# Antibacterial Mechanisms of the Lower Respiratory Tract

## I. IMMUNOGLOBULIN SYNTHESIS AND SECRETION

W. Lee Hand and J. Robert Cantey, with the technical assistance of Carl G. Hughes

*From the Medical and Research Services, Veterans Administration Hospital (Atlanta), Decatur, Georgia, 30033, and the Department of Medicine, Emory University School of Medicine, Atlanta, Georgia, 30303*

Abstract Immunoglobulin synthesis and secretion have been studied in the rabbit lower respiratory tract, both in the normal state and after infection with *Diplococcus pneumoniae* or *Listeria monocytogenes*. In vitro synthesis of immunoglobulin and specific antibody was assessed by incorporation of $^{14}$C-labeled amino acids into protein. Lower respiratory tract secretions and serum were analyzed for immunoglobulin and antibody against the infecting organism.

Normal respiratory tract produced small quantities of immunoglobulin, most of which was IgG. After bacterial infection of the lower respiratory tract, there was a marked increase in local synthesis of immunoglobulin, especially IgG. Specific antibody of IgG class was produced in all lungs infected with listeria by the 11th day, and in lungs infected with pneumococcus by the 8th day.

Secretions from all normal and infected lower respiratory tracts contained IgA and IgG. The IgA to IgG ratios in secretions of normal animals, and animals infected with listeria or pneumococcus, were 2.3, 2.5, and 2.6, respectively. Sera of animals infected with *L. monocytogenes* contained specific antibody of IgG class but lacked IgA antibody, whereas secretions had both IgA and IgG class antibody against listeria. Similarly, sera of animals infected with *D. pneumoniae* had IgG class antibody but no IgA antibody, whereas only IgA antibody was found in secretions. The evidence that locally synthesized immunoglobulin (especially IgA), including specific antibody, is secreted into the lower respiratory tract lumen is discussed. Further definition of the role of "local" antibacterial antibody in the respiratory tract is of considerable importance.

Dr. Hand is a Clinical Investigator, Veterans Administration Hospital (Atlanta).

During part of this study Dr. Cantey was a Postdoctoral Fellow, National Institute of Allergy and Infectious Diseases, United States Public Health Service (5 TO1 A100304).

*Received for publication 19 January 1973 and in revised form 17 September 1973.*

## INTRODUCTION

Intrinsic lower respiratory tract (LRT)[1] antibacterial mechanisms, such as alveolar macrophage and mucociliary function, have been extensively investigated and partially defined (e.g., 1, 2). However, relatively little is known of specific LRT immune mechanisms, such as immunoglobulin synthesis and secretion, that might contribute to antibacterial activity. Secretory IgA is the predominant immunoglobulin in several external secretions and in plasma cells of respiratory and gastrointestinal mucosa (3, 4). Increases in local secretory IgA virus-neutralizing activity have been associated with recovery or protection against certain viral infections (5–8), but data concerning the biological significance of secretory IgA class antibacterial antibody are conflicting (e.g., 9–13). Analyses of human tracheobronchial washings and sputum have revealed the presence of both IgA and IgG, usually with a predominance of the former immunoglobulin (14–16). However, there are no studies of the local synthesis or secretion of antibody after bacterial infection of the respiratory tract.

The present study was designed to compare immunoglobulin synthesis and secretion in the normal and infected LRT, and to evaluate the production of specific antibacterial antibody after infection.

## METHODS

*LRT infection.* Infection with a typical pyogenic organism, *Diplococcus pneumoniae* type III, or a facultative

[1] *Abbreviation used in this paper:* LRT, lower respiratory tract.

intracellular organism, *Listeria monocytogenes*, was produced in 5–6-lbs white, male New Zealand rabbits by exposure in an aerosol apparatus (Tri-R Airborne Infection Apparatus, Tri-R Instruments, Inc., Rockville Centre, N. Y.). Bacterial aerosols were monitored by means of a modified Andersen air sampler (Aerostatics Instrumentation & Research, Logan Utah). Between $5 \times 10^7$ and $1 \times 10^8$ organisms (infectious particles $< 2$ $\mu$m in diameter) were delivered to the alveoli of each animal (17). In some animals pneumococcal infection was produced by intratracheal injection of $5 \times 10^7$–$1 \times 10^8$ organisms. Illness after infection was characterized by fever, weight loss, lethargy, rapid and often labored respirations, sniffles, and conjunctivitis.

Animals with overwhelming clinical illness required antibiotic therapy by the 4th or 5th day after infection. Otherwise they were treated just before study to eradicate infecting organisms that might still be present. Rabbits infected with pneumococci were injected with 25 mg/kg/day of penicillin (Upjohn Co., Kalamazoo, Mich.) and animals infected with *L. monocytogenes* received 50 mg/kg/day of ampicillin (Bristol Laboratories, Div., Bristol-Myers Co., Syracuse, N. Y.).

Normal and infected animals were anesthetized with sodium thiopental, exsanguinated, and studied in an identical manner. LRT washings were obtained by a modification of the method of Myrvik, Leake, and Fariss (18). The thoracic cavity was opened, the upper trachea ligated, and the tracheobronchial tree dissected free. The LRT was then lavaged with sterile saline. In experiments when no washings were obtained or when one lung was excluded from lavage, the tracheobronchial tree and lungs were removed in an aseptic manner for in vitro protein synthesis studies. The following normal tissues were also evaluated for production of protein and immunoglobulin: nasopharyngeal tissues were removed from two animals. In another animal, a segment of small intestine (ileum) was excised and thoroughly washed with sterile saline. Mammary glands from two rabbits were obtained on the fourth post-partum day.

*Lower respiratory tract secretions and serum.* Cells were removed from LRT washings by centrifugation. Supernatants were then dialyzed against water, lyophilized, and reconstituted in 2 ml of saline. Protein content of secretions was estimated by the Lowry, Rosebrough, Farr, and Randall method (19). Immunoglobulins were identified by immunoelectrophoresis and by the double diffusion technique, both methods utilizing 2% purified agar (Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.) in barbital buffer on microscope slides.

Immunoglobulins in serum and secretions were quantitated by single radial immunodiffusion and by electroimmunodiffusion. Both methods were satisfactory for determination of IgG and IgM concentrations and for quantitation of serum IgA, whereas we found only electroimmunodiffusion to be reliable in determination of IgA concentrations in respiratory secretions. Single radial immunodiffusion was carried out on $3\frac{1}{2} \times 4$-in glass plates covered with agar ($1\frac{1}{2}$% final concentration) containing antiserum against rabbit IgG, IgM, or colostral IgA (20). Electroimmunodiffusion was performed by a modification of the technique of Merrill, Hartley, and Claman (21). Precoated $2 \times 3$-in glass slides were covered with 1% purified agar containing the appropriate antiserum. Standardized wells were cut in the agar of each slide and filled with the solutions to be evaluated. Electrophoresis was run at a constant current of 10 mA/slide. Standards of appropriately diluted purified immunoglobulin were included on each single radial immunodiffusion and electroimmunodiffusion slide. The nitrogen content of these immunoglobulin preparations was quantitated by the micro-Kjeldahl technique (22).

Antibacterial antibody in secretions and serum was determined by an antigen-binding technique using radioactive bacterial antigen. *D. pneumoniae* or *L. monocytogenes* were grown overnight in defined media (23, 24) containing either 500 $\mu$Ci of [$^{14}$C]glucose/liter (Schwarz/Mann) or 1 mCi of [$^{32}$P]phosphoric acid/250 ml (Schwarz/Mann. The $^{14}$C- or $^{32}$P-labeled listeria were washed and extracted overnight with ether, and the antigen was precipitated from the aqueous layer with ethanol after removal of cell bodies by centrifugation. After washing with ethanol, the antigen was dried and then reconstituted in an appropriate concentration as needed (25). The $^{14}$C-labeled pneumococci were killed with 1% phenol, and cell debris removed by centrifugation. Polysaccharide was precipitated from the supernate with acetone, extracted with hot phenol, shaken with ether, dialyzed against water, and lyophilized (26, 27).

The ability of immunoglobulin in LRT secretions and serum to bind radiolabeled bacterial antigen (listeria or pneumococcus) was evaluated by the method of Newcomb and Ishizaka (28). An excess of radiolabeled bacterial antigen in borate buffered saline (pH 8) was added to 0.1 ml of serum diluted 1:10 (borate buffered saline) for determination of serum IgG and IgM antigen binding activity. Evaluation of serum IgA antigen-binding ability and studies with LRT secretions utilized undiluted specimens. Control mixtures contained normal serum or secretions. Four tubes of each antigen and serum (or LRT secretion) mixture were incubated for 4 h at 37°C, after which an excess of appropriate anti-rabbit immunoglobulin serum was added to three of the four tubes in each series. (The fourth tube was a control for spontaneous precipitation of antigen by the antibody in secretion or serum alone.) After additional incubation at 37°C the tubes were allowed to sit at 4°C overnight. The precipitates were removed by centrifugation, and after washing three times with borate buffered saline, radioactivity was measured in a liquid scintillation counter (Packard Tri-Carb Model 2425, Packard Instrument Co., Inc., Downers Grove, Ill.). The average counts of the control tubes (normal serum or LRT secretions) were subtracted from the average values of tubes containing test specimen to give the counts attributed to specifically bound radiolabeled bacterial antigen. By comparison with the radioactivity of a set of tubes containing known quantities of the appropriate antigen, the amount of bound antigen was calculated. The results are expressed as the micrograms of antigen bound per 100 micrograms N of the immunoglobulin being evaluated.

*Histological examination of tissues.* Tissue obtained at the time of study was fixed in formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

*In vitro protein, immunoglobulin, and specific antibody synthesis.* This method has previously been described in detail (29–31). Minced tissue (lung, tracheobronchial tree [trachea and main stem bronchi], nasopharynx, mammary gland, or ileum) was incubated in modified Eagle's medium containing 4 $\mu$Ci of a mixture of algal protein hydrolysate [$^{14}$C]amino acids/g tissue (Schwarz/Mann). The incubation mixture was ultracentrifuged, and the supernate was then dialyzed to remove unincorporated radioactivity, fractionated on DEAE cellulose, concentrated by lyophilization, and reconstituted in sterile, glass-distilled water.

Aliquots of each DEAE fraction were evaluated in the following manner: (*a*) estimation of total protein syn-

TABLE I

*Specific Antibody Content of LRT Secretions and Serum*

| Experimental group | Day of infection | Antigen bound by antibody in secretions or serum: IgG Secretions | IgG Serum | IGA Secretions | IGA Serum |
|---|---|---|---|---|---|
| | | *μg antigen bound/100 μg N of IgA or IgG* | | | |
| *L. monocytogenes* infection | 12 | None | 65.8 | 6.9 | None |
| | 14 | 104.2 | 348.6 | 76.4 | None |
| | 15 | 7.1 | 49.0 | None | None |
| | 15 | 44.1 | 47.6 | None | None |
| | 16 | 94.3 | 84.0 | None | None |
| | 19 | 33.6 | 382.9 | 54.1 | None |
| | 20 | 8.3 | ND* | None | ND |
| | 21 | None | 98.6 | 4.4 | None |
| | 28 | None | 38.5 | 5.3 | None |
| | 69 | None | 85.7 | None | None |
| *D. pneumoniae* infection | 8 | None | None | 3.8 | None |
| | 12 | None | 8.2 | None | None |
| | 26 | None | 21.4 | None | None |
| | 28 | None | 219.7 | 5.8 | None |

* Not done.

thesis by precipitation with 10% trichloroacetic acid; (*b*) quantitation of synthesized immunoglobulin by immune precipitation in antibody excess, with goat anti-rabbit specific immunoglobulin sera (coprecipitation with ovalbumin-antiovalbumin served as a control for nonspecific radioactivity); and (*c*) identification of synthesized specific antibody against *L. monocytogenes* and *D. pneumoniae* by addition of antigen ($10^9$ heat-killed organisms) to DEAE fractions containing only or predominantly a single immunoglobulin, IgG, IgA, or IgM. *Pseudomonas aeruginosa* antigen served as a control for nonspecific adsorption of radioactivity. After liquid scintillation counting, values were expressed as counts per minute per gram of incubated tissue.

*Preparation of antisera.* Rabbit IgG was purified from rabbit gamma globulin (Miles Laboratories Inc., Miles Research Division, Kankakee, Ill.) by elution from DEAE cellulose, with 0.01 M phosphate buffer, pH 7. Rabbit colostrum was collected during the first 72 h after delivery, and colostral IgA was purified by the gel filtration and ion exchange chromatography method of Cebra and Robbins (32). Rabbit IgM was prepared from normal rabbit serum by a modification of the technique of VanDalen, Seijen, and Gruber (33).

Antisera to IgG, colostral IgA, and IgM were prepared in goats by repeated injections of the appropriate antigen incorporated into Freund's complete adjuvant. Antisera were absorbed by passage through columns containing the appropriate Sepharose-immunoadsorbent. The immunoadsorbents were prepared by coupling cyanogen bromide activated Sepharose 4B (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) with antigen (IgG, IgA, IgM, or Cohn fraction IV) (34, 35).

*Procedural controls.* The presence of pneumococcal polysaccharide in recently infected tissue or in LRT secretions from infected animals would interfere with determination of antibody against the type-specific polysaccharide. Consequently, secretions and tissue incubation fractions from animals infected with *D. pneumoniae* type III were assayed for the type-specific polysaccharide. Counterimmunoelectrophoresis, with antiserum against *D. pneumoniae* type III (Center for Disease Control, Atlanta, Ga.) and type III polysaccharide, was performed by a modification of the method of Coonrod and Rytel (36). Precoated 2 × 3-in glass slides were covered with 1% agarose (Schwarz/Mann). Double rows of wells, 3 mm in diameter, were cut so that the edges of the wells in the two rows were 2 mm apart. 20 μl of appropriate reagent was added to the wells. Electrophoresis for 1 h was carried out at a constant current of 10 mA/slide. A concentration of 0.01 μg/ml of type III pneumococcal polysaccharide could be detected in this manner. None of the LRT secretions or tissue (lung or tracheobronchial tree) incubation fractions contained detectable polysaccharide.

Similarly, the presence of C-reactive protein in LRT secretions, tissue incubation fractions, or immunologic reagents theoretically might lead to false positive results in the assays for antibody against pneumococcal polysaccharide. Thus secretions and tissue incubation fractions from infected animals, as well as the anti-immunoglobulin sera and purified immunoglobulin preparations used in the immune precipitations, were assayed for C-reactive protein by means of the Hyland CR Test (Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif.). None of these preparations contained C-reactive protein.

## RESULTS

*Lower respiratory tract secretions and serum.* Secretions washed from the LRT of all normal and infected animals contained both IgG and IgA by immunoelectrophoresis and double diffusion in agar, with specific antisera. IgM was only occasionally detected by these techniques and thus will not be presented in the quantitative results. The lower level of sensitivity of IgM detection by electroimmunodiffusion was $< 0.03$ mg/ml. (The least concentrated IgM standard included on the slides was 0.03 mg/ml.)

The mean quantities of immunoglobulin washed from the LRT of eight normal animals were 3.4 mg (SEM± 0.68) of IgA and 1.5 mg (±0.28) of IgG. In secretions of seven animals infected with pneumococci, the mean quantity of IgA was 2.7 mg (±0.29) and of IgG was 1.0 mg (±0.27). The secretions of animals infected with listeria contained 4.2 mg (±0.42) of IgA and 1.7 mg (±0.16) of IgG. The ratios of IgA to IgG in secretions of normal, listeria-infected, and pneumococcal-infected animals were 2.3, 2.5, and 2.6, respectively. Immunoglobulin accounted for a mean of 57%, 69%, and 74% of total protein in the secretions of these same groups of animals.

Table I exhibits data from all infected animals whose secretions contained antibody against the infecting organism. For comparison, the antigen-binding capacities of sera from these animals and from a few comparable infected animals without antibody in secretions were

determined. Six listeria-infected animals were found to contain IgG antibody against *L. monocytogenes* (mean of 48.6 μg antigen bound/100 μg N of IgG) in secretions. IgA class specific antibody (mean of 29.4 μg antigen bound/100 μg N of IgA) was detected in secretions from five animals infected with listeria. All of the animals infected with *L. monocytogenes* had circulating specific antibody of IgG class but lacked IgA-specific antibody.

Small quantities of IgA class-specific antibody were found in respiratory tract secretions of two animals infected with type III pneumococcus. No IgG class antibody against the pneumococcus was detected in secretions. However, serums from three of the four animals tested for antigen-binding activity contained IgG class-specific antibody, and all four serums had IgM specific antibody (not included in Table I). In contrast, none of the serums contained IgA class antibody against *D. pneumoniae.*

*Histological examination of tissues.* The usual histologic picture in infected animals was one of peribronchial mononuclear infiltration and bronchopneumonia. During the 1st wk of infection, a large number of polymorphonuclear leukocytes were observed in the inflammatory infiltrates. In keeping with other studies of pneumococcal infection produced by exposure of normal animals to bacterial aerosols, no true lobar consolidation occurred during these infections (e.g., 37). Microabscesses and granuloma formation were commonly observed in the lungs of animals with listeria infection.

*In vitro protein, immunoglobulin, and specific antibody synthesis.* Protein and Ig synthesis by normal lung tissues is recorded in Table II. Normal lung synthesized small quantities of immunoglobulin, most of which was IgG (57% of total Ig synthesis), with lesser amounts of IgM (26% of total Ig synthesis) and IgA (17% of total Ig synthesis).

Incubation of normal tracheobronchial tree from two animals yielded similar results (Table III). The predominant immunoglobulin synthesized in these tissues was IgG (mean of 66% of total Ig synthesis), followed by smaller amounts of IgM and IgA.

For comparison with LRT tissues, synthesis of immunoglobulin in upper respiratory (nasopharyngeal) tissue of two animals was also examined. Again IgG synthesis was predominant (48% of total Ig synthesis), but IgA synthesis (29% of total Ig synthesis) was proportionately increased over that found in the LRT.

As a methodological control, in vitro synthesis of immunoglobulin in mammary glands and intestinal tissue was evaluated, since these tissues have been demonstrated to produce considerable IgA in other studies (38–40). IgA production accounted for a mean of 51% of Ig synthesis in two mammary glands, followed by IgM (28% of Ig synthesis) and IgG (21% of Ig synthesis). Approximately equal amounts of each immunoglobulin were synthesized by a segment of normal small intestine (ileum) from another animal.

In lungs of animals aerosolized with *L. monocytogenes,* a marked increase in synthesis of total protein and

TABLE II

*Protein and Immunoglobulin Synthesis by Normal and Infected Lung*

| | | | Immunoglobulin | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Total | | | | % of immunoglobulin synthesis | | |
| | Day | Total protein | Immunoglobulin | IgG | IgA | IGM | IgG | IgA | IgM |
| | | | *cpm/g incubated tissue* | | | | | % | |
| Normal (6) | | 8,905 | 5,070 | 2,865 | 885 | 1,320 | 57 | 17 | 26 |
| ±1 SEM | | ±575 | ±840 | ±285 | ±285 | ±345 | | | |
| Listeria infection | 7 | 12,530 | 8,205 | 3,710 | 2,200 | 2,295 | 45 | 27 | 28 |
| | 8 | 9,165 | 5,750 | 3,550 | 1,060 | 1,140 | 62 | 18 | 20 |
| | 11 | 20,710 | 17,175 | 10,150 | 2,930 | 4,095 | 59 | 17 | 24 |
| | 15 | 21,180 | 10,480 | 6,700 | 1,480 | 2,300 | 64 | 14 | 22 |
| | 20 | 13,160 | 7,690 | 6,025 | 550 | 1,115 | 78 | 7 | 15 |
| | 20 | 15,280 | 9,550 | 7,140 | 935 | 1,475 | 75 | 10 | 15 |
| | 21 | 22,290 | 14,325 | 11,255 | 1,075 | 1,995 | 79 | 8 | 14 |
| | 28 | 25,245 | 17,325 | 14,245 | 1,190 | 1,890 | 82 | 7 | 11 |
| | 69 | 14,335 | 13,075 | 7,535 | 2,370 | 3,170 | 58 | 18 | 24 |
| Mean of days 11–69 | | 18,885* | 12,805* | 9,005* | 1,505 | 2,290 | 70 | 12 | 18 |
| (7) ±SEM | | ±1,740 | ±1,415 | ±1,130 | ±320 | ±390 | | | |

* Significantly greater than normal ($P < 0.01$).

TABLE III

*Protein and Immunoglobulin Synthesis by Normal and Infected Tracheobronchial Tree*

| | Day | Total protein | Immunoglobulin: Total: Immunoglobulin | Immunoglobulin: Total: IgG | Immunoglobulin: Total: IgA | Immunoglobulin: Total: IgM | % of immunoglobulin synthesis: IgG | % of immunoglobulin synthesis: IgA | % of immunoglobulin synthesis: IgM |
|---|---|---|---|---|---|---|---|---|---|
| | | | *cpm/g incubated tissue* | | | | % | | |
| Normal | | 14,905 | 1,850 | 1,490 | 120 | 240 | 81 | 6 | 13 |
| | | 12,470 | 2,010 | 1,040 | 310 | 660 | 52 | 15 | 33 |
| Pneumococcal infection | 4 | 9,155 | 2,640 | 2,095 | 185 | 360 | 79 | 7 | 14 |
| | 8 | 13,325 | 1,680 | 1,020 | 660 | 0 | 61 | 39 | 0 |
| | 8 | 13,010 | 7,330 | 5,610 | 815 | 905 | 77 | 11 | 12 |
| | 14 | 21,980 | 4,395 | 2,265 | 755 | 1,375 | 52 | 17 | 31 |
| | 19 | 19,075 | 8,615 | 6,850 | 890 | 875 | 80 | 10 | 10 |

immunoglobulin, especially IgG, was apparent by 11 days after infection (Table II). During the period 11 through 69 days after aerosol exposure, synthesis of protein and total immunoglobulin was twice as great, and production of IgG was three times normal. Although increased amounts of IgA and IgM were synthesized in infected lungs as compared to normal lungs, the increases were not statistically significant.

Similar results were obtained in animals infected with *D. pneumoniae*. Lungs of these animals, sacrificed 15–22 days after bacterial infection, produced three times as much protein and total immunoglobulin, and more than four times as much IgG as the normal (Table IV). Again, the increase in IgA and IgM synthesis was not statistically significant.

Incubation of tracheobronchial tissue from three animals studied 8–19 days after pneumococcal infection demonstrated an increase in production of immunoglobulin, especially IgG (Table III).

Normal lungs synthesized no antibody against pneumococci or against listeria. However, IgG class antibody against the infecting organism was produced in all lungs

TABLE IV

*Protein and Immunoglobulin Synthesis by Normal and Infected Lung*

| | Day | Total protein | Immunoglobulin: Total: Immunoglobulin | Immunoglobulin: Total: IgG | Immunoglobulin: Total: IgA | Immunoglobulin: Total: IgM | % of immunoglobulin synthesis: IgG | % of immunoglobulin synthesis: IgA | % of immunoglobulin synthesis: IgM |
|---|---|---|---|---|---|---|---|---|---|
| | | | *cpm/g incubated tissue* | | | | % | | |
| Normal (6) ±SEM | | 8,905 ±575 | 5,070 ±840 | 2,865 ±285 | 885 ±285 | 1,320 ±345 | 57 | 17 | 26 |
| Pneumococcal infection | 4 | 13,245 | 4,445 | 3,495 | 390 | 560 | 79 | 9 | 13 |
| | 8 | 9,840 | 7,635 | 3,710 | 1,740 | 2,185 | 49 | 23 | 29 |
| | 15 | 23,650 | 16,575 | 12,945 | 3,170 | 460 | 78 | 19 | 3 |
| | 19 | 53,510 | 29,815 | 20,170 | 2,515 | 7,130 | 68 | 8 | 24 |
| | 19 | 21,300 | 8,200 | 7,090 | 90 | 1,020 | 86 | 1 | 12 |
| | 19 | 37,235 | 33,015 | 24,370 | 2,500 | 6,145 | 74 | 8 | 19 |
| | 20 | 13,625 | 7,355 | 5,915 | 855 | 585 | 80 | 12 | 8 |
| | 22 | 22,765 | 10,465 | 5,505 | 1,635 | 3,325 | 53 | 16 | 32 |
| Mean of days 15–22 (6) ±SEM | | 28,680* ±5,865 | 17,570* ±4,590 | 12,665‡ ±3,275 | 1,795 ±475 | 3,110 ±1,200 | 72 | 10 | 18 |

* Significantly greater than normal ($P < 0.01$).

‡ Significantly greater than normal ($P < 0.02$).

infected with *L. monocytogenes* by the 11th day and in all lungs infected with *D. pneumoniae* by the 8th day (Table V). No synthesis of IgA class antibody against the infecting organism was detected by the methods used. Antibody against listeria accounted for a mean of 8% of IgG synthesized in lungs 11–69 days after infection with that organism. Similarly, antibody against the appropriate pneumococcal strain constituted 6% of IgG produced in lungs 8–22 days after pneumococcal infection.

Two tracheobronchial tissues (8 and 14 days after pneumococcal infection) also produced IgG class antibody against the infecting organism. This antibody represented 3% and 20% of IgG synthesis in these two tracheobronchial tree incubations.

TABLE V

*Specific Antibody Synthesis by Normal and Infected Lung*

| | Day | IgG | Specific antibody: Total | Specific antibody: Percent of IgG |
|---|---|---|---|---|
| | | *cpm/g incubated tissue* | | % |
| Normal (6) ±SEM | | 2,865 ±285 | None | None |
| Listeria infection | 7 | 3,710 | 0 | |
| | 8 | 3,550 | 0 | |
| | 11 | 10,150 | 1,421 | |
| | 15 | 6,700 | 402 | |
| | 20 | 6,025 | 241 | |
| | 20 | 7,140 | 428 | |
| | 21 | 11,255 | 1,463 | |
| | 28 | 14,245 | 855 | |
| | 69 | 7,535 | 377 | |
| Mean of days 11–69 (7) ±1 SEM | | 9,005 ±1,130 | 740 ±195 | 8 |
| Pneumococcal infection | 4 | 3,495 | 0 | |
| | 8 | 3,710 | 111 | |
| | 15 | 12,945 | 2,071 | |
| | 19 | 20,170 | 403 | |
| | 19 | 7,090 | 142 | |
| | 19 | 24,370 | 975 | |
| | 20 | 5,915 | 177 | |
| | 22 | 5,505 | 440 | |
| Mean of days 15–22 (6) ±1 SEM | | 12,665 ±3,275 | 700 ±300 | 6 |

## DISCUSSION

The organisms utilized for production of respiratory infection in this study were chosen because of their contrasting properties. The pneumococcus is an extracellular, pyogenic organism, whereas *Listeria monocytogenes* is a facultative intracellular bacterium. Humoral antibody against specific capsular polysaccharide and phagocytosis by polymorphonuclear leukocytes have been identified as important host defenses against *D. pneumoniae* (e.g. 41). On the other hand, cell-mediated immunity is the major protection against listeria (42). The latter organism, unlike the pneumococcus, is not a frequent cause of human pulmonary infection. However, listeria infection has proved to be a useful prototype for the study of the systemic immune response to facultative intracellular organisms. Thus information obtained from observations on the host response to LRT infection with these two organisms might be applicable to infections with similar bacteria.

The discovery that IgA is the major immunoglobulin in rabbit LRT secretions is not unexpected, since secretory IgA is the predominant immunoglobulin in several external secretions of many mammalian species. Furthermore, previous studies have revealed that IgA-containing cells are equal to or exceed other Ig-containing cells in human bronchial mucosa (43, 44). In view of these findings, it was somewhat surprising to find that IgG was the predominant immunoglobulin synthesized in the normal LRT. However, a single previous investigation of human respiratory tract in vitro immunoglobulin synthesis, utilizing a semiquantitative autoradiographic technique, also concluded that IgA is not invariably the major immunoglobulin produced in these tissues. Synthesis of IgG and IgA was estimated to be approximately equal in bronchial wall and lung tissues (45).

Why is local synthesis of IgA less than might be expected from previous observations on the number of IgA-containing cells in respiratory tract mucosa and from our data on the quantity of IgA in LRT secretions? There is no definitive answer to this question, but several possibilities should be considered. First, there may be a species difference between the rabbit and certain other animals, including man, to account for our observations. Available information suggesting that the rabbit and human secretory immunoglobulin systems are similar would argue against this possibility (46). However, it must be noted that IgG represents a greater percentage (and IgA a lesser percentage) of total Ig-containing cells in rabbit spleen and lymph node and of total rabbit serum Ig than in man (47). Second, the rate of synthesis and catabolism of secretory IgA by mucosal lymphoid tissue is unknown. If, for example, the IgA synthesis rate were considerably slower than that of IgG, the resulting data might be similar to what we observed. Third, it is probable that considerable quantities of IgG and IgM are produced by respiratory tract lymphoid tissue other than that located in the mucosa, while production of secretory IgA is largely limited to the latter position (48). Nonmucosal lymphoid nodules are seen along the bronchial network and are especially prominent after infection. Fourth, it is known that most

of the secretory (dimer) IgA is produced in plasma cells located near mucous membrane or glandular epithelial cells that produce "secretory piece" (44). Mucous-type epithelial cells, which contain especially large amounts of secretory piece, decrease in the distal bronchial tree and disappear at the bronchiolar level (49). This decrease in secretory piece-producing cells could account for diminished secretory IgA synthesis in the lung. Since each of these four possibilities is plausible, the question can only be answered by additional investigation.

Similarly, the response of the LRT after bacterial infection included a marked increase in production of IgG, some of which was antibody against the infecting organism. We are not aware of other local synthesis studies on bacterial respiratory infections, although Askonas and Humphrey studied the production of antibody in isolated lungs from rabbits hyperimmunized by intravenous administration of killed type III pneumococci. They observed the production of both specific antibody and other immunoglobulin, especially the latter. Unfortunately, their study, published in 1958, did not identify the class(es) of synthesized immunoglobulin (50). Our present observations are in keeping with a recent report that the local response of the hamster LRT to infection with *Mycoplasma pneumoniae* includes an increase in lymphoid cells containing IgM (early) and IgG class immunoglobulin (51).

Support for the belief that secretory IgA is preferentially secreted onto mucous membranes is found in our data. Although more IgG than IgA was synthesized in the LRT and serum IgG levels were 50 times those of IgA, the concentration of IgA was greater than that of IgG in lower respiratory secretions. The small quantity of IgA in rabbit serum (200 μg/ml) is unlikely to be the source of IgA in secretions, especially since the latter is presumably secretory IgA. In support of this concept, during immunoelectrophoresis a difference was noted in electrophoretic migration between the IgA in serum and that in respiratory secretions. This has been previously noted in comparison of rabbit serum IgA with rabbit secretory IgA from colostrum, intestinal secretions, and in vitro incubates of pyelonephritic kidneys (32, 40, 52). Furthermore, IgA class antibody against the infecting organism was found in secretions but not in the serum of infected animals. The IgA class antibody in secretions probably reflects the efficient secretion of, and thus concentration of, small amounts of antibody synthesized in the LRT. Unfortunately, we were unable to detect synthesis of such IgA class antibody in the LRT, presumably because these small amounts of IgA antibody could not be clearly separated from other immunoglobulin in the DEAE protein fractions containing IgA.

The origin(s) of the IgG antibody in respiratory secretion of animals infected with listeria can not be completely defined with the present data. Obviously, IgG class antibody was present in the serum of infected animals and could have been transported to secretions. However, there are indications that at least some of the IgG antibody in secretions was locally produced in the LRT. In these infected animals there was considerable variation in the ratios of antigen binding by IgG in serum as compared to secretions (Table I), in spite of the fact that the ratios of total IgG in serum to IgG in secretions were similar. For example, IgG in secretions of animals studied on days 15 and 16 bound antigen as well as or better than serum IgG. On the other hand, no IgG antibody was found in secretions of animals studied on days 12, 21, 28, and 69, in spite of serum IgG antibody activity. This lack of correlation between IgG antibody in serum and that in secretions suggests that in certain animals (especially days 15 and 16) locally synthesized IgG antibody contributed to the antibody in secretions. Inflammation of the infected LRT, with associated transudation of immunoglobulin into secretions, should also be considered as a possible determinant of IgG antibody in secretions. This process could transport both locally synthesized and serum antibody into secretions. Conversely, the absence of IgG antibody in secretions despite the presence of both locally produced and circulating antibody may be related to a waning of the inflammatory response (days 21, 28, and 69, Tables I and V).

The fact that only small amounts of IgA-specific antibody, or no antibody at all, is found in secretions of animals with *D. pneumoniae* infection, in spite of substantial quantities of IgG and IgM class circulating antibody, is of great interest. The possibility that "local" antibody in the LRT might be bound by pneumococcal polysaccharide persisting after the infection has been considered. Such persistence of polysaccharide after pneumococcal infection or injection of purified polysaccharide is well documented (e.g., 53). We have been unable to demonstrate the presence of pneumococcal polysaccharide in LRT secretions or in the lung incubation supernate after fractionation on DEAE cellulose. This does not, of course, entirely exclude the possibility that antibody in the LRT, either in the lungs or secretions, was bound by polysaccharide.

The biological significance of this immunoglobulin and specific antibody produced in response to bacterial infection of the LRT requires additional investigation. It is of obvious importance to determine if such antibody has opsonic or bactericidal activity or both, and whether it is related to recovery from infection or protection against reinfection with the same organism.

## ACKNOWLEDGMENTS

We are indebted to Ms. Betty Bradford for her patience and secretarial assistance.

This work was supported by a Veterans Administration Clinical Investigator award and by Veterans Administration institutional research funds.

Crystalline penicillin G sodium and sodium ampicillin were generously supplied by The Upjohn Company and Bristol Laboratories, respectively.

## REFERENCES

1. Green, G. M., and E. H. Kass. 1964. The role of the alveolar macrophage in the clearance of bacteria from the lung. *J. Exp. Med.* **119**: 167.
2. Green, G. M. 1968. Pulmonary clearance of infectious agents. *Annu. Rev. Med.* **19**: 315.
3. Tomasi, T. B., Jr., E. M. Tan, A. Solomon, and R. A. Pendergast. 1965. Characteristics of an immune system common to certain external secretions. *J. Exp. Med.* **121**: 101.
4. Tomasi, T. B., Jr., and J. Bienenstock. 1968. Secretory immunoglobulins. *Adv. Immunol.* **9**: 1.
5. Smith, C. B., R. H. Purcell, J. A. Bellanti, and R. M. Chanock. 1966. Protective effect of antibody to parainfluenza type I virus. *N. Engl. J. Med.* **275**: 1145.
6. Alford, R. H., R. D. Rossen, W. T. Butler, and J. A. Kasel. 1967. Neutralizing and hemagglutination-inhibiting activity of nasal secretions following experimental human infection with $A_2$ influenza virus. *J. Immunol.* **98**: 724.
7. Cate, T. R., R. D. Rossen, R. G. Douglas, Jr., W. T. Butler, and R. B. Couch. 1966. Role of nasal secretions and serum antibody in the rhinovirus common cold. *Am. J. Epidemiol.* **84**: 352.
8. Ogra, P. L., D. T. Karzon, F. Righthand, and M. MacGillivray. 1968. Immunoglobulin response in serum and secretions after immunization with liver and inactivated polio vaccine and natural infection. *N. Engl. J. Med.* **279**: 893.
9. Adinolfi, M., A. A. Glynn, M. Lindsay, and C. M. Milne. 1966. Serological properties of gamma A antibodies to *Escherichia coli* present in human colostrum. *Immunology.* **10**: 517.
10. Eddie, D. S., M. L. Schulkind, and J. B. Robbins. 1971. The isolation and biologic activities of purified secretory IgA and IgG anti-*Salmonella typhimurium* "O" antibodies from rabbit intestinal fluid and colostrum. *J. Immunol.* **106**: 181.
11. Wilson, I. D. 1972. Studies on the opsonic activity of human secretory IgA using an in vitro phagocytosis system. *J. Immunol.* **108**: 726.
12. Reynolds, H. Y., and R. E. Thompson. 1972. Pulmonary host defenses: role of antibody and phagocytosis by alveolar macrophages, Abstracts of Twelfth Interscience Conference on Antimicrobial Agents and Chemotherapy, 26–29 September, 1972, Atlantic City, N. J. Abstract 44, page 21.
13. Burdon, D. W. 1973. The bactericidal action of immunoglobulin A. *J. Med. Microbiol.* **6**: 131.
14. Keimowitz, R. I. 1964. Immunoglobulins in normal human tracheobronchial washings. A qualitative and quantitative study. *J. Lab. Clin. Med.* **63**: 54.
15. Masson, P. L., J. F. Heremans, and J. Prignot. 1965. Studies on the proteins of human bronchial secretions. *Biochim. Biophys. Acta.* **111**: 466.
16. Jurgensen, P. F., G. N. Olsen, J. E. Johnson, E. W. Swenson, E. M. Ayoub, and R. H. Waldman. 1972. Immunologic studies of human bronchoalveolar cells and secretions. *J. Clin. Invest.* **51**: 48*a*. (Abstr.)
17. Guyton, A. C. 1947. Analysis of respiratory patterns in laboratory animals. *Am. J. Physiol.* **150**: 78.
18. Myrvik, Q. N., E. S. Leake, and B. Fariss. 1961. Studies on pulmonary alveolar macrophages from the normal rabbit: a technique to procure them in a high state of purity. *J. Immunol.* **86**: 128.
19. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265.
20. Fahey, J. L., and E. M. McKelvey. 1965. Quantitative determination of serum immunoglobulins in antibody-agar plates. *J. Immunol.* **94**: 84.
21. Merrill, D., T. F. Hartley, and H. N. Claman. 1967. Electroimmunodiffusion (EID): a simple, rapid method for quantitation of immunoglobulins in dilute biological fluids. *J. Lab. Clin. Med.* **69**: 151.
22. Mayer, Manfred M. 1961. Kjeldahl nitrogen determination. *In* Experimental Immunochemistry. E. A. Kabat and M. M. Mayer, editors. Charles C Thomas Publisher, Springfield, Ill. 476.
23. O'Meara, R. A. Q., and A. M. Brown. 1936. On growing the pneumococcus with a view to the production of cultures of standard maximal density, virulence, and polysaccharide content. *J. Pathol. Bacteriol.* **42**: 679.
24. Green, G. M., and E. Goldstein. 1966. A method for quantitating intrapulmonary bacterial inactivation in individual animals. *J. Lab. Clin. Med.* **68**: 669.
25. Muraschi, T. F., and V. N. Tompkins. 1963. Somatic precipitinogens in the identification and typing of *Listeria monocytogenes. J. Infect. Dis.* **113**: 151.
26. Howard, J. G., G. H. Christie, M. J. Jacob, and J. Elson. 1970. Studies on immunological paralysis. III. Recirculation and antibody-neutralizing activity of $^{14}C$-labeled type III pneumococcal polysaccharide in paralysed mice. *Clin. Exp. Immunol.* **7**: 583.
27. Sutherland, I. W. 1967. The separation and purification of bacterial antigens. *In* Handbook of Experimental Immunology. D. M. Weir, editor. Blackwell Scientific Publications Ltd., Oxford, England. 355.
28. Newcomb, R. W., and K. Ishizaka. 1967. Human diphtheria antitoxin in immunoglobulin classes IgG and IgA. *J. Immunol.* **99**: 40.
29. Smiley, J. D., C. Sachs, and M. Ziff. 1968. In vitro synthesis of immunoglobulin by rheumatoid synovial membrane. *J. Clin. Invest.* **47**: 624.
30. Lehmann, J. D., J. W. Smith, T. E. Miller, J. A. Barnett, and J. P. Sanford. 1968. Local immune response in experimental pyelonephritis. *J. Clin. Invest.* **47**: 2541.
31. Hand, W. L., J. W. Smith, T. E. Miller, J. A. Barnett, and J. P. Sanford. 1970. Immunoglobulin synthesis in lower urinary tract infection. *J. Lab. Clin. Med.* **75**: 19.
32. Cebra, J. J., and J. B. Robbins. 1966. Gamma-A-immunoglobulin from rabbit colostrum. *J. Immunol.* **97**: 12.
33. VanDalen, A., H. G. Seijen, and M. Gruber. 1967. Isolation and some properties of rabbit IgM immunoglobin. *Biochem. Biophys. Acta.* **147**: 421.
34. Porath, J., R. Axen, and S. Ernback. 1967. Chemical coupling of proteins to agarose. *Nature (Lond.).* **215**: 1491.
35. Omenn, G. S., D. A. Ontjes, and C. B. Anfinsen. 1970. Fractionation of antibodies against staphylococcal nu-

clease on 'Sepharose' immunoadsorbents. *Nature (Lond.)*. **225**: 189.
36. Coonrod, J. D., and M. W. Rytel. 1973. Detection of type-specific pneumococcal antigens by counterimmunoelectrophoresis. I. Methodology and immunologic properties of pneumococcal antigens. *J. Lab. Clin. Med.* **81**: 770.
37. Stillman, E. G., and A. Branch. 1924. Experimental production of pneumococcus pneumonia in mice by the inhalation method. *J. Exp. Med.* **40**: 733.
38. Lawton, A. R., R. Asofsky, and R. G. Mage. 1970. Synthesis of secretory IgA in the rabbit. II. Production of alpha, light, and T. chains by *in vitro* cultures of mammary tissue. *J. Immunol.* **104**: 388.
39. Cohen, J. H., and M. Kern. 1969. Synthesis and secretion of γ-globulin by lymph node cells. VI. Characteristics of the structure of immunoglobulin A and its pattern of secretion by appendix cell suspensions. *Biochem. Biophys. Acta.* **188**: 255.
40. Smith, J. W., and W. L. Hand, and J. P. Sanford. 1971. Synthesis and secretion of local immunoglobulin by the intestinal tract of newborn rabbits. *J. Infect. Dis.* **124**: 494.
41. Wood, W. B., Jr. 1951. Studies on the cellular immunology of acute bacterial infections. *Harvey Lect.* **47**: 72.
42. Mackaness, G. B. 1969. The influence of immunologicalley committed lymphoid cells on macrophage activity in vivo. *J. Exp. Med.* **129**: 973.
43. Martinez-Tello, F. J., D. G. Braun, and W. A. Blanc. 1968. Immunoglobulin production in bronchial mucosa and bronchial lymph nodes, particularly in cystic fibrosis of the pancreas. *J. Immunol.* **101**: 989.
44. Tourville, D. R., R. H. Adler, J. Bienenstock, and T. B. Tomasi, Jr. 1969. The human secretory immunoglobin system: immunohistological localization of γ A, secretory "piece," and lactoferrin in normal human tissues. *J. Exp. Med.* **129**: 411.
45. van Furth, R., and F. Aiuti. 1969. Immunoglobulin synthesis by tissues of the gastro-intestinal and respiratory tracts. *Protides Biol. Fluids Proc. Colloq. Bruges.* **16**: 479.
46. Cebra, J. J., and J. A. O'Daly. 1971. Rabbit secretory IgA: molecular structure, isolation of its secretory component, and cellular localization of its parts. The Secretory Immunologic System. Proceedings of a Conference on the Secretory Immunologic System (Vero Beach, Fla.), December 10–13, 1969. U. S. Government Printing Office, Washington, D. C., 29.
47. Cebra, J. J. 1969. Immunoglobulins and immunocytes. *Bacteriol. Rev.* **33**: 159.
48. Tomasi, T. B., Jr. 1970. Structure and function of mucosal antibodies. *Annu. Rev. Med.* **21**: 281.
49. Bloom, W., and D. W. Fawcett. 1968. A Textbook of Histology. W. B. Saunders Company, Philadelphia. 9th edition. 629.
50. Askonas, B. A., and J. H. Humphrey. 1958. Formation of antibody by isolated perfused lungs of immunized rabbits. *Biochem. J.* **70**: 212.
51. Fernald, G. W., W. A. Clyde, Jr., and J. Bienenstock. 1972. Immunoglobulin-containing cells in lungs of hamsters infected with *Mycoplasma pneumoniae*. *J. Immunol.* **108**: 1400.
52. Smith, J. W., W. L. Hand, and J. P. Sanford. 1972. Local synthesis of secretory IgA in experimental pyelonephritis. *J. Immunol.* **108**: 867.
53. Stark, O. K. 1955. Studies on pneumococcal polysaccharide. Mechanism involved in production of "immunological paralysis" by type I pneumococcal polysaccharide. *J. Immunol.* **74**: 130.