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Antibody to *Mycoplasma pneumoniae* in Nasal Secretions and Sputa of Experimentally Infected Human Volunteers

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After experimental infection with *Mycoplasma pneumoniae*, 42% of 67 volunteers developed a threefold or greater rise in antibody in nasal secretions as measured by radioimmunoprecipitation. Development of an antibody increase in sputum was detected more often, i.e., in 73% of the volunteers. Each of the antibody increases involved immunoglobulin (Ig) A. Twelve rises in IgG antibody were detected in the specimens which exhibited a rise in IgA antibody. In almost every instance the rise in IgA antibody exceeded that seen with IgG antibody. Analysis of the response to experimental challenge with *M. pneumoniae* of volunteers with different levels of preexisting respiratory tract IgA antibody suggested that this secretory antibody was related to host resistance to *M. pneumoniae* disease. Further, respiratory tract IgA antibody appeared to be more directly related to host resistance than was antibody in serum.

During infection with *Mycoplasma pneumoniae* the site of localization of the organism appears to be superficial, involving only the epithelial layer of the respiratory passages (8). Studies in hamsters performed by Fernald and Clyde (11) suggest that local respiratory tract immune mechanisms play a greater role in resistance to the organism than do systemic immune mechanisms. Taken together, these findings suggest that evidence of specific *M. pneumoniae* immunity should be demonstrable in the respiratory tract or its secretions. Previous attempts to demonstrate local antibody to *M. pneumoniae* in the respiratory tract of experimentally infected volunteers failed, probably due to the relative insensitivity of the methods employed (2, 10, 20). However, Biberfeld and Sterner (3) reported the presence of immunoglobulin(Ig) A, IgG, and IgM antibodies in bronchial secretions of patients with lower respiratory illness due to *M. pneumoniae*, by use of immunofluorescence and complement fixation techniques.

This report describes the development of *M. pneumoniae* antibody in nasal secretions and sputa of a significant proportion of volunteers after intranasal inoculation of the organism. These antibodies were found to be predominantly of the IgA class. Furthermore, the presence of such antibody prior to challenge appeared to be correlated with resistance to illness. The possible implications of these findings to the problem of immunoprophylaxis of *M. pneumoniae* infections are discussed.

MATERIALS AND METHODS

Volunteers were healthy adult male inmates of the federal prison system or the Texas Department of Correction. The two studies included in this report were conducted to evaluate two different preparations of killed *M. pneumoniae* vaccine in man. The first study was performed in 1965-1966 and has been described in detail previously (20). The inactivated vaccine used in this study was prepared from a culture of the FH strain of *M. pneumoniae* grown in a medium consisting of a chemically defined solution of amino acids and vitamins enriched with a chloroform extract of egg yolk. The organisms were inactivated with Formalin and then concentrated by centrifugation. Twenty-two men, 16 vaccinees and 6 controls, were examined for local antibody prior to and at various time intervals after challenge with wild-type *M. pneumoniae*. Wild-type challenge was performed 6 weeks after the first of two injections of inactivated vaccine. Three of the vaccinees developed pneumonia, two developed febrile respiratory disease, and one de-

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veloped afebrile respiratory disease. In the unvaccinated control group two developed febrile respiratory disease and one developed afebrile respiratory disease. The second study was performed recently (1971). Forty-five men, 23 vaccinees and 22 controls, were studied for their local immune response after challenge with wild-type *M. pneumoniae*. Wild-type challenge was performed 4 weeks after the injection of inactivated vaccine. In this study two vaccinees developed febrile respiratory disease and 6 developed afebrile respiratory disease. In the control group of unvaccinated volunteers, six developed febrile respiratory disease whereas three had an afebrile illness. In the second study the vaccine was prepared by growing *M. pneumoniae* on the glass surface of 5-liter Povitsky bottles (23). The medium contained bovine serum fraction instead of horse serum. The cell sheet adhering to the glass surface was washed several times and scraped into distilled water. The organisms were then inactivated with Formalin.

Organisms and cultural conditions. *M. pneumoniae* strain PI 1428 in its second passage on artificial medium was used as the challenge inoculum. The same organism suspension was used in both studies. The growth medium has been described in detail previously (6). *M. pneumoniae* was grown on the glass surface of 5-liter Povitsky bottles containing 500 ml of medium, and the organisms were harvested after incubation at 37 C for 11 days by scraping the cell sheet into the broth medium. The suspension was distributed in glass ampoules and stored at −70 C until used. Volunteers were inoculated into the nasopharynx with 10^6 to 10^7 colony-forming units of this strain.

Collection and processing of specimens. Nasal secretions and sputa from the first study were collected and concentrated by lyophilization as described elsewhere (16). The nasal washings in study 2 were concentrated using Sephadex G200. The concentrated specimens were adjusted to approximately 10 to 20 mg of IgA per 100 ml (as determined with serum IgA standard).

Ig determinations. IgA, IgG, and IgM in nasal secretions and sputa were determined by use of the radial immunodiffusion method of Mancini et al. (17). Serum standards were used as reference. The diffusion plates and standards were obtained from Meloy Laboratories, Springfield, Va. The mean level of IgA was 32.5 mg/100 ml for sputa from study 1, 21.2 mg/100 ml for nasal washings from study 1 and 15.2 mg/100 for nasal washings from study 2. IgG levels were 19.6 mg/100 ml for sputa from study 1, 26.4 mg/100 ml for nasal washings from study 1, and 0.9 mg/100 ml for nasal washings from study 2. The reason for the low levels of IgG in the second study is not understood. Levels of IgM were low in each instance: 1.2 mg/100 ml for sputa from study 1, 4.5 mg/100 ml for nasal washings from study 1, and <1 mg/100 ml for nasal washings from study 2.

Antisera. Antisera to human IgA, IgG, and IgM produced in goats or sheep were obtained from Meloy Laboratories. The specificity of the antisera was determined in Ouchterlony double diffusion tests by use of whole human serum as antigen (18). Antiserum to whole human serum produced in a burro was also used. The antisera to human IgA and to whole human sera were shown to contain antibody to secretory IgA in double diffusion tests by use of purified human secretory IgA obtained from pooled, concentrated nasal washings as an antigen. The purified nasal IgA was kindley supplied by J. C. Perkins, formerly of The National Institutes of Health, and A. Jackson of Meloy Laboratories. Anti-IgG or anti-IgM did not show a precipitation line with the purified secretory IgA. The potency of the Ig antisera was assayed in a preliminary manner by immunodiffusion in agar. The IgA antiserum produced a visible precipitin line when diluted 1:32 and tested against purified nasal secretion IgA. Similarly the anti-IgG produced a precipitin reaction when diluted 1:16 and tested against human IgG (1 mg/ml). The anti-IgM was active at a dilution of 1:32 when tested against human IgM (1 mg/ml).

RIP test. The method for determination of radioimmunoprecipitation (RIP)-antibody to *M. pneumoniae* has been described in detail previously (H. Brunner and R. M. Chanock, Proc. Soc. Exp. Biol. Med., in press). Briefly, ^{14}C-oleic acid- and ^{14}C-palmitic acid-labeled *M. pneumoniae* organisms were filtered (450-nm pore size membrane filter, Millipore Corp.) and diluted to an activity of 500 to 1,000 dpm per 0.025 ml. Fourfold dilutions of nasal secretions or sputa were prepared in microtiter plates, and these diluted materials were incubated with 0.025 ml of labeled antigen for 60 min at 37 C and overnight at 4 C. Antigen-antibody complexes were precipitated by a 1:4 dilution of antiserum to human IgA, IgG, IgM, or a 1:8 dilution of antiserum for whole human serum. After centrifugation of the precipitates at 1,000 rpm for 10 min, the radioactivity remaining in the supernatant fluid was determined. The antibody titer was defined as the highest specimen dilution giving a 33% binding of antigen. Each specimen was tested in duplicate. Titers of secretions were calculated on the basis of 20 mg/100 ml of IgA.

MCT. The mycoplasmacidal test was based on the complement-mediated mycoplasma killing reaction originally described by Gale and Kenny (14). Details of the method were published earlier (5). For the study of large numbers of specimens which were available in small volume, the test was adapted to microtiter equipment. To 3 fourfold dilutions of the test specimen which were prepared in 0.05 ml of tris(hydrochloride)aminomethane - ethylenediaminetetraacetic acid-saline (TES)-buffered saline containing divalent cations and 0.1% gelatin, 0.1 ml of a filtered (450-nm pore size membrane filter, Millipore Corp.) suspension of *M. pneumoniae* organisms was added. After incubation for 60 min at 4 C, 0.05 ml of prediluted guinea pig serum which served as the source of complement was added and the plates were incubated for 120 min at 37 C. After vigorous shaking 0.1 ml of the reaction mixture was removed and diluted 1:100 in ice-cold TES-buffered saline to stop the reaction. After an additional 10-fold dilution, samples of each mixture were inoculated in triplicate on agar medium. The plates were incubated at 37 C for 8 to 12 days, and the number of colonies was counted by use of a dissecting microscope at a mag-

nification of ×25. Test specimen alone (in the highest concentration used), fresh guinea pig serum alone, and diluent alone were included in each assay as controls. Based on the average number of viable organisms in the controls, the extent of killing by test serum in the presence of complement was determined. The highest dilution of the best specimen which produced a 0% decrease in viability was calculated after logit transformation.

CF and MI. Complement fixation (CF) and metabolism inhibition (MI) procedures for measurement of serum antibodies were performed as described previously (25, 26).

RESULTS

Adequacy of anti-globulin antisera employed in RIP reaction. As shown in Table 1, RIP test performed with sera and nasal secretions from volunteers infected with *M. pneumoniae* (study number 2) yielded different antibody titers for IgG, IgA, and IgM. The predominant serum RIP antibody was found in the IgG fraction, whereas the antibody activity in nasal secretions appeared to be mainly IgA. These findings indicated that the IgG and IgA antisera were sufficiently potent for use in detecting these immunoglobulins by the RIP technique. Similarly, the IgM antiserum was capable of detecting IgM with *M. pneumoniae* specificity.

Antibody in respiratory tract secretions. *M. pneumoniae* antibody was detected in the majority (74%) of convalescent respiratory tract secretions tested. Antibody was also found in a smaller proportion (43%) of acute phase specimens. Consistent with the data shown in Table 1, most of the antibody activity was associated with IgA; each of the nasal secretion or sputum specimens which contained antibody activity had demonstrable IgA antibody for *M. pneumoniae*. IgG antibody was detected in 23% of 233 nasal secretion and sputum samples tested, but only 5 of these specimens contained IgM *M. pneumoniae* antibody.

TABLE 1. *Representative titers of IgG, IgA, and IgM antibody in serum and sputum as detected by RIP using anti-globulin antisera*

Anti-globulin used in RIP procedure	Pre serum	Post serum	Pre nasal section	Post nasal section
Volunteer A				
Anti-IgG	8,192	32,768	<2	<2
Anti-IgA	128	128	2.8	32
Anti-IgM	64	64	<2	<2
Volunteer B				
Anti-IgG	4,096	65,536	<2	<2
Anti-IgA	16	<16	<2.0	10.0
Anti-IgM	32	64	<2	<2

After experimental challenge with wild-type *M. pneumoniae*, 42% of 67 volunteers developed a threefold or greater rise in nasal secretion RIP antibody during convalescence (Table 2). Development of an antibody increase in sputum was observed more frequently, i.e., 73%. A threefold increase was considered significant because 12 replicate assays of a single nasal secretion yielded RIP antibody titers which varied less than twofold; the 95% confidence limits for this replicate assay were 1.15-fold. In addition, variation in replicate assays of several secretions performed on different days was never more than twofold. Previously, a similar reproducibility had been demonstrated for RIP serum antibody.

Each of the 43 antibody rises observed in nasal secretions or sputum involved IgA. Twelve rises in IgG antibody were detected in the specimens which exhibited a rise in IgA antibody. In almost every instance the rise in IgA antibody was greater than that seen with IgG. In no instance was an IgG antibody rise detected in the absence of an IgA response. A rise in IgM antibody was not detected. The specificity of the antibodies detected in postchallenge nasal secretions and sputa was further investigated by attempting to block antibody activity by incubation of secretions or sputum with mycoplasma broth, containing horse serum and yeast extract, prior to performance of the RIP test. This approach was taken since the volunteers were challenged with *M. pneumoniae* organisms grown in broth containing horse serum and yeast extract. Conceivably, the challenge inoculum could have stimulated antibodies against horse serum or yeast extract proteins. In addition, the RIP antigen could have contained horse serum or yeast extract antigens adsorbed to the surface of *M. pneumoniae* organisms, although this is unlikely since the organisms were grown on glass and were washed extensively. Nevertheless, the secretory antibodies which developed after challenge could have been directed against horse serum or yeast extract protein rather than *M. pneumoniae*. For this reason we incubated five postchallenge sputa and three postchallenge nasal secretions with an equal volume of a 1:10 or 1:20 dilution of complete mycoplasma broth for 1 h at 37 C, and then for 18 h at 4 C, thus providing an excess of horse and yeast proteins for absorption of antibodies against these antigens. As a control, the nasal secretions or sputa were preincubated with buffer for 1 h at 37 C, and then for 18 h at 4 C. After the 18-h incubation interval the nasal secretions or sputa, now diluted 1:2, were tested by the standard RIP procedure. In no instance did prior absorption

TABLE 2. *Secretory-antibody response to experimental infection with M. pneumoniae*

Study	Inactivated *M. pneumoniae* vaccine prior to challenge	No. of men	No. with ≥3-fold increase in IgA antibody in		Geometric mean titer				Serum MI antibody		
			Nasal secretion	Sputum	Nasal secretion		Sputum		No. with ≥4-fold increase	Geometric mean titer	
					Prechallenge	Postchallenge	Prechallenge	Postchallenge		Prechallenge	Postchallenge
1	Yes	16	4	10	1:1.4	1:2.3	1:3.3	1:20.4	15	1:1.8	1:51.3
	No	6	3	6	1:1.6	1:2.8	1:2.8	1:19.5	6	1:1.0	1:61.0
2	Yes	23	11		1:2.2	1:8.5			8	1:10.7	1:24.8
	No	22	9		1:1.5	1:4.3			20	1:1.4	1:15.4

TABLE 3. *Representative antibody responses of volunteers infected with M. pneumoniae (PI 1428 passage 2) (study 1)*

Volunteer	Specimen	Procedure	Antibody titer (reciprocal)[a] at indicated week after experimental infection with *M. pneumoniae*				
			Before infection	1	2	3	4
1	Sputum	RIP (IgA)	<2.0	NS[b]	2.4	45.7	NS
		RIP (IgG)	2.0	NS	2.0	4.0	NS
		MCT	<2.0	NS	<2.0	<2.0	NS
	Serum	MI	48				64
2	Sputum	RIP (IgA)	<2.0	11.4	9.4	3.8	53.3
		RIP (IgG)	<2.0	4.7	<2.0	<2.0	<2.0
		MCT	<2.0	<2.0	<2.0	<2.0	<2.0
	Serum	MI	2				24
3	Nasal secretion	RIP (IgA)	<2.0	2.5	<2.0	10.0	<2.0
		RIP (IgG)	<2.0	<2.0	<2.0	3.2	<2.0
	Sputum	RIP (IgA)	7.0	14.0	17.8	45.7	NS
		RIP (IgG)	<2.0	4.4	7.2	<2.0	NS
		MCT	<2.0	<2.0	<2.0	<2.0	NS
	Serum	MI	3				24
4	Sputum	RIP (IgA)	NS	<2.0	38.8	301.2	NS
		RIP (IgG)	NS	<2.0	4.4	<2.0	NS
		MCT		<2.0	<2.0	<2.0	
	Serum	MI	1.5				128

[a] Antibody titers adjusted to 20 mg of IgA per 100 ml.
[b] No specimen.

with mycoplasma broth reduce the titer of secretory antibody as measured with antiserum against IgA or whole human serum. These findings suggest that the secretory antibodies which we measured after experimental challenge were directed against *M. pneumoniae* antigens.

Representative antibody responses of the volunteers in the two studies are shown in Tables 3 and 4. IgA secretory antibody responses were usually more pronounced in sputum than in nasal secretions. Of interest was the dissociation of systemic and local respiratory antibody responses shown by volunteers 3 and 8 in Table 4. These men developed a nasal secretion antibody rise without an accompanying serum antibody response.

As shown in Table 3, MCT antibody was not detected in sputum. This supports the view that IgG and IgM antibodies to *M. pneumoniae* were not present in appreciable concentration in sputum since antibody measured in MCT is

TABLE 4. *Representative antibody responses of volunteers infected with M. pneumoniae (PI 1428 passage 2) (study 2)*

Volunteer	Reciprocal of preinfection (above) and 4 weeks postinfection (below) antibody titer			
	Nasal secretion		Serum	
	RIP[a] (IgA)	RIP (IgG)	CF	MI
1	<2.0	<2	4	<2
	16.0	<2	128	64
2	5.0	<2	16	16
	12.8	<2	16	16
3	2.6	<2	64	12
	17.7	<2	64	16
4	2.8	<2	32	<2
	32.0	<2	>128	16
5	<2.0	<2	32	<2
	10.0	<2	16	24
6	6.4	<2	128	64
	6.6	<2	128	24
7	<2.0	<2	16	12
	7.0	<2	32	24
8	<2.0	<2	32	12
	13.2	<2	32	6
9	<2.0	<2	4	<2
	7.6	<2	64	6
10	<2.0	<2	32	<2
	8.9	<2	64	12

[a] Nasal antibody titers adjusted to 20 mg of IgA per 100 ml.

complement dependent and therefore should be primarily of the IgG and IgM classes.

As shown in Fig. 1, the geometric mean titer of IgA antibody to *M. pneumoniae* in sputum increased during the first 3 weeks after infection and apparently decreased thereafter. IgG antibody was detected in some sputa, but an increase in geometric mean titer was not observed. The values in the figure represent geometric means obtained on the 22 volunteers of the first study. Figures 2 and 3 represent the data obtained on the nasal washings from study 1. As shown in Fig. 2, IgA antibody to *M. pneumoniae* in nasal secretions increased during the first week, remained at this level for the following 2 weeks, and showed a tendency to decrease thereafter. When an antiserum to whole human serum was employed to precipitate antigen-antibody complexes, the geometric mean titer of antibody in nasal secretions showed a more pronounced increase which continued over the 4-week observation period (Fig. 3). The increased reaction of the anti-whole human serum may have been due to its stronger antibody activity as compared to the anti-IgA or IgG serum.

Role of local antibody in protection against M. pneumoniae disease. After the development of the RIP test it was possible to assess the role of local antibody in resistance to *M. pneumoniae* disease. For this type of analysis we used the data obtained from the second volunteer study, since prechallenge specimens were not available from all participants in the first study. After challenge with 10^6 to 10^7 colony-forming units of virulent, wild-type *M. pneumoniae*, 42 of 45 volunteers became infected. The men who became ill tended to have lower nasal IgA antibody titers than the volunteers who failed to develop disease (Fig. 4). ($P < .01$; Wilcoxon-Mann-Whitney-test) (15). The volunteers from this study were divided into two

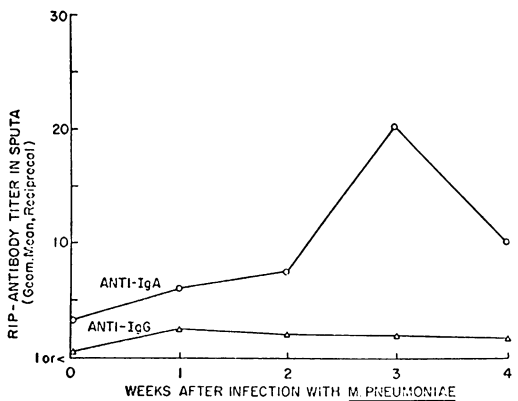

FIG. 1. *Geometric mean RIP titer of IgA and IgG M. pneumoniae antibody in sputum at indicated time after experimental infection (study 1).*

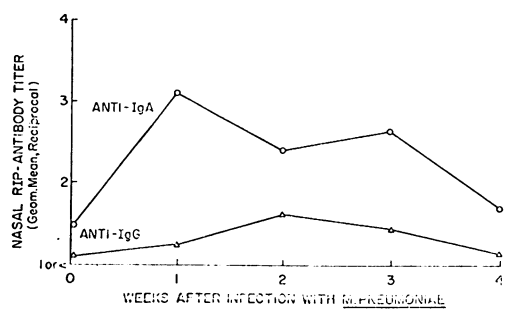

FIG. 2. *Geometric mean RIP titer of IgA and IgG M. pneumoniae antibody in nasal secretions at indicated time after experimental infection (study 1).*

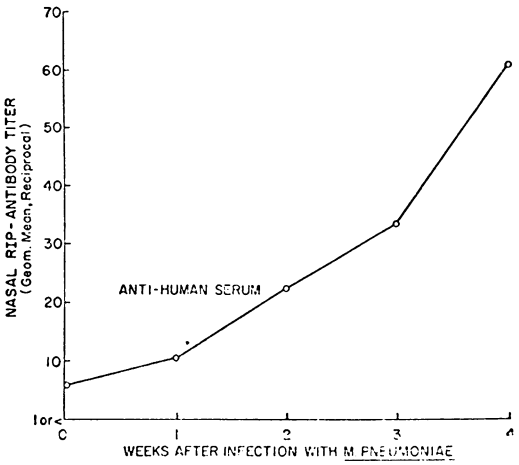

FIG. 3. *Geometric mean RIP titer of total M. pneumoniae antibody in nasal secretions at indicated time after experimental infection (study 1).*

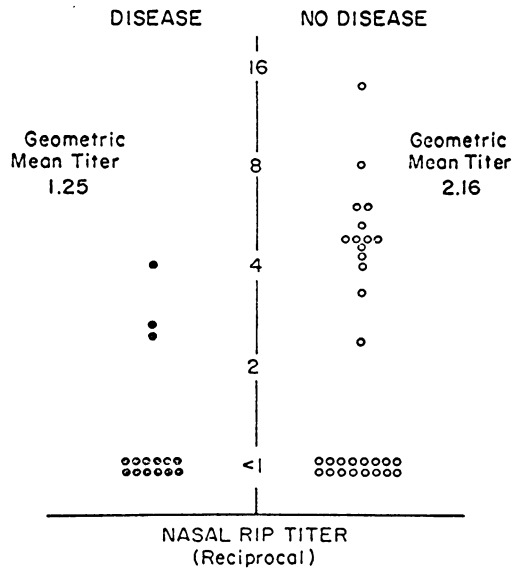

FIG. 4. *Prechallenge RIP titer of IgA M. pneumoniae antibody in nasal secretions of volunteers in study 2.*

approximately equal groups of men, one with low serum MI antibody (1:8 or less) and the other with high serum antibody (>1:8). These groups were then subdivided into subgroups of men with low (<1:3) or high (>1:3) nasal secretion antibody. Men with low levels of RIP nasal antibody (<1:3) developed *M. pneumoniae* disease with higher frequency than men with a high antibody titer (>1:3), irrespective of serum titer (Table 5). The correlation of nasal antibody with resistance was significant for the group of men with a serum antibody titer of 1:8 or more ($P = 0.04$; Fisher exact test). In contrast, a correlation of serum antibody with resistance was not evident ($P > 0.30$). These findings suggest that local IgA antibody, as measured by the RIP test, was related to resistance to *M. pneumoniae* disease.

Correlation of rise in local antibody with disease. There was also a correlation observed between the occurrence of illness and the development of a local secretory antibody response as shown in Table 6. Men with *M. pneumoniae* disease developed a rise in local antibody significantly more frequently than men who failed to become ill.

Correlation of nasal secretion RIP antibody and serum CF and MI antibodies. Volunteers who possessed nasal secretion RIP antibody also tended to have serum CF and MI antibodies (Fig. 5 and 6). Individuals with high levels of RIP antibody generally had high levels of serum antibodies.

DISCUSSION

Although reinfection with *M. pneumoniae* occurs with appreciable frequency under epidemic conditions, the decrease in incidence of

TABLE 5. *Relationship of preexisting antibodies in serum and nasal secretions to resistance to experimental challenge with M. pneumoniae (strain PI 1428, passage 2)*

RIP (IgA) antibody titer in nasal secretions	Serum MI antibody titer			
	≤1:8		>1:8	
	No. with disease	No. without disease	No. with disease	No. without disease
<1:3	9[a]	9[a]	5[b]	7[b]
>1:3	1[c]	5[c]	0[d]	9[d]

[a, b, c, d] Significance values: *a* vs *b*, $P > 0.3$; *c* vs *d*, $P > 0.3$; *a* vs *c*, $P = 0.18$; *b* vs *d*, $P = 0.04$.

TABLE 6. *Relationship of clinical response to M. pneumoniae to development of local nasal secretion IgA antibody*[a]

Clinical response	No. of men with indicated response in nasal IgA antibody titer after challenge with *M. pneumoniae* (PI 1428 passage 2)	
	Rise (≥3-fold)	No rise
Respiratory tract disease	12	3
No disease	8	22

[a] $P < 0.01$ (Chi square with Yates correction).

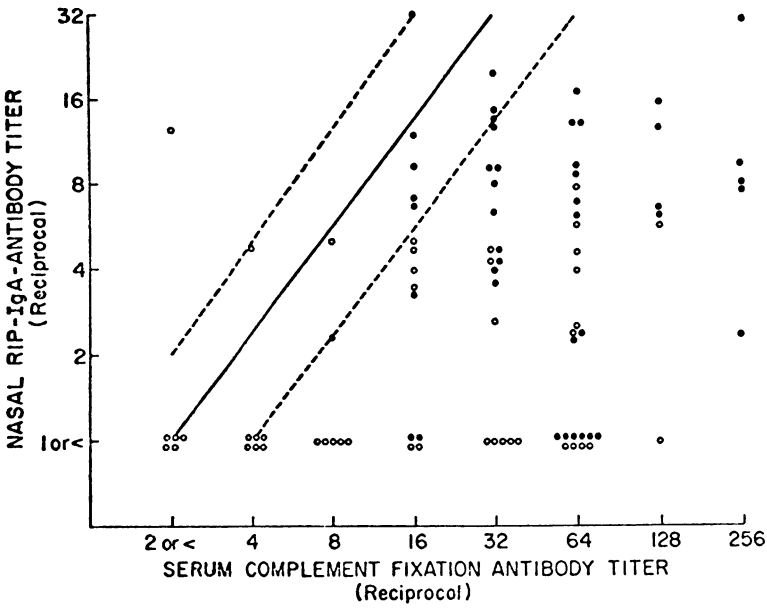

FIG. 5. *Relationship of RIP titer of IgA antibody in nasal secretions to titer of serum complement fixation antibody. Open circles denote preinfection values, and closed circles denote postinfection values.*

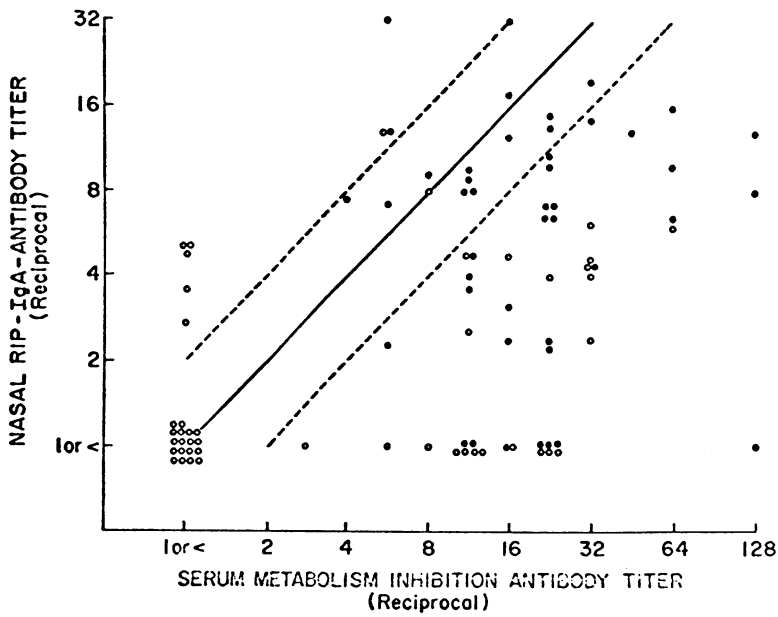

FIG. 6. *Relationship of RIP titer of IgA antibody in nasal secretions to titer of serum metabolism inhibition antibody. Open circles denote preinfection values and closed circles postinfection values.*

M. pneumoniae disease which occurs with increase in age after the third decade suggests that infection stimulates host defense mechanisms which are relatively effective in protecting against this type of illness (13, 24). In the experimentally infected hamster, *M. pneumoniae* localizes on the surface of the ciliated respiratory epithelium of the bronchi (8). Invasion of the lung parenchyma does not appear to occur. This type of superficial infection suggests

that the most effective protection would be provided by immunological defense mechanisms which operate at the epithelial surface of the respiratory tract. For this reason, it was not surprising to detect IgA antibody for *M. pneumoniae* in respiratory tract secretions. Previously Biberfeld and Sterner (3) had described the presence of antibody in sputum after natural infection with *M. pneumoniae*. These antibodies were detected in IgA, IgG, and IgM globulins in comparable amounts, and for this reason the possibility that the sputum specimens were contaminated with serum could not be excluded. In the present study significant increases in antibody activity in nasal secretion and sputum were predominantly in the IgA class of globulins. These results are in good agreement with the nature of the local respiratory tract antibody response to viruses (9, 19, 21, 27).

Failure to detect *M. pneumoniae* antibody in respiratory tract secretions in previous studies can be attributed in part to the relative insensitivity of the methods used when compared to the RIP procedure (2, 10, 20). In addition, the test methods previously used included the metabolism-inhibition antibody assay which measures complement-dependent antibody.

In the present study an increase in local IgA antibody was detected after experimental infection of a majority of the volunteers studied. Furthermore, analysis of the response to experimental *M. pneumoniae* challenge of volunteers with different levels of preexisting respiratory tract IgA antibody suggested that this secretory antibody was related to host resistance to *M. pneumoniae* disease.

Fernald et al. (12) recently reported that hamsters infected with *M. pneumoniae* developed a peribronchial mononuclear cell response in the lung which involved primarily IgM-containing cells although some IgA-containing cells were seen. Subsequently this was followed by the accumulation of non-immunoglobulin-containing mononuclear cells which were interpreted as a cell-mediated immune response. Our findings are not necessarily at variance with those of Fernald et al. since antibodies produced by IgM cells in the respiratory tract are probably destined for circulation in serum. In contrast, the product of IgA cells would be expected to be found primarily in the local secretions of the respiratory tract. In any case, at this point is is not possible to decide whether cellular immunity is more important than that associated with local secretory antibody. It is clear, however, that secretory antibody is correlated in some manner, either directly or indirectly, with resistance to *M. pneumoniae* disease.

If local antibody does not kill *M. pneumoniae* how could it be effective in protecting the host? We have suggested previously that the organism damages lung tissue by the production and release of H_2O_2 (7, 22). This type of epithelial cell damage is made possible by the ability of the organism to adsorb to the surface of respiratory epithelium and thus H_2O_2 can be delivered directly to the target area without inactivation from extracellular catalase and peroxidase. In this situation antibody might have an effect by preventing adsorption of organisms onto the respiratory epithelium. This type of effect has recently been described for certain oral bacteria which attach to the buccal epithelial surface (28). It is also possible that IgA antibody might be opsonic and aid in phagocytosis of *M. pneumoniae*. This possibility is rather unlikely, since IgA does not appear to be efficient in opsonization (4).

Our findings in this study have several implications to current efforts for the prevention of *M. pneumoniae* disease by immunoprophylaxis. First, the existence of a local secretory IgA *M. pneumoniae* antibody system in the respiratory tract offers additional hope for the success of a live vaccine. Presumably, attenuated live organisms introduced into the upper respiratory passages should be able to stimulate a local IgA antibody response. Second, the correlation of local antibody with resistance to *M. pneumoniae* disease suggests that this type of antibody should be measured when experimental vaccines, live or inactivated, are being evaluated for evidence of antigenicity. It is likely that the RIP measurement of local antibody will provide a helpful new parameter to vaccine evaluation.

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