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Comparison of Enzyme-Linked Immunosorbent Assay and Radioimmunoprecipitation Test for Detection of Immunoglobulin A Antibodies to *Mycoplasma pneumoniae* in Nasal Secretions

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An immunoglobulin A (IgA) enzyme-linked immunosorbent assay was developed and compared with the radioimmunoprecipitation test in determinations of IgA antibodies to *Mycoplasma pneumoniae*. Of 45 nasal secretions obtained from infected volunteers, 42 (93.4%) were positive and 2 (4.4%) were negative in both tests. The IgA enzyme-linked immunosorbent assay is at least as sensitive as the IgA radioimmunoprecipitation test but is simpler and safer and should therefore be preferred.

Mycoplasma pneumoniae is a common etiological agent of respiratory diseases in humans (4, 5, 10). Previous studies have revealed that local immunoglobulin A (IgA) antibodies are more important than serum antibodies in resistance to infection with these organisms (2, 7). To detect these protective antibodies in secretions from the respiratory tract, immunofluorescence tests (1) and radioimmunoassays (2, 3) have been developed and used in the past. This report compares the results of an enzyme-linked immunosorbent assay (ELISA) with those of a radioimmunoprecipitation test (RIP) (2) in detection of IgA antibodies to *M. pneumoniae* in nasal secretions of volunteers inoculated with this organism.

Samples of nasal secretions were obtained from 45 volunteers inoculated intranasally with the temperature-sensitive mutant H43 of *M. pneumoniae* (8). Samples, kindly provided to us by R. M. Chanock, National Institutes of Health, Bethesda, Md., were collected and processed as described previously (2, 8).

Control specimens collected from 10 healthy individuals were processed identically. Total IgA levels were determined in nasal secretions by the Mancini radial immunodiffusion method (9). The RIP was performed as previously described in detail (2). The ELISA, described by Engvall and Perlmann (6) and Van Weemen and Schuurs (13) was adapted for detection of *M. pneumoniae*-specific IgA antibodies. By using the FH strain of *M. pneumoniae*, kindly provided by J. G. Tully (National Institutes of Health), organisms were cultivated in SP-4 broth medium (12) and lysed in the presence of digitonin, and the cell membranes were collected (11). ELISA plates (no. 76-381-04; Linbro; Flow Laboratories, Inc., McLean, Va.) were coated with 100-μl portions of *M. pneumoniae* antigens at 20 μg/ml. Nasal secretions were tested in serial twofold dilutions, beginning with a dilution ratio of 1:4. IgA antibodies were detected by using affinity-purified goat anti-human IgA antibodies (α-chain specific) conjugated to alkaline phosphatase (no. 2491; Tago Inc.). A reaction was considered positive in the ELISA if the absorbance obtained in a well was twice that of the mean absorbance exhibited in eight wells containing a pool of 10 nasal secretions from healthy individuals. Individual IgA titers were determined as the highest dilution of each sample yielding a positive reaction.

The specificity of the IgA ELISA in determinations of IgA antibodies to *M. pneumoniae* was examined in a series of competition experiments. Samples from 10 nasal secretions positive for IgA anti-*M. pneumoniae* antibodies and from 10 negative control samples were pooled to construct positive and negative controls. To IgA ELISA plates coated with antigens prepared from *M. pneumoniae* FH, samples of positive and negative nasal secretions were added with various concentrations of antigens prepared from the following organisms: *M. pneumoniae* FH, *M. pneumoniae* PI 1428, *M. pneumoniae* B16, *M. hominis*, *M. salivarium*, *M. fermentans*, *Ureoplasma urealyticum*, *M. orale*, *M. pulmonis*, and *Acholeplasma laidlawii*. Sp-4 medium and phosphate-buffered saline were used for controls. Competition was observed only among the antigens from the three strains of *M. pneumoniae* and those of the FH strain used for coating the IgA ELISA plates. All other antigens prepared from different mycoplasma species did not affect the reactions between IgA antibodies and *M. pneumoniae* antigens, thus confirming the specificity of the assay. Furthermore, when IgA ELISA plates were coated with equal concentrations of antigens prepared from all these mycoplasma strains and species, negative IgA ELISA results were obtained with the IgA-positive nasal secretions and all the non-*M. pneumoniae* antigens, while the plates exhibited almost identical positive titers with all three *M. pneumoniae* strains.

These data and those reported previously (2, 8) demonstrate that IgA antibodies present in nasal secretions or sputa of individuals inoculated with the temperature-sensitive mutant H43 or with the wild-type FH strain of *M. pneumoniae* can be detected in either the RIP or the ELISA performed with antigens from the FH and PI 1428 strains of this organism.

Specimens of nasal secretions from infected and control individuals were examined in the two different assays for IgA antibodies to *M. pneumoniae* and for their total IgA levels. The RIP was performed in Germany (laboratory of H.B.), and the ELISA was performed in Israel (laboratory of Y.N.). Individual titers of each sample were subsequently normalized to a basis of 20 mg of total IgA per 100 ml (2, 3).

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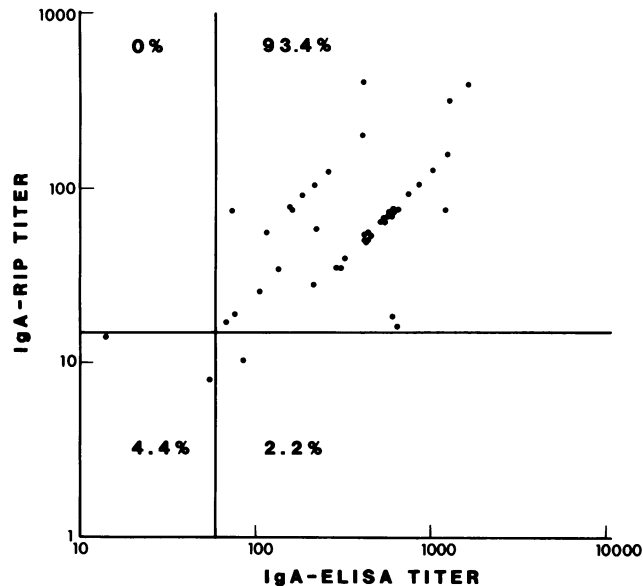

FIG. 1. Scattergram comparison of *M. pneumoniae*-specific IgA antibody titers determined in 45 samples of nasal secretions by using the IgA RIP and the IgA ELISA. Titers presented are normalized to 20 mg of IgA per 100-ml sample. Normalized IgA RIP titers of >1:15 and IgA ELISA titers of >1:60 were considered positive. The percentage of the samples which fall in each quadrant is indicated.

IgA RIP and IgA ELISA titers obtained were normalized by the following equation: IgA titer × 20/sample IgA level (mg) per 100 ml.

The scattergram shown in Fig. 1 compares the normalized IgA antibody titers obtained in the RIP to those obtained in the ELISA in 45 nasal secretions from infected individuals.

Forty-two (93.4%) of the samples were positive in both tests with corresponding normalized titers of greater than 1:15 and greater than 1:60 in the IgA RIP and IgA ELISA, respectively. One sample (2.2%) was positive in the IgA ELISA but negative in the IgA RIP. This sample, which contained 7.5 mg of total IgA per 100 ml, had an IgA ELISA titer of 1:32 (corresponding to a normalized titer of 1:85.3) and was negative (<1:4) in the IgA RIP with a normalized titer of less than 1:10.6. Two (4.4%) other samples of nasal secretions from infected individuals were negative (titers of less than 1:4) in both tests. These two samples contained only 4.2 and 5.7 mg of total IgA per 100 ml, compared with 12.7 ± 7.7 mg of IgA per 100 ml (the mean of all samples). The normalized titers of these two negative samples were less than 1:15 in the IgA RIP and less than 1:60 in the IgA ELISA. All control samples of nasal secretions from healthy individuals when tested individually had normalized IgA titers of less than 1:15 in the IgA RIP and less than 1:60 in the IgA ELISA. The IgA ELISA titers were generally severalfold higher than those determined in the IgA RIP, suggesting that the ELISA is more sensitive than the RIP in detection of IgA antibodies to *M. pneumoniae* (Fig. 1). Previous studies with the RIP have demonstrated rises in local IgA antibody titers in sputa, as well as in nasal secretions obtained from volunteers infected with the wild-type FH strain of *M. pneumoniae* (2) or with the temperature-sensitive mutant H43 of *M. pneumoniae* (8) and have demonstrated that local IgA antibodies to *M. pneumoniae* are more important than systemic antibodies in resistance to infection. With this organism it is necessary to provide sensitive and specific assays for determination of this class of antibodies for appropriate evaluation of the protective immunity exhibited by individuals previously exposed to this organism by natural infection or vaccination.

Our data demonstrate a clear agreement between the IgA RIP results and the IgA ELISA results, since 44 (97.8%) of the nasal secretions tested were either positive or negative in both tests.

However, the at least comparable sensitivity of the IgA ELISA, the simplicity of the test, and the advantages of avoiding the use of radioactive isotopes lead to the conclusion that the newly developed IgA ELISA should be preferred for determinations of local IgA antibodies to *M. pneumoniae*.

To determine the exact relationships between local IgA ELISA titers and resistance to *M. pneumoniae* disease, further large-scale studies should be performed with nasal secretions obtained from individuals infected naturally with *M. pneumoniae*.

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