LETTER TO THE EDITOR

NEW INSIGHTS INTO *CHLAMYDIAE* PERSISTENCE: AN ENERGY METABOLISM STRATEGY?

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***Chlamydiaceae* is a family of obligate intracellular bacteria generally considered energy parasites. Several studies have suggested that Chlamydiae are capable of independently producing energy and, more importantly, several genes involved in the energy metabolism are up-regulated during the persistent state. Thus, it has been suggested that chlamydial persistence could be a complex and flexible metabolic strategy designed to favor a lengthy survival in the host cell by evading the immune response. In conclusion, more detailed studies on the shift in the chlamydial energy metabolism, from the active to the persistent form, may be helpful in future to determine whether chlamydial persistence observed *in vitro* does occur *in vivo* and whether chronic sequelae of chlamydial diseases may be related to the persistence.**

Chlamydiaceae is a family of obligate intracellular bacteria, generally considered energy parasites since they depend upon the ATP produced by the host cell in order to survive and to multiply. The human pathogens include *Chlamydia trachomatis, Chlamydia pneumoniae* and *Chlamydia psittaci* that cause, respectively, genital and ocular infections, community-acquired pneumonia and psittacosis.

Chlamydiae are characterized by a biphasic developmental cycle alternating between the extracellular infectious form [Elementary Body, (EB)] and the intracellular replicative and non-infectious form [Reticulate Body, (RB)]. It has been demonstrated that *Chlamydiae* can alter their biological state to generate a viable but non-cultivable form called persistent form. As a result, normal RB transform into enlarged and morphologically aberrant RB [Aberrant Body, (AB)], thus stopping the production of infectious EB (1). Persistent chlamydial forms have been reported to play a role in the development of chronic sequelae since they can evade the host immune response and are more difficult to eradicate by antibiotics (2-10).

Chlamydial persistence has been extensively investigated by using several *in vitro* models concerning mainly *C. trachomatis* but also *C. pneumoniae* and *C. psittaci*. Persistent forms have been induced by iron or essential amino acid starvation (1, 11, 12), IFN-γ or antibiotic treatment (1, 11-14), and monocyte/macrophage culture (1, 12).

The presence of persistent chlamydial forms have been confirmed by the reduced or absent production of infectious progeny and the visualization of the aberrant inclusions by Transmission Electron Microscopy (12).

Several studies have investigated the transcriptional profile of the persistent chlamydial form in different *in vitro* persistence models. Specifically, numerous genes have been studied, such

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as genes implied in DNA replication (*polA, dnaA* and *mutS*), cell division (*ftsK* and *ftsW*), stress response (*ompA* and *groEL*) and RB to EB differentiation (*omcB*), whose expression has resulted in different patterns both between the various persistence models and between the chlamydial species of medical interest (1, 11-21) (Table I). As a matter of fact, only few genes have a relatively uniform expression pattern in the different persistence models and chlamydial species, such as *hctA* and *hctB* involved in RB to EB differentiation and *htrA* involved in the stress response (11, 13-16, 20, 21). Therefore, the lack of a unique transcriptional profile regardless of the chlamydial species and the persistence system actually weakens the hypothesis that the persistent form could be a genetically-defined state.

Interestingly, a large-scale microarray analysis of *C. pneumoniae* gene expression in the developmental cycle and in the iron starvation-induced persistence suggested that the shift from RB to persistent form was more likely a mid-cycle arrest during the development rather than a completely distinct gene expression pattern. More interestingly, during the persistent state, several genes involved in the energy metabolism were up-regulated (including *atpE* for the ATP synthase subunit, *tpiS, pkg* and *dhnA* responsible for the glycolysis and *glgA* involved in the sucrose and starch metabolism) (21), suggesting that *C. pneumoniae* could rely on a self-energy production and consequently become almost autonomous. Recently, it was suggested that *Chlamydiae* are capable of independently producing energy in addition to the uptake of ATP and other essential nutrients from the host cell, since they possess a nearly complete tricarboxylic acid (TCA), glycolysis and pentose phosphate pathways for glucose catabolism (21-23). In fact, the quantitative proteomic analysis of *C. trachomatis* EB (22)

Table I. *Chlamydiae gene expression patterns during persistence.*

References	Strain	Cells	Persistence Inducer	Genes (+ up regulation; – down regulation; = unchanged)										
				ompA	omcB	hctA	hctB	polA	dnaA	mutS	ftsK	ftsW	groEL	htrA
Byrne GI, 2001 *	*C. pneumoniae* TW-183	HEp-2	INF-γ					=	=	=	–	–		
Mathews S, 2001 *	*C. pneumoniae* IOL-207	HEp-2	INF-γ	+	=								=	
Nicholson T, 2002 *	*C. trachomatis* D	n/a	Penicillin G	=			–		=	+	+	=	=	+
Belland RJ, 2003 *	*C. trachomatis* D	HeLa	INF-γ (24 h)	–	–	–	–	–	–	–	=	–	=	–
Belland RJ, 2003 *	*C. trachomatis* D	HeLa	INF-γ (48 h**)	+	=	+	+	=	–	–	=	–	=	=
Hogan RJ, 2003 *	*C. pneumoniae* TW-183	HEp-2	Continuous infection	+	+						=		=	
Slepenkin A, 2003 *	*C. pneumoniae* CM-1	HEp-2	INF-γ	+	+						–		+	
Goellner S, 2006 (13)	*C. psittaci*	HEp-2	INF-γ	–	–[1]	–						–	–	
Goellner S, 2006 (13)	*C. psittaci*	HEp-2	Iron depletion	=	–	–						–	=	
Goellner S, 2006 (13)	*C. psittaci*	HEp-2	Penicillin G	+	–	–						–	=	
Mukhopadhyay S, 2006 (15)	*C. pneumoniae* A-03	HEp-2	INF-γ ***	+	–								+	+
Mukhopadhyay S, 2006 (15)	*C. pneumoniae* A-03	HEp-2	Iron depletion ***	+	=								+	+
Polkinghorne A, 2006 (16)	*C. pneumoniae* A-03	HEp-2	INF-γ		+						–	+	–	+
Maurer AP, 2007 (21)	*C. pneumoniae* CWL-029	HEp-2	Iron depletion	=	–	=	–	=	=		=	=	=	+
Huston WM, 2008 (14)	*C. trachomatis* L2	HEp-2	INF-γ ***											–
Huston WM, 2008 (14)	*C. trachomatis* L2	HEp-2	Penicillin G ***											+
Klos A, 2009 (17)	*C. pneumoniae* CWL-029	HeLa	INF-γ					=			–	–	+	
Klos A, 2009 (17)	*C. pneumoniae* CWL-029	HeLa	Iron depletion					–			=	–	–	
Klos A, 2009 (17)	*C. pneumoniae* CWL-029	HeLa	Penicillin G					–			–	–	–	
Timms P, 2009 (11)	*C. pneumoniae* A-03	HEp-2	INF-γ	–	–		–				–		–	+
Timms P, 2009 (11)	*C. pneumoniae* A-03	HEp-2	Iron depletion	–	–		–				–		–	+
Kokab A, 2010 (18)	*C. trachomatis* E	HEp-2	INF-γ	=							–	+		
Di Pietro M, 2012 (20)	*C. pneumoniae* AR-39	HEp-2	Penicillin G	–		–	–						–	+

** Hogan J et al., 2004; ** reactivated culture; [1] up-regulated at 12 h p.i.; *** proteomic analysis*

revealed that it expresses several proteins involved in the energy metabolism, such as the ATP synthase subunit A, B, D, E, I and K for the ATP biosynthesis and enzymes belonging to the TCA and pentose phosphate pathways. More importantly, a recent study on *C. trachomatis* metabolic activity demonstrated, for the first time, that EB are able to use glucose 6-phosphate (G6P) in order to produce ATP (24). Also, a previous proteomic assay of *C. pneumoniae* EB (23) showed the presence of analogous proteins for ATP biogenesis, glycolysis, TCA and pentose phosphate pathways. Furthermore, also *C. psittaci* could be capable of producing energy since the sequences of *C. trachomatis* energy metabolism genes showed an identity rate with *C. psittaci* genome ranging from 68% to 75% by using Blast algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Consequently, the hypothesis has been forwarded that chlamydial persistence could be a complex and flexible metabolic strategy designed to favor a lengthy survival in the host cell by evading the immune response (17, 21).

Currently, the data from chlamydial persistence are not sufficiently uniform and complete to allow a full understanding of the mechanisms underlying this condition. More detailed studies on the shift in the chlamydial energy metabolism, from the active to the persistent form, may be helpful to determine whether chlamydial persistence observed *in vitro* does occur *in vivo* and whether chronic sequelae of chlamydial diseases may be related to the persistence.

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