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Examination of *Haemophilus pleuropneumoniae* for Immunoglobulin A Protease Activity

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***Haemophilus pleuropneumoniae*, the etiological agent of porcine contagious pneumonia, was examined for the ability to produce an immunoglobulin A (IgA) protease specific for porcine IgA. No IgA protease activity against either porcine or human IgA was detected. Furthermore, no sequence homology was found between *H. pleuropneumoniae* chromosomal DNA and the gene which specifies IgA protease in *Haemophilus influenzae*.**

Immunoglobulin A (IgA) proteases, extracellular bacterial enzymes that are specific for human IgA1, are characteristic of *Haemophilus influenzae*, a pathogen which causes respiratory disease and meningitis in humans, but not of other species within the genus *Haemophilus*, which are part of the normal nasopharyngeal microflora (4, 9, 12). Because these enzymes are apparently linked to virulence (6, 12; M. H. Mulks, *in* I. A. Holder (ed.), *Bacterial Enzymes and Virulence*, in press), there has been interest in the development of an animal model to evaluate the actual role of IgA proteases in pathogenesis. The minimal requirements of a biologically relevant model should include the induction of disease at a mucosal surface with an IgA protease-producing pathogen whose enzyme specifically cleaves the IgA of the host.

Kilian et al. (4) have reported that *Haemophilus pleuropneumoniae*, which causes a highly contagious and often fatal respiratory infection in pigs (8, 13, 14), produces an IgA protease which can cleave porcine but not human IgA. Thus, *H. pleuropneumoniae* disease in pigs seemed a promising model for testing the role of IgA proteases in the pathogenesis of respiratory infection. We have examined several strains of *H. pleuropneumoniae* and found no detectable IgA protease activity against either porcine or human IgA. Furthermore, the chromosomal DNA of this organism contains no sequences homologous with the gene which specifies IgA protease in *H. influenzae*.

H. influenzae serotype b (ATCC 9795), *H. influenzae* serotype c (ATCC 9007), *H. pleuropneumoniae* serotype 1 (ATCC 27088), and *H. pleuropneumoniae* serotype 2 (ATCC 27089) were purchased from the American Type Culture Collection, Rockville, Md. *H. pleuropneumoniae* serotype 3, serotype 5, K-17, and 158 were kindly provided by V. Rapp, Iowa State University. All cultures were grown at 37°C on brain heart infusion broth or agar (Difco Laboratories, Detroit, Mich.) plus 5% Fildes enrichment (Difco) under 5% CO_2 in air. Purified porcine colostral secretory IgA (from D. Leadbeatter, Unilever Research, Bedford, England), human colostral secretory IgA (7), and purified human serum IgA (2) were extrinsically labeled with ^{125}I (Amersham Co., Arlington Heights, Ill.) by the chloramine-T method (3). IgA protease cleavage of these substrates was assayed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (11).

H. influenzae type 1 and type 2 IgA proteases, prepared from serotype b and c isolates, respectively, cleaved both human serum and secretory IgA to yield intact Fabα and Fcα fragments but did not cleave porcine secretory IgA (Fig. 1). In comparison, *H. pleuropneumoniae* isolates, including the strain studied by Kilian et al. (4), cleaved neither human nor porcine IgA. Porcine IgA exposed to *H. pleuropneumoniae* showed a slight size reduction on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 1, lanes 10 through 12) as compared with a control sample (Fig. 1, lane 7); however, this alteration, which is not typical of IgA protease hydrolysis of the α chain, might be more readily explained as either carbohydrase activity or nonspecific proteolysis.

Chromosomal DNA was purified from *Haemophilus*

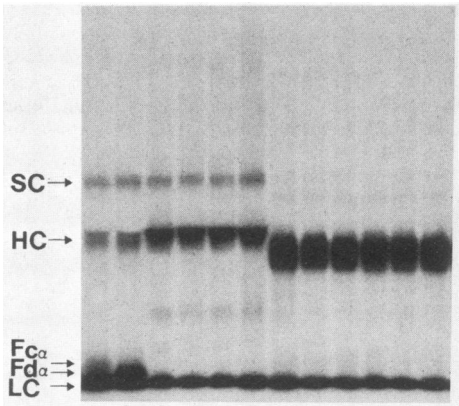

FIG. 1. Autoradiograph of sodium dodecyl sulfate-polyacrylamide gel electrophoresis of ^{125}I-labeled human and porcine secretory IgA digested with the following: lanes 1 and 8, *H. influenzae* type 1 IgA protease; lanes 2 and 9, *H. influenzae* type 2 IgA protease; lanes 3 and 10, *H. pleuropneumoniae* 27088; lanes 4 and 11, *H. pleuropneumoniae* 27089; lanes 5 and 12, *H. pleuropneumoniae* 158; and lanes 6 and 7, buffer control. Lanes 1 through 6 contain human secretory IgA; lanes 7 through 12 contain porcine secretory IgA. SC, Secretory component; HC, heavy chain; Fcα, Fcα fragment of IgA1; Fdα, heavy chain component of Fabα fragment of IgA1; LC, light chain.

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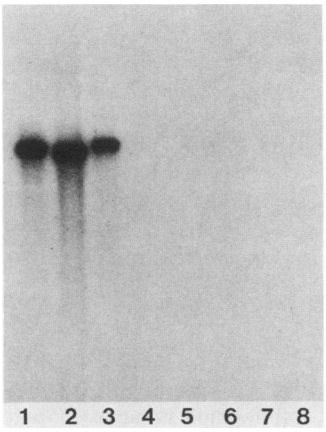

FIG. 2. Restriction and Southern blot analysis of *Haemophilus* DNA. Bacterial chromosomal DNA was digested with *Eco*RI, fractionated on a 1% agarose submarine gel, blotted onto nitrocellulose paper, and hybridized to ^{32}P-labeled probe DNA. DNA was purified from the following: lanes 1 through 3, three different *H. influenzae* serotype b isolates; lanes 4 through 8, *H. pleuropneumoniae* isolates, serotype 1, serotype 3, serotype 5, K17, and 158, respectively. A single fragment of 6.9 kilobases is homologous with the probe in all three *H. influenzae* isolates; no homology is seen with any of the *H. pleuropneumoniae* isolates.

strains, digested with *Eco*RI restriction endonuclease (New England Biolabs), and fractionated on a 1% agarose (Marine Colloids, Rockland, Maine) gel, as previously described (1). DNA was transferred from the gel to a nitrocellulose filter by the Southern technique (15) and hybridized to probe DNA (10). The probe was a 3.4-kilobase fragment of DNA containing the structural region of the *iga* gene specifying IgA1 protease activity in *H. influenzae* d; isolation of the gene and preparation of ^{32}P-labeled probe DNA has been previously described (1; J. Bricker, M. H. Mulks, A. G. Plaut, and A. Wright, manuscript in preparation). This probe hybridizes strongly under highly stringent conditions (65°C; 0.33 M salt) to chromosomal DNA from all *H. influenzae* isolates tested to date, regardless of protease type produced. This probe also hybridizes under conditions of low stringency (52°C; 1 M salt) to DNA isolated from *Neisseria gonorrhoeae* and *Neisseria meningitidis*. Examination of chromosomal DNA isolated from seven *H. pleuropneumoniae* strains with this probe, under conditions of either high or low stringency, showed no homology with the *iga* gene probe (Fig. 2).

We have been unable to detect IgA protease-like activity against either human or porcine IgA in any *H. pleuropneumoniae* isolates which we have examined. In addition, an *H. influenzae* gene probe, which has been shown to be at least partially homologous with DNA from all gram-negative bacteria which produce IgA proteases, failed to demonstrate the presence of homologous chromosomal DNA in *H. pleuropneumoniae*. Similar results have recently been reported by Koomey and Falkow (5). We conclude that *H. pleuropneumoniae* is not a promising candidate model to study the role of IgA proteases in virulence.

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