (ed.), Biology of endothelial cells. Martinus Nijhoff, Publisher, Boston.
19. **Keusch, G. T.** 1979. Specific membrane receptors: pathogenetic and therapeutic implications in infectious diseases. Rev. Infect. Dis. **1**:517–529.
20. **Lalezari, P.** 1986. Neutrophil and platelet antibodies in immune neutropenia and thrombocytopenia, p. 630–634. *In* H. R. Rose, H. Friedman, and J. L. Fahey (ed.), Manual of clinical laboratory immunology, 3rd ed. American Society for Microbiology, Washington, D.C.
21. **Maciag, T., G. A. Hoover, M. B. Stemerman, and R. Weinstein.** 1984. Factors which stimulate the growth of human umbilical vein endothelial cells in vitro, p. 87–96. *In* E. A. Jaffe (ed.), Biology of endothelial cells. Martinus Nijhoff, Publisher, Boston.
22. **Montesano, R., A. Mossaz, J. E. Ryser, L. Orci, and P. Vassalli.** 1984. Leukocyte interleukins induce cultured endothelial cells to produce a highly organized, glycosaminoglycan-rich pericellular matrix. J. Cell Biol. **99**:1706–1715.
23. **Montesano, R., L. Orci, and P. Vassalli.** 1985. Human endothelial cell cultures: phenotypic modulation by leukocyte interleukins. J. Cell Physiol. **122**:424–434.
24. **Moscatelli, D., M. Presta, J. Joseph-Silverstein, and D. B. Rifkin.** 1986. Both normal and tumor cells produce basic fibroblast growth factor. J. Cell Physiol. **129**:273–276.
25. **Moscatelli, D., M. Presta, and D. B. Rifkin.** 1986. Purification of a factor from human placenta that stimulates capillary endothelial cell protease production, DNA synthesis, and migration. Proc. Natl. Acad. Sci. USA **83**:2091–2095.
26. **Ogawa, S. K., E. R. Yurberg, V. B. Hatcher, M. A. Levitt, and F. D. Lowy.** 1985. Bacterial adherence to human endothelial cells in vitro. Infect. Immun. **50**:218–224.
27. **Parsons, C. L.** 1982. Prevention of urinary tract infection by the exogenous glycosaminoglycan sodium pentosanpolysulfate. J. Urol. **127**:167–169.
28. **Parsons, C. L.** 1986. Bladder surface glycosaminglycan: efficient mechanism of environmental adaptation. Urology **27**(Suppl.): 9–14.
29. **Reisberg, B. E.** 1979. Infective endocarditis in the narcotic addict. Prog. Cardiovasc. Dis. **22**:193–203.
30. **Ryan, U. S.** 1986. Metabolic activity of pulmonary endothelium: modulations of structure and function. Annu. Rev. Physiol. **48**:263–277.
31. **Ryan, U. S., and J. W. Ryan.** 1984. The ultrastructural basis of endothelial cell surface functions. Biorheology **21**:155–170.
32. **Schleimer, R. P., and B. K. Rutledge.** 1986. Cultured human vascular endothelial cells acquire adhesiveness for neutrophils following stimulation with interleukin-1, endotoxin, and tumor-promoting phorbol diesters. J. Immunol. **136**:649–654.
33. **Schwartz, S. M., and C. M. Gajdusek.** 1982. Growth factors and the vessel wall, p. 85–112. *In* T. H. Spaet (ed.), Progress in hemostasis and thrombosis, vol. 6. Grune and Stratton, New York.
34. **Shrom, S. H., C. L. Parsons, and S. G. Mulholland.** 1977. Role of urothelial surface mucoprotein in intrinsic bladder defense. Urology **9**:526–533.
35. **Vercellotti, G. M., D. Lussenhop, P. K. Peterson, L. T. Furcht, J. B. McCarthy, H. S. Jacob, and C. F. Moldow.** 1984. Bacterial adherence to fibronectin and endothelial cells: a possible mechanism for bacterial tissue tropism. J. Lab. Clin. Med. **103**:34–43.
36. **Zar, U. H.** 1984. *Biostatistical analysis*, p. 150–152. Prentice Hall, Inc., Englewood Cliffs, N.J.