Quantitative Screening of Clinical Isolates for Immunoglobulin A Protease Production

LUTHER E. LINDLER AND FRED J. STUTZENBERGER*

Department of Microbiology, Clemson University, Clemson, South Carolina 29631

Received 3 September 1982/Accepted 14 April 1983

The production of immunoglobulin A (IgA) protease is a potentially useful marker in differentiating pathogenic from nonpathogenic species of clinical isolates; however, current quantitative assay methods are too tedious for routine application. A simple quantitative method was developed to screen clinical isolates for IgA protease production. This method is based on the specificity of reaction between IgA and alpha chain-specific antiserum in an immunochemistry analyzer (Beckman Instruments, Inc., Brea, Calif.). Colonies of IgA protease producers (*Streptococcus sanguis*, *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Haemophilus influenzae*) were picked from solid media, transferred to brain heart infusion containing IgA1, and incubated at 37°C for at least 2 h to provide a detectable decrease in IgA concentration. The standard deviation for randomly picked colonies within a species was about $\pm 15\%$. Several IgA protease-negative species caused no detectable reduction in the IgA content of the system. The specificity of the IgA measurement eliminates the requirements for extensive purification and radiolabeling of substrate and provides the basis for a well-defined IgA protease activity unit (micrograms of IgA1 cleaved per minute per milliliter of culture).

Immunoglobulin A (IgA) is the predominant immunoglobulin present on human mucosal surfaces; about 80% of all antibody-secreting plasma cells in mucosal tissues produce IgA (7). IgA has antibody activity against bacteria, viruses, toxins, and enzymes and provides host protection against infection by preventing adherence and colonization on mucosal surfaces (29, 31). The ratio of A1 to A2 idiotype-producing plasma cells of bronchial, salivary, and tonsillar tissue is about 2:1 (1). However, the A1 idiotype of both serum and secretory IgAs is specifically cleaved to intact Fab and Fc fragments by IgA proteases liberated from a variety of human pathogens. At present, this list includes *Streptococcus sanguis*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Bacteroides* species (6, 13, 16, 20, 21). Production of IgA protease has not been demonstrated in nonpathogens despite extensive screening (5, 7). Although IgA protease destroys the antibody function of IgA1 immunoglobulins (24), its role as a virulence factor has yet to be defined. Nevertheless, studies of these unique enzymes will provide information on the genetic interrelationships of various serotypes within species of clinically relevant pathogens (7). In this regard, correlations have already been observed between serotype and IgA cleavage patterns in *H. influenzae* (16) and *N. menin-*

gitidis (19). Furthermore, IgA protease production may be used as a characteristic distinguishing pathogenic from harmless clinical isolates of the *Neisseriaceae* (18). All such studies have been only qualitative, and no attempts have been made to compare the IgA protease activities of various clinical isolates. The major detriment to such studies has been the lack of a rapid quantitative screening method for IgA protease production. Methods currently in use require lengthy purification and radiolabeling of IgA substrate, followed by electrophoresis and autoradiography (22), measurement of decreases in binding of IgA to streptococcal cell walls (10), or a 3-day immunodiffusion (9). Recently, we published a rapid quantitative assay for kinetic studies on the protease of *S. sanguis*, based on the measurement of IgA concentrations with the Beckman immunochemistry analyzer (Beckman Instruments, Inc., Brea, Calif.) (11). We have now developed a rapid quantitative screening system for the IgA protease producers, using the ability of the analyzer to detect depletion of IgA1 in the culture medium during growth.

MATERIALS AND METHODS

Clinical isolates. Cultures of *H. influenzae*, *S. pneumoniae*, *Neisseria perflava*, *N. meningitidis*, *N. gonorrhoeae*, and *Branhamella catarrhalis* were obtained from Anderson Memorial Hospital (Anderson, S.C.)

and the Kansas City Health Department (Kansas City, Mo.). *S. sanguis* was ATCC strain 10556. All others used in this study came from the stock culture collection of the Department of Microbiology, Clemson University.

Preparation of IgA1 substrate. In the Beckman immunochemistry system (BIS), the specificity of the reaction (in this case, between alpha chains and alpha chain-specific antiserum supplied by Beckman) eliminated the necessity of using highly purified IgA1 as previously required (10, 22). In the present study, IgA1 from myeloma serum (generously donated by Jiri Mestecky, University of Alabama, Birmingham, and Richard Wistar, Naval Medical Research Institute, Bethesda, Md.) was precipitated by 60% saturation with ammonium sulfate, redissolved in 0.05 M phosphate buffer (pH 7.2) to a concentration of 10 mg/ml, and used without further purification.

Cultural conditions. Cultures were streaked for isolation on blood agar or chocolate agar and incubated for 24 h at 37°C in a 10% CO₂ atmosphere. After growth on the appropriate solid medium, individual colonies were transferred with a sterile loop to 1 ml of sterile brain heart infusion (BHI) containing filter-sterilized IgA1 (concentrations of 1 to 2 mg/ml as noted in Results) in 1.5-ml sterile Eppendorf tubes. Before inoculation, a zero-time sample (200 µl) was removed, mixed with an equal volume of 100 mM EDTA (pH 7.2), and assayed for initial IgA concentration in the BIS. After 4 to 8 h of incubation at 37°C, the remaining mixture was clarified by centrifugation. A sample (200 µl) was mixed with EDTA, and its IgA1 concentration was compared with that at zero time. The proteases of *Haemophilus* and *Neisseria* species (which are less EDTA sensitive than those of *Streptococcus* species) may be inactivated by heating samples at 56°C for 30 min if the IgA assay is not run immediately after sampling.

Measurement of IgA concentration in the BIS. The BIS measures the rate of complex formation between an antigen (in this case, IgA) and specific antibody (in this case, alpha chain-specific antiserum supplied in the Beckman immunoglobulin A reagent test kit). As the antigen-antibody reaction proceeds, the light-scattering ability of the mixture increases. To measure this increase, the BIS utilizes a tungsten-iodine lamp, focusing input optics, a detection optics system at a 70° forward angle, a photomultiplier tube detector, and a photodiode for sensing a trigger dye. Light in the wavelength range of 400 to 550 nm is used for scatter measurements, and longer wavelengths are used for the triggering. The scattered light signal is amplified, filtered to remove aberrations from bubbles or particulates, and sent to a microprocessor. The scatter signal is differentiated in the microprocessor to obtain a digitally filtered first-derivative signal. This nephelometric rate signal is used in a curve-fitting algorithm for the direct calculation of sample concentration based on the maximum rate signal (28).

In preliminary experiments to determine the usefulness of the BIS in the IgA protease detection system, we compared the observed reactivity of intact IgA1, with that of cleavage products resulting from attack by the IgA protease from *S. sanguis*. The IgA1 used in these experiments was purified to electrophoretic homogeneity from IgA1 myeloma serum by the method of Mestecky et al. (15). The IgA protease was partially

purified and concentrated by 65% saturation of cell-free culture fluid with ammonium sulfate. Samples were taken from reaction mixtures of protease and IgA1 (buffered to pH 7.0 with 0.05 M phosphate containing 5 mM MgCl₂). These were fractionated on a column (1.75 by 53 cm) of Sephadex G-200 which had been previously calibrated with proteins of the following molecular weights (in parentheses): RNase A (13,700), chymotrypsinogen A (25,000), ovalbumin (43,000), bovine serum albumin (67,000), and aldolase (158,000). The reactivity of each fraction with the commercial alpha chain-specific antiserum was determined in the BIS and compared with relative protein concentration (absorbance at 280 nm). The low reactivity of the cleavage products (as described in Results) allowed easy detection of IgA1 cleavage as the protease reaction proceeded. This ease of detection provided the basis for the colony-screening method for IgA protease described below.

The differences between IgA concentrations of samples taken before and after incubation in the colony-screening method were determined by mixing 42 µl of EDTA-sample mixture with an equal volume of the commercial antiserum and 0.6 ml of phosphate-buffered saline at pH 7.2 in the 1.5-ml analyzer cell. The rate of antigen-antibody complex formation was measured for only about 10 s. IgA depletion in the screening method was recorded as micrograms per minute per milliliter of culture.

In some cases it is necessary to separate effects on enzyme production from effects on rates of enzyme activity. In these cases, the isolate may be grown in the absence of the IgA in the medium, and samples of culture fluid, clarified by centrifugation, can be used as the enzyme source for a separate reaction mixture. Routine IgA protease reaction mixtures (200 µl) were prepared by mixing equal volumes of clarified culture fluid with a concentrated IgA1 stock solution to yield a final IgA concentration of 10 mg/ml. Mixtures were buffered with 0.05 M phosphate, pH 7.0, containing 5 mM MgCl₂. Samples (50 µl) were taken at zero time and after 4 h of incubation at 37°C. Each sample was mixed with an equal volume of EDTA as previously described. The IgA concentrations of the zero-time and 4-h samples were determined in the BIS, and units of IgA protease activity were expressed as micrograms of IgA cleaved per minute per milliliter of reaction mixture.

Other methods. Protein concentrations in pooled concentrated chromatography fractions were estimated by the dye-binding method of Bradford (3), with bovine gamma globulin (Bio-Rad Laboratories, Richmond, Calif.) as the standard. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed according to the method of Weber and Osborn (30). The molecular weight standards were lysozyme (14,400), soybean trypsin inhibitor (21,500), carbonic anhydrase (31,000), ovalbumin (45,000), bovine serum albumin (66,200), and phosphorylase B (92,500), all supplied by Bio-Rad.

RESULTS

Since the IgA proteases effect such limited cleavage of the IgA1 molecule by hydrolyzing only a single peptide bond in the hinge region, it was important to determine how much detect-

FIG. 1. (a) Elution patterns of IgA protease reaction mixture samples (partially purified *S. sanguis* protease with initial IgA1 concentration of 11.1 mg/ml) chromatographed on a Sephadex G-200 column buffered with 0.01 M phosphate (pH 7.0) containing 0.05% NaN_3 . The upper pattern is that of the zero-time sample; the lower pattern is that of a sample taken after 5 h at 37°C. The IgA protease in both samples was inactivated by addition of 30 mM EDTA and heating at 51°C for 30 min before application to the column. The void volume of the column was 19 ml. Symbols: absorbances (Abs.) at 280 nm (○); IgA concentration detected on the BIS (△) (b) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis pattern formed by concentrated cleavage products from fractions 35 to 45 in the lower elution pattern.

able antigenic reactivity the cleavage products retained in the BIS. We used the *S. sanguis* IgA protease in the reaction test system fractionated by gel permeation chromatography to separate cleavage products from intact substrate. The elution patterns shown in Fig. 1a are from zero-time and 5-h reaction mixture samples. The zero-time pattern shows the emergence of the intact IgA1 substrate directly after the void volume. There was good correlation between protein concentration and IgA1 detectable in the BIS. This main peak was followed much later by a small peak which probably contained protein contaminants from the partially purified IgA

protease. The elution pattern of the 5-h sample showed a marked decrease (about 40%) in the area of the intact IgA1 peak and the appearance of a new protein peak (estimated molecular weight, 58,000) which had little detectable reactivity in the BIS. To quantitate the extent of this decreased reactivity in the BIS, we concentrated fractions 35 to 45 by ultrafiltration on the Amicon YM10 membrane. After 10-fold concentration, the apparent IgA1 concentration per milligram of protein in the reaction products was only about 7% of that in the intact substrate when measured in the BIS. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis anal-

FIG. 2. Growth (optical density at 610 nm [OD₆₁₀], ○) and depletion of intact IgA1 (Δ) by *S. pneumoniae* after transfer of a single colony into the IgA protease screening system.

ysis of a sample from the peak after concentration revealed only two closely running bands (Fig. 1b) having estimated molecular weights of 26,800 and 31,200. These two bands probably represent light chains and heavy fragments, respectively, based on their apparent molecular weights (17). This large difference in reactivity between intact IgA1 and cleavage products, as detected during the brief measurement of initial complex formation in the BIS, provided a valid basis for the use of this system as a rapid, quantitative, and specific assay for IgA protease activity in clinical isolates.

Preliminary experiments on the growth of the more fastidious IgA protease producers (such as the *Haemophilus* and *Neisseria* species) revealed the need for additional growth factors in the BHI-IgA1 culture medium used in colony screening. We found that the addition of 1% supplement C (Difco Laboratories, Detroit, Mich.) fulfilled this need and supported adequate growth for detectable IgA protease production.

The depletion of intact IgA1 during the growth of IgA protease-producing cultures in the screening system was not proportional to incubation time. An example of the depletion time course is shown in Fig. 2. After transfer of a colony of *S. pneumoniae* into BHI containing 1.6 mg of IgA per ml, the depletion occurred more rapidly during early log phase and decreased gradually as the culture went into stationary phase. Similar results were obtained with the other IgA protease producers, and we found that little was to be gained by allowing

TABLE 1. IgA1 cleavage by 10 randomly picked colonies of *S. pneumoniae* and *N. gonorrhoeae* in the IgA protease screening system^a

Colonies of:	Cleavage (μg/ml per min)
<i>S. pneumoniae</i> ^b	0.56
	0.58
	0.50
	0.56
	0.79
	0.60
	0.69
	0.67
	0.64
	0.54
	0.61
	Avg
	SD
<i>N. gonorrhoeae</i> ^c	±0.08
	0.89
	0.68
	0.75
	0.98
	1.06
	0.83
	0.92
	0.65
	0.73
	0.99
	0.85
	SD
	±0.13

^a *S. pneumoniae* was streaked for isolation on two blood agar plates and incubated for 24 h at 37°C before picking colonies. *N. gonorrhoeae* was streaked for isolation on two chocolate agar plates and incubated for 24 h at 37°C in a 10% CO₂ atmosphere before picking colonies.

^b The IgA-containing medium was BHI.

^c The IgA-containing medium was BHI with 1% supplement C.

growth of our protease-producing isolates beyond 8 h. Incubations of 2 to 4 h were sufficient to provide detectable decreases.

The technique of transferring a single colony from a solid medium to the screening system raised the question of reproducibility. To test this point, we randomly picked isolated colonies from plates streaked with pure cultures of the IgA protease producers and measured the reduction in detectable IgA levels after 8 h. The variation between colonies was lower than expected. Examples are shown in Table 1 for 10 colonies picked from plates streaked with either *N. gonorrhoeae* or *S. pneumoniae*. The standard deviations calculated from these data were ±15 and ±13%, respectively, for *N. gonorrhoeae* and *S. pneumoniae*. None of the standard deviation values calculated for any of our IgA protease producers exceeded ±24%.

We found the following cultural conditions to be suitable for IgA protease production by all enzyme-positive species tested: incubation for 8

TABLE 2. Comparison of IgA protease production in various species, using a quantitative screening system

Organism	μg of IgA1 cleaved/ min per ml
<i>S. sanguis</i> (ATCC 10556)	0.90
<i>S. pneumoniae</i>	0.81
<i>N. gonorrhoeae</i>	0.69
<i>N. meningitidis</i>	1.00
<i>N. perflava</i>	0
<i>H. influenzae</i>	0.43
<i>E. coli</i>	0
<i>P. aeruginosa</i>	0
<i>S. pyrogenes</i>	0
<i>B. catarrhalis</i>	0

h at 37°C in BHI with 1% supplement C in a 10% CO₂ atmosphere. In this standardized system, we compared rates of IgA cleavage by some acknowledged IgA protease producers as well as some IgA protease-negative species (Table 2). The proven IgA protease producers cleaved the IgA at rates of 0.48 to 1.00 $\mu\text{g}/\text{minute}$ per ml of culture. *H. influenzae* was the least active; this may have been due to the relatively small diameter (about 0.4 mm) of the *H. influenzae* colonies after only 24 h of incubation. The protease-negative strains caused no detectable depletion of IgA concentration.

We also explored the alternative method, expressing differences in units of enzyme activity per milliliter of culture fluid. In such studies, IgA protease producers can be cultivated in the absence of IgA, since biosynthesis of this enzyme is constitutive (7). The activity in the spent culture fluid can then be measured under optimal conditions in a separate reaction mixture without regard for growth requirements of the organism being tested. An example of results obtained in this system is shown in Fig. 3. IgA protease activity of culture fluid of *S. sanguis* ATCC 10556 paralleled growth (measured as increase in optical density at 610 nm) in BHI at 37°C. This time course of IgA protease secretion is similar to that observed in earlier studies that used other methods for estimation of IgA protease activity (22, 23) and therefore substantiates the validity of the method.

DISCUSSION

The importance of microbial proteases as virulence factors has only been recently recognized. Protease production may be required for virulence in certain strains of *Vibrio cholerae* (26), *Pseudomonas aeruginosa* (8), and *Serratia marcescens* (12). The unique IgA proteases, secreted by a rather small group of pathogens, have attracted the most attention due to their specificity of cleavage sites (7, 21) and their

FIG. 3. Growth (optical density at 610 nm [OD₆₁₀, ○] and IgA protease production (Δ) after inoculation of a single colony of *S. sanguis* into BHI without IgA1.

potential usefulness as immunochemical tools in studies on IgA1 structure and function (25). There is ample circumstantial evidence (reviewed by Kornfeld and Plaut [7]) for a role of IgA proteases as virulence factors, but a full definition of that role must await a better understanding of the host-protective function served by IgA. In the meantime, there is need for further studies on all of the IgA proteases, since little is known about genetic control of biosynthesis, chemical characterization, or their validity as markers in clinical evaluations of pathogenicity. If the problems of the IgA protease assay can be resolved, progress in these studies will be greatly facilitated (7). Various assays for either detection or measurement have been described in the literature; for routine assay, they are too slow (as in the case of the 3-day radial immunodiffusion [9]) or too complicated and tedious in that they require extensive purification of substrate, followed by radiolabeling with ¹²⁵I, electrophoresis (22), autoradiography (17), or measurement of reduction in intact IgA1 binding to streptococcal cell walls (10). For routine studies in clinical labs which do not have facilities for extensive purification of proteins, such methods have little usefulness. In the present study, we have developed a method for the screening of clinical isolates for IgA protease activity which has several advantages over previously used methods: (i) its specificity for IgA eliminates the need for extensive purification of substrate; (ii) the requirement for radiolabeling of substrate (and the precautions in use and disposal) are eliminated; (iii) the incubation, measurement of IgA depletion, and calculation of results are expressed in well-defined absolute terms of IgA1

cleavage and may be obtained in a single working day for a number of isolates; (iv) the method utilizes the BIS, which is widely used for quantitation of a variety of substances in clinical samples.

The decrease in reactivity of the cleavage products compared with the intact IgA1 when measured with the alpha chain-specific antiserum in the BIS is more than what is predicted by a decrease in the mass of the primary antigen-antibody complex. As an antigen-antibody reaction proceeds, eventually resulting in lattice formation to the point of insolubility, the turbidity of the mixture increases. When a beam of light is directed through the reaction mixture, the ratio of scattered light (I_θ , detected at an angle θ) to incident light (I_0) is equal to HNm^2 , where H is a constant, N is the molar concentration of molecules in the reaction mixture, and m is the average molecular weight of the complex (4). The rate of scattering increase (R) as antibody reacts with antigen is obtained as dI_θ/dt (I_θ/I_0). Marrack and Richards (14) found that the aggregation of antigen-antibody complexes was accelerated by change in the structure of the antibody molecules induced by combination with antigen. This change depends on the size of the antigen; rates of aggregation are slow with small antigens and more rapid with large ones. It would then follow that if an antigen is cleaved into fragments, the rate of light-scattering increase during their reaction with specific antibody would be less than the rate observed in reaction of antibody with intact antigen, even if all antigenic determinants were conserved on the fragments. Such a situation would exist in the case of IgA cleavage by the IgA proteases to yield intact Fab and Fc fragments. Even if all of the antigenic determinants survive the single-site cleavage, the rate of scattering increase would be less when antibody reacts with immunoglobulin fragments compared with that reaction with comparable concentrations of intact molecules.

The presence of a mixture of antigenic fragments in such a reaction presents obvious difficulties in analysis. However, if we assume that all antigenic determinants of the IgA molecules are contained in the Fc portion (27) and confine the discussion to the simplest complex (one antibody and one antigen), then the following calculation is appropriate. Assuming that the molecular weight of an intact IgA1 molecule is 160,000 and that the Fc fragment after cleavage is 53,000 molecular weight, then the complex of one intact IgA molecule with specific antibody would be 320,000 molecular weight, and the complex of one Fc fragment with another molecule of that specific antibody would be 213,000. The rate of scatter increase with the Fc fragment

(R_c) divided by the rate for the intact IgA1 (R_i) is expressed as: $R_c/R_i = [(dI_\theta/dt)N_c m_c^2] / [(dI_\theta/dt)N_i m_i^2] = 0.44 \{[(dI_\theta/dt)N_c] / [(dI_\theta/dt)N_i]\}$. Therefore, the rate of scattering increase observed with the Fc fragment should theoretically be 44% of that observed with the intact IgA1 molecule. In practice, we observed it to be only 7%. This difference may be explained by studies such as that of Atassi and Singhal (2) which showed that fragments released from molecules after proteolytic cleavage assumed configurations greatly altered in both secondary and tertiary structure. Therefore, the changes of reactivity observed in the BIS may be the result from a combination of molecular weight reduction and reduced affinity of the Fc fragment for the alpha chain-specific antibody.

Although we have compared the extent of IgA1 cleavage for several of the IgA protease producers, some caution should be exercised in drawing conclusions about the relative rates of biosynthesis of this enzyme. Since IgA protease levels in culture fluids parallel cell number during logarithmic growth, growth rates in a screening system will determine the enzymatic performance of a given species. Therefore, it would seem appropriate to recommend that if comparisons between strains are to be done routinely, they should be expressed as IgA protease units per milligram of cells or culture optical density unit. This might be particularly important if short incubation times (2 to 4 h) are used. For example, in this study, colonies of *H. influenzae* after 24 h of incubation on chocolate agar, reached a diameter only about half that of *S. pneumoniae*. Picking a colony of each would probably result in a heavier *S. pneumoniae* inoculum. The use of a different medium such as Levinthal's enrichment agar (32) or incubating the plates with *H. influenzae* for 48 rather than 24 h might provide colonies of more comparable size for transfer into the IgA-containing incubation medium.

At present, there is no apparent explanation as to why rates of IgA cleavage decrease as the cultures age (such as shown in Fig. 2). Despite the fact that IgA protease secretion only occurs during cell proliferation, maximum rates of cleavage should occur during early stationary phase when extracellular enzyme levels are at their peak. Reasons for this decrease may include the following: gradual denaturation of the enzyme in the extracellular fluid, inactivation by other extracellular proteases, depletion of available substrate, or inhibition of enzyme activity by IgA1 cleavage products. The influence of all of these factors could be minimized by shortening the incubation time to about 4 h.

In summary, we have developed a simple quantitative screening system for IgA protease-

producing isolates which is suitable for clinical labs having access to an immunochemistry analyzer. The use of this system should facilitate attempts to correlate pathogenicity with IgA protease production in clinical isolates as well as serve as a useful tool in a variety of genetic studies.

ACKNOWLEDGMENTS

We thank Eugene Baille and Brenda Sexton of the Department of Pathology, Anderson Memorial Hospital, Anderson, S.C., for valuable assistance during this study.

LITERATURE CITED

- Andre, C., F. Andre, and M. C. Fargier. 1978. Distribution of IgA1 and IgA2 plasma cells in various normal human tissues and in the jejunum of plasma IgA-deficient patients. *Clin. Exp. Immunol.* 33:327-331.
- Atassi, M. Z., and R. P. Singhal. 1970. Conformational studies on modified proteins and peptides. *J. Biol. Chem.* 245:5122-5128.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248-254.
- Bull, H. B. 1971. An introduction to physical chemistry, 2 ed., pp. 197-199. F. A. Davis Co., Philadelphia.
- Genco, R. J., A. G. Plaut, and R. C. Moellering, Jr. 1975. Evaluation of human oral organisms and pathogenic *Streptococcus* for production of IgA protease. *J. Infect. Dis.* 131:S17-S21.
- Kilian, M. 1981. Degradation of immunoglobulins A1, A2, and G by suspected principle periodontal pathogens. *Infect. Immun.* 34:757-765.
- Kornfeld, S. J., and A. G. Plaut. 1981. Secretory immunity and the bacterial IgA proteases. *Rev. Infect. Dis.* 3:521-534.
- Kreger, A. S., and L. D. Gray. 1978. Purification of *Pseudomonas aeruginosa* protease and microscopic characterization of pseudomonal protease-induced rabbit corneal damage. *Infect. Immun.* 19:630-648.
- Labib, R. S., N. J. Calvanico, and T. B. Tomasi, Jr. 1978. Studies on extracellular proteases of *Streptococcus sanguis*: purification and characterization of a human IgA₁ specific protease. *Biochim. Biophys. Acta* 526:547-559.
- Lindahl, L., C. Schalen, and P. Christensen. 1981. New method that uses binding of Immunoglobulin A to Group A streptococcal Immunoglobulin A Fc receptors for demonstration of microbial Immunoglobulin A protease activity. *J. Clin. Chem.* 13:991-993.
- Lindler, L. E., and F. J. Stutzenberger. 1982. Nephelometric assay for the immunoglobulin A1-protease produced by the oral bacterium *Streptococcus sanguis*. *Arch. Oral Biol.* 27:853-859.
- Lyerly, D., and A. S. Kreger. 1979. Purification and characterization of a *Serratia marcescens* metalloprotease. *Infect. Immun.* 24:411-421.
- Male, C. J. 1979. Immunoglobulin A protease by *Haemophilus influenzae* and *Streptococcus pneumoniae*. *Infect. Immun.* 26:254-261.
- Marrack, J. R., and C. B. Richards. 1971. Light scattering studies of the formation of aggregates in mixtures of antigen and antibody. *Immunology* 20:1019-1040.
- Mestecky, J., W. J. Hammack, R. Kulhavy, G. P. Wright, and M. Tomana. 1977. Properties of IgA myeloma proteins isolated from sera of patients with the hyperviscosity syndrome. *J. Lab. Clin. Med.* 89:919-927.
- Mulks, M. H., S. J. Kornfeld, M. E. Lamm, and A. G. Plaut. 1980. Immunoglobulin A protease specificity among *Haemophilus influenzae* serotypes. *Clin. Res.* 28:513A.
- Mulks, M. H., S. J. Kornfeld, and A. G. Plaut. 1980. Specific proteolysis of human IgA by *Streptococcus pneumoniae* and *Haemophilus influenzae*. *J. Infect. Dis.* 141:450-456.
- Mulks, M. H., and A. G. Plaut. 1978. IgA protease production as a characteristic distinguishing pathogenic from harmless *Neisseriaceae*. *N. Engl. J. Med.* 299:973-976.
- Mulks, M. H., A. G. Plaut, H. A. Feldman, and B. Frangione. 1980. IgA proteases of two distinct specificities are released by *Neisseria meningitidis*. *J. Exp. Med.* 152:1442-1447.
- Plaut, A. G., R. J. Genco, and T. B. Tomasi, Jr. 1974. Isolation of an enzyme from *Streptococcus sanguis* which specifically cleaves IgA. *J. Immunol.* 113:289-291.
- Plaut, A. G., J. V. Gilbert, M. S. Artenstein, and J. D. Capra. 1975. *Neisseria gonorrhoeae* and *Neisseria meningitidis*: extracellular enzyme cleaves human immunoglobulin A. *Science* 190:1103-1105.
- Plaut, A. G., J. V. Gilbert, and I. Heller. 1978. Assay and properties of IgA protease of *Streptococcus sanguis*, pp. 489-495. In J. R. McGhee, J. Mestecky, and J. L. Babb (ed.), *Secretory immunity and infection*. Plenum Publishing Co., New York.
- Plaut, A. G., J. V. Gilbert, and A. H. Rule. 1978. Isolation and properties of the immunoglobulin A proteases of *Neisseria gonorrhoeae* and *Streptococcus sanguis*, pp. 279-284. In G. F. Brooks, E. C. Gotschlich, K. K. Holmes, W. D. Sawyer, and F. E. Young (ed.), *Immunobiology of Neisseria gonorrhoeae*. American Society for Microbiology, Washington, D.C.
- Plaut, A. G., J. V. Gilbert, and R. Wistar, Jr. 1977. Loss of antibody activity in human immunoglobulin A exposed to extracellular immunoglobulin A proteases of *Neisseria gonorrhoeae* and *Streptococcus sanguis*. *Infect. Immun.* 17:130-135.
- Putnam, F. W., Y. V. Liu, and T. L. K. Low. 1979. Primary structure of a human IgA1 immunoglobulin. IV. Streptococcal IgA1 protease digestion, Fab and Fc fragments, and the complete amino acid sequence of the alpha heavy chain. *J. Biol. Chem.* 254:2865-2874.
- Schneider, D. R., S. P. Sigel, and C. D. Parker. 1981. Characterization of *Vibrio cholerae* protease activities with peptide digest analysis. *J. Clin. Microbiol.* 13:80-84.
- Spiegelberg, H. L. 1974. Biological activities of immunoglobulins of different classes and subclasses. *Adv. Immunol.* 19:259-294.
- Sternberg, J. C. 1977. A rate nephelometer for measuring specific proteins by immunoprecipitin reactions. *Clin. Chem.* 23:1456-1464.
- Waldman, R. H., and R. Ganguly. 1974. Immunity to infections on secretory surfaces. *J. Infect. Dis.* 130:419-440.
- Weber K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* 244:4406-4412.
- Williams, R. C., and R. J. Gibbons. 1972. Inhibition of bacterial adherence by secretory Immunoglobulin A: a mechanism of antigen disposal. *Science* 177:697-699.
- Young, V. M. 1974. *Haemophilus*, p. 302-307. In E. H. Lennette, E. H. Spaulding, and J. P. Truett (ed.), *Manual of clinical microbiology*, 1st ed. American Society for Microbiology, Washington, D.C.