JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1988, p. 2505–2509
0095-1137/88/122505-05$02.00/0

Vol. 26, No. 12

Staphylococcus aureus Capsular Types and Antibody Response to Lung Infection in Patients with Cystic Fibrosis

ANNE ALBUS,[1] JEAN-MICHEL FOURNIER,[2] CHRISTIANE WOLZ,[1] ALAIN BOUTONNIER,[2] MICHAEL RANKE,[3] NIELS HØIBY,[4] HEINZ HOCHKEPPEL,[5] AND GERD DÖRING[1]*

Hygiene-Institute[1] and Children's Hospital,[3] University of Tübingen, D-7400 Tübingen, Federal Republic of Germany; Institut Pasteur, F-75724 Paris Cedex 15, France[2]; Statens Seruminstitut, DK-2200 Copenhagen N, Denmark[4]; and CIBA-GEIGY, CH-4002 Basel, Switzerland[5]

Received 13 May 1988/Accepted 24 August 1988

Chronic respiratory tract infections caused by *Staphylococcus aureus* are common in patients with cystic fibrosis (CF). Recently, it was shown in a few CF patients that *S. aureus* isolates produce capsular polysaccharides (CPs). However, it is not known whether this is a common feature and whether an immune response to CPs in CF is detectable. Therefore, we examined 170 *S. aureus* isolates from CF patients and healthy individuals for production of CP types 5 and 8 by using monoclonal antibodies. We found that CP-producing staphylococcal isolates were randomly distributed among CF patients and healthy carriers. Eighty-five percent of all isolates produced CPs, 77% of which were type 8. Examination of one sputum sample by an immunofluorescence technique suggested that production of CPs is not an in vitro phenomenon. *S. aureus* isolates from various sites of a single person often yielded more than one CP type. A random distribution of *S. aureus* strains with CP type 5 or 8 from the skin and respiratory tracts of patients and from the skin of healthy individuals was found. Antibody response to CP types 5 and 8, measured by enzyme-linked immunosorbent assay, was not elevated in CF patients with chronic *S. aureus* lung infection in comparison with healthy carriers. On the contrary, in CF patients the ratios of antibodies to CP 8 were significantly lower ($P < 0.005$; $\alpha = 0.025$). The ratios of antibodies to CP types did not change when monitored longitudinally over several months. This study suggests that the production of CPs is a universal property of *S. aureus* and that infected CF patients do not have elevated ratios of antibodies to these antigens.

Cystic fibrosis (CF) is the most common hereditary disease of Caucasians, with an incidence of one case in every 2,000 newborns in Europe (24, 33). More than 80% of the patients suffer from chronic bacterial lung infections (24), and besides *Pseudomonas aeruginosa*, *Staphylococcus aureus* is the predominant organism isolated from CF sputum (14). Chronic *P. aeruginosa* infection usually correlates with poor clinical status, whereas patients with chronic or intermittent *S. aureus* infection alone reveal high clinical scores and a mild disease state (16). It is unclear which factors determine these differences in pathogenicity, since virulence of both pathogens is multifactorial. Virulence of *S. aureus* includes factors attached to the cell wall, such as protein A (20), clumping factor (12), and fibronectin- or collagen-binding proteins (28, 29), as well as several soluble extracellular enzymes and toxins (3). Some *S. aureus* strains (M and Smith) are highly encapsulated and require both complement and specific anticapsular antibodies for efficient phagocytosis (31). Nevertheless, *S. aureus* may survive within polymorphonuclear leukocytes and alveolar macrophages (5, 15).

In CF patients only little is known about the production of capsular polysaccharides (CPs) by *S. aureus* (1), which may act as an additional virulence factor. This is of special interest, since in CF patients production of mucoid exopolysaccharides by *P. aeruginosa* is common. Additionally, nothing is known about whether CF patients respond to *S. aureus* CPs with specific antibodies. Recently, a significantly altered immune response to *P. aeruginosa* lipopolysaccharides in these patients was reported, which is thought to contribute to bacterial virulence by impairing opsonophagocytosis (23; R. B. Moss, Y. P. Hsu, N. J. Lewiston, and G. deLange, Pediatr. Res. 21:A940, 1987).

Therefore, the object of this study was to investigate the presence of CP-producing *S. aureus* isolates on the skin and in the respiratory tracts of CF patients and to determine whether CP type 5 or 8 is predominant and whether infected CF patients have elevated immune responses to the CP type. The purification of *S. aureus* CP types 5 (9) and 8 (10) and the development of monoclonal antibodies (MAb5, MAb8) to these CPs (13) have facilitated investigations in this field.

MATERIALS AND METHODS

Patients. Thirty-seven CF patients (mean age, 10 years; 15 females and 22 males) and 29 healthy individuals (mean age, 10 years; 12 females and 17 males) were investigated for skin colonization with *S. aureus*. Samples from the forehead, forearm, back, shoulder, and thigh were collected with contact agar plates for *S. aureus* isolation. Sputum was collected from 101 patients with CF (age range, 3 to 30 years; 40 females and 61 males) suffering from chronic or intermittent *S. aureus* lung infection. *S. aureus* infection was defined as chronic when multiple sputum samples collected over at least half a year yielded positive cultures. Serum samples from 29 CF patients (mean age, 15 years; 7 females and 22 males) were investigated for antibody response to CP types 5 and 8. Fourteen patients had chronic *P. aeruginosa* infection at the same time. Twenty healthy adult blood donors whose staphylococcal carrier status was not known served as controls. The CF patients attended the Cystic Fibrosis Clinic G, Rigshospitalet, Copenhagen, Denmark, and the Children's Hospital, University of Tübingen, Tübingen, Federal Republic of Germany. Diagnosis of CF was based on accepted criteria (33), including a typical history of CF with

* Corresponding author.

marked elevated sweat chloride levels in repeated tests and altered pulmonary function. Informed consent was obtained from all patients or their parents.

Bacteria. *S. aureus* strains were isolated on mannitol agar (Oxoid, Wesel, Federal Republic of Germany) and identified by using routine methods (19). Strains were propagated on 1.5% Columbia agar (Oxoid) supplemented with 4% NaCl for 20 h in a 5% CO_2 atmosphere. Strains were kept in nutrient broth supplemented with 15% glycerine at −70°C until use. For CP typing, bacteria were harvested from Columbia agar in 2 ml of phosphate-buffered saline (PBS), autoclaved at 121°C for 1 h, and centrifuged for 10 min at 10,000 × *g*. The culture supernatants were stored at −20°C until CP typing.

Purification of *S. aureus* CP types 5 and 8. CPs were purified as described previously from *S. aureus* Becker (prototype 8) and Reynolds (prototype 5) (9, 10). Briefly, strains were cultivated on Columbia agar, suspended in PBS (pH 7.5), autoclaved, and centrifuged at 25,000 × *g*. The culture supernatant was treated with DNase, RNase, and protease. CP was purified on DEAE-Trisacryl M (Réactifs IBF, Villeneuve-la-Garenne, France). Contaminating teichoic acid was removed by Sepharose CL-4B (Pharmacia, Freiburg, Federal Republic of Germany) gel filtration. The purified CP contained 1% protein, 0.5% nucleic acids, and 0.01% teichoic acid. Structure analysis revealed that CP types 5 and 8 are distinct sugar polymers composed of about 90% *N*-acetylamino sugars (9).

Typing of *S. aureus* CPs and detection of serum antibodies to CPs. For CP typing, a two-step inhibition enzyme-linked immunosorbent assay (ELISA) was performed with CP-specific MAb5 and MAb8 (13), as previously described (25). Detection of serum antibodies to CP types was performed with purified CP of *S. aureus* with a newly developed CP-specific ELISA. Sera were diluted 1:2,000, 1:4,000, 1:8,000, and 1:16,000 in PBS, and 100 μl of the dilutions was placed into CP-coated microdilution wells. After 1 h at 37°C, the trays were washed five times and incubated with 100 μl of peroxidase-conjugated sheep anti-human immunoglobulins (heavy and light chain specific; Diagnostics Pasteur, Paris, France) for 45 min at 37°C. After being washed with PBS, bound antibodies were visualized with 100 μl of 0.4% 1,2-phenylenediamine dihydrochloride (Dakopatts, Hamburg, Federal Republic of Germany) in 0.1 M sodium citrate, pH 5.2, and 0.06% H_2O_2. The reaction was stopped after 10 min with 50 μl of 3 N HCl, and the trays were read at an optical density of 492 nm (OD_{492}) in a spectrophotometer (Micro-ELISA reader; Dynatech Laboratories, Inc., Alexandria, Va.). Antibody titration curves were obtained by plotting the OD_{492} versus serum dilutions. Antibody quotients were calculated by dividing the OD_{492} of patient or normal serum by the OD_{492} of the positive control serum at the same dilution. These antibody ratios were determined when the serum dilution curves were linear and parallel (≤10%) to the control serum curve. The positive control, present on each plate as an internal standard, was derived from a blood donor hospitalized because of severe staphylococcal infection. Antibody ratios are expressed in $\log_{10}$. The upper normal limit for antibodies was defined as the mean value of the normal control group + 2 standard deviations. Thus, none of the 20 healthy individuals was positive. Consequently, quotients greater than 0.33 for CP 5 and 0.59 for CP 8 were judged to be positive.

Immunofluorescence. For direct detection of microencapsulated *S. aureus* in the sputum of a CF patient, samples of sputum material which had been kept at −70°C were solubilized by adding 10 volumes of sterile physiological saline in a Vortex mixer. After centrifugation for 5 min at 1,000 × *g*, the supernatant was again centrifuged for 5 min at 4,700 × *g*. The pellet was distributed on immunofluorescence slides (Biomérieux, Charbonnières les Bains, France), heat fixed, and incubated with fluorescein isothiocyanate (FITC)-labeled MAb5 and MAb8 (immunoglobulin M; IgM). *S. aureus* subcultured on agar plates served as the control. After being washed, slides were mounted with PBS and examined with a fluorescence light microscope (Zeiss, Oberkochen, Federal Republic of Germany). Labeling of MAbs was accomplished by the dialysis method. Briefly, FITC (Serva, Heidelberg, Federal Republic of Germany) solubilized in 0.025 M sodium-hydrogen-carbonate buffer (pH 9.5) was incubated with MAbs at 4°C overnight. Thereafter, the solution was centrifuged at 4,000 × *g* through a Centricon 10 (Amicon Corp., Danvers, Mass.) tube until the dialysate was free of FITC.

Statistical analysis. Data were analyzed with the U test by the method of Wilcoxon, Mann, and Whitney.

TABLE 1. CP types in human *S. aureus* isolates

CP type[a]	No. (%) of *S. aureus* isolates from[b]:			
	All sites	CF sputum	CF skin	Normal skin
5	33 (19.4)	30 (21.3)	2 (14.3)	1 (6.7)
8	112 (65.8)	91 (64.5)	10 (71.4)	11 (73.3)
NT[c]	25 (14.7)	20 (14.2)	2 (14.3)	3 (20.0)

[a] CP types were determined by ELISA.
[b] One hundred seventy strains were isolated from the sputum and skin of 101 patients with CF and from the skin of 8 healthy carriers. Included are multiple strains isolated over a 1-year period from 21 CF patients, as well as strains isolated from multiple skin sites of 13 CF patients and 8 normal individuals.
[c] NT, Nontypeable.

RESULTS

***S. aureus* CP typing.** Of 170 *S. aureus* strains isolated from CF patients and normal individuals, 145 (85%) produced CP (Table 1). Sixty-six percent of all isolates yielded CP type 8. Random distribution of encapsulated *S. aureus* strains between patients and healthy carriers was found, and random occurrence of CP types 5 and 8 was seen between both groups. Therefore, production of exopolysaccharides is universal among *S. aureus* strains and not restricted to clinical isolates but also found in skin isolates from normal individuals and nonhospitalized CF patients. The percentage of *S. aureus* skin isolates from CF patients (35%) was similar and not significantly elevated compared with that of normal individuals (28%). A single person may harbor more than one *S. aureus* CP type at the same time on the skin or in the respiratory tract; skin and sputum CP types differed in three of eight cases, and one patient had *S. aureus* with CP types 5 and 8 on the forearm and forehead. Therefore, it is not surprising that repeated isolation of *S. aureus* from a single patient yielded different CP types in eight cases.

By using direct immunofluorescence, evidence was found that production of CPs is not an in vitro phenomenon secondary to subculturing. Examination of multiple preparations from the sputum of one CF patient revealed bright staining with MAb8 but not with MAb5. A typical picture is shown in Fig. 1. Subsequent isolation of *S. aureus* and staining, as well as characterization by ELISA, confirmed the presence of CP type 8 (Fig. 1C). These results also demonstrated that MAb5 and MAb8 bind specifically to the

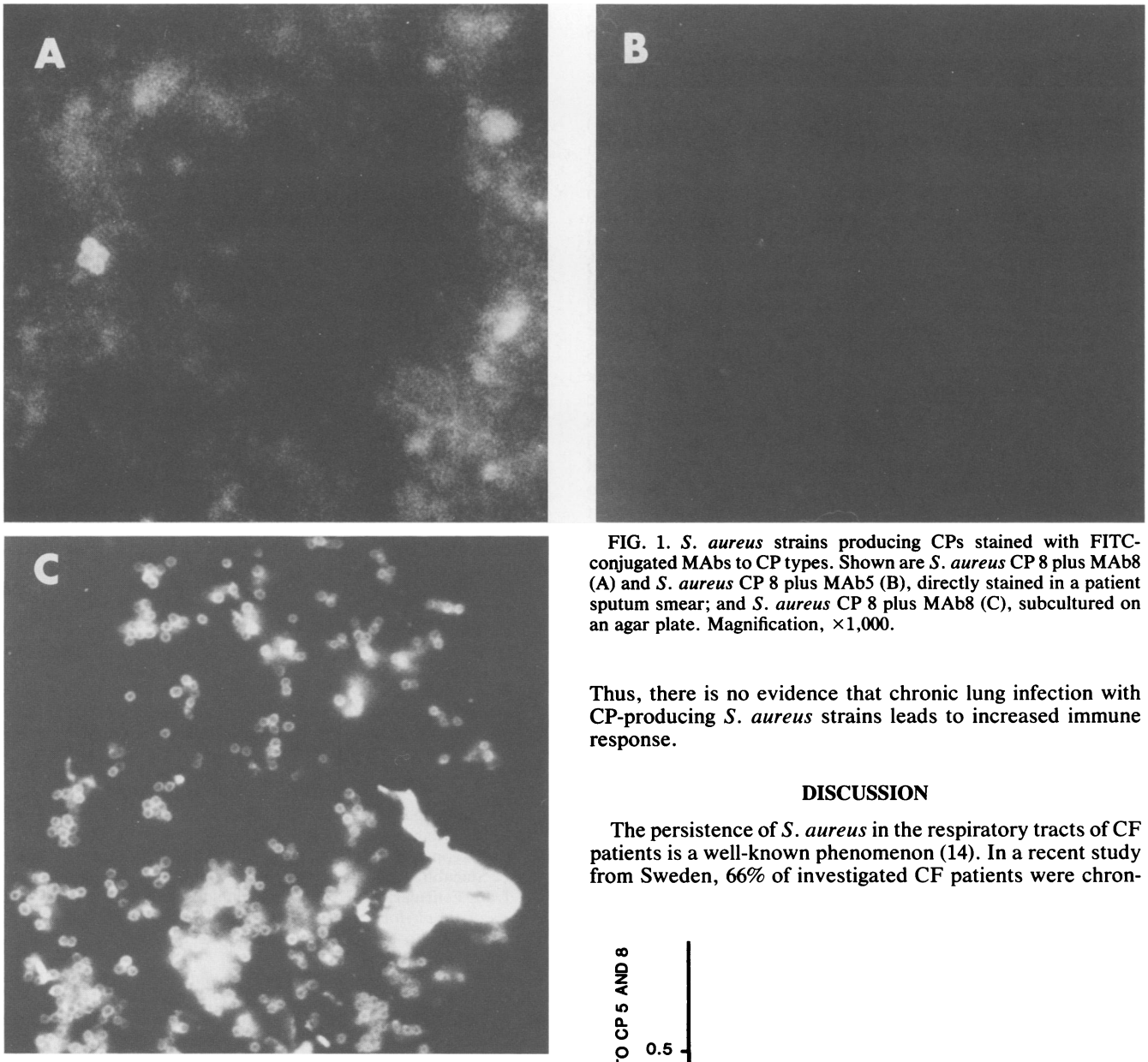

FIG. 1. *S. aureus* strains producing CPs stained with FITC-conjugated MAbs to CP types. Shown are *S. aureus* CP 8 plus MAb8 (A) and *S. aureus* CP 8 plus MAb5 (B), directly stained in a patient sputum smear; and *S. aureus* CP 8 plus MAb8 (C), subcultured on an agar plate. Magnification, ×1,000.

CPs and do not bind to protein A. The determination of the number of *S. aureus* organisms in the immunofluorescence stained preparation and in a Gram-stained preparation from the same sputum yielded comparable counts.

Serum antibodies to *S. aureus* CP types 5 and 8 in CF patients and healthy controls. A positive reaction in the ELISA with a serum dilution of 1:2,000 to 1:8,000 in all 77 specimens of the 49 individuals suggested that immunological response to CP is considerable in healthy individuals and CF patients. There was no difference in antibody response to CP 5 between CF patients and healthy controls (Fig. 2). Furthermore, CF patients had significantly lower ratios of antibodies to CP 8 than controls had ($P < 0.005$; $\alpha = 0.025$). Only 10% (2 of 20) of the chronically infected CF patients had elevated ratios of antibodies to both antigens. The longitudinal study of CP antibody ratios up to 14 months in 15 mostly chronically infected patients revealed that ratios did not change during chronic infection (data not shown). Thus, there is no evidence that chronic lung infection with CP-producing *S. aureus* strains leads to increased immune response.

DISCUSSION

The persistence of *S. aureus* in the respiratory tracts of CF patients is a well-known phenomenon (14). In a recent study from Sweden, 66% of investigated CF patients were chron-

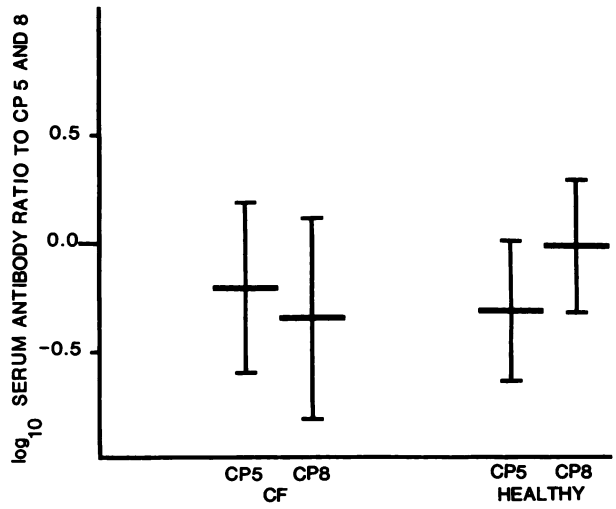

FIG. 2. Serum antibody response of CF patients and healthy individuals to *S. aureus* CP types 5 and 8, measured by ELISA. Bars represent means ± 1 standard deviation of CP antibody ratios. The antibody ratio of a single patient was determined from at least quadruplicate values. Twenty chronically and nine intermittently infected CF patients and twenty healthy individuals were tested.

ically infected with this pathogen (6). In the present study, we have investigated the possibility that *S. aureus* strains in CF produce CPs. The development of MAbs to CP types of *S. aureus* has provided an excellent means to study this factor, which may contribute to *S. aureus* virulence.

A high percentage of encapsulated *S. aureus* strains (85%) was found in the present study, corroborating results with other clinical isolates of *S. aureus* (1, 13, 27). Detection of microencapsulated *S. aureus* in a sputum smear by an immunofluorescence technique supports the notion of others (2) that production of CP occurs during in vivo infection and is not an in vitro phenomenon. An important finding of this study is that production of CPs is not restricted to clinical isolates. *S. aureus* isolates from healthy individuals as well as nonhospitalized CF patients produced CPs. The capsule was not detected until MAbs were developed, since it was not demonstrable by traditional methods such as India ink preparation or by the specific capsule reaction (32) because of its small size. Until recently, encapsulated *S. aureus* has been considered rare, and peptidoglycan, teichoic acid, and protein A were regarded as the major *S. aureus* cell wall components. An additional factor now has to be taken into consideration as being part of the outer surface. It is most likely that the microcapsule of *S. aureus* has no significant influence on phagocytic uptake of the bacterium (8, 21). However, it may participate in *S. aureus* adherence. Furthermore, the chemical nature of the bacterial outer surface may influence the host immune response.

In the majority of the CF patients with chronic *S. aureus* lung infection, elevated levels of antibody to CP in comparison with levels in healthy controls were not found. Several factors may account for this phenomenon. Firstly, immunological response to polysaccharides may be limited, as was shown for the type b vaccine of *Haemophilus influenzae* (22) and the group C meningococcal polysaccharides (11). Whether this is also true for *S. aureus* CP types remains to be investigated. Secondly, specific antibodies to CP may be bound to the antigen in circulating immune complexes, leading to decreased serum antibody detection. Thirdly, the outer surface of *S. aureus*, a cross-linked polymeric sugar, classically requires an IgG2 response (4). Recently, significantly decreased IgG2 titers in response to vaccination with a bacterial polysaccharide were reported in CF patients (Moss et al., Pediatr. Res. **21**:A940, 1987). Similarly, the low antibody response to CP 8 in the majority of patients may be due to decreased IgG2. IgG subclass distribution among CP types in infected CF patients and normal individuals awaits further investigation. Finally, immune response to *S. aureus* antigens in patients with severe staphylococcal disease is in general not very different from that of normal persons of comparable age (17, 18, 26, 30). Determinations of antibodies to *S. aureus* antigens in CF patients are in accordance with this view (7). This raises the possibility that repeated infections since early infancy may maintain a sufficiently large antigenic mass to result in immunological unresponsiveness (26).

In summary, this study suggests that CP is universally expressed by *S. aureus*. Whether CP may be regarded as a virulence factor of *S. aureus* which facilitates bacterial colonization and supports the chronicity of infection remains to be studied.

ACKNOWLEDGMENT

This work was supported by a grant for A. Albus from the Ministerium für Wissenschaft und Kunst, Baden-Würtemberg, Federal Republic of Germany.

LITERATURE CITED

1. **Arbeit, R. D., W. W. Karakawa, W. F. Vann, and J. B. Robbins.** 1984. Predominance of two newly described clinical isolates of *Staphylococcus aureus*. Diagn. Microbiol. Infect. Dis. **2**:85–91.
2. **Arbeit, R. D., and M. J. Nelles.** 1987. Capsular polysaccharide antigenemia in rats with experimental endocarditis due to *Staphylococcus aureus*. J. Infect. Dis. **155**:242–246.
3. **Arbuthnott, J. P.** 1981. *Staphylococcal* toxins: past and future. Zentralbl. Bakteriol. Mikrobiol. Hyg. **10**(Suppl.):215–222.
4. **Berger, M.** 1987. Immunoglobulin G subclass determination in diagnosis and management of antibody deficiency syndromes. J. Pediatr. **110**:325–328.
5. **Buggy, B. P., D. R. Schaberg, and R. D. Swartz.** 1984. Intraleukocytic sequestration as a cause of persistent *Staphylococcus aureus* peritonitis in continuous ambulatory peritoneal dialysis. Am. J. Med. **76**:1035–1040.
6. **Ericsson, A., M. Granström, R. Möllby, and B. Strandvik.** 1986. Antibodies to staphylococcal teichoic acid and α-toxin in patients with cystic fibrosis. Acta Paediatr. Scand. **75**:139–144.
7. **Ericsson-Hollsing, A., M. Granström, and B. Strandvik.** 1987. Prospective study of serum staphylococcal antibodies in cystic fibrosis. Arch. Dis. Child. **62**:905–911.
8. **Falcieri, E., P. Vaudaux, E. Huggler, D. Lew, and F. Waldvogel.** 1987. Role of bacterial exopolymers and host factors on adherence and phagocytosis of *Staphylococcus aureus* in foreign body infections. J. Infect. Dis. **155**:524–531.
9. **Fournier, J.-M., K. Hannon, M. Moreau, W. W. Karakawa, and W. F. Vann.** 1987. Isolation of type 5 capsular polysaccharide from *Staphylococcus aureus*. Ann. Inst. Pasteur Microbiol. **138**: 561–567.
10. **Fournier, J.-M., W. F. Vann, and W. W. Karakawa.** 1984. Purification and characterization of *Staphylococcus aureus* type 8 capsular polysaccharide. Infect. Immun. **45**:87–93.
11. **Goldschneider, I., M. L. Lepow, E. D. Gotschlich, F. T. Mauck, F. Bachl, and M. Randolph.** 1973. Immunogenicity of group A and group C meningococcal polysaccharides in human infants. J. Infect. Dis. **128**:769–776.
12. **Hasche, K. D., J. Brückler, W. Schaeg, and H. Blobel.** 1975. Einfluß des "Clumping Factors" von *Staphylococcus aureus* auf die Phagozytose. Zentralbl. Bakteriol. Mikrobiol. Hyg. Abt. 1 Orig. Reihe A **232**(Suppl. 10):446–453.
13. **Hochkeppel, H. K., D. G. Braun, W. Vischer, A. Imm, J. Sutter, U. Staeubli, R. Guggenheim, E. L. Kaplan, A. Boutonnier, and J. M. Fournier.** 1987. Serotyping and electron microscopy studies of *Staphylococcus aureus* clinical isolates with monoclonal antibodies to capsular polysaccharide types 5 and 8. J. Clin. Microbiol. **25**:526–530.
14. **Høiby, N.** 1982. Microbiology of lung infections in cystic fibrosis patients. Acta Paediatr. Scand. Suppl. **301**:33–54.
15. **Hoidal, J. R., D. Schmeling, and P. K. Peterson.** 1981. Phagocytosis, bacterial killing, and metabolism by purified human lung phagocytes. J. Infect. Dis. **144**:61–71.
16. **Huang, N. N., D. V. Schidlow, T. H. Szatrowski, J. Palmer, L. R. Laraya-Cuasay, W. Yeung, K. Hardy, L. Quitell, and S. Fiel.** 1987. Clinical feature, survival rate, and prognostic factors in young adults with cystic fibrosis. Am. J. Med. **82**:871–879.
17. **Jarlov, J. O., F. Espersen, B. Christensson, B. A. Jensen, S. A. Hedström, and J. B. Hertz.** 1987. Antibody response to whole cells and teichoic acid of *Staphylococcus aureus* strain E 1369 in human sera. Acta Pathol. Microbiol. Immunol. Scand. Sect. B **95**:115–120.
18. **Julander, I. G., M. Granström, S. A. Hedström, and R. Möllby.** 1983. The role of antibodies against α-toxin and teichoic acid in the diagnosis of *Staphylococcus aureus*. Infection **2**:77–83.
19. **Kloos, W. E., and K. H. Schleifer.** 1975. Simplified scheme for routine identification of human *Staphylococcus* species. J. Clin. Microbiol. **1**:82–88.
20. **Langone, J. J.** 1982. Protein A of *Staphylococcus aureus* and related immunoglobulin receptors produced by *Streptococci* and *Pneumococci*. Adv. Immunol. **32**:157–252.
21. **Lee, J. C., J. M. Betley, C. A. Hopkins, N. E. Perez, and G. B. Pier.** 1987. Virulence studies, in mice, of transposon-induced mutants of *Staphylococcus aureus* differing in capsule size. J.

Infect. Dis. **156**:741–750.
22. **Mäkelä, P. H., H. Peltola, H. Käythy, H. Jousimies, O. Pettay, E. Ruoslahti, A. Sivonen, and O. V. Renkonen.** 1977. Polysaccharide vaccines of group A *Neisseria meningitidis* and *Haemophilus influenzae* type b: a field trial in Finland. J. Infect. Dis. **136**(Suppl.):43–50.
23. **Moss, R. B., Y. P. Hsu, M. M. Sullivan, and N. J. Lewiston.** 1986. Altered antibody isotype in cystic fibrosis: possible role in osponic deficiency. Pediatr. Res. **20**:453–459.
24. **Organisation mondiale de la Santé.** 1985. La mucoviscidose. Bull. W.H.O. **63**:203–213.
25. **Poutrel, B., A. Boutonnier, L. Sutra, and J.-M. Fournier.** 1988. Prevalence of capsular polysaccharide types 5 and 8 among *Staphylococcus aureus* isolates from cow, goat, and ewe milk. J. Clin. Microbiol. **26**:38–40.
26. **Quie, P. G., and L. W. Wannamaker.** 1964. Serum antibodies in staphylococcal disease. Pediatrics **33**:63–70.
27. **Sompolinsky, D., Z. Samra, W. W. Karakawa, W. F. Vann, R. Schneerson, and Z. Malik.** 1985. Encapsulation and capsular types in isolates of *Staphylococcus aureus* from different sources and relationship to phage types. J. Clin. Microbiol. **22**: 828–834.
28. **Speziale, P., M. Höök, and T. Wadström.** 1985. Binding of type II collagen to *Staphylococci*. Zentralbl. Bakteriol. Mikrobiol. Hyg. **14**(Suppl.):191–196.
29. **Świtalski, L. M., C. Rydén, K. Rubin, Å. Ljungh, M. Höök, and T. Wadström.** 1983. Binding of fibronectin to *Staphylococcus* strains. Infect. Immun. **42**:628–633.
30. **Verbrugh, H. A., R. Peters, M. Rozenberg-Arska, P. K. Peterson, and J. Verhoef.** 1981. Antibodies to cell wall peptidoglycan of *Staphylococcus aureus* in patients with serious staphylococcal infections. J. Infect. Dis. **144**:1–9.
31. **Verbrugh, H. A., P. K. Peterson, B. Y. T. Nguyen, S. P. Sisson, and Y. Kim.** 1982. Opsonization of encapsulated *Staphylococcus aureus*: the role of specific antibody and complement. J. Immunol. **1**:1681–1687.
32. **Wilkinson, B. J.** 1983. Staphylococcal capsules and slime, p. 481–523. *In* C. S. F. Easmon and C. Adlam (ed.), Staphylococci and staphylococcal infections, vol. 2. Academic Press, Inc., San Diego, Calif.
33. **Wood, R. E., T. F. Boat, and C. F. Doershuk.** 1976. State of the art: cystic fibrosis. Am. Rev. Respir. Dis. **113**:833–878.