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Nucleotide Sequence Homology Between the Immunoglobulin A1 Protease Genes of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Haemophilus influenzae*

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Isolated DNA fragments encoding the immunoglobulin A1 (IgA1) protease of *Neisseria gonorrhoeae* were used as hybridization probes to search for homologous sequences in whole cell DNA from *Neisseria meningitidis* and *Haemophilus influenzae*. Significant homology was detected. That the detected homology represented IgA1 protease-specific sequences was confirmed by the cloning of these sequences in *Escherichia coli* HB101 and demonstrating the expression of IgA1 protease by these transformed cells. Molecular probing of commensal *Neisseria* and *Haemophilus* species, which do not elaborate IgA1 protease activity, revealed that they were devoid of sequence homology with the cloned IgA1 protease gene DNA.

The human mucosal surface is the major portal of entry of microbial pathogens and represents a critical first point of microbe-host interaction. Immunoglobin A (IgA) in several forms represents the major component of specific immunity at mucosal sites. Certain bacteria that are etiological agents of human infectious disease have been shown to elaborate a class of extracellular proteases that specifically cleaves the heavy chain of human IgA1 (16). Such organisms include virtually all isolates of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* (13, 17, 21, 25). Nonpathogenic members of these genera are uniformly IgA1 protease negative (13, 17, 21, 22). Some oral bacteria implicated in periodontal disease, dental plaque formation, and occasional systemic disease also produce an IgA protease (8, 24). The correlation of IgA1 protease activity with pathogenic potential, their striking substrate specificity, and their enzymatic destruction of the functional integrity of a major class of human immunoglobulin (27) suggest that these enzymes may be important determinants of microbial virulence.

All IgA1 proteases have a number of common properties in addition to their unique substrate specificity. These properties include their extracellular nature (12, 25), the type of peptidyl bond hydrolyzed (12, 25), and their molecular mass (16). Biochemical examination of these endopeptidases from various microorganisms has revealed differences in their isolelectric point (26), the precise site of proteolytic cleavage within the IgA1 alpha-1 heavy immunoglobulin chain (12, 23, 25), and their inhibition by metal chelators (12). At present, insufficient amounts of purified enzyme have hindered attempts at a comparative analysis of their primary structure.

In an attempt to elucidate the biological significance of IgA1 protease activity in the pathogenesis of gonorrhea, we cloned the gene encoding gonococcal protease activity in *Escherichia coli* K12 and used this cloned gene to construct mutants of *N. gonorrhoeae* that were protease negative (15). The investigation reported here was undertaken to examine the genetic relatedness of gonococcal IgA1 protease-specific genes with those possessed by *N. meningitidis* and *H. influenzae*. Hybridization experiments were performed with the cloned gonococcal IgA1 protease gene as a molecular probe to search for sequence homology with DNA from these bacteria. Similarly, we addressed the genetic basis for the absence of these endopeptidases in closely related commensal species.

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MATERIALS AND METHODS

Strains and culture conditions. The cloning vector pBR325 (5) and *E. coli* K12 strain HB101 (2) were used in all molecular cloning experiments. The derivation and molecular organization of pVD105 has been described previously (15). Strains of various *Neisseria* species were obtained from Joan Knapp, Neisseria Reference Laboratory, Seattle Public Health Hospital, Seattle, Wash. *H. influenzae* and *Haemophilus parainfluenzae* strains were obtained from Arnold Smith and Marilyn Roberts, Children's Orthopedic Hospital, Seattle. *Haemophilus somnus* and *Haemophilus pleuropneumoniae* isolates were kindly provided by Ernst Biberstein, Department of Veterinary Microbiology, University of California at Davis. All strains of *Neisseria* were grown on GC agar base plus 1% IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.); *Haemophilus* spp. were grown on chocolate agar prepared from GC agar base plus 5% sheep blood cells.

Enzymes. Restriction endonucleases and DNA ligase were obtained from New England Biolabs Inc., Boston, Mass. Calf intestinal alkaline phosphatase was the gift of T. St. John, Department of Pathology, Stanford University. Aminophenylthioether paper was prepared as described previously (29).

DNA isolation and manipulation. Chromosomal DNA from appropriate bacterial strains were prepared as described previously (15). Plasmid DNA was isolated by a previously published procedure (3). Electroelution of fragments of DNA from agarose gels was performed as reported previously (30). For molecular cloning experiments, all restriction endonuclease-digested pBR325 DNA was treated with calf alkaline phosphatase to prevent intramolecular ligation. Transformation of *E. coli* HB101 was done as detailed previously (6).

DNA hybridization. Purified restriction endonuclease-generated DNA fragments were nick translated (18) to a specific activity of 2×10^7 cpm/μg of DNA with ^{32}P-deoxynucleotides (New England Nuclear Corp., Boston, Mass.). Conditions for colony hybridization (10), agarose gel electrophore-

sis (15), and Southern blot hybridization with aminophenylthioether paper (15) have been reported previously. Stringent hybridization reactions (allowing 20% or less nucleotide sequence mismatching) were performed in 50% formamide–5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 37°C. Reduced stringency hybridization reactions (allowing 33% or less nucleotide sequence mismatching) were performed in 25% formamide–5× SSC at 37°C. Filters were washed in 5× SSC–0.1% sodium dodecyl sulfate at the appropriate temperature before autoradiography.

IgA1 protease assay. The IgA1 protease assay system employed sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography to monitor the structural integrity of ^{125}I-labeled myeloma immunoglobulin after incubation with the appropriate bacterial cell suspensions (15).

RESULTS

We have previously cloned the IgA1 protease gene from *N. gonorrhoeae* strain F62 and identified its gene product in *E. coli* as a 140,000-dalton polypeptide (15). A recombinant plasmid, pVD105, that expressed IgA1 protease activity in *E. coli* was mapped through the analysis of Tn*5* insertion sequences, deletion derivatives, and the examination of specific polypeptides synthesized in minicells (15; unpublished data). These results showed that the DNA sequences specific for IgA1 protease expression began some 1,000 base pairs to the left of a single *Ava*I site in the cloned insert of pVD105 (Fig. 1). The gene is transcribed leftward (relative to the orientation shown in Fig. 1) and ends just proximal to the leftward *Hin*dIII site.

We employed two restriction endonuclease-generated fragments from pVD105 as gonococcal IgA1 protease gene-specific probes for sequence homology experiments. One probe consisted of a 4.2-kilobase (kb) fragment derived by *Hin*dIII cleavage (Fig. 1). This DNA fragment contained the bulk of the IgA1 protease gene and lacked only the sequences encoding the amino terminus of the peptide. The other DNA probe consisted of a 2.7-kb fragment resulting from a *Hin*dIII-*Cla*I double enzyme digestion (Fig. 1). This DNA sequence was adjacent to the previously described 4.2-kb *Hin*dIII fragment and contained 400 to 500 base pairs specifying the amino terminus of the protease as well as 2.2 kb of DNA sequences known not to be related to IgA1 protease expression in *E. coli*.

IgA1 protease-specific sequences in members of the genus *Neisseria*. DNA colony hybridization provides a rapid means to determine whether a microorganism carries a particular DNA sequence of interest (10). We employed this method to learn which other members of the genus Neisseria harbored sequences homologous to the gonococcal IgA1 protease gene. Reference strains of *Neisseria* species, as well as gonococcal strain F62 from which the gene was cloned, were grown on nitrocellulose filters. Duplicate filters were then

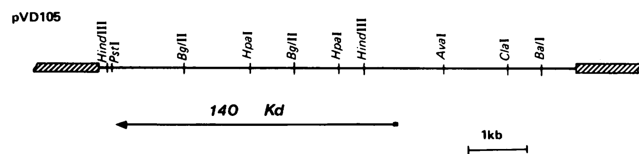

FIG. 1. Restriction map of pVD105 showing the position and orientation of the gonococcal IgA1 protease gene and the restriction fragments used for DNA homology experiments. The hatched areas represent pBR322 sequences.

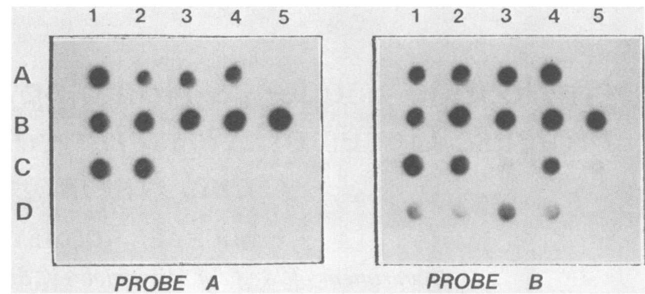

FIG. 2. Detection of DNA sequence homology with the gonococcal IgA1 protease gene by colony hybridization. Duplicate filters were prepared with *Neisseria* spp. strains and a negative control, *E. coli* K12 HB101. Spots: (A1) *N. gonorrhoeae* F62, (A2) *N. gonorrhoeae* MS11, (A3) *N. gonorrhoeae* 5766, (A4) *N. gonorrhoeae* 7155, (A5) *E. coli* HB101, (B1) *N. menigitidis* 16123 (serogroup A), (B2) *N. meningitidis* 15894 (serogroup B), (B3) *N. meningitidis* 15980 (serogroup C), (B4), *N. meningitidis* 16021 (serogroup E), (B5) *N. meningitidis* 16042 (serogroup Z), (C1) *N. meningitidis* 15916 (serogroup 29E), (C2) *N. meningitidis* 15902 (serogroup W-135), (C3) *N. flava* 30008, (C4) *N. lactamica* 30011, (C5) *N. perflava* 30015, (D1) *N. sicca* 30016, (D2) *N. subflava* 30017, (D3) *N. cinerea* 30003, (D4) *N. mucosa* 9998, (D5) *B. catarrhalis* 30018. Probe A is the 4.2-kb *Hin*dIII-generated fragment of pVD105. Probe B is the 2.7-kb *Hin*dIII-*Cla*I–derived fragment of pVD105. Hybridizations and washes were performed under high-stringency conditions.

hybridized under stringent conditions with the two radiolabeled IgA1 protease gene-specific DNA probes. Representative autoradiograms from these experiments are shown in Fig. 2. All strains of gonococci and meningococci tested showed sequence homology with the 4.2-kb *Hin*dIII probe. None of the commensal *Neisseria* spp. showed sequence homology with this probe, however. Results obtained with the 2.7-kb *Hin*dIII-*Cla*I DNA probe showed a clear-cut hybridization signal with all gonococci and meningococci as well as with the *Neisseria lactamica*, *Neisseria cinerea*, and *Neisseria sicca* strains. There was also a weak hybridization signal found with other species of *Neisseria* tested using the 2.7-kb *Hin*dIII-*Cla*I probe. This slight background hybridization was not detected with *E. coli* or *Branhamella catarrhalis* DNA, and we believe that this low-level homology represents some sequence within the 2.7-kb restriction fragment common to the *Neisseriaceae*. It thus appeared that only the IgA1 protease-producing *Neisseria* spp. possessed sequence homology with the 4.2-kb *Hin*dIII gene-specific probe. Although the *N. lactamica*, *N. cinerea*, and *N. sicca* strains possessed sequences homologous to either the amino terminus of the IgA1 protease gene or sequences immediately adjacent to this gene as evidenced by its reaction with the 2.7-kb *Hin*dIII-*Cla*I DNA probe, none of the other commensal *Neisseria* species tested possessed significantly homologous sequences to the IgA1 protease gene or to the vast majority of adjacent sequences.

We used Southern blot hybridization (31) to further investigate the homology of meningococcal DNA with the gonococcal IgA1 protease gene. *Hin*dIII and *Cla*I restriction endonuclease digests of chromosomal DNA from *N. gonorrhoeae* F62, *N. meningitidis* 16123 (serogroup A), and *N. meningitidis* 15894 (serogroup B) were electrophoretically separated and denatured, and the fragments were transferred to aminophenylthioether paper. The paper was then hybridized with the radiolabeled 4.2-kb *Hin*dIII fragment of pVD105, washed, and then autoradiographed. After removing the annealed 4.2-kb sequences by denaturation, the paper was rehybridized with the 2.7-kb *Hin*dIII-*Cla*I frag-

ment. Both hybridizations were carried out under conditions of high stringency (Fig. 3).

Both radiolabeled probes hybridized to the same 11-kb *ClaI* fragment of *N. gonorrhoeae* F62 (Fig. 3A and B, lanes 1). Similarly both DNA probes reacted with the same 14-kb fragment of *ClaI*-digested *N. meningitidis* 15894 DNA (Fig. 3A and B, lanes 3). In contrast, each DNA probe hybridized to a different fragment of *ClaI*-cleaved *N. meningitidis* 16123 chromosomal DNA (Fig. 3A and B, lanes 2); the 4.2-kb gonococcal sequences reannealed to a 12-kb chromosomal fragment (Fig. 3A, lane 2), whereas the 2.7-kb gonococcal sequence reacted with a 4-kb meningococcal chromosomal fragment (Fig. 3B, lane 2).

Hybridization of the two gonococcal probes to *HindIII*-cleaved chromosomal DNA digests from the three bacterial strains showed comparable results. Each probe detected a separate *HindIII*-cleaved *N. gonorrhoeae* F62 chromosomal fragment, as one would expect for the homologous strain from which the probes were derived (Fig. 3A and B, lanes 4). Distinct *N. meningitidis* 15894 DNA fragments were also detected with the two gonococcal probes; the 4.2-kb gonococcal fragment reacted with a 30-kb meningococcal chromosomal fragment (Fig. 3A, lane 6), whereas the other gonococcal probe hybridized with a 24-kb meningococcal chromosomal fragment (Fig. 3B, lane 6). The two gonococcal probes hybridized to the same 19-kb *HindIII* fragment of the *N. meningitidis* 16123 chromosomal digest (Fig. 3A and B, lanes 5).

These hybridization data show that meningococci and gonococci share homologous sequences in the IgA1 protease regions of their respective genomes. Moreover, the homologous sequences shared by the two meningococcal strains are likely contiguous since they could be found under appropriate hybridization conditions on a single restriction fragment (Fig. 3A and B, lanes 3; Fig. 3A and B, lanes 5). Nevertheless it is clear that the homologous sequences of *N. meningitidis* 16123 possessed a *ClaI* site, and not an *HindIII* site, defining the two regions hybridizing with the gonococcal 4.2-kb *HindIII* probe and the 2.7-kb *HindIII-ClaI* probe.

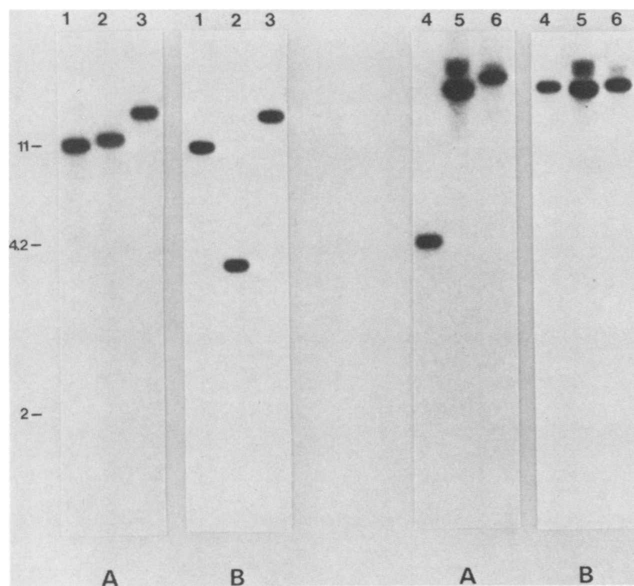

FIG. 3. Detection of meningococcal IgA1 protease genes by Southern hybridization. Autoradiograms shows the restriction fragments of chromosomal DNA which hybridized with the probes. Lanes: 1, *N. gonorrhoeae* F62, *ClaI*; 2, *N. meningitidis* 16123, *ClaI*; 3, *N. meningitidis* 15894, *ClaI*; 4, *N. gonorrhoeae* F62, *HindIII*; 5, *N. meningitidis* 16123, *HindIII*; 6, *N. meningitidis* 15894, *HindIII*. Filters were hybridized with the nick-translated 4.2-kb *HindIII* fragment of pVD105 (A) and then the 2.7-kb *HindIII-ClaI* fragment of pVD105 (B) under conditions of high stringency. The smears at the top of lanes 5 and 6 are the result of incomplete digestion. Sizes of restriction fragments (in kb) were determined by comparison with λ DNA cleaved with *EcoRI*, *HindIII*, and *SmaI*.

Molecular cloning of the IgA1 protease gene of *N. meningitidis* 15894. We wished to establish that the sequence homology detected between gonococcal and meningococcal DNA actually reflected the IgA1 protease genetic material. Chromosomal DNA of *N. meningitidis* 15894 was cleaved with *ClaI* and separated by electrophoresis in a preparative agarose gel, and the region of the gel containing 12- to 16-kb fragments as excised and isolated by electroelution. These fragments were ligated with appropriately treated pBR325 DNA and transformed into *E. coli* strain HB101. A clone containing the fragment of interest was identified by colony hybridization with the radiolabeled gonococcal probes. The plasmid DNA in this hybridization-positive clone, designated pVD113, was isolated and found to contain a 14-kb meningococcal chromosomal DNA fragment. This *E. coli* clone was then tested for IgA1 protease activity by using an ^{125}I-labeled myeloma protein–sodium dodecyl sulfate gel assay (15) and was found to elaborate a proteolytic activity identical to that produced by *N. meningitidis* 15894 (data not shown).

Figure 4 shows a restriction map of the cloned meningococcal chromosomal DNA fragment of pVD113 associated with IgA1 activity. The sequences directly associated with IgA1 protease activity were localized to some extent by construction of two deletion derivatives (Fig. 4). Plasmid pVD114 was created by *EcoRI* digestion of pVD113 DNA followed by intramolecular ligation. pVD114 was deleted for 3.5 kb of DNA from one end of the insert DNA, and *E. coli* transformants of this plasmid still exhibited IgA1 protease activity. Plasmid pVD115 was constructed by *HindIII* digestion of pVD113 followed by intramolecular ligation. pVD115 was deleted for 4 kb of DNA at one end of the inserted DNA, and its transformants no longer synthesized protease activity. The inability of pVD115 transformants to produce the protease was consistent with the Southern hybridization data, which showed that the *N. meningitidis* 15894 DNA sequences sharing homology with the gene probes could be found on two distinct *HindIII* fragments.

Sequence homology between the IgA1 protease genes of *N. gonorrhoeae* and *H. influenzae*. Southern blot hybridization was employed to determine whether there was sequence homology between the IgA1 protease genes of the *N. gonorrhoeae* and *H. influenzae*. Chromosomal DNAs from *N. gonorrhoeae* F62 and *H. influenzae* Rd$^-$/b$^+$, a genetically well characterized Rd$^-$ strain transformed to type b capsule production (1), were cleaved with appropriate restriction endonucleases, electrophoresed, denatured, and transferred to aminophenylthioether paper. The paper was then hybridized sequentially under conditions of high stringency with the two IgA1-specific gonococcal DNA fragments described earlier. Both of the gonococcal probes hybridized with the same restriction fragment of the homologous strain F62. Hybridization occurred with a 19-kb *EcoRI* fragment, an 11-kb *ClaI* fragment, and 30-kb *Pst* fragment (Fig. 5A and C, lanes 1, 3, and 5). There was no detectable homology between the gonococcal probes and *H. influenzae* DNA (Fig. 5A and C, lanes 2, 4 and 6).

At the conditions used for these hybridization experiments

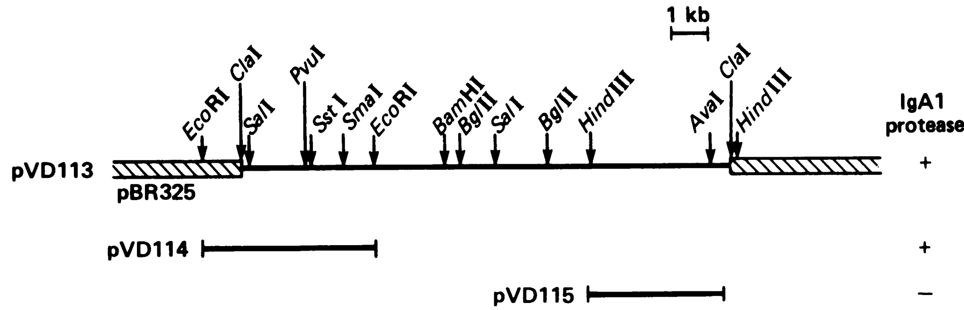

FIG. 4. Restriction maps of pVD113 and its derivatives pVD114 and pVD115. Solid bracketed lines denote regions deleted from pVD113. The IgA1 protease phenotype of strain HB101 bearing these plasmids is shown on the right.

only DNA-DNA duplexes with a high degree of sequence homology were expected to be stable. Previous investigations have established that highly stringent hybridization conditions may not be appropriate for examining the relationship of nucleotide sequences of heterogeneous origin. Although there is little data concerning the homology between the chromosomal DNA of *N. gonorrhoeae* and *H. influenzae*, the disparity in the overall guanine plus cytosine content of their DNA (50 and 39%) (7, 11) probably reflects a similar variation in overall nucleotide sequence for most genes. We therefore repeated the hybridizations with conditions of reduced stringency.

Under conditions of reduced stringency the 4.2-kb *Hin*dIII gonococcal probe with the same F62 DNA fragments as under conditions of higher stringency. In addition, a number of other gonococcal DNA fragments were detected which appeared to be of less intensity on the autoradiographs (Fig. 5B, lanes 1, 3, and 5). The 4.2-kb probe detected specific restriction fragments in *H. influenzae* DNA digests, a 7-kb *Eco*RI fragment, an 11-kb *Cla*I fragment, and two *Pst*I fragments of 8.4 kb and 4 kb (Fig. 5B, lanes 2, 4 and 6).

They hybridization results obtained with the radiolabeled 2.7-kb *Hin*dIII-*Cla*I probe under reduced conditions of stringency were quite similar (Fig. 5D). The appropriate gonococcal fragments were seen, as well as other gonococcal sequences of lowered intensity. The number of these extra hybridizing fragments appeared to be increased over that seen with the 4.2-kb gonococcal probe. In the *H. influenzae* digests nearly identical results to that seen with the 4.2-kb probe were seen. The same 7-kb *Eco*RI, 11-kb *Cla*I, and 8.4-kb *Pst*I fragments were present, although the 4-kb *Pst*I fragment was absent. The observation that the two gonococcal probes hybridized to the same *H. influenzae* restriction fragment implied that the homology was directed toward an

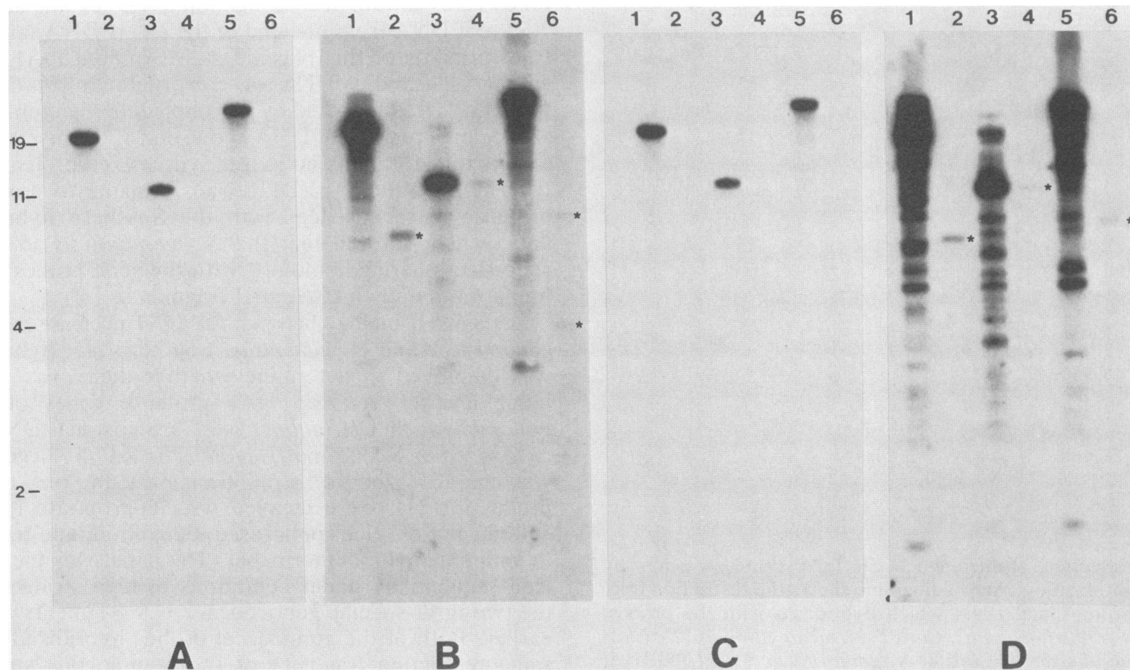

FIG. 5. Detection of the IgA1 protease gene of *H. influenza* Rd$^-$/b$^+$ by Southern hybridization. Autoradiograms show the restriction fragments of chromosomal DNA that hybridized with the probes. Lanes: 1, *N. gonorrhoeae* F62, *Eco*RI; 2, *H. influenza* Rd$^-$/b$^+$, *Eco*RI; 3, *N. gonorrheae* F62, *Cla*I; 4, *H. influenzae* Rd$^-$/b$^+$, *Cla*I; 5, *N. gonorrheae* F62, *Pst*I; 6, *H. influenzae* Rd$^-$/b$^+$, *Pst*I. The filter was hybridized sequentially with the 4.2-kb *Hin*dIII fragment under stringent conditions (A) and reduced-stringency conditions (B) and then with the 2.7-kb *Hin*dIII-*Cla*I fragment under stringent conditions (C) and reduced-stringency conditions (D). *H. influenzae* restriction fragments hybridizing with the DNA probes are indicated by asterisks. The sizes of the restriction fragments (in kb) were determined by comparison with λ DNA cleaved with *Eco*RI, *Hin*dIII, and *Sma*I.

IgA1 protease gene. However, the heterogeneous pattern seen within the gonococcal digests under these hybridization conditions raised some doubt as to the specificity of the DNA duplexes.

We addressed the nature of the observed homology between *H. influenzae* and the gonococcal IgA1 protease gene by cloning the 7-kb *Eco*RI *H. influenzae* DNA fragment that hybridized with the probes. Preparative gel electrophoresis of *Eco*RI-cleaved *H. influenzae* DNA and electroelution of DNA fragments in the 6 to 8-kb size range yielded a population of chromosomal sequences enriched for the 7-kb fragment. These fragments were ligated to *Eco*RI-cleaved pBR235 vector DNA and transformed into *E. coli* HB101. The clones were screened with a [^{125}I]IgA1 myeloma protein–sodium dodecyl sulfate-polyacrylamide gel assay to determine whether any of them exhibited IgA1 protease activity. Of 45 clones tested in this matter, 2 elaborated an endopeptidase activity indistinguishable in its action on IgA1 from the protease synthesized by *H. influenzae* Rd$^-$/b$^+$ strain from which the cloned DNA was derived (data not shown).

Restriction endonuclease digestion of the plasmid DNA in these two protease-positive clones showed they contained the same 7-kb *Eco*RI fragment and that this fragment comigrated with the *Eco*RI *H. influenzae* chromosomal fragment detected in the hybridization experiments. The plasmid containing this fragment and encoding the *H. influenzae* IgA1 protease was designated pVD116. A preliminary restriction endonuclease digestion map of this cloned DNA is shown in Fig. 6. Further localization of the IgA1 protease gene within the 7-kb insert was performed by deletion analysis. Plasmid pVD117 was prepared by *Cla*I cleavage and intramolecular ligation to yield a 2.2-kb deletion (Fig. 6). In a similar way, pVD118 was derived by *Pst*I cleavage to yield a 2.8-kb deletion. *E. coli* transformants for these two plasmids were prepared, and we observed that those clones receiving pVD117 showed protease activity, whereas *E. coli* harboring pVD118 were devoid of protease activity. These results were in accord with the hybridization data, which showed that both gonococcal gene probes hybridized to a single 11-kb *Cla*I *H. influenzae* chromosomal fragment, whereas two *Pst*I chromosomal fragments reacted with the probes. In the same vein, the fact that the 2.7-kb gonococcal *Cla*I-*Hin*dIII fragment (encoding the amino-terminal portion of the IgA1 protease) annealed to the 8.4-kb *Pst*I fragment, whereas the other probe that encoded the remaining part of the gonococcal IgA1 protease reacted with both the 8.4- and the 4-kb *H. influenzae Pst*I fragments, implied that the sequences specifying the amino terminus of the *H. influenzae* polypeptide were in the 8.4-kb piece and the remainder was in the 4-kb fragment. Superimposing this hybridization mapping data to the structure of pVD116 one can see that the amino-terminal sequences lie between the *Pst*I and *Cla*I sites and that the gene is transcribed from right to left as represented in Fig. 6.

The distribution of the IgA1 protease gene within members of the genus *Haemophilus* was determined by hybridization experiments with a ^{32}P-nick translated 7-kb *Eco*RI DNA fragment of pVD116 as a probe against various *Haemophilus* species (data not shown). Under high-stringency hybridization conditions, all strains of *H. influenzae* tested (five capsular type b strains, two type c strains, and three nontypable strains) reacted with the probe, whereas other *Haemophilus* species (three strains of *H. parainfluenzae*, two strains of *H. somnus*, and two strains of *H. pleuropneumoniae*) that do not elaborate an IgA1 protease activity failed to show DNA sequence homology. Kilian et al. have reported that strains of *H. pleuropneumoniae*, a porcine pathogen, produce an extracellular protease that cleaves porcine IgA (13). Two such strains were tested and were not observed to hybridize with the *H. influenzae* IgA1 protease-specific DNA probe. In addition, we have been unable to demonstrate a proteolytic activity for porcine IgA with these two strains.

DISCUSSION

Previous research has demonstrated that *N. gonorrhoeae* and *N. meningitidis* are genetically related, as evidenced by interspecific DNA transformation (32, 33) and a high level of shared nucleotide sequences (14). It was not surprising then that our studies showed that the IgA1 protease genes of gonococci and meningococci were homologous by DNA hybridization. Comparison of the cloned meningococcal IgA1 protease gene of pVD113 with the cloned gonococcal equivalent with restriction endonuclease cleavage has revealed a high level of shared genetic organization as well. However, the distribution of shared restriction endonuclease sites flanking the IgA1 protease structural gene appears to be less conserved between these two species (unpublished data). Moreover these shared sites are not seen in the IgA1 protease gene of *N. meningitidis* 16123. This strain possessed a *Cla*I site (rather than an *Hin*dIII site) defining two continuous regions identified by hybridization with DNA probes isolated from the cloned pVD105 gonococcal IgA1 protease. In this regard it is of interest that the IgA1 proteases of *N.* spp. can be differentiated into two groups based on the specificity of proteolytic cleavage within the hinge region of the alpha-1 heavy chain and that the type of protease elaborated correlates with certain meningococcal serogroups (23). Hence serogroup A meningococci produce type 1 enzyme, whereas most gonococci produce type 2 IgA protease (23). Moreover, type 1 and type 2 IgA protease can be distinguished antigenically on the basis of their relative inactivation by sera from patients recovering from menin-

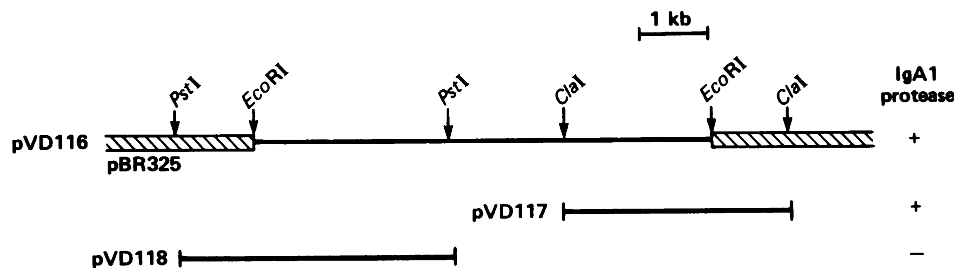

FIG. 6. Restriction map of the *H. influenzae* IgA1 protease clone pVD116 and its derivatives pVD117 and pVD118. Solid bracketed lines denote regions deleted from pVD116. The IgA1 protease phenotype of HB101 bearing these plasmids is shown on the right.

gococcal infection by either type 1 or type 2 protease-producing organisms (D. C. Stafford and A. G. Plaut, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, B125, p. 38). It is intriguing to speculate that the commonality evidenced by the protease genes of *N. gonorrhoeae* F62 and *N. meningitidis* 15894 are representative of type 2 IgA protease (23), whereas the distinctive restriction patterns of the gene in the serogroup A meningococcal strain 16123 are reflective of type 1 protease genes. The substantiation of this speculation requires more extensive analysis however.

Our initial attempts to show nucleotide homology between the IgA protease genes of *N. gonorrhoeae* and *H. influenzae* were not successful. These experiments were performed under conditions in which only closely homologous nucleotides (>78% nucleotide sequence homology) would be detected. We repeated the hybridization experiments with a reduced formamide concentration to permit the detection of less highly conserved homologous DNA sequences. These results showed clearly that the gonococcal IgA protease gene probes hybridized to specific restriction fragments of the *H. influenzae* genome. Our data do not permit a precise determination of the degree of sequence homology shown between the genes. However an approximate estimate of 67 to 75% homology can be made based on the conditions required to detect the homologous sequences by hybridization. The cloning of these sequences in *E. coli* and the concomitant expression of IgA protease by these clones confirmed that the homologous sequences carried the *H. influenzae* IgA protease genes. Hence, despite the apparent distant relatedness between *Haemophilus* spp. and *Neisseria* spp. as evidenced by their significantly different overall DNA composition, their related IgA protease genes presumably reflect a common origin. In this respect other gene families of bacteria, including the tryptophan operon of the *Enterobacteriaceae* (4), the heat-stable enterotoxins of *E. coli* (20), and the *E. coli* heat-labile enterotoxin and cholera toxin (19), all show divergence, and their relatedness can be shown only under reduced stringency of hybridization.

An unexpected finding stemming from the hybridization experiments performed under reduced stringency was the detection of a large number of gonococcal chromosomal genetic sequences that reacted with the labeled IgA protease-specific DNA probes. Identical results were seen in digests of meningococcal DNA. In addition, although strains of commensal *Neisseria* spp. failed to react with the gonococcal gene probes under conditions of stringent hybridization, under less stringent conditions similar cross-reacting sequences were readily observed. The patterns observed could not be explained by partial restriction endonuclease digestion or by extraneous restriction endonuclease activity. The fidelity of the detected DNA-DNA hybrids seems assured since the only *H. influenzae* DNA sequences reacting with the probes under conditions of reduced hybridization stringency were those that contained the analogous IgA protease gene. Moreover, Southern blot hybridization with a radiolabeled *H. influenzae* IgA protease gene probe under reduced stringency hybridization conditions revealed that, although the heterologous IgA protease-specific gonococcal DNA sequences could be detected, no broad cross-reactivity was seen in the gonococcal or *H. influenzae* chromosomal digests. Thus the "extra" homologous sequences were not related to the IgA protease gene itself, but appeared to be unique to *Neisseria* spp. Recently we observed that analogous broad reactivity can be seen under conditions of high stringency hybridization when the gonococcal probes were of a relatively small size (about 60 bases). We believe that these data can be best explained by the presence of a repetitive DNA sequence in the gonococcal gene probes. A candidate for this repetitive sequence is the putative DNA-specific uptake sequence found in gonococcal DNA (9). This sequence is presumably present on the cloned gonococcal IgA protease gene since the cloned genes can be transferred from *E. coli* into *N. gonorrhoeae* by transformation (15).

Most commensal or nonpathogenic species of the genera *Neisseria* and *Haemophilus* do not contain DNA sequences homologous to the IgA protease genes of pathogenic *Neisseria* spp. or *H. influenzae*. Thus these nonpathogenic organisms fail to produce the enzyme because they do not contain the genetic potential to do so rather than carrying an allelic counterpart that is "silent" or defective. Preliminary evidence suggests that the DNA homology detected in the *N. lactamica*, *N. cinerea*, and *N. sicca* strains with the 2.7-kb *Hin*dIII-*Cla*I DNA probe does not represent the presence of IgA1 protease gene sequences. The limited distribution of the IgA protease sequences is not restricted to the structural gene itself, but also to adjacent genetic sequences. Both the amino terminal-encoding IgA protease gonococcal gene probe and the *H. influenzae* gene probe contained DNA sequences not involved in protease expression. Yet *Haemophilus* spp. nonpathogenic for humans and commensal *Neisseria* spp. (with the possible exception of the *N. lactamica*, *N. cinerea*, and *N. sicca* strains) were devoid of these sequences as well. It appears therefore that in gonococci and *H. influenzae* there are DNA sequences linked to the IgA1 protease gene that are limited in their distribution only to pathogenic members of their respective genera.

The presence of homologous IgA1 protease genes in species of two distantly related genera raises the question of the origin of these enzymes. Convergent evolution is one possibility. One can also envision a mechanism whereby members of both genera obtained the protease gene from a common source, perhaps some other microorganism or even the human host. Alternatively the gene may have been passed between *Neisseria* spp. and *Haemophilus* spp. by genetic exchange followed by nonhomologous (illegitimate) recombination. In this latter regard, gonococci and *H. influenzae* have been shown, at least, to share common plasmid species (28). Whatever the mechanism, a kind of paradox exists because of the absence of the sequences from commensal *Neisseria* and *Haemophilus* species. If one assumes that the IgA protease genes were disseminated by intergeneric transfer, surely intraspecific genetic exchange would subsequently occur at even a higher frequency. In any event this investigation provides another striking example, like that of the *E. coli* heat-labile enterotoxin and cholera toxin, in which distantly related microorganisms share common genes and gene products. These virulence-associated gene families are not apparently restricted in their dissemination by normal taxonomic boundries, but rather are constrained by the presence of ancillary factors (adhesins, agressins, and other determinants) that are needed for the concerted expression of the virulent phenotype.

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