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Lack of Cleavage of Immunoglobulin A (IgA) from Rhesus Monkeys by Bacterial IgA1 Proteases

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Bacterial immunoglobulin A1 (IgA1) proteases cleaving IgA1 and secretory IgA1 molecules in the hinge region are believed to be important virulence factors. Previous studies have indicated that IgA of humans, gorillas, and chimpanzees are the exclusive substrates of these enzymes. In a recent study, IgA from the rhesus monkey was found to be susceptible to the IgA1 protease activity of *Streptococcus pneumoniae*. In an attempt to reproduce this observation, we found that neither five isolates of *S. pneumoniae* nor other IgA1 protease-producing bacteria representing different cleavage specificities caused cleavage of rhesus monkey IgA. Hence, the rhesus monkey does not appear to be a suitable animal model for studies of IgA1 proteases as virulence factors.

Bacterial immunoglobulin A1 (IgA1) proteases are endopeptidases capable of cleaving IgA1 and secretory IgA1 molecules in the hinge region, thereby leaving them as intact Fab (monomeric) and Fc fragments. IgA1 proteases have been found to be produced as constitutive enzymes by a number of pathogenic bacteria, including the three leading causes of bacterial meningitis (*Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus pneumoniae*) and the cause of gonorrhea, *Neisseria gonorrhoeae*. In addition, several oral bacteria produce IgA1 protease. Among these are three streptococcal species that initiate the colonization of tooth surfaces (*Streptococcus sanguis*, *Streptococcus oralis*, and *Streptococcus mitis* biovar 1) as well as *Prevotella*, *Porphyromonas*, and *Capnocytophaga* species that appear to be involved in the pathogenesis of destructive periodontal disease. Comprehensive lists of bacteria found to possess IgA1 protease are provided by several reviews (6, 11, 12).

In accordance with their exquisite specificity for IgA1 molecules, individual IgA1 proteases have been found to cleave one particular Pro-Ser (protease type 1) or Pro-Thr (protease type 2) peptide bond within a sequence which is present in the α1 chain but deleted in the α2 chain of IgA2 molecules. An exception is the protease of a strain of *Clostridium ramosum* isolated from a patient with inflammatory bowel disease. This protease cleaves a peptide bond located outside the sequence (Fig. 1) and is capable of cleaving not only IgA1 but also molecules belonging to the A2m(1) allotype of the A2 subclass (3).

A considerable amount of data indicates that IgA1 proteases play a role as virulence factors (5, 6). Hypothetical models for the development of invasive infection due to IgA1 protease-producing bacteria (7) and for protease-facilitated colonization of oral surfaces (1) are currently being investigated in our laboratory. However, the role of IgA1 proteases in vivo has been difficult to evaluate because of the lack of an appropriate animal model. Previous screening of a large number of animal species including 20 species of nonhuman primates (9) led to the conclusion that IgA of humans and the two great apes *Gorilla gorilla* and *Pan troglodytes* (the chimpanzee) were the exclusive substrates of bacterial IgA1 proteases. It was therefore of great interest when, in a recent study by Proctor and Manning (13), IgA of the rhesus monkey was found to be susceptible to the IgA1 protease activity of *S. pneumoniae*.

Against this background we have examined the susceptibility of rhesus monkey IgA to the IgA protease activity of human isolates of *S. pneumoniae* and other bacterial species representing different sites of cleavage within the hinge region of human IgA.

Bacteria. The following bacteria served as sources of IgA1 protease: *S. pneumoniae* ATCC 27336 (noncapsulated) and four clinical isolates of serotypes 3, 9A, 14L, and 18A; *S. sanguis* ATCC 10556; *S. oralis* ATCC 10557; two clinical isolates of *N. meningitidis*, (HF124 [serogroup C, IgA1 protease type 1] and HF161 [serogroup B, IgA1 protease type 2]); *N. gonorrhoeae* NG74 (IgA1 protease type 2) (received from T. F. Meyer, Tübingen, Germany); two clinical isolates of *H. influenzae*, HK368 [biotype 1, serotype b, IgA1 protease type 1] and HK224 [biotype 1, nonencapsulated, IgA1 protease type 2]); and *C. ramosum* AK183 (received from Y. Fujiyama, Tsukinowa, Japan). Streptococci were cultured on Todd-Hewitt agar, and strains of *N. meningitidis*, *N. gonorrhoeae*, *H. influenzae*, and *C. ramosum* were cultured on chocolate agar plates in an atmosphere of 10% carbon dioxide, 10% hydrogen, and 80% nitrogen at 35°C. Bacteria harvested from the agar plates were used as sources of IgA1 protease except for *S. pneumoniae* serotypes 9A, 14L, and 18A, in which crude enzyme preparations prepared as described elsewhere (14) were employed. The sites at which IgA1 proteases of the individual bacteria cleave human α1 chains are indicated in Fig. 1.

IgA proteins. Two preparations of IgA from individual rhesus (*Macaca mulatta*) monkeys were employed. One serum was obtained from an animal (no. 9190) kept by Vetdiagnostics, Small Dole, West Sussex, United Kingdom, whereas another was a gift from M. Schiødt, Royal Dental College, Copenhagen, Denmark, and originated from California Primate Research Center, Davis. Human IgA1 (Fri) was obtained from the serum of an individual with myelomatosis. Monkey and human IgAs were purified by a procedure involving ammonium sulfate precipitation, size exclusion, and anion-exchange chromatography (10). IgA-containing column eluent fractions were identified by an

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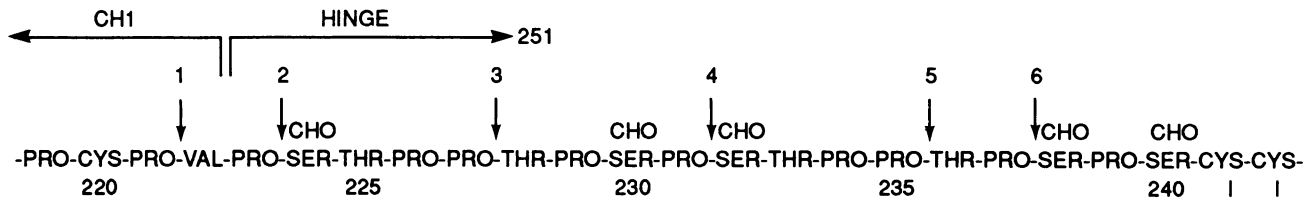

FIG. 1. Primary structure of the hinge region of human IgA1. The sequence Ser-224 to Thr-236 is deleted in IgA2 molecules. The arrows indicate the peptide bonds cleaved by the individual IgA1 proteases as follows: 1, *C. ramosum*; 2, *Prevotella melaninogenica* and *Capnocytophaga ochracea*; 3, *S. pneumoniae*, *S. oralis*, *S. sanguis*, and *S. mitis* biovar 1; 4, *H. influenzae* protease type 1; 5, *H. influenzae* protease type 2, *N. gonorrhoeae* protease type 2, and *N. meningitidis* protease type 2; 6, *N. gonorrhoeae* protease type 1 and *N. meningitidis* protease type 1. CHO, carbohydrate side chains.

enzyme-linked immunosorbent assay using rabbit antibodies against human α chains for capture and development (8).

Assay for IgA1 protease activity. One loopfull of bacteria or a 10-μl volume of crude bacterial enzymes was mixed with 25 μl of IgA protein at 0.5 mg/ml in 0.01 M phosphate-buffered saline (0.15 M) containing 0.02% sodium azide. After incubation at 35°C overnight, the mixture was centrifuged and the supernatant was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions and then electroblotted onto Immobilon P (Millipore Corp., Bedford, Mass.) as previously described (1). Blots were stained with affinity-purified rabbit antibodies against human α chains (Dakopatts, Glostrup, Denmark) followed by affinity-purified, alkaline phosphatase-conjugated swine anti-rabbit immunoglobulin antibodies and developed in 5-chromo-4-chloro-3-indolyl-phosphate–Nitro Blue Tetrazolium staining solution (1). Lanes of blotted M_r markers (14 to 97 kDa; Bio-Rad, Richmond, Calf.) were stained separately with amido black.

Immunoblots of IgA1 (Fri) incubated with the individual bacteria or crude enzymes confirmed that all strains possessed IgA1 protease active against human IgA. Protease activity was revealed by the presence of bands corresponding to Fc_α and Fd_α fragments in combination with the complete or partial disappearance of bands representing intact α chains (Fig. 2). Differences in apparent M_r values of Fc_α and Fd_α fragments (Fig. 2) can be ascribed to differences in the site of cleavage of the corresponding IgA1 proteases and, in the case of *S. pneumoniae*, to the additional activity of bacterial glycosidases. When IgA from rhesus monkeys instead of IgA1 (Fri) was exposed in similar experiments to the bacterial enzymes, no signs of cleavage were observed. Thus, with all strains and with IgA from either of the two animals, immunoblots demonstrated a band of intact α chains similar to that obtained from the control IgA substrate (Fig. 2).

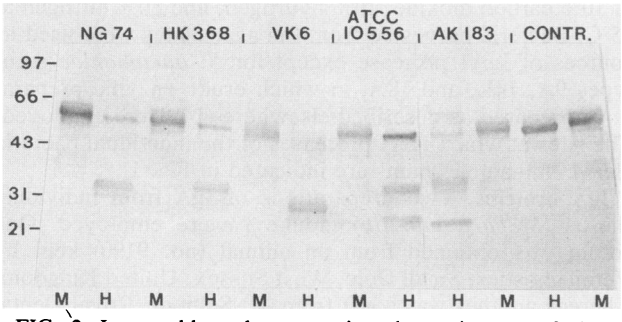

FIG. 2. Immunoblots demonstrating the resistance of rhesus monkey IgA along with the susceptibility of human IgA1 (Fri) to the IgA1 protease activities of *N. gonorrhoeae* NG74, *H. influenzae* HK368, *S. pneumoniae* VK6, *S. sanguis* ATCC 10556, and *C. ramosum* AK183. Control lanes (CONTR.) of sham-incubated IgA substrates are included. At the bottom of each lane is indicated the type of IgA substrate (M, monkey; H, human). Molecular weight markers are in kilodaltons.

Additional experiments were done to examine whether protease-inhibiting antibodies might account for the lack of cleavage of monkey IgA. A mixed substrate containing 0.4 mg of monkey IgA and 0.1 mg of human IgA1 (Fri) per ml was incubated with the individual strains. The immunoblots obtained (not shown) displayed bands of α-chain fragments similar to those resulting from the use of pure IgA1 (Fri) substrate, indicating that protease-inhibiting antibodies were not responsible for the resistance of monkey IgA to the IgA1 proteases.

Proctor and Manning (13) found that IgA from the rhesus monkey was susceptible to IgA protease activity in strains of *S. pneumoniae* isolated from humans and from several other animals, including the rhesus monkey itself. Concerning susceptibility to human isolates, our results are at variance with those of these investigators. Using a closely similar method of testing, we found that rhesus monkey IgA was resistant to the protease activity of all five human isolates of *S. pneumoniae*, including the human isolate ATCC 27336 employed by Proctor and Manning (13). Furthermore, we found that rhesus IgA was resistant to the activity of other bacteria representing the same or other sites of cleavage within the hinge region of human IgA1. Our results are in accordance with the notion by Plaut and Kornfeld (9, 12) that IgA from humans, gorillas, and chimpanzees are the exclusive substrates of bacterial IgA1 proteases.

Although studies characterizing the structure of rhesus monkey IgA have not been published, there are reasons to doubt that a molecular conformation similar to that present in the hinge region of human IgA1 is represented in IgA from the rhesus monkey. Thus, epitopes recognized by monoclonal antibodies specific for human IgA1 have been found not to be represented in rhesus monkey serum (2). In addition, *N*-acetylgalactosamine, which is a characteristic component of carbohydrate side chains in the hinge region of human IgA1 molecules, cannot be detected in purified rhesus monkey IgA (15). Unlike the study of Proctor and Manning, our study did not include experiments with strains of *S. pneumoniae* isolated from rhesus monkeys. Therefore, our results do not contradict the observation by these investigators that rhesus monkey IgA is substrate to the IgA protease activity of homologous isolates of *S. pneumoniae*. However, in the light of our observations, such a situation is unlikely, as it would imply that isolates from rhesus monkeys but not

from humans had the dual capacity to cleave human as well as rhesus monkey IgA.

An interesting observation made by Proctor and Manning (13) and ourselves (4) is that IgA1 protease-producing bacteria may be isolated from animal species whose IgA is apparently not susceptible to IgA1 protease. Considering that IgA1 proteases have been found to be constitutive bacterial enzymes, this observation may indicate the existence of alternative substrates for IgA1 proteases. In the light of the observation that the susceptibility of human IgA1 to protease is influenced by the composition of its carbohydrate constituents (14), it should, however, stimulate experiments examining the effect of IgA1 protease on animal IgA in the presence of relevant microbial glycosidases. It is conceivable that such experiments will lead to the identification of an appropriate animal model for the study of IgA1 proteases as virulence factors.

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