Lack of Immunoglobulin A1 Protease Production by Branhamella catarrhalis

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Received 1 November 1985/Accepted 22 January 1986

Clinical isolates of Branhamella catarrhalis from the sputum of 20 patients with acute bronchopulmonary infection were examined for synthesis of immunoglobulin A1 protease by immunoelectrophoresis. Ten strains produced ß-lactamase, and 10 were ß-lactamase negative. None of the strains demonstrated immunoglobulin A1 protease activity despite the fact that three different culture media were used.

Mucous membranes have an elaborate protective mechanism, part of which is the secretion of immunoglobulin A (IgA) by mucosal plasma cells into mucosal fluid where antibody specificity to microorganisms prevents their binding to host cells. A number of pathogens, including Streptococcus pneumoniae, Haemophilus influenzae, Neisseria meningitidis, and Neisseria gonorrhoeae, are known to produce IgA1 protease, a trypsinlike enzyme which cleaves the antibody into inactive Fab and Fc fragments (7, 13). It seems likely, therefore, that the elaboration of IgA1 protease confers some pathogenic advantage to the organism. The genus Neisseria is composed of a number of different species, both pathogenic and commensal, but the factors that distinguish these two subgroups are still poorly understood. In the past decade, it has been increasingly recognized that Branhamella catarrhalis, previously considered a nasopharyngeal commensal, behaves more as an opportunistic pathogen, particularly in patients with otitis media (6) or chronic pulmonary disease (9, 12, 14) or who are immune suppressed (4). The reason for this is not yet clear. Two other members of the Neisseriaceae family, N. gonorrhoeae and N. meningitidis, are well-recognized human pathogens, and both elaborate IgA1 protease. As only two strains of B. catarrhalis have been studied to date, and these were commensal nasopharyngeal isolates (11), we studied 20 clinical isolates of B. catarrhalis to see whether these pathogenic strains differ from the commensals by virtue of their ability to elaborate IgA1 protease (13).

Clinical isolates of B. catarrhalis from the sputum of 20 patients presenting with symptoms of acute lower respiratory tract infection were identified by standard criteria (3). ß-Lactamase production was detected by using chromogenic cephalosporin. DNase production was confirmed (3). B. catarrhalis strains were subcultured onto Columbia blood agar (Oxoid CM331) and incubated for 18 h in 8% CO2 at 36°C. Five colonies from the pure culture obtained were transferred into 5 ml of nutrient broth no. 2 (Oxoid CM67), capped, and incubated at 36°C for 24 h. The broth was then centrifuged at 1,410 × g for 20 min, and sterile filtrates were obtained by passing the supernatant through a Millipore membrane (pore size, 0.22 µm). In a pilot study to select the best organism for a positive control, fresh clinical isolates of N. meningitidis, N. gonorrhoeae, and S. pneumoniae type 6 were tested. N. meningitidis was grown on heated Columbia blood agar (chocolate). N. gonorrhoeae was cultured on New York City medium and subcultured onto heated Columbia blood agar, and S. pneumoniae was cultured on Columbia blood agar. All were grown in 8% CO2 for 24 h. Each species was then grown in a medium known to support vigorous growth. Five N. meningitidis colonies were transferred to 5 ml of Mueller-Hinton broth (Oxoid CM405), five N. gonorrhoeae colonies were transferred to GC medium base with the agar removed (Difco 0289), and five S. pneumoniae colonies were transferred to Todd-Hewitt serum glucose broth (Oxoid CM189 with 5% serum and 1% glucose). All were incubated for 24 h, and a sterile supernatant was prepared as described above. In addition, 10 B. catarrhalis isolates (five ß-lactamase positive and five ß-lactamase negative) were grown in Todd-Hewitt serum glucose broth and in Mueller-Hinton broth to determine whether the culture medium in any way induces enzyme production. Monoclonal IgA1 (myeloma) (from the Edinburgh Blood Transfusion Service) was diluted by addition to an equal volume of phosphate-buffered saline (pH 7.3). A 50-µl sample of the resultant solution was mixed with 50 µl of each sterile broth supernatant for 18 h at 36°C. Cleavage proteins were sought by immunoelectrophoresis of the substrate. Immunoelectrophoresis was performed on Gelbond film coated with 1% agarose in 25% glycerine-Tris-barbitone buffer. The antiserum used to visualize the IgA protease substrates was goat anti-human IgA of appropriate heavy- and light-chain specificity (Miles Scientific, Slough, England). On all slides, N. meningitidis digests were used as positive controls, and IgA plus buffer was used as the negative control. Two hundred volts were applied for 2 h. The gel was fixed, stained with Ponceau S, and dried overnight in air at room temperature. Two experienced independent observers assessed the resultant curves in a blind fashion.

None of the 20 B. catarrhalis isolates cultured in nutrient broth cleaved IgA1. The two additional media failed to induce enzyme production. N. meningitidis, N. gonorrhoeae, and S. pneumoniae type 6 all cleaved IgA1, although N. meningitidis gave the clearest lines.

The function of IgA1 proteases has not been clearly established, although the detection of Fc fragments in stools (10) and in secretions from vaginal tracts infected with N. gonorrhoeae (1) confirms that cleavage of IgA1 does occur in vivo. Certainly, the establishment of the fact that S. pneumoniae, N. meningitidis, and H. influenzae (the three major etiological agents of bacterial meningitis) cleave IgA1 indicates that IgA1 protease production may represent a significant virulence factor. S. pneumoniae and H. influenzae are...
also the commonest respiratory tract pathogens. In contrast, bacteria associated with nosocomial pneumonias do not produce IgAl and rarely cause respiratory infection in uncompromised hosts. The mechanism of pathogenicity which distinguishes a commensal strain of \textit{B. catarrhalis} from a pathogenic strain is still poorly understood, although several markers have been proposed: DNase, which is capable of inflaming mucous membranes, is produced; production of $\beta$-lactamase first noted in 1977 (8) was proposed as a marker of pathogenicity (5), although $\beta$-lactamase-negative strains do cause disease (9, 14), which would tend to defeat this contention; a monoclonal antibody that recognizes an outer membrane antigen common to the pathogenic \textit{Neisseria} species but not to most nonpathogenic \textit{Neisseria} species has been described (2). The production of IgAl protease among members of the \textit{Neisseriaceae} has also been proposed as a marker of pathogenicity (11), but only two strains of \textit{B. catarrhalis} from nasopharyngeal isolates have been studied. Our study of clinical isolates causing bronchopulmonary infection (i.e., pathogenic strains) failed to demonstrate IgAl protease production, suggesting that in \textit{B. catarrhalis}, which appears to be an opportunistic pathogen, the elaboration of IgAl protease is not an important factor. Further studies of the pathogenesis of \textit{B. catarrhalis} infection are clearly indicated.

LITERATURE CITED