Reactivity of *Chlamydia pneumoniae* Strains in the IDEIA CHLAMYDIA® Test Kit Designed for Detection of *Chlamydia trachomatis*

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Abstract

The intensity of reaction in the IDEIA CHLAMYDIA® test kit to lipopolysaccharide of seven strains of *Chlamydia pneumoniae* was examined by using highly purified elementary bodies (EBs). The strains were divided into two groups according to whether the EBs were pear-shape or round. The group with the pear-shaped EBs consisted of TW-183, AR-39 and AR-388 strains; the group with the round EBs consisted of IOL-207, Kajaani-6, YK-41 and KKpn-1 strains. The number of EBs at the cutoff level of the test kit were determined as follows: TW-183, 7.0×10^3 ; AR-388, 8.4×10^3 ; AR-39, 2.4×10^4 ; IOL-207, 6.0×10^3 ; Kajaani-6, 1.2×10^4 ; KKpn-1 2.8×10^4 ; and YK-41, 4.0×10^4 per assay. The number of EBs of *C. trachomatis* L₂/434/Bu and *C. psittaci* Cal 10 were 1.0×10^3 and 2.7×10^4 per assay, respectively. The results demonstrated that the EBs of *C. pneumoniae* were detected with the test kit at different reaction intensities ranging from 6.0×10^3 to 4.0×10^4 per assay and that there was no correlation between EB number and morphology.

Introduction

The IDEIA CHLAMYDIA® test kit (IDEIA; Dako diagnostics Co.) is designed for the detection of *Chlamydia trachomatis* lipopolysaccharide (LPS) antigen in clinical specimens, such as urogenital swabs and urine specimens of patients¹⁾. Chlamydial LPS is a major genus-specific antigen^{2,3,4)}. Therefore, LPS of *C. psittaci* and *C. pneumoniae* react in the test kit, but the reaction intensity of *C. trachomatis* is higher than that of *C. psittaci* and *C. pneumoniae*^{5,6)}. This suggests that the antigenic structure of *C. trachomatis* LPS is somewhat different from that of *C. psittaci* and *C. pneumoniae* LPS.

C. pneumoniae is recognized as one of the important etiological agents of pneumonia and acute respiratory tract infections^{7,8)}. Based on similarities in many biochemical and immunological aspects, such as structural proteins, genomic DNA restriction patterns, seroreactivity in the micro-immunofluorescence test and immunoblotting, *C. pneumoniae* was identified as a single strain or serovar^{8,9,10)}. Morphological examination by Chi et al.¹¹⁾ demonstrated that elementary bodies (EBs) of *C. pneumoniae* had unique "pear-shaped" profiles in thin sections. This EB morphology was regarded as one of the criteria for classification of *C. pneumoniae* species⁸⁾. However, our previous observations on the morphology of EBs of several *C. pneumoniae* strains revealed that the EBs of some strains were round rather than pear-shaped^{12,13)}, suggesting possible diversity not only in morphology, but also in antigenic properties. This suggestion may be supported by Black et al. who reported diversity in protein antigens of *C. pneumoniae* strains¹⁴⁾. In this study, we examined the reactivity of highly purified EBs of several *C. pneumoniae* strains

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in the IDEIA test kit and then compared their reactivity at the detection limit (cutoff level) to the number of EBs as a parameter of the reaction intensity.

Materials and Methods

Chlamydial strains

C. pneumoniae TW-183, AR-39, AR388⁷⁾, IOL-207¹⁵⁾, Kajaani-6¹⁶⁾, YK-41¹⁷⁾, KKpn-1¹³⁾, *C. trachomatis* L₂/434/Bu¹⁸⁾ and *C. psittaci* meningopneumonitis Cal 10¹⁹⁾ strains were used throughout this study. Strains TW-183, AR-39 and AR-388 were purchased from the Washington Research Foundation, Seattle, WA, U.S.A.; strains IOL-207 and Kajaani-6 were supplied by P. Saikku, University of Helsinki, Finland; strain YK-41 was supplied by Y. Kanamoto, Hiroshima Prefectural Institute of Public Health, Japan, and strain KKpn-1 was isolated at the Kawasaki Medical School Hospital. All strains were cultured in HL cells or HeLa 229 cells by the method of Kuo et al.²⁰⁾ and harvested on day 3 postinoculation. Our previous electron microscopic observations revealed that the EBs of the TWAR strains were pear-shaped, and those of strains IOL-207, Kajaani-6, YK-41 and KKpn-1 were round and were morphologically indistinguishable from those of *C. trachomatis* and *C. psittaci* EBs^{12,13,16,21)} (Table 1). Strain L₂ was supplied by S. Yamasaki, National Institute of Health, Japan, and maintained continuously in HeLa 229 or L 929 cells. The Cal 10 strain has been maintained in our laboratory for more than 20 years in monolayer or suspension cultures of the L 929 cell line²²⁾, and harvested on day 2 or 3 postinoculation.

Purification of EBs

The Cal 10 EBs were purified by the method of Tamura and Higashi²²⁾, but trypsin treatment was omitted to exclude any possible change in the antigenic property of the EB surface. The EBs of L₂ and all *C. pneumoniae* strains were purified by the method described previously⁶⁾, and the quality of the preparations were determined by transmission electron microscopy (Hitachi H-500).

EB counting

The EBs in suspension, prepared by two fold serial dilution, were counted under a Hitachi S-570 scanning electron microscope by the method described previously⁶⁾.

Immunolabeling of LPS on the surface of intact EBs and EB envelopes

Electron microscopy revealed that EB preparations of the *C. pneumoniae* strains were mixtures of intact EBs and a few EB envelopes, which might have been formed during the purification process. Since the target antigen of the IDEIA is chlamydial LPS, it was necessary to confirm the presence of LPS on the

Table 1 Chlamydial strains

Species	Strain	Origin	Reference
<i>C. pneumoniae</i>	pear-shape	TW-183	conjunctiva/Taiwan/1965 (7, 11)
		AR-39	pharyngitis/U.S.A./1983 (7, 11)
		AR-388	ARD ^{a)} /U.S.A./1984 (7, 11)
	round	IOL-207	trachoma/Iran/1967 (15, 21)
		Kajaani-6	pneumonia/Finland/1987 (16)
		YK-41	bronchitis/Japan/1991 (12, 17)
		KKpn-1	bronchitis/Japan/1992 (13)
<i>C. trachomatis</i>	L ₂ /434/Bu	LGV ^{b)} /U.S.A./1967	(18)
<i>C. psittaci</i>	Cal 10	meningopneumonitis/U.S.A./1938	(19)

^{a)} ARD : Acute respiratory disease.

^{b)} LGV : Lymphogranuloma venereum.

EB envelopes. We previously reported the presence of LPS on EB envelopes as well as on intact EBs^{5,6}. To confirm these findings with intact EBs and envelopes of the *C. pneumoniae* strains, immunogold labeling was performed with a primary anti-LPS monoclonal antibody (supplied by K. Hirai, Faculty of Agriculture, Gifu University) and protein A-gold particles (15 nm in diameter, Amersham Co.) by the method of Birkelund et al.²³.

Fig. 1 Shadowcast preparations of purified EBs of *C. pneumoniae*. EBs were air-dried, mounted on specimen grids and shadowcast with Pt-palladium alloy. (A) *C. pneumoniae* IOL-207. (B) *C. pneumoniae* AR-39. The AR-39 EBs showed a pear-shaped morphology with a wide outer membrane area, whereas EBs of IOL-207 had a round fried-egg shape. A few envelopes (arrowheads) are seen in the *C. pneumoniae* EB preparations. Bars indicate 1 μ m.

Fig. 2 Immunolabeling on the surface of intact EBs and EB envelopes of *C. pneumoniae* strains. (A) *C. pneumoniae* Kajaani-6. (B) *C. pneumoniae* AR-388. The samples were exposed to monoclonal antibody directed to chlamydial LPS, then labeled with protein A-gold particles. Intact EBs and EB envelopes (arrowheads) are heavily labeled with the gold particles. Bars indicate 0.5 μ m.

Assay with the IDEIA test kit

A series of EB suspensions was prepared by two fold serial dilution, and 100 μ l of each suspension was added to the same volume of transport medium to prepare a second two fold dilution series. A 200- μ l sample of each dilution was used for the assay. All steps in the assay were performed in accordance with the instructions provided in the manual for the IDEIA test kit. In the IDEIA system, chlamydial LPS is solubilized in the transport medium contained in the kit, and captured with anti-LPS monoclonal antibody fixed in the wells of a microtiter plate. The captured LPS is then assayed by the uniquely enhanced colorimetric reaction¹⁾.

Results

Purity of EB preparations

Electron microscopy demonstrated that the L₂ and Cal 10 EB suspensions contained no impurities derived from the host cells or reticulate bodies and consisted of almost 100% intact EBs (data not shown). In contrast, the *C. pneumoniae* pear-shaped and round EB suspensions contained both intact EBs and a few EB envelopes. Intact EBs were present at 97 and 98% for the pear-shaped and round-shaped strains, respectively (Fig. 1). No debris derived from the host cells and RBs was encountered.

Immunolabeling of LPS

When the immunolabeling method was applied to EB suspensions of the *C. pneumoniae* pear-shaped and round-shaped strains, both intact EBs and EB envelopes were heavily labeled with the protein A-gold particles (Fig. 2), suggesting that EB envelopes are also involved in the reaction in the IDEIA assay. This indicates that elimination of EB envelopes from any of the EB preparations is not required when the IDEIA sensitivity is evaluated.

Reaction intensity of EBs

Figure 3 shows the intensity of reaction to the EBs of seven *C. pneumoniae* strains tested in the kit. The EB numbers at the cutoff level were calculated in the manner described previously⁶⁾ and are summarized in Table 2. The most sensitive strain was L₂. The numbers of TW-183, AR-388 (both EBs are pear-shaped) and IOL-207 (round) ranged from 6.0×10^3 to 8.4×10^3 EBs per assay, and those of AR-39,

Fig. 3 Relationship between the number of EBs and reactivity of *C. pneumoniae* strains.
O.D.: Optical density.

Table 2 Comparison of the number of EBs at cutoff level of the IDEIA test kit

Species	Strain	EB number per assay
<i>C. pneumoniae</i>		
pear-shape	TW-183	7.0×10^3
	AR-388	8.4×10^3
	AR-39	2.4×10^4
round	IOL-207	6.0×10^3
	Kajaani-6	1.2×10^4
	KKpn-1	2.8×10^4
	YK-41	4.0×10^4
<i>C. trachomatis</i>	L ₂ /434/Bu	1.0×10^3
<i>C. psittaci</i>	Cal 10	2.7×10^4

KKpn-1 and Cal 10 were almost the same, although they are different in morphology and species. The Yk-41 was less sensitive at 4.0×10^4 EBs per assay at the cutoff level. The results indicate the presence of wide-ranging diversity of the LPS antigen on the EBs of *C. pneumoniae* strains without correlation with the EB morphology.

Discussion

Since the target antigen of the IDEIA test kit is *C. trachomatis* LPS, which is regarded as a major genus-specific antigen^{2,3,4)}, it is reasonable to assume that the EBs of *C. pneumoniae* strains used in the present study would be reactive in the test kit. In our previous study on evaluation of the test kit, it was demonstrated that the numbers of EBs per assay at the cutoff level were 9.6×10^2 , 6.5×10^3 and 2.5×10^4 for the L₂, TW-183 and Cal 10, respectively⁶⁾. These numbers were determined for EB suspensions prepared by 10-fold serial dilution. In the present study, in which the EBs were newly purified and the EB suspensions were prepared by two fold dilution, the EB numbers at the cutoff level were 1.0×10^3 , 7.0×10^3 and 2.7×10^4 per assay for the L₂, TW-183 and Cal 10, respectively. These numbers agreed well with those obtained in the previous study⁶⁾, indicating that the EB counting method is reproducible and therefore reliable for evaluation of the IDEIA test kit. An additional finding was that the antigenic diversity of LPS may be detected when the reaction intensity at the cutoff level was expressed with the EB number as a parameter. Accordingly, we determined the EB number of seven strains of *C. pneumoniae* at the cutoff level. The results demonstrated that the EBs of all strains used were reactive at different magnitudes, ranging from 6.0×10^3 to 4.0×10^4 per assay, and that there was no correlation between the EB number and morphology (Table 2). What is not known, however, is whether the different reactivities of *C. pneumoniae* strains resulted from differences in quality or quantity of LPS on the EB surface, since the chemical structure of the antigenic epitope in LPS has been proposed³⁾. If this is true for the *C. trachomatis* species, it seems that the reaction magnitude of the *C. trachomatis* strains is also different among 18 serovars and that the IDEIA sensitivity to *C. trachomatis* is different for different serovars relative to one another.

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Chlamydia trachomatis LPS を対象とした IDEIA CHLAMYDIA[®]
test kit に対する *Chlamydia pneumoniae* 株の反応強度

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要 旨

Chlamydia trachomatis の LPS を対象として開発された IDEIA CHLAMYDIA[®] (Dako Diagnostics 社; 以下 IDEIA) に対する *C. pneumoniae* 7 株の反応強度を高度精製基本小体 (EB) を用いて比較検討した. *C. pneumoniae* 株を EB の形態によって pear-shape 群 (TW-183, AR-39, AR-388) および round shape 群 (IOL-207, Kajaani-6, YK-41, KKpn-1) の 2 群に分けてそれぞれの株の IDEIA kit の cutoff 値における EB 個数を決定した. その結果, assay 当たりの EB 個

数は pear-shape 群; TW-183 7.0×10^3 , AR-388 8.4×10^3 , AR-39 2.4×10^4 , round-shape 群; IOL-207 6.0×10^3 , Kajaani-6 1.2×10^4 , KKpn-1 2.8×10^4 , YK-41 4.0×10^4 , 同時に検討した *C. trachomatis* L₂/434/Bu および *C. psittaci* Cal 10 ではそれぞれ 1.0×10^3 , 2.7×10^4 個であった. 以上の結果は, EB の形態との相関は認められないものの, *C. pneumoniae* における LPS の抗原性が 7 株間で異なることを示している. しかしこの反応の相違が LPS の質的相異によるのか量的相違によるのかは判然としない.