

Pneumonia with Serological Evidence of Acute Infection with the Chlamydia-like Microorganism "Z"

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"Z" is a recently discovered microorganism that may belong to a new genus in the family Chlamydiaceae. Using an ELISA test we developed, we measured levels of serum antibody against "Z" for 308 paired sera obtained from adult patients hospitalized with community-acquired pneumonia (CAP). In 114 patients (37%), serological evidence of past infection with "Z" was found. In eight patients (2.6%) there was serological evidence of acute infection with this pathogen. In four of these eight patients, no other pathogen for CAP was identified despite an intensive serological investigation encompassing 13 etiological agents. The four patients were about 30 yr old, and three of them had no history of chronic illness. Their illness was characterized by high fever, a nonproductive cough, gastrointestinal symptoms, a shift to the left in the white blood cell count, and a prompt, dramatic response to erythromycin therapy. We conclude that the microorganism "Z", or a close variant, is infectious for humans, in some cases causing CAP. In these cases the disease is mild and responds quickly to treatment with erythromycin. Lieberman D, Kahane S, Lieberman D, Friedman MG. Pneumonia with serological evidence of acute infection with the chlamydia-like microorganism "Z".

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The microorganism "Z" is a chlamydia-like bacterium that was discovered as a contaminant in laboratory cell cultures and was first characterized by Kahane and colleagues in 1993 (1). Its replication cycle is similar to that of chlamydia and some rickettsia, but it differs from chlamydia in the OMP-1 and OMP-2 components of the outer membrane. Glucose, which cannot be utilized by chlamydia but is utilized by some rickettsiae (2), is required by "Z" for growth. "Z", like rickettsia, is resistant to penicillin, while all known chlamydia are sensitive *in vitro* to penicillin (3). The 16S ribosomal DNA sequence found in "Z" (GenBank accession number L27666, Simkania Z) has an 83% sequence identity with chlamydial species and a 73% identity with certain rickettsia-like organisms (4).

Our objective was to determine whether "Z", like some chlamydia and rickettsia species, causes or contributes to the development of community-acquired pneumonia (CAP). To this end we developed a specific ELISA to determine serum antibody levels against this microorganism. With this assay we tested paired sera of 308 patients who were included in a comprehensive prospective study of adult patients hospitalized with CAP at the Soroka Medical Center, as described previously (5).

In this paper we present the results of screening for antibodies to "Z" among these CAP patients and a detailed description of four CAP patients in whom no recognized etiol-

ogy was identified, despite the comprehensive serological testing, but who had evidence of an acute infection with "Z" based on comparisons of antibody levels in the acute phase and convalescent phase sera.

METHODS

The Community-Acquired Pneumonia Study

We conducted a prospective study of the cause of CAP in 346 adult patients who were admitted to the hospital with this diagnosis at the Soroka Medical Center in Beer-Sheva, Israel, for the year between November 1, 1991, and October 31, 1992. The study was approved by the review board for human research (the Helsinki committee) of the Soroka Medical Center, and all patients gave their informed consent to participate.

The mean age of the patients was 49.3 yr (SD 19.5, range 17-94). Of the 346 patients initially studied, 187 (54%) were men. Sixteen patients (4.6%) died in the hospital. All other patients were alive at least 6 wk after admission to the hospital. During the course of their hospitalization the patients were diagnosed and treated by the medical staffs of the internal medicine wards, without intervention by the investigators. At discharge, the patients were referred to the investigators at the pulmonary disease clinic of the hospital for clinical and radiological follow-up.

Community-acquired pneumonia was diagnosed by the presence of an acute febrile disease, acute pulmonary infiltrate on chest radiogram, and a clinical and radiological course that confirmed the diagnosis. Exclusion criteria included positive blood tests for HIV, lung malignancies, and having been discharged from the hospital less than 21 d before the present admission with pneumonia.

In addition to routine hospital blood tests (complete blood count, biochemistry, and blood cultures), a serum sample was obtained within the first 48 h of admission for serological testing. A second (convalescence) serum was obtained from 308 patients (89%), usually at the follow-up appointment. The mean interval between the two se-

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rum samples was 31.7 d (SD 12.1, range 17–45). All sera were separated immediately and stored at -70°C until tested.

The diagnosis of bacterial pathogens was based on positive blood cultures and/or positive serological tests. In patients with a positive blood or pleural fluid culture, the isolated bacterium was considered to be the causative agent of CAP. Bacterial etiologies for *S. pneumoniae* pneumolysin, *Hemophilus influenzae*, and *Moraxella (Branhamella) catarrhalis* were also determined by serological testing, which was also used to identify infection with *M. pneumoniae*, *C. pneumoniae*, *Legionella* spp., *C. burnetii*, and six respiratory viruses (influenza A, influenza B, adenovirus, respiratory syncytial virus [RSV], parainfluenza 1, and parainfluenza 3). Details of the various serological methods used in this study and of the criteria for positive serological diagnoses for each of these causative agents have been published previously (5).

Serologic Tests for Infection with "Z" and Chlamydia

All 308 paired sera were tested for antibodies to "Z" and to chlamydia. The test for "Z" antibodies was carried out using a modification of the ELISA test originally developed for differentiating between antibodies against *Chlamydia trachomatis* and those against *Chlamydia pneumoniae*. The same test was also used in this study for determination of antibodies to the two Chlamydia species. This assay was shown to have an excellent correlation with the gold standard microimmunofluorescence test (MIF): a sensitivity of 94% and specificity of 99% with respect to the detection of antibodies to *C. trachomatis*, and a sensitivity of 95% and specificity of 91% for antibodies to *C. pneumoniae* (unpublished data). Briefly, "Z" was grown in Vero cells as described previously (4). Elementary and reticulate bodies were purified from infected cells and resuspended in sucrose-phosphate glutamate buffer (SPG) (4). After inactivation with 0.3% formalin for 1 h at room temperature, they were diluted to a concentration of 1 $\mu\text{g}/\text{ml}$ in carbonate-bicarbonate buffer and used to coat the wells of microtiter plates (Step 1). Following deoxycholate treatment of the antigen (2% deoxycholate monohydrate in 5 mM EDTA, preserved with 0.1% sodium azide, for 1 h at 37°C) as Step 2, a blocking solution consisting of 0.5% gelatin and 5% bovine serum albumin in phosphate-buffered saline (PBS), pH 7.2, was added to all wells (200 λ) for 2 h at room temperature (Step 3). Serum samples were applied to duplicate wells at dilution of 1:100 for immunoglobulin (IgG) testing (1:30 for IgA) and incubated for 1 h at room temperature. After a 15-min wash with 6 M urea (United States Biochemical Co., Cleveland, OH), followed by thorough washing with PBS containing 0.05% Tween-20 (PBS-Tween), conjugated second antibody was applied as required (rabbit antihuman IgG or rabbit antihuman IgA) and incubated for 0.5 h at room temperature. Washing was carried out after steps 1, 2, and 3 with PBS and thereafter with PBS-Tween. Bound second antibody was detected with orthophenylenediamine dihydrochloride (OPD) in citrate-phosphate buffer, pH 5.0. The reaction was stopped by the addition of 50 λ of 3 N H_2SO_4 and absorbance was read at 492 nm with an Organon Teknika ELISA reader, against a reference wavelength of 650 nm. Average optical density (OD) levels for each serum after background subtraction were normalized with respect to the absorption of a reference serum tested in twelve different experiments. This serum had an average absorption of 0.619 ± 0.066 at OD 492 nm. In addition to the reference serum, a control negative serum was included in each experiment as well. A level of > 0.60 adjusted OD was taken as evidence of past infection with "Z" (this was well above the grey level of 0.4–0.5 OD). Current infection was indicated by very high (> 1.2 OD) or rising (increase of 0.5 OD units between paired sera) levels of "Z"-specific IgA and/or rising levels of "Z"-specific IgG.

RESULTS

In 112 (37%) of the 308 CAP patients tested, serological evidence for past infection with "Z" was found. In eight patients (2.6%), there was serological evidence for acute infection with "Z". In four of these eight patients, serological evidence was found for at least one other etiological agent known to cause CAP. In the other four patients, no other known pathogen for CAP was identified. Details relating to these eight patients

TABLE 1
CHARACTERISTICS OF PATIENTS WITH SEROLOGICAL EVIDENCE OF ACUTE INFECTION WITH "Z", AND ADDITIONAL CAP PATHOGENS IDENTIFIED

Patient	Age (yr)	Sex	Additional CAP Etiologies
1	28	M	
2	31	F	
3	32	M	
4	32	M	
5	42	F	<i>C. burnetii</i> , <i>S. pneumoniae</i>
6	60	F	<i>L. pneumophila</i> (serogroup 6)
7	54	M	<i>L. jordanii</i>
8	67	F	<i>L. pneumophila</i> (serogroup 1), <i>S. pneumoniae</i> , <i>H. influenzae</i>

and their additional diagnoses are presented in Table 1. Antibody levels for "Z", *C. trachomatis*, and *C. pneumoniae* in paired sera of these eight patients are presented in Table 2. An additional four patients had elevated levels of IgA to "Z" in their first serum sample; however, they all had proven acute *C. pneumoniae* infection or high levels of IgA antibody to that organism as well and are not presented here.

The four patients (numbers 1–4 in Table 1) who had serological evidence for acute infection with "Z" and were negative for a broad spectrum of other etiologies for CAP are described in detail below

Case Descriptions

Case 1. A 28-year-old male electrician was hospitalized with a 2-wk fever that reached 39°C . A nonproductive cough began a week prior to hospitalization; 2 d later he developed left pleuritic chest pain that gradually increased until his admission. He denied nausea, vomiting, or diarrhea and received no antibiotic therapy before hospitalization. The patient had never smoked and his past medical history was unremarkable.

On physical examination at admission the man's temperature was 39.4°C , his blood pressure was 131/69, his pulse rate was 96 bpm, and his respiratory rate was 20/min. There were no findings on lung auscultation, and the rest of the physical

TABLE 2
COMPARISON OF ELISA (ADJUSTED OD 492 nm) ANTIBODY LEVELS TO *C. trachomatis*, *C. pneumoniae*, AND "Z" IN PAIRED SERUM SAMPLES OF THE EIGHT PATIENTS WITH SEROLOGICAL EVIDENCE OF ACUTE INFECTION WITH "Z"

Patient	Serum Sample	<i>C. trachomatis</i>		<i>C. pneumoniae</i>		"Z"	
		IgG	IgA	IgG	IgA	IgG	IgA
1	I	0.03	0.11	0.28	0.47	1.30	0.23
	II	0.09	0.14	0.45	0.49	1.85	0.79
2	I	0.23	0.23	0.43	0.54	1.02	2.03
	II	0.13	0.27	0.51	0.56	1.24	2.09
3	I	0.07	0.14	0.09	0.27	0.33	0.56
	II	0.10	0.15	0.18	0.30	0.96	1.28
4	I	0.19	0.34	0.21	0.39	1.33	1.80
	II	0.16	0.34	0.24	0.40	1.41	1.34
5	I	0.13	0.33	0.48	0.52	1.15	1.31
	II	0.18	0.31	0.72	0.60	1.61	1.16
6	I	0.22	0.15	0.36	0.26	2.35	1.75
	II	0.25	0.11	0.38	0.26	2.04	1.63
7	I	0.31	0.26	0.44	0.35	0.40	2.00
	II	0.21	0.38	0.48	0.40	0.62	2.14
8	I	0.10	0.13	0.32	0.32	0.56	0.26
	II	0.12	0.25	0.34	0.55	1.82	0.41

examination and the electrocardiogram were normal. The chest X-ray showed a small, peripheral, nonhomogeneous infiltrate in the left upper lobe.

Laboratory results were hemoglobin 13.3 g/100 dl, 6,300 leukocytes/mm³ (of which 11% were band cells, 65% were segmented cells, 20% were lymphocytes, and 4% were eosinophils), and 216,000 thrombocytes/mm³. The ambient Pa_{O₂} was 77 mm Hg, and the Pa_{CO₂} was 32 mm Hg. Biochemistry blood tests were within normal limits. Blood and urine cultures were sterile and the urine sediment was normal.

During his hospitalization, the patient was treated with oral erythromycin base at a dose of 500 mg every 8 h. His fever gradually decreased and disappeared on the third hospital day, together with a gradual alleviation of the pleuritic pain. The patient was discharged after 4 d of hospitalization with the recommendation that he continue the same treatment for another week. He recovered fully and returned to work a week later.

Forty-three days after discharge from the hospital the patient was examined at the follow-up clinic. He was asymptomatic and the physical examination, laboratory tests, and chest X-ray were normal. At this opportunity blood was drawn for convalescence serology.

Case 2. A 31-year-old woman, a hospital nurse, was hospitalized for 4 d with a fever of 40° C and lower abdominal pain. A light nonproductive cough began 2 day before hospitalization and the abdominal pain worsened, with diarrhea and an isolated incident of vomiting. She was not treated with antibiotics before hospitalization. The patient's medical history was unremarkable except for a 5 pack-year smoking history.

On physical examination at admission her temperature was 38.5° C, her blood pressure was 120/80, her pulse rate was 92 bpm, and her respiratory rate was 16/min. Inspiratory rales were heard over the posterior aspect of the right lower lung field. There was bilateral lower abdominal tenderness, which was more marked on the right, with normal peristalsis. The pelvic examination and sonogram showed no abnormalities in the uterus or adnexa. The electrocardiogram was normal. A nonhomogeneous infiltrate was seen in the basal segments of the right lower lobe on chest X-ray.

Laboratory results were hemoglobin 13.3 g/100 dl, 7,400 leukocytes/mm³ (of which 9% were band cells, 72% were segmented cells, 17% were lymphocytes, and 2% were eosinophils), and 195,000 thrombocytes/mm³. The ambient Pa_{O₂} was 82 mm Hg, and the Pa_{CO₂} was 28 mm Hg. Biochemistry blood tests were within normal limits. Blood, urine, and stool cultures were sterile and the urine sediment was normal.

Upon admission, intravenous therapy with erythromycin lactobionate was begun at a dose of 600 mg every 8 h. Her fever decreased over 48 h, together with a significant reduction of the abdominal pain. On the third day of hospitalization, the treatment was changed to oral erythromycin base at a dose of 250 mg every 6 h. The patient was discharged after 4 d of hospitalization with the recommendation that she continue the same oral treatment for another week. She recovered fully after 6 d and returned to work 4 d after that.

Thirty-three days after discharge from the hospital, the patient was examined at the follow-up clinic. She was asymptomatic and the physical examination, laboratory tests, and chest X-ray were normal. At this opportunity, blood was drawn for convalescence serology.

Case 3. A 32-year-old male construction worker was hospitalized with weakness, a nonproductive cough, left pleuritic chest pain, diarrhea, vomiting, loss of appetite, and a fever of 38.5–40° C that had begun 4 d before hospitalization. Over the 3 d before hospitalization, he was treated with erythromycin

(as stearate) at a dose of 500 mg every 6 h, without any improvement in his condition. The patient's medical history was unremarkable, except for a 20 pack-year smoking history.

On physical examination at admission, the man's temperature was 36.8° C, his blood pressure was 107/63, his pulse rate was 100 bpm, and his respiratory rate was 18/min. The base of the left thorax at the back was dull to percussion with increased fremitus. The respiratory sounds on auscultation were normal except for abundant inspiratory rales. A homogeneous infiltrate was seen in the basal segments of the left lower lobe on chest X-ray, without presence of pleural fluid.

Laboratory results were hemoglobin 13.8 g/100 dl, 10,800 leukocytes/mm³ (of which 23% were band cells, 51% were segmented cells, 21% were lymphocytes, and 5% were eosinophils), and 234,000 thrombocytes/mm³. The ambient Pa_{O₂} was 70 mm Hg, and the Pa_{CO₂} was 37 mm Hg. Biochemistry blood tests were within normal limits except for an elevated serum glutamic oxaloacetic transaminase (SGOT or AST) level of 78 IU (normal < 40). Blood and urine cultures were negative. The urine sediment had traces of protein, and a few red blood cells without casts were seen.

During hospitalization the patient was treated with intravenous crystalline penicillin followed by oral penicillin. He was afebrile throughout his hospitalization and his symptoms gradually improved. The patient was discharged after 5 d of hospitalization with the recommendation that he continue the same oral treatment for another week. He returned to work and full daily activity a week later.

Twenty-eight days after discharge from the hospital, the patient was examined at the follow-up clinic. He was asymptomatic and the physical examination, laboratory tests, and chest X-ray were normal. At this opportunity, blood was drawn for convalescence serology.

Case 4. A 32-year-old male worked with heavy machinery in a phosphate plant. He was hospitalized with a fever of 40° C accompanied by weakness, diarrhea, a nonproductive cough, and right pleuritic chest pain that started 2 d before hospitalization. He received no antibiotic therapy before he was hospitalized. The patient suffered from familial Mediterranean fever (FMF) from childhood and was treated with colchicine at a dose of 0.5 mg twice a day. He was hypersensitive to penicillin and had a 15 pack-year smoking history.

On physical examination at admission, his temperature was 39.2° C, his blood pressure was 107/47, his pulse rate was 108 bpm, and his respiratory rate was 20/min. A few inspiratory rales were heard over the upper right anterior thorax. Peristaltic sounds were increased, and the rest of the physical examination and the electrocardiogram were normal. A homogeneous infiltrate was seen in the anterior segment of the right upper lobe on chest X-ray.

Laboratory results were hemoglobin 15.4 g/100 dl, 15,000 leukocytes/mm³ (of which 16% were band cells, 62% were segmented cells, 18% were lymphocytes, 2% were eosinophils, and 2% were atypical monocytes), and 231,000 thrombocytes/mm³. The ambient Pa_{O₂} was 76 mm Hg, and the Pa_{CO₂} was 36 mm Hg. Biochemistry blood tests were within normal limits. Blood, urine, and stool cultures were sterile and the urine sediment was normal.

During hospitalization, the patient was treated with oral erythromycin base at a dose of 250 mg every 6 h. From the day after admission he was afebrile with a significant improvement in his symptoms. The patient was discharged after 3 d of hospitalization with the recommendation to continue the same oral treatment for another week. He was fully recovered 3 d after discharge and returned to work 4 d later.

Forty days after discharge from the hospital, the patient

was examined at the follow-up clinic. He was asymptomatic and the physical examination, laboratory tests, and chest X-ray were normal. At this opportunity, blood was drawn for convalescence serology.

DISCUSSION

In previous reports of the frequency distribution of the various etiologies for CAP among hospitalized patients, despite comprehensive serological and microbiological testing, there were many patients whose disease was defined as being of unknown etiology. One of the possible explanations for these results is that there are pathogens for CAP that have not yet been identified, much as was the case for *Legionella* species until 1976 and for *C. pneumoniae* until the last decade. This study, in which we have attempted to establish an etiological connection between a new microorganism related to Chlamydiae (4) and CAP, is part of the effort to minimize the group of CAP patients classified as patients with unknown etiologies in future studies.

There is no gold standard for "Z" serology, since the organism was only recently discovered. However, because of its apparently closer phylogenetic relationship to chlamydia than to other microorganisms (4), we adapted an ELISA assay previously developed to distinguish between antibodies to *C. trachomatis* and *C. pneumoniae* for detection of antibodies to "Z".

It is clear from Table 2 that the assay does not detect only cross-reactive antibodies. The patients described in this paper had either very low levels or no antibodies against *C. trachomatis* and *C. pneumoniae*. Thus, the "Z" antibody levels observed could not be due to infection with chlamydia. In this study we did not investigate the possible presence of "Z"-specific IgM in the CAP sera because we found that the ELISA method we developed did not reliably distinguish between IgM specific for *C. trachomatis* and IgM specific for *C. pneumoniae*. We therefore assumed that its ability to reliably detect IgM produced in response to "Z" infection would be limited.

The presence of IgA antibodies specific for an organism is generally accepted to imply recent of current exposure to the relevant antigens (6). Although some have taken issue with this thesis, more recently support for the concept in regard to chlamydia has increased, especially with respect to *C. pneumoniae* (7, 8).

The appreciable rate of patients with evidence of past infection with "Z" (37%) demonstrates that much of the adult population in our region has been exposed to this microorganism in the past and confirms data indicating that about 32% of blood donors in our region show evidence of past infection with "Z" (unpublished data). We do not have data regarding the clinical manifestations of this exposure.

Eight patients had serological features that provided evidence of acute infection with "Z" that could not be attributed to chlamydial infection. It is noteworthy that although the study lasted for 12 mo, all eight patients were hospitalized in the 40 mo period between mid-February and mid-June. In four of the eight cases, there was serological evidence of acute infection with at least one additional pathogen known to cause CAP. The co-existence of more than one serologically proven pathogen for CAP is not surprising. In our series, this was the case for 38.4% of the CAP patients and included all possible combinations of etiological agents. A detailed report on this phenomenon is presented, together with analyses and possible explanations, in our paper on the distribution of etiological agents for CAP (5). The cases of the four CAP patients who had serological evidence of acute infection with "Z" and at

least one other etiological agent were not appropriate for defining the clinical manifestations of acute infection with "Z", since we could not differentiate with certainty between the clinical expressions of each of the pathogens. For this reason we did not describe these four patients in detail.

The other four patients, whose cases are presented here, had serological evidence of acute infection with "Z" only, without evidence of infection by another known etiological agent for CAP despite comprehensive serological testing for 13 CAP pathogens. We see this as indirect evidence of the etiological connection between CAP and "Z", much like the generally accepted etiological connection between CAP and known CAP pathogens in the presence of serological evidence of acute infection with those pathogens.

Assuming a true etiological association between CAP and "Z" in the four patients described above, several unique clinical features are noteworthy. All four patients were close to 30 yr of age and three of them had no chronic comorbidity. In addition to high fever and a nonproductive cough, three of the patients complained of pleuritic chest pain and three had gastrointestinal symptoms including diarrhea, vomiting, and/or lower abdominal pain. Although the pleuritic pain can be considered a coincidental result of the anatomic proximity of the infected lung and the pleura, the assumption that the gastrointestinal symptoms were coincidental is improbable. All four patients were treated with erythromycin, which has the potential to cause gastrointestinal side effects, but in the three patients who had gastrointestinal tract symptoms they appeared before erythromycin therapy was initiated. The radiological manifestations of CAP were not uniform. In two patients the infiltrate was homogeneous, while in the other two it was nonhomogeneous. All four patients had mild hypoxemia. None had abnormal biochemical blood tests except for a slight elevation of SGOT (AST) levels in patient 3. The leukocyte was not uniform, ranging from 6,300 wbc/mm³ to 15,000 wbc/mm³ among the four patients. The differential white blood cell count was similar in all four patients: a shift to the left, band cells ranging from 9–23%, and low levels of eosinophils.

All four patients had quick recoveries under treatment with erythromycin. Patient 3, who was treated with penicillin during his hospital stay, received erythromycin during the 3 d before admission and can be considered to have responded to erythromycin therapy, at least in regard to the drop in body temperature. Although there is no definitive proof that the patient's disease responded to erythromycin therapy, the temporal association between the initiation of erythromycin therapy and the disappearance of fever, together with the improvement in the other symptoms, provides strong support for this contention. The disease course in all four patients was relatively mild and in retrospect hospitalization was not indicated. The recovery and return to full activity was relatively quick and uncomplicated.

We conclude that the microorganism "Z", or a close variant of it, can infect human beings, with one of the clinical manifestations of this infection being CAP. The disease caused by the infection is relatively mild, produces gastrointestinal symptoms, and responds well to treatment with erythromycin.

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