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Immunoglobulin A from Bronchopulmonary Secretions Blocks Bactericidal and Opsonizing Effects of Antibody to Nontypable *Haemophilus influenzae*

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Patients with chronic bronchitis are colonized by and may develop acute bronchopulmonary infection due to nontypable *Haemophilus influenzae* (NTHI) despite the presence of bactericidal and opsonizing antibody to the infecting organism. To test the hypothesis that secretory immunoglobulin A (IgA) interferes with host defense mechanisms, we extracted secretory IgA from bronchopulmonary secretions of five patients with NTHI pneumonia. NTHI was incubated with IgA before or during incubation with each patient's own serum or normal human serum. IgA from four of these individuals blocked the bactericidal and opsonizing effects of normal human serum and/or their own serum against their own and/or other NTHI. IgA from bronchopulmonary secretions of patients not infected with NTHI or from the serum of a patient with an IgA myeloma had no such effect. Blocking appeared to result from a direct interaction between IgA and the bacteria. The presumed mechanism is an interaction with bacterial surface antigens, although it is not known whether this occurs at antigenic sites responsible for bactericidal and opsonizing activity or whether interaction with adjacent antigenic sites and subsequent steric interference is responsible. This blocking effect of IgA may be one mechanism that allows for the development of NTHI colonization or pneumonia in an individual who already has seemingly adequate antibody against the infecting organism.

Patients with chronic bronchitis may have colonization of the bronchial tree (8, 9) or a spectrum of acute infection including purulent febrile tracheobronchitis and pneumonia (2, 11, 12, 15, 20) due to nontypable *Haemophilus influenzae* (NTHI). Colonization and infection appear to occur despite the presence of substantial bactericidal activity and variable opsonizing activity in the serum of normal subjects (14) or those hospitalized for acute lower respiratory NTHI infection (15). Specific antibody to (presumably nontypable) *H. influenzae* has been demonstrated in sputum of patients with chronic bronchitis (7). Recent studies have shown that immunoglobulin and complement are both present in bronchopulmonary secretions in sufficient concentrations (1) to be bactericidal for NTHI and to opsonize these organisms for ingestion by polymorphonuclear leukocytes (PMN) (14). A number of factors may contribute to bacterial persistence and proliferation under these circumstances. In the experiments described in this paper we chose to investigate the hypothesis that secretory immunoglobulin A (IgA) present in respiratory secretions contributes to colonization and/or infection by blocking the interaction between bactericidal or opsonizing antibody and NTHI.

MATERIALS AND METHODS

Bacteria. Pleomorphic gram-negative coccobacilli were seen in Gram stain as the overwhelmingly predominant organism, and *H. influenzae* predominated in cultures of bronchopulmonary secretions from five patients with acute pneumonia (16). *H. influenzae* formed grayish colonies on chocolate but not on sheep blood agar, required hemin and NAD for growth, and did not ferment sucrose (17). Serotyping was carried out by counterimmunoelectrophoresis (14) with antisera to *H. influenzae* types a through f, provided by the Centers for Disease Control, Atlanta, Ga.; each study included one *H. influenzae* of each serotype and several NTHI as positive and negative controls, respectively. The five isolates of *H. influenzae* used in these studies were determined to be nontypable based on their failure to form precipitin lines with any of the six antisera. Bacteria were grown overnight in tryptic soy broth to which 10 μg of hemin and NAD per ml had been added. Bacteria were radiolabeled for phagocytosis by the addition of [^{3}H]thymidine (specific activity, 80 Ci/mmol; New England Nuclear Corp., Boston, Mass.) at the start of the incubation to yield 1 μCi/ml; the usual uptake was 30,000 to 50,000 cpm/10^7 bacteria.

Bronchopulmonary secretions. Purulent bronchopulmonary secretions were obtained from five patients within 2 days of hospitalization for acute pneumonia due to NTHI (15). Bronchopulmonary secretions were also obtained from four additional patients (non-NTHI-infected controls) who were hospitalized in a medical intensive care unit for a variety of complicated medical illnesses and were not infected with *H. influenzae*, although the possibility that they had previously been infected with NTHI could not be excluded.

Extraction of IgA. Bronchopulmonary secretions were homogenized by dropwise addition of 20% *N*-acetylcysteine with vigorous agitation, and particulate matter was removed by centrifugation at 1,500 × *g*. IgA was assayed by radioimmunodiffusion (Meloy, Springfield, Va.); the concentration of secretory IgA (referred to hereafter as IgA concentrations) was calculated by using serum IgA standards and multiplying by a conversion factor of 5.6 (5). IgA concentrations in these homogenized samples ranged from 1 to 4 mg/ml.

IgA was extracted in accordance with accepted methods (19). An equal volume of a saturated solution of NH_4SO_4 was added to homogenized bronchopulmonary secretions with continuous stirring, and this suspension was allowed to stand overnight at room temperature; centrifugation at

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17,000 × *g* was used to collect precipitated immunoglobulins. The precipitate was resuspended in phosphate-buffered saline (PBS; pH 7.4) and dialyzed at 4°C for 24 to 48 h against PBS with repeated changes of the dialyzing solution. The dialyzed material was put over columns of Sephacryl S-300 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.). Ouchterlony diffusion with goat antiserum to human IgA was used to detect IgA-containing fractions, which were then pooled, placed in dialysis tubing, and concentrated back to the starting volume by treatment of the external surface with Sephadex G-200 (Pharmacia). Total protein was measured (Bio-Rad Laboratories, Richmond, Calif.), and IgA was found to constitute 60 to 90% of the protein present; IgG was not detectable (<0.01 mg/ml; radioimmunodiffusion assay; Meloy).

After incubation with these IgA preparations, NTHI bound IgA as demonstrated by the subsequent binding of ^{125}I-labeled rabbit anti-human IgA. Neither IgG nor IgM could be detected on the NTHI using radiolabeled antisera specific for those isotypes. When necessary, IgA solutions were adjusted to yield final concentrations of 1 mg/ml.

Serum. Pooled normal human serum (NHS) was obtained by separating and pooling the serum from the blood of 10 normal, healthy adults. Serum was obtained from patients on admission and 2 to 3 weeks later, after the completion of a course of antibiotics for pulmonary infection. Sera were stored in 0.5-ml aliquots at −70°C.

Bactericidal assay. Various concentrations of IgA were added to suspensions of 10^5 to 10^6 CFU of NTHI per ml in PBS that contained 2.5% heat-treated fetal calf serum and incubated for 30 min at 37°C; this treatment was shown not to reduce CFU. Fetal calf serum was added because NTHI lost viability in PBS in the absence of such a buffering solution. NHS or the patient's own serum was then added to yield a final concentration of 10%. In some experiments, bacteria were collected by centrifugation and washed three times in PBS to remove nonadherent IgA before the addition of serum. Samples were removed just before the serum was added (time zero) and thereafter at 30-min intervals, diluted serially when indicated, and plated in duplicate on chocolate agar. CFU were counted after overnight incubation at 37°C under 5% CO_2.

To determine a blocking effect of IgA, CFU per milliliter were converted to $\log_{10}$, and the results of all experiments were averaged. Significant suppression was defined as ≥1 $\log_{10}$ reduction in the bactericidal effect of serum with IgA compared with serum without IgA, indicating ≧90% suppression of the bactericidal activity.

Opsonization. Radiolabeled bacteria were washed three times, resuspended to yield 5 × 10^7 CFU/ml in Hanks balanced salt solution, and incubated with the desired concentration of IgA at 37°C for 30 min. Serum was then added to yield a 10% concentration, and the incubation was continued for 30 additional min. Bacteria were collected by centrifugation at 1,700 × *g* and resuspended in Hanks balanced salt solution that contained 0.1% gelatin.

Phagocytosis. Phagocytosis was studied by techniques we have previously described (16). PMN were separated from peripheral blood of normal human donors by dextran sedimentation and centrifugation over Ficoll and resuspended in Hanks balanced salt solution that contained 0.1% gelatin. Phagocytosis was studied by the addition of 10^6 PMN to 10^7 CFU of preopsonized, radiolabeled bacteria in a total volume of 0.4 ml and vigorous shaking in a water bath at 37°C. At various time intervals the reaction was stopped by the addition of 2 ml of iced PBS. Non-cell-associated bacteria were removed by a series of three washings in cold PBS, each with centrifugation for 5 min at 160 × *g*, yielding a final centrifuged pellet that contained PMN and cell-associated bacteria. Total bacteria present in the incubation mixture was obtained by centrifuging a duplicate set of reaction tubes for 10 min at 1,700 × *g*. Centrifuged pellets were suspended in 2 ml of scintillation fluid (ACS; Amersham Corp., Arlington Heights, Ill.), and discharges were counted in a scintillation spectrometer (model 3375; Packard Instruments, Downers Grove, Ill.). Cell-associated bacteria were reported as a percentage of the total ^{3}H-labeled NTHI that had been present during incubation. Electron microscopic studies (F. Gyorkey, unpublished observations) showed that virtually all bacterial forms present at the end of the differential centrifugation procedure were contained within phagocytic vacuoles.

A blocking effect of IgA on phagocytosis was calculated as follows. The uptake of NTHI after preincubation in IgA and opsonization with 10% serum was subtracted from the uptake after opsonization in serum alone; this result was then divided by the uptake after opsonization in serum alone. The degree of suppression in each experiment was thus calculated as a percentage. The overall degree of suppression is reported as the average of results obtained in every individual experiment. A mean difference of ≤10% is considered not significant.

Adherence of IgA to bacterial surfaces. An indirect immunofluorescence assay was used to verify the interaction between NTHI and IgA. NTHI were washed three times in PBS, resuspended to a concentration of about 10^7/ml,

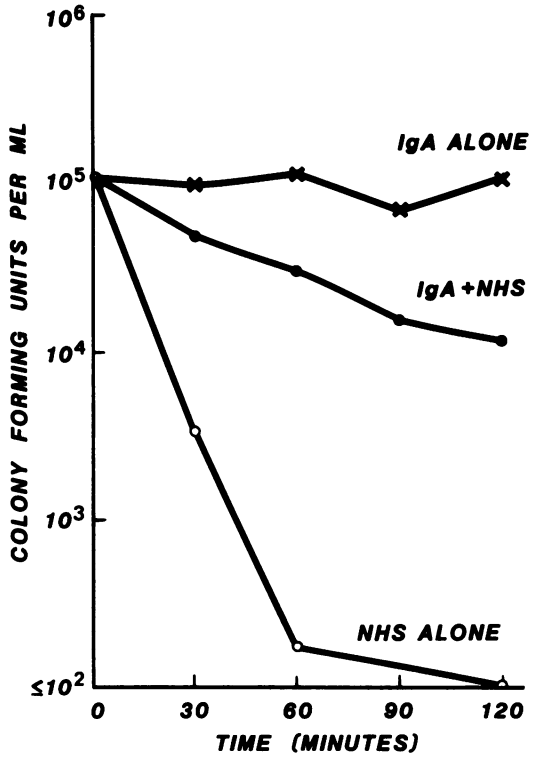

FIG. 1. NTHI (10^5/ml) from a patient with NTHI pneumonia (patient 1) was incubated with 0.4 mg of IgA per ml extracted from the patient's own sputum, after which 10% NHS was added. Bacteria were also incubated with IgA alone and with 10% NHS alone.

streaked onto glass slides, and allowed to dry at room temperature. An appropriate dilution of IgA was overlaid, and slides were incubated in a moist chamber for 30 min at room temperature. The slides were then washed three times in PBS for 10 min and flooded with dilutions of fluorescein-conjugated goat anti-human IgA (Cappel Laboratories, Downington, Pa.) that had previously been adsorbed twice with 10^{10} CFU of NTHI per ml. After an additional 30 min of incubation, the slides were washed copiously. Coded slides were examined with a fluorescent microscope (Leitz Dialux 20 EB) with Ploem optics. Bacteria incubated with unadsorbed NHS or PBS alone served as positive or negative controls, respectively.

RESULTS

Bactericidal activity. Incubating NTHI with 10% pooled NHS generally caused 99 to 99.9% killing in 1 to 2 h. Preincubation with IgA extracted from bronchopulmonary secretions of four of the five subjects with NTHI pneumonia blocked the bactericidal activity of pooled NHS and/or the patients' own serum. Figure 1 gives results from a representative experiment in which NTHI was incubated in PBS containing 0.5 mg of IgA per ml or in PBS alone, followed by the addition of 10% NHS; prior incubation with IgA caused a 2.1 $\log_{10}$ (99.2%) decrease in bacterial killing at 1 h. The NTHI and IgA in this experiment had both been derived from one patient with NTHI pneumonia. Similar results were observed when this patient's own serum was used in place of NHS (Patient 1, Table 1). The bactericidal effect of NHS was blocked in a dose-related fashion at concentrations ranging from 0.025 to 0.4 mg of IgA per ml (Fig. 2). No decrease in bacterial viability resulted from incubating NTHI in 1 mg of IgA per ml with or without 10% guinea pig serum.

Table 1 summarizes data from all the experiments that studied the effect of secretory IgA from these five patients on serum bactericidal activity. IgA blocked the bactericidal activity of NHS and/or autologous serum on NTHI in two of the first three patients listed in Table 1. IgA from patient 4 blocked the bactericidal effect of NHS or autologous serum against another patient's NTHI, indicating cross-reactivity of the interaction. Preincubation with IgA from patient 5 failed to alter the bactericidal effect of serum against his own or other isolates of NTHI. Incubation with IgA extracted from bronchopulmonary secretions of four non-NTHI-infected controls or from serum of a patient with IgA myeloma produced no blocking effect. When IgA and serum were added to NTHI together (i.e., without preincubation), similar results were obtained (data not shown).

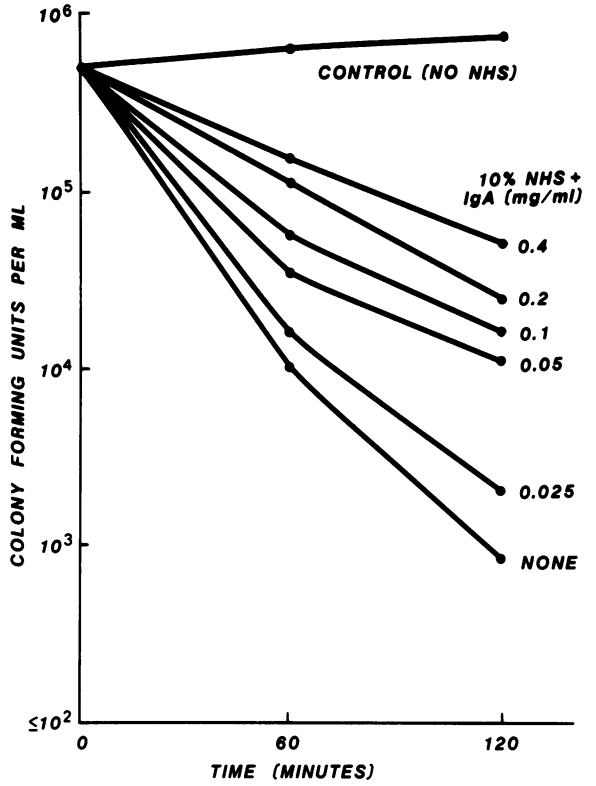

FIG. 2. NTHI (5×10^5/ml) from patient 1 was incubated with increasing concentrations of the patient's IgA, after which 10% NHS was added. NTHI was also incubated without NHS or IgA and without IgA but with the addition of 10% NHS.

TABLE 1. Blocking of bactericidal activity of human serum against NTHI by secretory IgA[a]

IgA source	No. of expts	Serum from patient	Organism from patient	Mean $\log_{10}$ decrease in bactericidal activity[b]
Patient 1	9	NHS	1	1.7
	2	1	1	1.6
Patient 2	4	NHS	2	1.6
	2	2	2	NS
Patient 3	3	NHS	3	NS
	2	3	3	2.1
Patient 4	2	NHS	4	—[c]
	2	NHS	1	3.1
	1	1	1	3.2
Patient 5	4	NHS	5	NS
	3	5	5	NS
	2	1	1	NS

[a] IgA, serum, and NTHI were obtained from five individual subjects with NTHI pneumonia. The IgA concentration was usually 0.5 mg/ml (range, 0.4 to 0.6 mg/ml), and the serum concentration was 10%.

[b] Data are reported as mean $\log_{10}$ differences in CFU from all experiments that compared incubation with and without IgA. Mean differences less than 1 $\log_{10}$ (i.e., <90% suppression of bactericidal activity) were regarded as not significant (NS).

[c] —, NHS had no bactericidal effect against this isolate.

Several experiments were undertaken to verify that a direct interaction between IgA and NTHI was responsible for the observed blocking effect. Studies with fluorescein-labeled goat antiserum to IgA or ^{125}I-labeled rabbit anti-human IgA documented that incubation of NTHI with secretory IgA led to deposition of IgA on the bacterial surface. The possibility that blocking resulted from a direct interaction between IgA and serum IgG or IgM was excluded by experiments in which NTHI was incubated with IgA and washed repeatedly before the addition of NHS; the blocking effect was still present. Preincubation of IgA with immobilized Fab' fragments also failed to reduce the degree of blocking, thereby excluding the possibility that IgA acted by interfering with immunoglobulin antigen-binding sites (3, 4).

Opsonizing activity. IgA isolated from bronchopulmonary secretions of three patients blocked opsonization of their own NTHI by NHS and their own serum. As shown in the

TABLE 2. Blocking effect of IgA on the opsonization of NTHI by human serum[a]

IgA source	No. of expts	Source of:		% Reduction of opsonization[b]
		Serum	NTHI	
Patient 1	4	NHS	Patient 1	35
	2	Patient 1	Patient 1	16
Patient 2	3	NHS	Patient 2	14
	2	Patient 2	Patient 2	16
Patient 3	5	NHS	Patient 3	14
	4	Patient 3	Patient 3	20
Patient 5	2	NHS	Patient 5	NS
	2	Patient 5	Patient 5	NS
Patient 1	2	NHS	Patient 2	NS
	1	Patient 2	Patient 2	NS
Patient 2	2	NHS	Patient 1	17
	1	Patient 1	Patient 1	23
Patient 3	3	NHS	Patient 2	14
	3	Patient 2	Patient 2	NS
Patient 3	3	NHS	Patient 1	NS
	3	Patient 1	Patient 1	NS
Patient 4	1	NHS	Patient 1	27
	1	Patient 1	Patient 1	45

[a] IgA, serum, and NTHI were obtained from five individual subjects with NTHI pneumonia.

[b] Data are reported as the mean percentage decrease in opsonizing effect by human serum (i.e., blocking effect) after preincubation with IgA. Differences of ≤10% are considered not significant (NS).

upper half of Table 2, phagocytosis was reduced by 14 to 35%. IgA from patient 5 that had failed to block bactericidal activity of human serum did not reduce opsonization, and no blocking resulted from incubating NTHI with IgA from non-NTHI-infected controls. Studies of the specificity of the IgA blocking effect using IgA from one subject with NTHI from others gave mixed results, with reduction in uptake of up to 45% being observed (Table 2, lower half).

DISCUSSION

Of the mechanisms that might protect the human host against bronchopulmonary infection with NTHI, the capacity of humoral antibody and complement to kill these microorganisms or to opsonize them for phagocytosis by alveolar macrophages or PMN is likely to play a major role. The presence of bactericidal and opsonizing antibody in the serum of patients at the time they come to medical attention for pneumonia due to NTHI (15), together with the likelihood that these substances are also present in sputum (1, 7), raises the possibility that some factor(s) might inhibit the antibody-bacterium interaction.

Previous studies have demonstrated that certain nonbactericidal antibodies, termed blocking antibodies, may interact with bacterial surfaces and prevent the subsequent interaction with bactericidal antibody. Thus, hyperimmunization of rabbits with *Brucella abortus* leads to the emergence of serum IgA that blocks the bactericidal activity of human serum (10), and removing IgA from the serum of some young adults acutely ill with meningococcal meningitis allows expression of bactericidal antibody that might otherwise have protected the host against such severe infection (6). Normal human serum also contains IgG that blocks bactericidal activity against *Neisseria gonorrhoeae* (13); serum resistance of isolates that cause disseminated infection is thought to result, at least in part, from this blocking effect (18). A preliminary communication has suggested a possible role for IgA in blocking the protective effect of immune serum in mice challenged with *Streptococcus pneumoniae* (D. E. Briles, C. Forman, S. Hudak, and L. Claflin, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, E40, p. 83).

The results of the present study show that secretory IgA blocks the effect of bactericidal and opsonizing antibody against NTHI. This report is the first to show that IgA from bronchopulmonary secretions can block bactericidal or opsonizing activity against a common pulmonary pathogen. These studies utilized secretory IgA, serum, and NTHI that had all been obtained from individual patients at the time they were hospitalized for acute NTHI pneumonia, thereby giving further clinical relevance to the findings.

Binding of IgA to the surface of NTHI was thought to be responsible for the blocking effect, based on (i) the demonstration by immunofluorescent and radioimmune techniques of IgA on the surface of NTHI after incubation and washing and (ii) the failure of repeated washings to remove the blocking effect. The mechanism for the observed blocking, namely, whether IgA actually binds antigenic sites that might otherwise interact with IgG or IgM or whether it attaches at other sites but then blocks by steric interference remains to be elucidated. The possibility that proteins other than IgA or degradation products of IgA may have been responsible for the observed blocking cannot be excluded, although some of our IgA preparations appeared to have ≥95% purity. Since the antigenic components of NTHI that interact with antibody and lead to killing or opsonization have not yet been identified, it is difficult to hypothesize which site(s) is involved in the observed blocking effect.

The specificity of the IgA-bacterial interaction did not follow a clear-cut pattern, probably reflecting a substantial degree of antigenic similarity among NTHI (14). Thus, one could not conclude that IgA was more likely to block antibody present in NHS or in a patient's own serum, nor could the cross-reactivity among different isolates predicted. Nevertheless, IgA from bronchopulmonary secretions of four uninfected patients and serum IgA from a patient with an IgA myeloma demonstrated no blocking effect, indicating that an antigenic relation between IgA and the colonizing NTHI is necessary for blocking to take place.

In summary, these studies suggest that blocking of the interaction between IgG or IgM and NTHI by secretory IgA may contribute to the occurrence of NTHI colonization pneumonia in individuals who already have demonstrable bactericidal and opsonizing antibody to these organisms.

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ADDENDUM IN PROOF

A recent report has shown that homogenized whole sputum from patients with cystic fibrosis inhibits the bactericidal effect of normal human serum for mucoid *Pseudomonas aeruginosa* (N. L. Schiller and R. L. Millard, Pediatr. Res. **17**:747–752. 1983).

LITERATURE CITED

1. **Bell, D. Y., J. A. Haseman, A. Spock, G. McLennan, and G. E. R. Hook.** 1981. Plasma proteins of the bronchoalveolar surface of the lungs of smokers and nonsmokers. Am. Rev. Respir. Dis. **124**:72–79.

2. **Berk, S. L., S. A. Holtsclaw, S. L. Wiener, and J. K. Smith.** 1982. Nontypeable *Haemophilus influenzae* in the elderly. Arch. Intern. Med. **142**:537–539.
3. **Birdsall, H. H., M. D. Lidsky, and R. D. Rossen.** 1983. Anti-Fab' antibodies in rheumatoid arthritis. Measurement of the relative quantities incorporated in soluble immune complexes in sera and supernatants from cultured peripheral blood lymphocytes. Arthritis Rheum. **26**:1481–1492.
4. **Birdsall, H. H., and R. D. Rossen.** 1983. Characterization of anti-Fab' antibodies in human sera: identification of soluble immune complexes that contain hidden anti-KLH and blocking anti-immunoglobulins following immunization with keyhole limpet haemocyanin. Clin. Exp. Immunol. **53**:497–504.
5. **Greenberg, S. B., R. D. Rossen, H. R. Six, B. D. Baxter, and R. B. Couch.** 1978. Determination of immunoglobulin A concentrations in human nasal secretions with a serum immunoglobulin A standard. J. Clin. Microbiol. **8**:465–467.
6. **Griffiss, J. M., and M. A. Bertram.** 1977. Immunoepidemiology of meningococcal disease in military recruits. II. Blocking of serum bactericidal activity by circulating IgA early in the course of invasive disease. J. Infect. Dis. **136**:733–739.
7. **Gump, D. W., W. A. Christmas, B. R. Forsyth, C. A. Phillips, and W. H. Stouch.** 1973. Serum and secretory antibodies in patients with chronic bronchitis. Arch. Intern. Med. **132**:847–851.
8. **Gump, D. W., C. A. Phillips, B. R. Forsyth, K. McIntosh, K. R. Lamborn, and W. H. Stouch.** 1976. Role of infection in chronic bronchitis. Am. Rev. Respir. Dis. **113**:465–474.
9. **Haas, H., J. F. Morris, S. Samson, J. P. Kilbourn and P. J. Kim.** 1977. Bacterial flora of the respiratory tract in chronic bronchitis: comparison of transtracheal, fiberbronchoscopic, and oropharyngeal sampling methods. Am. Rev. Respir. Dis. **116**:41–47.
10. **Hall, W. H., R. E. Manion, and H. H. Zinneman.** 1971. Blocking serum lysis of *Brucella abortus* by hyperimmune rabbit serum immunoglobulin A. J. Immunol. **107**:41–46.
11. **Hirschmann, J. V., and E. D. Everett.** 1979. Haemophilus influenzae infections in adults: report of nine cases and a review of the literature. Medicine **58**:80–94.
12. **Levin, D. C., M. I. Schwarz, R. A. Matthay, and F. M. LaForce.** 1977. Bacteremic Hemophilus influenzae pneumonia in adults: a report of 24 cases and a review of the literature. Am. J. Med. **62**:219–224.
13. **McCutchan, J. A., D. Katzenstein, D. Norquist, G. Chikami, A. Wunderlich, and A. I. Braude.** 1978. Role of blocking antibody in disseminated gonococcal infection. J. Immunol. **121**:1884–1888.
14. **Musher, D. M., M. Hague-Park, R. E. Baughn, R. J. Wallace, Jr., and B. Cowley.** 1983. Opsonizing and bactericidal effects of normal human serum on nontypable *Haemophilus influenzae*. Infect. Immun. **39**:297–304.
15. **Musher, D. M., K. R. Kubitschek, J. Crennan, and R. E. Baughn.** 1983. Pneumonia and acute febrile tracheobronchitis due to *Haemophilus influenzae*. Ann. Intern. Med. **99**:444–450.
16. **Musher, D. M., H. A. Verbrugh, and J. Verhoef.** 1981. Suppression of phagocytosis and chemotaxis by cell wall components of *Staphylococcus aureus*. J. Immunol. **127**:84–88.
17. **Oberhofer, T. R., and A. E. Back.** 1979. Biotypes of *Haemophilus influenzae* encountered in clinical laboratories. J. Clin. Microbiol. **10**:168–174.
18. **Rice, P. A., and D. L. Kasper.** 1982. Characterization of serum resistance of *Neisseria gonorrhoeae* that disseminate: roles of blocking antibody and gonococcal outer membrane proteins. J. Clin. Invest. **70**:157–167.
19. **Rossen, R. D., R. H. Alford, W. T. Butler, and W. E. Vanner.** 1966. The separation and characterization of proteins intrinsic to nasal secretion. J. Immunol. **97**:369–378.
20. **Tillotson, J. R., and A. M. Lerner.** 1968. *Haemophilus influenzae* bronchopneumonia in adults. Arch. Intern. Med. **121**:428–432.