Precipitating Antibodies in Mycoplasma Infection

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The effectiveness of counterimmunoelectrophoresis (CIEP) for detecting human precipitating antibodies to mycoplasma antigen was compared with the conventional complement fixation (CF) method in a double-blind experiment. Fifty-one sera from patients suspected of having acute mycoplasma infection were tested by both techniques. Dense precipitin lines to mycoplasma antigen developed in 28 sera with CIEP. Twenty-six of 28 had elevated CF titers to this antigen. No precipitin bands were observed in sera with low antibody titers to mycoplasma. These findings indicate that the CIEP test is a specific method for reliably detecting elevated serum CF antibody levels in patients with acute or recent mycoplasma infection.

Recently, we reported a counterimmunoelectrophoresis (CIEP) test for detecting human precipitating antibody to mycoplasma antigen (4). The results indicated that the CIEP test was highly specific for serum mycoplasma antibody, but its general applicability could not be fully ascertained due to the limited number of mycoplasma cases available.

This study was carried out to determine the reliability and sensitivity of CIEP for the serological diagnosis of acute mycoplasma infection. A large number of individuals with a clinical syndrome consistent with mycoplasma infection, and controls, were tested for complement-fixing (CF) and precipitating antibodies (by CIEP) to mycoplasma antigen in a double-blind experiment.

MATERIALS AND METHODS

Sera and serology. Fifty-one sera were collected over a 15-month period from patients who presented symptoms suggestive of an acute mycoplasma infection (7) and submitted to the viral diagnostic laboratory of St. Luke's Hospital Center, New York. Sera were stored at -20°C , and only samples for CIEP were heat inactivated prior to use. A standard CF microtiter technique, as described by Lennette (6) with commercially prepared mycoplasma antigen (Flow Biologics, Rockville, Md.), was performed with 8 U of antigen and 1.7 U of complement and incubated overnight at 4°C .

The experiment was double blind. Test samples were assigned a code number at St. Luke's Hospital Center where the CF test was performed. The coded sera were sent to the East Orange Veterans Administration Hospital where the CIEP test was performed independently without knowledge of the clinical or CF findings.

CIEP. Mycoplasma CF antigen, lot no. 7514, and control media, no. 5125, obtained from Microbiological Associates, Bethesda, Md., were stored at -80°C in small 250- μl portions in Beem capsules. The CF antigen titer was 1:8. Unused portions of thawed antigen were discarded. Human hyperimmune serum was used as a positive control since commercial high-titered sera prepared in animals gave multiple precipitin bands to control media as well as to test antigen.

CIEP was carried out on 0.75% agarose gels, prepared by boiling 750 mg of agarose in 100 ml of barbital buffer (pH 8.6; ionic strength 0.05), and layered on glass microscope slides (5 by 7.5 cm). Gels were stored overnight in a moist chamber at 4°C and used within 3 weeks. Paired wells 5 and 3 mm in diameter were made 3 mm apart with a metal punch to accommodate 21 serum samples per slide, including a positive and negative control. A two-step electrophoresis technique was used. First, 25 μl of serum samples were placed in the 5-mm anodal wells, and an initial electrophoresis step was performed for 10 min with 30 mA of current (12 V/cm of agar surface) at room temperature. Without removing the gel from the chamber, 15 μl of antigen was added to the 3-mm cathodal wells, and electrophoresis was carried out for an additional 50 min. Electrophoresis of the sera prior to the addition of antigen resulted in a greatly sharpened precipitin line more centrally positioned between the paired wells. A dense precipitin band between the paired wells was best viewed in indirect light against a black background. Specificity of the precipitin lines was confirmed by retesting positive sera against both antigen and control media.

RESULTS

Fifty-one coded sera were tested by the CF and CIEP tests. Figure 1 shows that 28 of the 51

FIG. 1. *Mycoplasma* complement fixation antibody titers in grouped CIEP-positive and -negative sera. The positive group has markedly elevated complement fixation titers compared with those of the negative group.

sera were positive in the CIEP test, and the 23 remaining sera were interpreted as negative. When the code was broken, the CIEP-positive group of sera had markedly elevated CF titers to mycoplasma antigen (mean, 1:350). The test successfully identified all sera with a CF titer of 1:128 or greater and was negative in all sera with CF titers of 1:16 or less. The only discrepancy between the two serological methods appeared with sera of intermediate CF titers in the range of 1:32 to 1:64. Two samples were positive by CIEP, and two others were interpreted as negative.

DISCUSSION

The CIEP test has been used in the rapid diagnosis of a number of infectious diseases (3, 8). Recently, we developed a CIEP test for detecting precipitating antibody to mycoplasma antigen (4). A similar test for mycoplasma precipitating antibodies has been described by Low (Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, D30, p. 56).

The aim of this study was to confirm and extend our recent preliminary report showing

that the CIEP test can reliably identify sera containing elevated CF antibody titers to mycoplasma antigen. The data presented show that the CIEP method successfully recognized all 26 sera with elevated CF antibody titers of 1:128 or higher to mycoplasma antigen. In contrast, all sera with low (1:16) or no CF titers were negative. Only four sera, with intermediate CF titers of 1:32 and 1:64, were at variance with the CF findings. Two samples were positive by CIEP, and two were negative. If a patient is seen and mycoplasma infection is a prime consideration, the CIEP and/or CF test may be negative when low levels of antibody are present. In this situation, a diagnostic laboratory would have to rely on more elaborate, time-consuming techniques of metabolic inhibition, mycoplasmicidal testing, and radioimmunoassay, all of which require sophisticated equipment and skilled laboratory personnel (1). In contrast to CF, and the more sensitive "cidal" assay for detecting mycoplasma antibodies, CIEP is an extraordinarily rapid method, requiring only 60 min to perform. It requires no expertise and utilizes readily available commercial reagents and inexpensive routine laboratory electrophoretic equipment. Thus, the CIEP test appears to be ideally suited for the conventional hospital laboratory.

In a previous communication (4), we found that we were unable to serially dilute sera more than 1:4 from patients with mycoplasma precipitating antibodies without loss of visible precipitin bands, indicating that CIEP was far less sensitive than CF testing for antibody detection. In a CIEP test for detecting cytomegalovirus precipitating antibody, we were similarly unable to dilute strongly positive sera more than 1:4 (2). By contrast, detection of cytomegalovirus and mycoplasma antigen by CIEP was only slightly less sensitive than CF techniques. This also has been noted for herpes type I and type II viruses (5).

In this study, the excellent correlation between high CF titers and CIEP positivity indicates that the latter test is reliable and sensitive for detecting elevated antibody titers to mycoplasma in sera from patients with acute or recent mycoplasma infection. However, in patients who do not generate high CF antibody responses, the CIEP test will be of limited value.

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