Survey of Immunoglobulin A Protease Activity Among Selected Species of *Ureaplasma* and *Mycoplasma*: Specificity for Host Immunoglobulin A

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Because immunoglobulin A (IgA) is the predominant immunoglobulin at mucosal surfaces, IgA proteases produced by pathogenic bacteria are considered potential virulence factors for organisms that cause disease or gain entry at mucous membranes. To determine the role of IgA protease in the pathogenicity of mycoplasmal disease, a variety of human and animal mycoplasma and ureaplasma species were examined for IgA protease activity with human, murine, porcine, and canine IgA. None of the mycoplasma species examined showed detectable IgA protease activity with any of the IgAs tested. Twenty-eight strains of *Ureaplasma urealyticum* isolated from human urogenital tissues cleaved human IgA1, but no cleavage of human IgA2 or murine, porcine, or canine IgA was observed. Ureaplasmas isolated from nonhuman hosts (feline, canine, avian, and bovine [*Ureaplasma diversum*]) did not cleave human IgA1. Two strains of canine ureaplasmas were able to cleave canine IgA, but not murine IgA. Thus, ureaplasmas from other species can produce IgA protease, but the specificity of the enzyme was restricted to the IgA of the appropriate host. This finding suggests that IgA proteases could play a role in the selective host specificity of mucosal pathogens.

Immunoglobulin A1 (IgA1) protease activity has been detected in a number of pathogenic bacteria responsible for human disease. IgA1 proteases specifically cleave the IgA1 isotype at a single Pro-Thr or Pro-Ser peptide bond in the hinge region of the immunoglobulin, releasing intact Fc and Fab fragments (reviewed in references 12, 13, 14, and 19). These enzymes are released by pathogens that cause bacterial meningitis, pneumonia, gonorrhea, periodontal disease, and dental plaque (reviewed in references 12 and 19). Other reports have described IgA protease activity in organisms causing otitis media and pneumonia (31) and urinary tract infections (16). Because IgA is the predominant immunoglobulin at mucosal surfaces, the IgA proteases may be important virulence factors for organisms that cause disease or gain entry at mucous membranes. There is evidence to indicate that IgA immunoglobulins are involved in resistance to bacterial disease (29), and human IgA1 myeloma paraproteins of known antigen specificity were shown to lose antigen-binding activity when treated with IgA protease (20). Thus, these IgA-specific proteases may be capable of destroying pathogen-specific antibody activities.

A number of mycoplasma pathogens of humans and animals, including *Mycoplasma pneumoniae*, initiate infection by attaching to mucosal epithelial tissues, followed by damage to the infected tissue (5). Our laboratory has been involved in studies to define virulence components of *M. pneumoniae* such as attachment and ciliotoxic and chemotactic factors (2, 3; M. G. Gabridge, D. K. F. Chandler, and M. J. Daniels, *in* S. Razin and M. F. Barile, ed., *The Mycoplasmas*, vol. 4, *Mycoplasma Pathogenicity*, in press), because they represent potentially important immunogens for the prevention of disease. There is some evidence to indicate that IgA immunoglobulins against *M. pneumoniae* are associated with resistance to disease (1). It was of interest to establish whether a known human mycoplasmal pathogen such as *M. pneumoniae* contained specific IgA1

protease activity. We have also examined the IgA1 protease activity of reputed human pathogens, including *Ureaplasma urealyticum*, *Mycoplasma hominis*, and *Mycoplasma genitalium*, a newly identified species from humans isolated from the urethra of patients with nongonococcal urethritis (28). IgA protease activity in *U. urealyticum* has recently been described (10, 23), and thus we have conducted a survey of IgA protease activity of mycoplasmas and ureaplasmas obtained from human hosts.

Kilian et al. (11) reported that *Haemophilus pleuropneumoniae*, the causative agent of porcine pleuropneumonia, specifically cleaved porcine IgA, but not human IgA, although a recent study failed to confirm this observation (18). For this reason, a number of respiratory mycoplasma pathogens known to produce pneumonia in mice, swine, and cattle, as well as ureaplasmas isolated from animal hosts, were examined for IgA-specific protease activity. The results of these studies are presented in this report.

MATERIALS AND METHODS

Organisms. The mycoplasma species and strains examined and their sources of origin are given in Tables 1 and 2. The animal mycoplasmas were isolated from the respiratory tract, except for *Mycoplasma pulmonis* strain Barden (strain JB) and *Mycoplasma arthritidis*, which were recovered from arthritic joints, and the animal ureaplasmas were isolated from the urogenital tract. *M. pneumoniae*, *M. pulmonis*, *Mycoplasma gallisepticum*, and *M. genitalium* were grown attached to glass in Edward-Hayflick broth (2). Colonies were washed with phosphate-buffered saline, pH 7.4 (PBS), scraped with a rubber policeman into PBS, and concentrated by centrifugation at 10,000 × *g* for 30 min. The sedimented pellet was suspended in 200 to 300 μl of PBS. The other mycoplasma species were grown in Kimler bottles containing 100 to 200 ml of either Edward-Hayflick broth (*Mycoplasma mycoides* subsp. *mycoides*, *M. mycoides* subsp. *capri*, and *Mycoplasma hyopneumoniae*) or Edward-Hayflick broth plus arginine (1.5 g/liter) (*M. hominis* and *M.*

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TABLE 1. Human mycoplasmas examined for IgA1 protease activity

Species	Strain	Site of isolation	Source
Mycoplasma hominis	10144	Upper urinary tract	D. Taylor-Robinson
Mycoplasma hominis	11589	Hypogammaglobulinemic patient	D. Taylor-Robinson
Mycoplasma hominis	13408	Nongonococcal urethritis	D. Taylor-Robinson
Mycoplasma hominis	11085	Blood, postpartum fever	D. Taylor-Robinson
Mycoplasma hominis	MY13428	Blood	D. Taylor-Robinson
Mycoplasma hominis	LO1888	Cell culture	R. Del Giudice
Mycoplasma hominis	L1846	Cell culture	R. Del Giudice
Mycoplasma hominis	PG21	Rectal swab	Research Resources Section, NIAID
Mycoplasma hominis	LBD5	Blood	J. Tully
Mycoplasma hominis	W1456	Blood	C. Zeirdt
Mycoplasma hominis	F4238	Blood	C. Zeirdt
Mycoplasma hominis	M5039	Blood	C. Zeirdt
Mycoplasma hominis	H5488	Blood	C. Zeirdt
Mycoplasma hominis	1628	Septic arthritis	M. Barile
Mycoplasma genitalium	G37	Nongonococcal urethritis	J. Tully
Mycoplasma pneumoniae	PI1428	Atypical pneumonia	R. M. Chanock
Mycoplasma pneumoniae	FH[a]	Atypical pneumonia	R. M. Chanock
Mycoplasma pneumoniae	B176[a]	Atypical pneumonia	W. A. Clyde
Mycoplasma pneumoniae	M129	Atypical pneumonia	W. A. Clyde
Mycoplasma pneumoniae	Mac[a]	Atypical pneumonia	W. A. Clyde
Mycoplasma pneumoniae	TW1-6	Atypical pneumonia	J. Tully
Mycoplasma pneumoniae	TW8-6	Atypical pneumonia	J. Tully
U. urealyticum	U19	Urogenital tract	S. J. Lin
U. urealyticum	K2	Urogenital tract	S. J. Lin
U. urealyticum	K11	Urogenital tract	S. J. Lin
U. urealyticum	U23	Urogenital tract	S. J. Lin
U. urealyticum	U24	Urogenital tract	S. J. Lin
U. urealyticum	U38	Urogenital tract	S. J. Lin
U. urealyticum	T960	Urogenital tract	S. J. Lin
U. urealyticum	K393	Urogenital tract	S. J. Lin
U. urealyticum	U9	Urogenital tract	S. J. Lin
U. urealyticum	U30	Urogenital tract	S. J. Lin
U. urealyticum	Black I	Urogenital tract	S. J. Lin
U. urealyticum	Black II	Urogenital tract	S. J. Lin
U. urealyticum	Black III	Urogenital tract	S. J. Lin
U. urealyticum	Black IV	Urogenital tract	S. J. Lin
U. urealyticum	Black V	Urogenital tract	S. J. Lin
U. urealyticum	Black VI	Urogenital tract	S. J. Lin
U. urealyticum	Black VII	Urogenital tract	S. J. Lin
U. urealyticum	Black VIII	Urogenital tract	S. J. Lin
U. urealyticum	Vancouver (ATCC 33175)	Urogenital tract	J. Robertson
U. urealyticum (serovar 1)	Ford 7	Urogenital tract	M. Shepard
U. urealyticum (serovar 2)	Ford 23	Urogenital tract	M. Shepard
U. urealyticum (serovar 3)	Ford 27	Urogenital tract	M. Shepard
U. urealyticum (serovar 4)	Ford 58	Urogenital tract	M. Shepard
U. urealyticum (serovar 5)	Ford 354	Urogenital tract	M. Shepard
U. urealyticum (serovar 6)	Pi	Urogenital tract	M. Shepard
U. urealyticum (serovar 7)	Co	Urogenital tract	M. Shepard
U. urealyticum (serovar 8)	T-960	Urogenital tract	M. Shepard

[a] Laboratory strains.

arthritidis). Microorganisms were concentrated and washed with PBS by centrifugation as described above. Ureaplasma strains derived from human, bovine, feline, canine, and avian sources were grown in 300 ml of the U9B medium described by Shepard and Lunceford (24). After 18 h of incubation at 36°C, the organisms were concentrated by centrifugation at 100,000 × g for 1 h at 4°C. The sedimented pellet was washed once with PBS and suspended in 200 μl of PBS. The pellets from each strain tested were either used immediately or stored at −70°C until used. A non-encapsulated mutant derived from Neisseria meningitidis strain M986 (group B, serotype 2a) was obtained from Carl Frasch, Center for Drugs and Biologics, Food and Drug Administration. This strain was used as the IgA protease-producing positive control culture and was grown on Columbia blood

agar plates overnight at 37°C in 5% carbon dioxide–95% air. Colonies were collected and suspended in 200 to 300 μl of PBS and used immediately.

Immunoglobulins and antisera. Human myeloma IgA1 (kappa) was purchased from Cappel Laboratories, West Chester, Pa., and human IgA1 (kappa) Scillia, IgA1 (kappa) Stewart, and IgA2 were gifts from R. Wistar, Jr., Naval Medical Research Institute, Bethesda, Md. Mouse myeloma IgA (kappa) was from Walgene Laboratories, Arcadia, Calif., and mouse clarified ascites IgA (kappa) was from Bionetics Laboratories, Kensington, Md. Bovine secretory IgA and anti-bovine IgA were gifts from A. Guidry, U.S. Department of Agriculture, Beltsville, Md. The bovine immunoglobulin preparation was purchased from Calbiochem, La Jolla, Calif. Dog myeloma IgA was a gift from J. P. Vaerman,

International Institute of Cellular and Molecular Pathology, Brussels, Belgium, and porcine secretory IgA was a gift from Prem S. Paul, U.S. Department of Agriculture, Ames, Iowa.

Anti-human IgA (α-chain specific) and anti-human kappa light chains were obtained from Cappel Laboratories. The anti-mouse IgA (α-chain specific) and anti-kappa light chain were from Bionetics Laboratories. Anti-porcine IgA, anti-canine IgA, and anti-bovine IgA antisera were purchased from Miles Scientific Co., Elkhardt, Ind.

Detection of IgA protease activity. A 10-μl sample of human myeloma IgA (10 mg/ml) was added to 100 μl of the concentrated pellet of each human or animal ureaplasma or mycoplasma strain tested (Tables 1 and 2). In addition, 20 μl of mouse myeloma IgA (kappa) (1 mg/ml) or 10 μl of mouse clarified ascites fluid IgA (kappa) (5 mg/ml) was added to 100 μl of the concentrated pellet of mycoplasma or ureaplasma as indicated below. Canine, porcine, or bovine IgA (10 to 20 μl, 1 to 2 mg/ml) was added to 50 μl of the concentrated pellet of mycoplasma or ureaplasma tested. Culture supernatant fluids were not systematically examined due to the high concentration of serum proteins in the medium.

The IgA substrates were incubated with PBS or the cultures at 37°C for 18 h. Mycoplasma species that did not exhibit any cleavage of substrate initially were incubated with substrate for periods of up to 1 week. After incubation the mixtures were centrifuged for 10 min in an Eppendorf model 5414 microfuge, and the supernatant fluids were subjected to immunoelectrophoresis or gel electrophoresis.

Immunoelectrophoresis of the reaction mixtures was performed with 2% agarose (Seakem, Rockland, Maine) in 0.05 M barbital buffer, pH 8.2 (6), with the LKB Multiphor system (LKB, Bromma, Sweden). Electrophoresis was carried out for 1 h at 10 V/cm at 10°C, and then 50 μl of antisera specific for the A chain and kappa light chains were added to the troughs. Immunodiffusion was developed overnight at room temperature, and the plates were washed, dried, and stained with Coomassie brilliant blue R-250 (Bio-Rad Laboratories, Richmond, Calif.).

TABLE 2. Animal mycoplasmas examined for IgA protease activity

Host	Species	Strains	Source
Mice	M. pulmonis	Barden	J. Tully
	M. arthritidis	07	P. Smith
	M. arthritidis	PG27	P. Smith
Dogs	Ureaplasma sp.	D1M-C	K. Koshimizu
	Ureaplasma sp.	D29M	K. Koshimizu
	Ureaplasma sp.	D11N-A	K. Koshimizu
	Ureaplasma sp.	D6P-C	K. Koshimizu
Cats	Ureaplasma sp.	F45d-2	K. Koshimizu
	Ureaplasma sp.	F2	K. Koshimizu
Birds	Ureaplasma sp.	T9-1	K. Koshimizu
	Ureaplasma sp.	D6-1	K. Koshimizu
	M. gallisepticum	PG31	NIAID[a]
Cattle	U. diversum	A417	K. Koshimizu
	M. mycoides subsp. mycoides	B3	Z. Dinter
	M. mycoides subsp. mycoides	UM30847	R. Purcell
Goats	M. mycoides subsp. capri	PG3	D. F. Edward
Pigs	M. hyopneumoniae (M. suipneumoniae)	J	P. Whittlestone

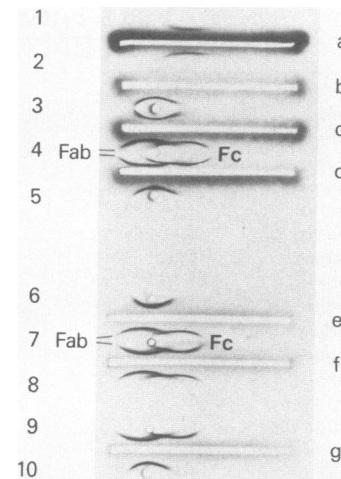[a] Research Resources Section, National Institute of Allergy and Infectious Diseases.

FIG. 1. Immunoelectrophoretic pattern of human IgA and mouse IgA incubated with suspensions of *U. urealyticum*, *N. meningitidis*, and *M. hominis*. Mouse myeloma IgA was incubated with (well 1) PBS or (well 2) *U. urealyticum* strain Vancouver. Human myeloma IgA1 was incubated with (well 3) *M. hominis* strain H5488, (well 4) *U. urealyticum* strain Vancouver, (wells 5 and 6) PBS, (well 7) *U. urealyticum* strain CO (serovar 7), (well 8) *U. urealyticum* strain T960 (serovar 8), (well 9) *N. meningitidis* strain M986, or (well 10) *M. hominis* strain PG21. Trough a contained rabbit antiserum to mouse immunoglobulin kappa light chain plus goat antiserum to mouse IgA; troughs b, c, d, e, f, and g contained rabbit antiserum to human kappa light chain plus goat antiserum to human serum IgA (alpha chain specific). The Fab and Fc fragments indicated at wells 4 and 7 are also seen at wells 8 and 9. The anode is to the right.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as follows. The microfuge supernatant fluid (30 μl) was mixed with 30 μl of buffer containing 1% SDS, 5% 2-mercaptoethanol, 1 mM EDTA, and 10 mM Tris-hydrochloride (pH 8.0), and the mixtures were boiled for 2 min. Electrophoresis of the reduced samples was performed on Pharmacia PAA 4/30 gradient gels (Pharmacia Fine Chemicals, Piscataway, N.J.) with electrophoresis buffer containing 0.04 M Tris-hydrochloride, 0.02 M sodium acetate, 2 mM EDTA, and 0.2% SDS (pH 7.4), and gels were stained with Coomassie brilliant blue R-250. Molecular weights (MW) of the immunoglobulin fragments were calculated as described by Weber and Osborn (30) with the following proteins as standards: phosphorylase b (MW, 94,000), albumin (MW, 67,000), ovalbumin (MW, 43,000), carbonic anhydrase (MW, 30,000), trypsin inhibitor (MW, 20,100), and alpha-lactalbumin (MW, 14,400) (Pharmacia).

RESULTS

IgA1 protease activity of human ureaplasmas and mycoplasmas. Twenty-eight strains of *U. urealyticum* isolated from the urogenital tract of patients were examined for IgA protease activity (Table 1). Each of the 28 strains exhibited IgA1 protease activity as determined by immunoelectrophoresis and SDS-PAGE procedures. Figure 1 illustrates the typical immunoelectrophoretic pattern of human myeloma IgA1 incubated with *U. urealyticum* (wells 4, 7, and 8). The immunoelectrophoretic pattern of the cleavage products was indistinguishable from that obtained with *N. meningitidis* strain M986, included as a positive control (well 9). The digested IgA showed two bands with different mobilities, determined to be Fab and Fc fragments by
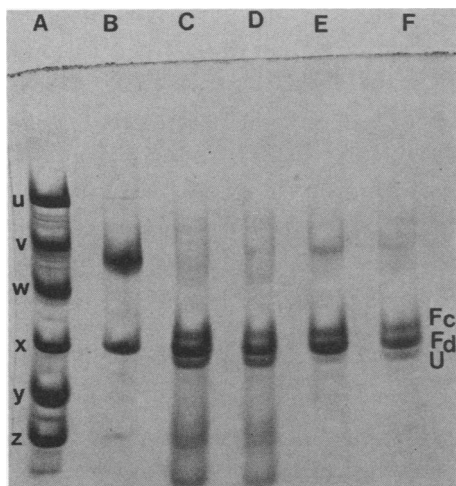
FIG. 2. SDS-PAGE pattern (reducing conditions) of human myeloma IgA incubated with suspensions of *U. urealyticum*. Lane A contains the following MW standards: u, phosphorylase *b* (94,000); v, albumin (67,000); w, ovalbumin (43,000); x, carbonic anhydrase (30,000); y, trypsin inhibitor (20,100); z, alpha-lactalbumin (14,400). Lanes: B, PBS; C, strain Ford 7 (serovar 1); D, strain Ford 23 (serovar 2); E, strain Ford 27 (serovar 3); F, strain Ford 58 (serovar 4). Abbreviations: Fc, Fc fragment; Fd, Fd fragment; U, ureaplasma protein.

reactivity with anti-light chain and anti-heavy chain (anti-alpha) antisera, respectively (data not shown). IgA1 incubated with PBS was not digested (wells 5 and 6), and *U. urealyticum* did not cleave murine IgA (well 2).

The SDS-PAGE pattern of human myeloma IgA incubated with suspensions of *U. urealyticum* is presented in Fig. 2. Most of the heavy chain was digested, and two bands of lower MW appeared instead. One band was about 33,000 MW, and the other band was 28,000 to 28,500 MW. This lower-MW band was not discernible from the light chain of the IgA due to the similarity in MW of the two components; a similar phenomenon has been reported by Male (15). The use of nongradient gels did not improve the resolution of the low MW cleavage fragment from the light chain. Other protein bands are derived from the ureaplasmas, as indicated by control incubations with ureaplasmas alone (See Fig. 4).

The capacity of the human *U. urealyticum* serovars to cleave non-human IgA was also examined. *U. urealyticum* strains Ford 7, Black V, and Vancouver were incubated with murine IgA, strains Ford 7 and Black I were incubated with porcine IgA, and strain Black II was incubated with canine IgA. However, none of these strains cleaved the nonhuman IgA. Thus, the human ureaplasma IgA proteases also demonstrate the marked host substrate specificity noted for the bacterial enzymes (14).

In addition to the strains of *U. urealyticum* tested, 14 strains of *M. hominis*, 7 strains of *M. pneumoniae*, and 1 strain of *M. genitalium* were screened for IgA protease activity. However, no cleavage of human IgA1 by *M. hominis* (Fig. 1, wells 3 and 10) or any of the other strains of human mycoplasmas was detected.

IgA protease activity of animal mycoplasmas and ureaplasmas. The animal ureaplasma and mycoplasma strains listed in Table 2 were examined for IgA protease activity with human IgA1 as the substrate. None of the animal mycoplasma strains tested was able to cleave human IgA. Moreover, each of the bovine, feline, canine, and avian

ureaplasma strains tested failed to cleave the human myeloma IgA. These results show that only human ureaplasmas were capable of cleaving human IgA immunoglobulins.

We examined the IgA protease activity in a variety of animal mycoplasmas by using the appropriate host IgA as the substrate. The murine pathogens *M. pulmonis* and *M. arthritidis* did not cleave mouse IgA. *M. hyopneumoniae*, the causative agent of enzootic pneumonia of pigs, did not cleave porcine secretory IgA. *M. mycoides* subsp. *mycoides*, the cause of contagious bovine pleuropneumonia, and *M. mycoides* subsp. *capri*, which causes pneumonia in goats, did not show IgA protease activity when examined with bovine IgA. Thus, none of the mycoplasmas examined exhibited protease activity, even with IgA from the host of origin.

When canine myeloma IgA was incubated with canine ureaplasma strain D1M-C, an additional band was obtained with immunoelectrophoresis (Fig. 3, well 4). Essentially identical results were obtained when canine IgA was incubated with strain D11N-A (data not shown). However, the canine IgA was not digested by *U. urealyticum* strain Black II, nor was human IgA1 cleaved by canine ureaplasma strains. Murine IgA was not digested by *U. diversum* strain A417, canine ureaplasma strains D1M-C and D11N-A, feline ureaplasma strains F45d-2 and F2, or avian ureaplasma strain T9-1. As reported above, murine IgA and porcine IgA were not digested by *U. urealyticum* strains. These results demonstrate a strict substrate specificity for the ureaplasma IgA proteases.

Analysis of the reaction products of the canine IgA after cleavage with canine ureaplasma strains D1M-C and D11N-A was carried out with SDS-PAGE (Fig. 4). The canine myeloma IgA incubated with PBS showed heavy and light chains of 58,000 and 27,500 MW, respectively (lane 2). There appeared to be four cleavage products of 36,000, 33,000, 31,500, and 25,500 MW when the canine IgA was incubated with canine ureaplasmas (lanes 4 and 6). Figure 4 also illustrates that *U. urealyticum* from human host does not cleave canine myeloma IgA (lane 7) or human IgA2 (lane 12).
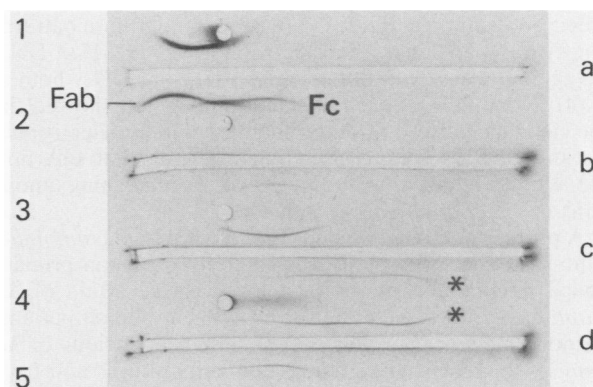

FIG. 3. Immunoelectrophoretic pattern of canine and human IgA incubated with suspensions of *U. urealyticum* and canine ureaplasmas. Human myeloma IgA1 was incubated with (well 1) PBS or (well 2) *U. urealyticum* strain Black II. Canine myeloma IgA was incubated with (well 3) *U. urealyticum* strain Black II, (well 4) canine ureaplasma strain D1M-C, or (well 5) PBS. Trough a contained rabbit antiserum to human kappa light chain plus goat antiserum to human serum IgA (alpha chain specific); troughs b, c, and d contained rabbit antiserum to canine IgA. The asterisk indicates the canine IgA cleavage fragment. The anode is to the right.

As a positive control, cleavage of human IgA1 by *U. urealyticum* is shown in lane 10 to identify IgA cleavage products.

DISCUSSION

Because IgA immunoglobulins have been associated with resistance to disease, this study was designed to determine whether selected human and animal mycoplasma pathogens contain specific IgA protease activity. The study has addressed essentially two questions concerning the IgA proteases of mycoplasmas: which mycoplasmas and ureaplasmas have the capacity to produce the IgA proteases, and what is the substrate specificity of these enzymes as related to host specificity of the pathogens? The results suggest that ureaplasmas from humans and dogs can cleave the IgA of the host of origin, but not nonhost IgA. No activity was found in any of the mycoplasmas tested, even with the host-specific IgA.

IgA protease activity was found in each of the 28 strains of human *U. urealyticum* examined with human IgA as the substrate. These results confirm the reports by other investigators that *U. urealyticum* produces IgA protease (10, 23; Opoku-Agyeman et al., Abstr. Annu. Meet. Am. Soc. Microbiol. G19, p. 99, 1983). The cleavage products were 28,000 to 28,500 MW and 33,000 MW, which correspond to the Fd and Fc fragments, respectively. These MW differ only slightly from the fragment MW of 29,000 and 33,500 reported by Kilian et al. (10). Thus, the bond cleaved by the *U. urealyticum* protease is likely between residues 235 and 236, which is cleaved by *H. influenzae* type B (13) and by *N. meningitidis* enzyme type 2 (14), or between residues 237 and 238, which occurs with *N. meningitidis* enzyme type 1 (14). Confirmation of this site of the protease-induced cleavage will require amino acid sequencing of purified Fc fragments.

U. urealyticum has been associated with urogenital tract infections of men and women, low-birth-weight infants, and respiratory disease in neonates (8, 21, 26). Although the role of specific serovars of *U. urealyticum* in disease processes has not been established, the serovars of *U. urealyticum* show marked phenotypic and genotypic heterogeneity based on their antigenic reactivities (25), protein migration patterns upon two-dimensional gel electrophoresis (9, 17), DNA cleavage patterns by endonucleases (22), and DNA homology (4). Nonetheless, each of the strains tested showed the same cleavage pattern as determined by immunoelectrophoresis and SDS-PAGE. The findings indicate that IgA protease activity is not a useful means of distinguishing among strains of *U. urealyticum*.

IgA protease activity was not found with *M. pneumoniae*, including low-passage clinical isolates from human primary atypical pneumonia, nor with a low-passage strain of *M. genitalium*, a newly identified species from human patients with nongonococcal urethritis (28). Fourteen strains of *M. hominis*, isolated from contaminated cell cultures and from blood, urogenital tract, and septic arthritic joint fluids of human patients, failed to show IgA protease activity. These results suggest that these human mycoplasmas do not produce IgA protease, although we can not rule out the possibility that the conditions of growth or assay did not allow expression of the enzyme. Other investigators have reported that *Mycoplasma* and *Acholeplasma* species fail to show IgA protease activity (10, 12, 23). None of the animal mycoplasmas examined for protease activity cleaved human IgA. Moreover, a number of animal respiratory pathogens (*M. mycoides* subsp. *mycoides*, *M. pulmonis*, *M. arthritidis*,
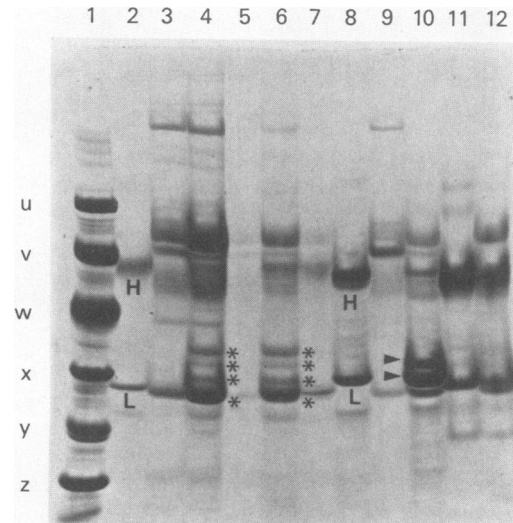

FIG. 4. SDS-PAGE pattern (reducing conditions) of canine and human IgA incubated with suspensions of canine ureaplasmas and *U. urealyticum*. Lanes: 1, MW standards (designations are the same as in Fig. 2); 2, canine myeloma IgA plus PBS; 3, canine ureaplasma strain D1M-C alone; 4, canine myeloma IgA plus canine ureaplasma strain D1M-C; 5, canine ureaplasma D11N-A alone; 6, canine myeloma IgA plus canine ureaplasma strain D11N-A; 7, canine myeloma IgA plus human *U. urealyticum* strain Black II; 8, human myeloma IgA1 plus PBS; 9, *U. urealyticum* strain Black II alone; 10, human myeloma IgA1 plus *U. urealyticum* strain Black II; 11, human myeloma IgA2 plus PBS; 12, human myeloma IgA2 plus *U. urealyticum* Black I. Heavy and light chains are indicated by H and L, respectively, beneath the bands. The canine IgA cleavage products are designated by asterisks, and the human IgA cleavage products are designated by arrow points.

and *M. hyopneumoniae*) also failed to cleave host-specific IgA. However, murine myeloma IgA is reported to consist primarily of the IgA2 isotype, which is known to be resistant to enzymatic cleavage (27).

Of special interest is the finding that ureaplasma strains isolated from dogs could cleave canine myeloma IgA, but not human or murine IgA. Cleavage was detected by an elongated immunoelectrophoresis pattern (Fig. 3, well 4) and by the appearance of additional bands on SDS-PAGE (MW of 36,000, 33,000, 31,500, and 25,500; Fig. 4, lanes 4 and 6). Thus, the canine ureaplasmas may express two enzymes that cleave at two different sites, as described for some strains of *H. influenzae* (13). Further verification of the IgA protease activity could be performed with ^{125}I-labeled canine IgA (23) or by isolating and characterizing the cleavage products. Alternatively, the use of antisera specific for the Fab and Fc fragments of canine IgA should further differentiate the cleavage fragments.

The fact that the IgA protease specificity of human serovars of *U. urealyticum* differs from that of ureaplasma obtained from canine origin provides additional support, indicating that the ureaplasmas from different hosts each represent distinct species (7a, 14a; E. B. Stephens, K. Koshimizu, R. Harasawa, and M. F. Barile, unpublished results). It has already been established that the bovine ureaplasmas (*U. diversum*) are quite distinct by serological procedures from the human *U. urealyticum*. If IgA proteases are also produced by ureaplasmas of other animal hosts and if these enzymes exhibit a similarly restricted substrate specificity for the IgA of the host of origin, such findings could indicate that these enzymes play a role in the selective host specificity of

certain mucosal pathogens. We attempted to examine the IgA protease activity of bovine ureaplasmas, but the results were inconclusive and require further study. We were unable to investigate the enzymatic activity of the feline and avian ureaplasmas against their homologous IgA because we were unable to obtain these reagents. Such studies have merit and should be conducted. Recently, Kilian and Freund (10a) have reported that the A417 strain of *U. diversum* could degrade bovine and human IgA, although release of intact Fab and Fc fragments was not observed.

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