Chlamydia pneumoniae sp. nov. for *Chlamydia* sp. Strain TWAR

J. THOMAS GRAYSTON,^{1,2*} CHO-CHOU KUO,¹ LEE ANN CAMPBELL,¹ AND SAN-PIN WANG¹

Departments of Pathobiology¹ and Epidemiology,² University of Washington, Seattle, Washington 98195

A third species, *Chlamydia pneumoniae*, is proposed for the genus *Chlamydia*. This bacterium is a human respiratory pathogen, which has been referred to as the TWAR strain of *Chlamydia*. Species identification is based on ultrastructural differences in the elementary bodies, deoxyribonucleic acid analysis, and serology.

The purpose of this paper is to classify chlamydial strain TWAR as a new species, *Chlamydia pneumoniae*.

The first *C. pneumoniae* isolate, TW-183^T (T = type strain), was isolated in 1965 from the eye of a child in Taiwan (6). The first pharyngeal isolate, AR-39, was obtained from a university student with pharyngitis in Seattle, Wash., in 1983 (hence, TWAR, TW-183^T and AR-39) (6). At first, these new organisms were thought to be *Chlamydia psittaci* (6, 9). To date, in addition to TW-183^T, we have obtained 27 isolates, 17 from Seattle and 10 from Finland (in collaboration with P. Saikku), all from pharyngeal swabs of patients with acute respiratory diseases. An isolate said to be similar to TW-183^T was isolated in England from a conjunctival specimen of a child in Iran (4).

C. pneumoniae was established as an important respiratory pathogen when a series of isolates were obtained from the throats of patients in Seattle with acute respiratory diseases, especially pneumonia, but also bronchitis and pharyngitis (6). *C. pneumoniae* have been shown to cause endemic and epidemic pneumonia (6–8, 13, 16).

C. pneumoniae was identified as a member of the genus *Chlamydia* on the basis of the following criteria: obligate intracellular parasitism, the unique developmental cycle (9), shared genus-specific lipopolysaccharide and complement fixation antigen (9), and comparable guanine-plus-cytosine content (3).

Justification for a new species. The justification for establishing a new species for this organism is based on the uniqueness of the ultrastructure of the elementary body, deoxyribonucleic acid (DNA) analysis, and serology.

C. pneumoniae has a pear-shaped elementary body (Fig. 1) (2), unlike the elementary bodies of other *Chlamydia* species, which are typically round (15). The reticulate bodies of *C. pneumoniae* are circular, like those of the other species. We examined eight additional *C. pneumoniae* isolates and found that all had identical morphology (data not shown).

A genetic analysis revealed 10% or less DNA relatedness between *C. pneumoniae* and the other two species of *Chlamydia*, while the level of homology was 94% or greater within the species (3). The restriction endonuclease patterns were the same among the *C. pneumoniae* isolates and were readily distinguishable from those of *Chlamydia trachomatis* and *C. psittaci* (1). *C. pneumoniae* can also be differentiated from the other chlamydiae by using cloned *C. pneumoniae* restriction fragments or whole chromosomal DNAs as probes (1).

C. pneumoniae can be differentiated serologically from

strains of *C. trachomatis* and *C. psittaci* by immunofluorescent antibody staining of elementary bodies or inclusions, using *C. pneumoniae*-specific monoclonal antibodies (9). While antibody responses to *C. pneumoniae* infection in humans may be detected by the *Chlamydia* group-reactive complement fixation test, a *C. pneumoniae*-specific serodiagnosis can be made by the micro-immunofluorescence test (6, 17).

Description of *Chlamydia pneumoniae* sp. nov. *Chlamydia pneumoniae* (Pneu.mo'ni.æ. Gr. n. *pneumonia*, pneumonia, inflammation of the lungs; M.L. gen. n. *pneumoniae*, of pneumonia). The description is based on 10 strains. The species has a chlamydial developmental cycle and in cultured cells has intracytoplasmic inclusions which are round and dense in appearance in Giemsa stain (9). The inclusions are iodine stain negative (they contain no glycogen). The elementary body is pleomorphic but typically pear shaped (Fig. 1) (2). The average size is 0.38 µm, with a long axis of 0.44 µm, a short axis of 0.31 µm, and a ratio of the long axis to the short axis of 1.42. The cytoplasmic mass is round, with an average diameter of 0.24 µm. There is a large periplasmic space. Small, round electron-dense bodies (diameter, 0.05 µm), which are attached to the cytoplasm by a stringlike structure, are present in the periplasmic space. The reticulate body is circular and has an average diameter of 0.51 µm. It shares the *Chlamydia* genus-specific antigen, as demonstrated by staining inclusions by immunofluorescence using genus-specific monoclonal antibodies (9). Serologically, it can be identified by the monoclonal antibody specific to *C. pneumoniae*. The guanine-plus-cytosine content of the DNA is 40 mol% (3), which is within the range for chlamydiae (15). No extrachromosomal DNA (plasmid) has been found (1). The organisms grow better at 35°C than at 37°C in cell cultures (11). Cell culture growth is enhanced by pretreatment of host cells with diethylaminoethyl-dextran before inoculation, centrifugation of inoculum onto cell monolayers, and incorporation of antimetabolites, such as cycloheximide, in Eagle minimum essential medium containing 10% fetal calf serum (9, 11). *C. pneumoniae* shows low virulence to chicken embryos as determined by yolk sac inoculation and to mice as determined by intracerebral, intranasal, and intravenous inoculations and does not cause follicular conjunctivitis in monkeys as determined by conjunctival inoculation (9). The spectrum of antibiotic susceptibility is similar to that of other chlamydiae, except that, like most *C. psittaci* strains, *C. pneumoniae* is resistant to sulfa drugs (10). The organism has been isolated from human throats and conjunctivas. Differentiation characteristics for the species are listed in Tables 1 and 2.

All of the isolates which have been studied are similar as

* Corresponding author.

FIG. 1. Electron micrographs showing the structure of two kinds of elementary bodies (E). The preparations are organisms that were purified on a linear gradient of Renografin. (A) *C. pneumoniae* TW-183^T. (B) *C. trachomatis* B/TW-5/OT. Note that the *C. pneumoniae* elementary bodies are pear shaped, have large periplasmic spaces, and contain small, round, electron-dense bodies (arrowheads). In contrast, the elementary bodies of the other two species, *C. psittaci* and *C. trachomatis*, as represented by strain B/TW-5/OT, are round with narrow or barely discernible periplasmic spaces. The reticulate bodies (R) are round in all *Chlamydia* species. The reticulate body in (A) is broken. om, Outer membrane. Bar = 0.5 μ m.

determined by ultrastructural, DNA, and serological analyses and thus are a single strain. This strain is known as the TWAR strain of *C. pneumoniae*. So far, only one serovar has been identified.

The type strain is the first isolate, TW-183, which came from the conjunctiva of a child in Taiwan. As with nine

subsequent throat isolates from humans, it has all of the properties described above for the species. The type strain has been made available to investigators by the Washington Research Foundation, Seattle, Wash. Strain TW-183^T has already been distributed to more than 50 investigators throughout the world.

TABLE 1. Differentiation of *Chlamydia* species^a

Species	Elementary body morphology	Inclusion morphology ^b	Glycogen in inclusions ^c	Folate biosynthesis ^d
<i>C. trachomatis</i>	Round	Oval, vacuolar	+	+
<i>C. psittaci</i>	Round	Variable shape, dense	—	—
<i>C. pneumoniae</i> ^e	Pear shaped	Oval, dense	—	— ^f

^a Modified from reference 15.

^b As determined by Giemsa staining of cultured cells.

^c As evidenced by positive iodine staining of inclusions (5).

^d As demonstrated by inhibition of chlamydial growth in chicken embryo yolk sacs by adding 1 mg of sulfadiazine per embryo (12).

^e See references 2, 6, and 9.

^f Based on in vitro susceptibility test in cell cultures showing resistance to sulfisoxazole (10). This test cannot be done in eggs due to the low virulence of *C. pneumoniae*.

TABLE 2. Some characteristics of the species and biovars of the genus *Chlamydia*^a

Characteristic	<i>C. trachomatis</i> biovars			<i>C. psittaci</i>	<i>C. pneumoniae</i> ^b
	Trachoma	Lymphogranuloma venereum	Mouse		
Guanine-plus-cytosine content of DNA (mol%)	40	42	43	41	40
% DNA homology to <i>C. pneumoniae</i>	10	10	NA ^c	10	100
Plasmid	+	+	NA	+/- ^d	-
No. of serovars as determined by microimmunofluorescence	12	3	NA	NA	1
Type specificity as determined by monoclonal antibodies ^e	+	+	NA	NA	+
Natural hosts	Humans	Humans	Mice	Birds, lower mammals	Humans
Preferred site of infection	Squamo-columnar epithelium (oculogenital, respiratory)	Lymph nodes	Lungs	Multiple sites	Squamo-columnar (respiratory)
Intracerebral lethality in mice	-	+	-	+	-
Follicular conjunctivitis in monkeys	+	-	-	-	-
Cell culture enhancement of infectivity by:					
Centrifugation of inoculum onto monolayer	+	-	-	-	+
Treatment of host cells with diethylaminoethyl-dextran	+	-	-	Weak	Weak
Plaque on L cells	-	+	+	+	NA

^a Modified from reference 15.^b See references 1, 3, 6, 9, 11, and 17.^c NA, Data not available.^d Some strains do not contain plasmids (1, 14).^e See references 9 and 18.

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LITERATURE CITED

- Campbell, L. A., C.-C. Kuo, and J. T. Grayston. 1987. Characterization of the new *Chlamydia* agent, TWAR, as a unique organism by restriction endonuclease analysis and DNA-DNA hybridization. *J. Clin. Microbiol.* **25**:1911-1916.
- Chi, E. Y., C.-C. Kuo, and J. T. Grayston. 1987. Unique ultrastructure in the elementary body of *Chlamydia* sp. strain TWAR. *J. Bacteriol.* **169**:3757-3763.
- Cox, R. L., C.-C. Kuo, J. T. Grayston, and L. A. Campbell. 1988. Deoxyribonucleic acid relatedness of *Chlamydia* sp. strain TWAR to *Chlamydia trachomatis* and *Chlamydia psittaci*. *Int. J. Syst. Bacteriol.* **38**:265-268.
- Dwyer, R. S. C., J. D. Trehan, B. R. Jones, and J. Herring. 1972. Chlamydia infection: results of microimmunofluorescence tests for the detection of type-specific antibody in certain chlamydial infections. *Br. J. Vener. Dis.* **48**:452-459.
- Gordon, F. B., and A. L. Quan. 1965. Occurrence of glycogen in inclusions of the psittacosis-lymphogranuloma venereum-trachoma agents. *J. Infect. Dis.* **115**:186-196.
- Grayston, J. T., C.-C. Kuo, S.-P. Wang, and J. Altman. 1986. A new *Chlamydia psittaci* strain called TWAR from acute respiratory tract infections. *N. Engl. J. Med.* **315**:161-168.
- Grayston, J. T., C.-C. Kuo, S.-P. Wang, M. K. Cooney, J. Altman, T. J. Marrie, J. G. Marshall, and C. H. Mordhorst. 1986. Clinical findings in TWAR respiratory tract infections, p. 337-340. *In* J. D. Oriel et al. (ed.), *Chlamydial infections. Proceedings of the Sixth International Symposium on Human Chlamydial Infections*. Cambridge University Press, Cambridge.
- Kleemola, M., P. Saikku, R. Visakorpi, S.-P. Wang, and J. T. Grayston. 1988. Epidemics of pneumonia caused by TWAR, a new chlamydia organism, in military trainees in Finland. *J. Infect. Dis.* **157**:230-236.
- Kuo, C.-C., H.-H. Chen, S.-P. Wang, and J. T. Grayston. 1986. Identification of a new group of *Chlamydia psittaci* strains called TWAR. *J. Clin. Microbiol.* **24**:1034-1037.
- Kuo, C.-C., and J. T. Grayston. 1988. In vitro drug susceptibility of *Chlamydia* sp. strain TWAR. *Antimicrob. Agents. Chemother.* **32**:257-258.
- Kuo, C.-C., and J. T. Grayston. 1988. Factors affecting the viability and the growth in HeLa 229 cells of *Chlamydia* sp. strain TWAR. *J. Clin. Microbiol.* **26**:812-815.
- Lin, H.-S., and J. W. Moulder. 1966. Patterns of response to sulfadiazine, D-cycloserine, and D-alanine in members of the psittacosis group. *J. Infect. Dis.* **116**:372-376.
- Marrie, T. J., J. T. Grayston, S.-P. Wang, and C.-C. Kuo. 1987. Pneumonia associated with the TWAR strain of *Chlamydia*. *Ann. Intern. Med.* **106**:507-511.
- McClenaghan, M., A. J. Herring, and I. D. Aitken. 1984. Comparison of *Chlamydia psittaci* isolates by DNA restriction endonuclease analysis. *Infect. Immun.* **45**:384-389.
- Moulder, J. W., T. P. Hatch, C.-C. Kuo, J. Schachter, and J. Storz. 1984. *Chlamydia* Jones, Rake and Stearns 1945,55, p. 729-735. *In* N. R. Krieg and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 1. The Williams & Wilkins Co., Baltimore.
- Saikku, P., S.-P. Wang, M. Kleemola, E. Brander, E. Rusanen, and J. T. Grayston. 1985. An epidemic of mild pneumonia due to an unusual *Chlamydia psittaci* strain. *J. Infect. Dis.* **151**:832-839.
- Wang, S.-P., and J. T. Grayston. 1986. Microimmunofluorescence serological studies with the TWAR organism, p. 329-332. *In* D. Oriel et al. (ed.), *Chlamydial infections. Proceedings of the Sixth International Symposium on Human Chlamydial Infections*. Cambridge University Press, Cambridge.
- Wang, S.-P., C.-C. Kuo, R. C. Barnes, R. S. Stephens, and J. T. Grayston. 1985. Immunotyping of *Chlamydia trachomatis* with monoclonal antibodies. *J. Infect. Dis.* **152**:791-800.