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Streptococcus pneumoniae Type 16A, a Hitherto Undescribed Pneumococcal Type

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Some properties of a newly recognized pneumococcal capsular serotype, type 16A, are described, bringing the number of capsular types characterized to 84.

Since the report of Lund in 1957 (5) listing 80 pneumococcal capsular serotypes, accounts of additional ones have appeared with decreasing frequency. Only three have been described in the past 28 years (7–9). This note recounts some properties of a newly recognized pneumococcal type, designated type 16A because of its serologic relatedness to the previously described type 16, which is redesignated here as type 16F.

Three strains of pneumococcus type 16A have been isolated. The first strain was recovered in 1974 from the sputum of a 67-year-old alcoholic male hospitalized in Philadelphia with osteomyelitis of the spine and delirium tremens. Although his lungs were clear on examination and his temperature and leukocyte count were normal, an X-ray of the chest showed a small infiltrate in the lower lobe of the left lung. Blood culture was sterile. Colonies resembling those of pneumococci were identified on the surface of a blood agar plate streaked with sputum.

The second strain was isolated in San Francisco in 1975 from the sputum of a 55-year-old male admitted to the hospital with chronic obstructive pulmonary disease and in mild congestive heart failure. He was afebrile and had a slightly elevated leukocyte count, no radiologic evidence of pneumonia, and a sterile blood culture. Pneumococci were isolated from the peritoneal washings from a mouse inoculated with the mucoid sputum of the patient.

Additional strains of the newly recognized type were not identified until 1984, when a pneumococcal isolate from the middle ear of a 1-year-old male in Beijing, People's Republic of China, designated untypable, was received in Philadelphia from Ding Shao-qing of the National Institute for the Control of Pharmaceutical and Biological Products. Like the two previous isolates, this strain showed an equivocal quellung reaction in typing pool D from the Danish Statens Seruminstitut. It gave a large and unequivocal reaction when exposed to antiserum to the isolate from San Francisco.

The pneumococcal isolates cited were identical in their properties. All grew diffusely in broth and had typical glistening, dome-shaped colonies surrounded by a zone of α-hemolysis on the surface of blood agar plates and showing central autolysis with time. In Gram-stained preparations, diplococcal forms characteristic of pneumococci predominated. The strains were inhibited by Optochin to the same degree as a strain of pneumococcus type 16F inoculated on the same plate, and the two initial isolates underwent lysis in 0.1% sodium deoxycholate (the third was not tested). Because of their limited discriminatory value, tests of the ability of the strains to ferment carbohydrates were not done. An injection of 1 ml of undiluted broth culture of each strain, which had been incubated overnight and was inoculated intraperitoneally, killed white mice regularly within 48 h; but 1 ml of a 10^{-1} dilution of the same culture killed only half the inoculated animals and required 2 to 4 days to do so. Higher dilutions caused no perceptible illness. Pneumococci of capsular type 16A are uncommon and evidently are also of low virulence for humans, because additional strains of this organism have not been identified from sputum since the recovery of the two initial isolates. The strain recovered from the middle ear in 1984 is the only one unequivocally associated with infection. Of interest, however, is the wide geographical occurrence of this uncommon capsular type.

DNA prepared from the first isolate transformed a noncapsulated pneumococcus, strain $S-_{III5}$ (2), to capsular type 16A in a transforming system described previously (10).

When examined in the quellung reaction with sera prepared by the Danish Statens Seruminstitut, none of the strains gave a positive reaction with pneumococcal Omniserum; however, a faintly detectable capsular precipitin reaction was observed in serum pool D, and a somewhat more pronounced reaction was observed with antiserum to pneumococcus type 16F. To explore further this cross-reaction, we prepared antisera to pneumococcal types 16F and 16A by immunizing rabbits intravenously with formalinized, heat-killed vaccine of either organism according to a protocol published previously (1). The results shown in Table 1 demonstrate both the cross-reactivity and distinctiveness of the two capsular serotypes in serogroup 16 in unabsorbed and in reciprocally absorbed rabbit antisera.

Tests of the cross-reactivity of pneumococcus type 16A with antisera to other pneumococcal capsular serotypes showed the absence of such reactivity with the 48 com-

TABLE 1. Capsular titers (quellung reactions) of unabsorbed and reciprocally absorbed rabbit antisera to pneumococcal types in serogroup 16

Antiserum	Titer with pneumococcal type:	
	16F	16A
16F	1:64	1:2
16F absorbed with 16A	1:16	Negative
16A	1:2	1:64
16A absorbed with 16F	Negative	1:32

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TABLE 2. Capsular (quellung) reactions of pneumococcal types 16F and 16A with various type-specific and factor antisera

Capsular type	Antiserum			
	16F	16A	11d	28b
16F	+++	+	+	+
16A	+	+++	−	−

mercially available type- or group-specific antisera and with factor sera used to distinguish individual capsular serotypes within the several serogroups (6) that were provided through the generosity of Erna Lund and Jørgen Henrichsen of the Danish Statens Seruminstitut. No reactions of the 83 known pneumococcal serotypes with antiserum to pneumococcus type 16A, other than that of type 16F, were observed.

Pneumococcus type 16F was assigned an antigenic formula that includes factors 16a, 16b, and 11d because of its previously identified cross-reactivity with pneumococcal types 11A and 28F (3, 4). Pneumococcus type 16A gave no detectable reaction to factor serum 28b, which quelled pneumococcus type 16F, or to factor serum 11d, which reacted also with the latter organism (Table 2). On the basis of these observations and of those described earlier, the two pneumococcal types in serogroup 16, in conformity with the Danish scheme of classification, were assigned the following antigenic formulas. The antigenic formula for pneumococcus type 16F is 16a,16b,11d. The formula for type 16A is 16a,16c. Sera for the identification of pneumococcus type 16A are not generally available at this time.

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