JOURNAL OF CLINICAL MICROBIOLOGY, July 2000, p. 2760–2762 Vol. 38, No. 7
0095-1137/00/$04.00+0

Immunoglobulin A1 Protease Activity in *Gemella haemolysans*

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Received 29 February 2000/Returned for modification 31 March 2000/Accepted 25 April 2000

The purpose of this study was to determine the occurrence and nature of immunoglobulin A1 (IgA1) protease activity in members of the genus *Gemella* and related taxa. Among a total of 22 *Gemella* strains belonging to the four species *Gemella haemolysans*, *Gemella morbillorum*, *Gemella sanguinis*, and *Gemella bergeriae* and four reference strains of the species *Helcococcus kunzii*, *Facklamia hominis*, and *Globicatella sanguinis*, IgA1 protease activity was an exclusive character of all nine isolates of *G. haemolysans*. The IgA1 protease of *G. haemolysans* appears to be a metallo-type IgA1 protease that cleaves the Pro_{227}-Thr_{228} peptide bond in the hinge region of the α1 chain like that of several *Streptococcus* species. Phenotypic characterization of the isolates demonstrates that screening for IgA1 protease activity provides a valuable means for species differentiation in this group of bacteria.

The genus *Gemella* currently comprises the species *G. haemolysans*, *G. morbillorum*, *G. bergeriae*, and *G. sanguinis* (2–4). While there is limited information on the natural occurrence of *G. bergeriae* and *G. sanguinis*, both *G. haemolysans* and *G. morbillorum* are part of the human resident microbiota of several mucosal surfaces (1, 4). Both species have been detected in the oropharynx, and *G. morbillorum* has, in addition, been detected in the gastrointestinal tract. The clinical significance of *Gemella* species is not entirely clear, though all four species have been isolated from blood of patients with subacute endocarditis. In addition, *G. haemolysans* and *G. morbillorum* have been cultured from cerebrospinal fluid of patients with meningitis and from infected eyes, *G. haemolysans* has been cultured from urine, and *G. morbillorum* has been cultured from bone and synovial fluid in a patient with septic arthritis (1–4).

Identification of *Gemella* isolates is notoriously difficult because of the easy decolorization in the Gram stain, the low growth rate, and the still not well defined biochemical characteristics of the individual species. This problem has been enhanced by the recognition of several related genera of gram-positive cocci, mainly based on distinct 16S rRNA sequences.

Members of the genus *Gemella* share many physiologic and biochemical properties with the viridans group streptococci, including the range of infections that they cause (4). Several commensal *Streptococcus* species, i.e., *Streptococcus sanguis*, *Streptococcus oralis*, *Streptococcus mitis* biovar 1, and important mucosal pathogens such as *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* have evolved specific immunoglobulin A1 (IgA1) proteases. By cleaving human IgA1 in the hinge region into monomeric Fab fragments that are capable of antigen binding but devoid of antibody effector functions, these proteases allow bacteria to evade the protective functions of the principal adaptive immune factor at mucosal surfaces (6, 10). A previous comprehensive screening of bacteria revealed IgA1-cleaving activity in two strains of *G. haemolysans* (7). However, apart from this preliminary observation, the occurrence and nature of this enzyme activity in members of the genus *Gemella* as presently defined and in more recently described, related taxa have never been examined.

Twenty-two *Gemella* strains originally isolated from humans were included in the study. Of these 19 were obtained from the Culture Collection of the University of Göteborg, Göteborg, Sweden: *G. haemolysans* strains CCUG 411, CCUG 4815, CCUG 28134, CCUG 33602, CCUG 37278, and CCUG 37985^T; *G. sanguinis* strains CCUG 37820^T, CCUG 24073, CCUG 37821, and CCUG 37970; *G. morbillorum* strains CCUG 15561, CCUG 18164^T, CCUG 18165, CCUG 32414, and CCUG 32957B; *G. bergeriae* strains CCUG 31456, CCUG 37817^T, CCUG 37818, and CCUG 37968. An additional three dental plaque isolates of *G. haemolysans* were from our own collection (SK940, SK912, and SK891). The identity of the final three isolates was verified by 16S rRNA gene sequence analysis (9). In addition, reference strains of the related catalase-negative, gram-positive cocci *Globicatella sanguinis* (strains CCUG 33367 and CCUG 36563), *Helcococcus kunzii* (strain CCUG 32213^T), and *Facklamia hominis* (strain CCUG 36813^T), were examined. *S. sanguis* strain SK1, *Streptococcus pneumoniae* strain 1416-93, and isolated IgA1 proteases of *H. influenzae* strains HK393 (cleavage type 1) and HK224 (cleavage type 2) served as references of different IgA1 protease cleavage specificities.

All strains of gram-positive cocci were propagated on 5% horse blood agar incubated in air plus 5% CO_2 and were biochemically characterized using the API-ZYM system (API bioMérieux, Marcy l'Etoile, France) supplemented with tests for pyrrolidonyl aminopeptidase activity, acetoin production (Voges-Proskauer [VP] test), and neuraminidase activity. Acid production from raffinose, mannitol, sorbitol, sucrose, lactose, maltose, and trehalose was examined in fluid medium. All tests were performed as previously described (8). IgA1 protease activity was detected by suspending several colonies of each strain in 100 μl of substrate solution containing 2.1 μg of myeloma IgA1 or IgA2m(1) in phosphate-buffered saline (PBS). After incubation for 20 h at 37°C bacteria were removed by centrifugation at 10,000 × *G* for 10 min, after which the supernatant was boiled for 5 min with reducing sample buffer before electrophoresis on a sodium dodecyl sulfate (SDS)-polyacrylamide gradient (4 to 20%) gel. Protein bands were visualized using 0.1% (wt/vol) Coomassie blue.

IgA1 protease activity was exclusively detected among the nine reference strains of *G. haemolysans*, including the type strain. Cleavage of IgA2m(1) was not observed for any of the

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TABLE 1. Differential biochemical activities of *Gemella*, *Globicatella*, *Facklamia*, and *Helcococcus* species

Species	No. of isolates positive in reactions[a]/total no. of isolates											
	Phosph	VP	Leu AA	Cys AA	Raffinose	Mannitol	Sorbitol	Sucrose	Lactose	Maltose	Trehalose	IgA1
G. haemolysans	9/9	8/9	0/9	0/9	1/9	1/9	1/9	7/9	0/9	8/9	0/9	9/9
G. morbillorum	0/5	0/5	0/5	0/5	0/5	0/5	0/5	5/5	0/5	3/5	0/5	0/5
G. sanguinis	4/4	0/4	0/4	0/4	3/4	4/4	4/4	4/4	0/4	4/4	1/4	0/4
G. bergeriae	0/4	0/4	3/4	4/4	2/4	2/4	3/4	0/4	4/4	1/4	4/4	0/4
G. sanguinis	0/2	0/2	0/2	0/2	2/2	1/2	2/2	2/2	2/2	2/2	2/2	0/2
F. hominis	0/1	0/1	1/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
H. kunzii	0/1	0/1	0/1	0/1	0/1	1/1	1/1	0/1	1/1	1/1	1/1	0/1

[a] Phosph, alcaline and acid phosphatases; Leu AA, leucine arylamidase; Cys AA, cystine arylamidase; IgA1, IgA1 protease activity. Activities of phosphatase, leucine arylamidase, and cystine arylamidase were detected in the API-ZYM kit. Other reactions in the API-ZYM kit were negative or indistinct. The remaining tests were performed in conventional test tube media.

bacterial strains. As illustrated in Fig. 1, SDS-polyacrylamide gel electrophoresis showed Fc_α and Fd_α fragments generated by the IgA1 protease of *G. haemolysans* that were indistinguishable from those produced by the *S. sanguis* IgA1 protease, suggesting the site of cleavage in the IgA1 hinge region is the peptide bond Pro_{227}-Thr_{228}, which is the target for the streptococcal enzyme. The similarity between IgA1 proteases of these species is further supported by the demonstrated inhibition of the *G. haemolysans* IgA1 protease by the metal chelator EDTA, which corresponds to a metallo-type proteinase (5).

All strains of the four *Gemella* species were alpha-hemolytic, produced acid in glucose broth, and were positive for esterase lipase C8, naphthol-AS-BI-phosphohydrolase, and pyrrolidonyl aminopeptidase activity, with the exception of *G. haemolysans* strain SK891. None of the isolates were positive for valine arylamidase, trypsin, α-galactosidase, β-galactosidase, β-glucoronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, or neuraminidase. Other biochemical activities are summarized in Table 1. As illustrated by the table, *G. haemolysans* could be distinguished from all other species by the ability to cleave IgA1. Most of the biochemical results shown in Table 1 are in agreement with those of other studies (1, 4). However, some reactions are at variance with those reported by Collins et al. (2, 3), in spite of the fact that the majority of strains are common to the two studies. While eight of nine strains of *G. haemolysans* examined by us were positive in the VP test in agreement with results reported by Berger (1), Collins and coworkers (3) reported this reaction to be negative. Interestingly, the one negative exception in our study was the type strain. Likewise, Collins et al. reported no acid production from sorbitol by *G. bergeriae*, while three of four strains were positive in our study, and no acid production by *G. sanguinis* from raffinose, while three of four strains were positive in our study. These discrepant results are likely to be due to different methods (conventional tube fermentation tests in this study in contrast to dehydrated substrates in commercial kits in the study reported by Collins et al. [2, 3]) and emphasize the problems associated with phenotypic differentiation of members of this group of bacteria. Thus, the test for IgA1 protease activity is a valuable differential test for *G. haemolysans* and *G. morbillorum* as it is for differentiation of some of species of the mitis group of streptococci (8).

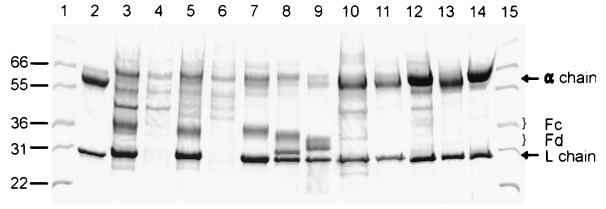

FIG. 1. SDS-polyacrylamide gel electrophoresis of human IgA1 and IgA2 myeloma proteins before and after incubation for 24 h with *G. haemolysans* or reference IgA1 proteases. Lane 1, molecular weight standards (in thousands); lane 2, intact IgA1 control; lane 3, IgA1 incubated with *G. haemolysans* cells; lane 4, PBS incubated with *G. haemolysans* cells; lane 5, IgA1 incubated with *S. pneumoniae* cells; lane 6, PBS incubated with *S. pneumoniae* cells; lane 7, IgA1 incubated with *S. sanguis* cells; lane 8, IgA1 incubated with cleavage type 1 IgA1 protease from *H. influenzae* HK393; lane 9, IgA1 incubated with cleavage type 2 IgA1 protease from *H. influenzae* HK224; lane 10, IgA1 incubated with *G. haemolysans* cells in the presence of 100 mM EDTA; lane 11, IgA1 incubated with *S. pneumoniae* cells in the presence of 100 mM EDTA; lane 12, IgA2 incubated with *G. haemolysans* cells; lane 13, IgA2 incubated with *S. pneumoniae* cells and 100 mM EDTA; lane 14, intact IgA2; lane 15, molecular weight standards. Lane 3 demonstrates that *G. haemolysans* cleaves IgA1 to yield Fc and Fd fragments identical in size to those released by *S. sanguis* (lane 7) and distinct from those released by *S. pneumoniae* (lane 5) and the two cleavage types of *H. influenzae* (lanes 8 and 9). Note that Fd fragments released by the protease activity of *G. haemolysans* and *S. sanguis* are close to the size of IgA1 light chains (L chain). The activity is inhibited by EDTA (lane 10). The distinct size of Fc fragments released from IgA1 by *S. pneumoniae* (lane 5), although cleaving the same peptide bond as *S. sanguis*, is due to glycosidase activities releasing carbohydrate side chains of the heavy chain (8). None of the bacteria cleave IgA2 (lanes 12 and 13).

Although phylogenetically related, members of the genus *Gemella* constitute a distinct branch separate from the genus *Streptococcus* (12). The finding that their IgA1 proteases are metallo-proteinases and appear to cleave the same peptide bond shows that they belong to the same family of IgA1 proteases. Although genetic studies are required to elucidate this question, it is conceivable that the gene encoding IgA1 protease in *G. haemolysans* shares a common ancestor with the mutually related *iga* genes of *S. sanguis*, *S. oralis*, *S. mitis*, and *S. pneumoniae* (11). Although IgA1 protease enables bacteria to evade the adherence-inhibitory activity of secretory IgA in vitro and is assumed to constitute an important virulence determinant in bacterial meningitis (6, 10), direct proof is still missing due to the lack of a suitable animal model. Why the activity has been conserved in *G. haemolysans* along with the mentioned *Streptococcus* species is an intriguing question.

This study was supported by a grant from the Danish Medical Research Council.

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