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Synovial *Chlamydia trachomatis* up-regulates expression of a panel of genes similar to that transcribed by *Mycobacterium tuberculosis* during persistent infection

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Running title: Gene expression in chlamydial persistence

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Abstract

Objective: Synovial tissues in patients with *Chlamydia*-associated arthritis are persistently infected by *C. trachomatis*, an organism for which genetic manipulation is not possible. *M. tuberculosis* also engages in persistent infection, and because this bacterium is genetically tractable many groups have been able to define transcriptional characteristics of mycobacterial growth and persistence. We investigated whether the pattern of gene expression underlying chlamydial persistence is similar to that underlying mycobacterial persistence.
Methods: A study from another group identified 194 genes in *M. tuberculosis* that are transcriptionally up-regulated to support *in vivo* growth and persistence of that organism. We compared each of those genes to the *C. trachomatis* genome to identify orthologs. Expression of selected chlamydial orthologs so identified was assessed by real time RT-PCR in an *in vitro* model of chlamydial persistence and synovial tissues from patients PCR-positive for *C. trachomatis* at that site.
Results: 67 *C. trachomatis* genes were identified as being orthologous to mycobacterial persistence-related genes, representing 35% of the genes tested. The chlamydial orthologs fall into similar metabolic and other categories as those in *M. tuberculosis*. Expression of a majority of selected chlamydial orthologs is strongly up-regulated in an *in vitro* model of chlamydial persistence and in synovial tissues of relevant patients, compared to their expression during active infection.
Conclusions: These observations provide new insight concerning the molecular genetic basis underlying chlamydial persistence, and they indicate that this information can be obtained in some instances by extrapolating observations made in other biologic systems and/or organisms.

Key Words: pathogenesis persistent infection gene expression reactive arthritis *Chlamydia trachomatis*

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Introduction

Chlamydia trachomatis is an obligate intracellular pathogen that is the most prevalent sexually transmitted bacterium in the US and other developed countries [1]. An often severe acute inflammatory arthritis develops in some individuals with a prior urogenital infection with this organism, and about half of all patients who develop acute arthritis progress to chronic disease [2,3 for reviews]. The reason(s) that only a limited number of patients with a genital chlamydial infection develop acute arthritis is/are poorly understood. We also do not understand why only a portion of individuals with acute disease progress to chronicity, although both issues relate to our lack of detailed understanding of host-pathogen interaction during synovial chlamydial infection.

C. trachomatis undergoes a biphasic developmental cycle at its sites of primary infection, the urethral or cervical epithelium [4 for review]. This organism can disseminate widely from those primary infection sites, and when it does so the vehicle of its dissemination is the monocytic cell, the cell type which is the primary synovial host in both acute and chronic *Chlamydia*-induced arthritis [4,5]. In contrast to the normal progression through the developmental cycle undergone during active infection of epithelial cells, however, *C. trachomatis* residing within monocytic cells in the joint transit rapidly to an unusual biologic state designated "persistence" [again, 3 for review]. In this state, the organisms are morphologically aberrant, non-culturable by standard laboratory methods, resistant to antibiotics, and they display an unusual transcript profile [6-11; 12 for review]. *C. pneumoniae*, a human respiratory pathogen related to *C. trachomatis*, also has been implicated in eliciting inflammatory arthritis, and this organism too has been shown to engage in persistent infection under some circumstances [13,14].

It seems clear that synovial pathogenesis in *Chlamydia*-induced arthritis is a function of persistent, rather than normal active, infection. However, details relating to the mechanism(s) by which chlamydiae enter, and thereafter maintain, the persistent infection state *in vivo*, as well as details concerning the means by which persistent chlamydiae elicit disease, remain to be elucidated. As mentioned, persistent chlamydiae display an unusual transcript profile compared to that of active infection. For example, expression of *omp1*, the gene encoding the major outer membrane protein, is severely attenuated in persistence [*e.g.*, 7]. Genes encoding products required for DNA replication (*e.g.*, *dnaA*, *polA*, others) are transcribed in persistently infecting *C. trachomatis* cells as they are during active infection, but expression of genes specifying products required for cytokinesis is severely down-regulated during persistence [*e.g.*, *ftsK*, *ftsW*; ref. 8]. Several other chlamydial genes of known function also are differentially expressed in persistent *vs.* active infection [9,10; 3 for review]. Full understanding of the genetic program underlying entry into and maintenance of chlamydial persistence will be a difficult task, since many coding sequences on the *C. trachomatis* and *C. pneumoniae* genomes specify products of unknown function [15,16]. To complicate matters further, no system for genetic manipulation of either pathogen exists to date.

Bacterial pathogens other than *C. trachomatis* and *C. pneumoniae* engage in persistent infection, and in some cases the mechanisms by which those organisms establish persistence have been studied extensively. *Mycobacterium tuberculosis*, in which genetic manipulation is possible and for which the full genome sequence is available, is one such organism [17,18 for review]. Studies from several laboratories have defined many critical aspects underlying mycobacterial persistence [*e.g.*, 19-21]. For example, one recent investigation of genes required for *in vivo* growth and persistence of *M. tuberculosis* in an animal model of disease identified 194 coding sequences required for those processes [22]. Virtually all of the genes identified in that study as being required

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for establishment of persistence were not expressed, or were expressed at only low level, during axenic growth of the organism.

Because genetic manipulation of *Chlamydia* is not available, identification of chlamydial genes orthologous to those shown to function in persistence in other organisms may be of value. We undertook a study to determine whether none, some, or all of the 194 genes shown to be transcriptionally up-regulated in support of growth and persistence *in vivo* for *M. tuberculosis* have orthologs in *C. trachomatis*. We report that 35% of the coding sequences identified in the mycobacterial study have related genes in *C. trachomatis*. We further show that a selected set of those chlamydial orthologs spanning several metabolic and other categories show up-regulation of expression during persistence, as in *M. tuberculosis*, in both an *in vitro* model of that infection state and in samples from arthritis patients PCR-positive for the organism in synovial tissue.

Patients and methods

Patient samples. Synovial biopsies were procured under an approved protocol from patients attending the Arthritis Clinics at the V.A. Medical Center and the University of Pennsylvania Hospital, Philadelphia PA USA; protocol approval was from the University of Pennsylvania School of Medicine and Wayne State University School of Medicine IRB committees. Synovial biopsies were obtained by the Parker-Pearson method and were immediately snap-frozen at -80°C until prepared for analyses [23]. The three patients studied were included solely because they were PCR-positive for *C. trachomatis* chromosomal DNA in synovial tissues, and because enough tissue was present from each to support the several molecular genetic analyses required. Diagnoses for the patients were made according to criteria of the American College of Rheumatology. The clinical characteristics of each patient from whom samples were obtained are summarized briefly in Table 1.

Table 1. Characteristics of patients included in the present study

Patient	Age/Sex	Diagnosis[1]	Disease Duration
1	M/62	RA	84 months
2	F/60	UO	72 months
3	M/48	ReA	36 months

[1]Abbreviations: RA, rheumatoid arthritis; UO, undifferentiated oligoarthritis; ReA, reactive arthritis

***Cell growth, in vitro infection with C. trachomatis**.* HEp-2 cells support active growth of *C. trachomatis* [8,14], *i.e.*, *Chlamydia* infecting this cell line progress normally through the developmental cycle. Nearly confluent monolayers of HEp-2 cells were infected with *C. trachomatis* K serovar at an MOI of 1:1 in the standard manner [8,9]. Infected cultures were harvested at 12 hr post-infection (p.i.), and cell pellets were snap-frozen at -80°C until processed for analysis. *C. trachomatis* infecting normal human peripheral monocytes in culture enter the persistent infection state by d 3 p.i. [7-10]. Blood samples were procured from volunteer donors under an approved protocol, and monocytic cells were

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prepared and put into culture from them as described by us [*e.g.*, 6-10]; the protocol was approved by the Wayne State University School of Medicine IRB. Nearly confluent monolayers of monocytes were infected at an MOI of 1:1 with *C. trachomatis* serovar K, and infected cultures were harvested at d 5 p.i.; cell pellets were snap-frozen at -80°C until processed. To be certain that chlamydiae infecting monocytes in these experiments had entered the persistent state, transcripts from the *C. trachomatis dnaA*, *polA*, *omp1*, *ftsK*, *pyk*, and *gap* genes were assessed by RT-PCR (see just below); all such transcripts were absent as expected except those from *dnaA* and *polA*, which are expressed during both active and persistent infection [8].

Preparation and analysis of RNA/cDNA. Total nucleic acid preparations were made from *Chlamydia*-infected HEp-2 and human monocyte cell pellets, and from synovial tissue samples, as described [8,9]. Pure RNA was prepared from aliquots of each preparation *via* treatment with DNaseI (RQ1 DNase; Promega Biotech, Madison WI USA), followed by extraction in phenol:chloroform and collection by ethanol precipitation. Reverse transcription of total RNA preparations to cDNA was done using the MuLV enzyme and random hexamers as primers (Invitrogen, Carlsbad CA USA), as described [10,14].

cDNA preparations were subjected to quantitative real time RT-PCR analyses for 16 targeted *C. trachomatis* genes, using the SYBR green method [10,14]. The coding sequences of interest and the primer systems used in the analyses are given in Table 2. Each real time RT-PCR assay for each targeted gene was run in triplicate three times independently. Data were normalized to chlamydial 16S rRNA, as described [8,10,14]; data for specific transcripts in all monocyte- and patient-derived assays were indexed to the level of that transcript in *C. trachomatis* actively-growing for 12 hr in HEp-2 cells. Assays were done using a PE Biosystems (Foster City, CA USA) model 7700 sequence detector; data were analyzed using v1.9 sequence analysis software from PE Biosystems.

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Table 2. *C. trachomatis* genes targeted and primer sequences for real time RT-PCR .

16S rRNA
5'-aaaggagagaaagaccgacc-3' us
5'-cgccgtttagcttaactcc-3' ds
Ct776 / aas
5'-tgctgccgatcatcctt-3' us
5'-taaggaatcgcttctacctgat-3 ds
Ct468 / atoC
5'-catctcacatcctctgatcgc-3' us
5'-agatcgaggggagtgcttgt-3'ds
Ct130 / glnQ
5'-tcttcaaatcgttctgtctcta-3' us
5'-catgcagactatcgtaagca-3' ds
Ct653 / yhbG
5'-atcgagcgcgccttg-3' us
5'-agcatttaataggtgcgtt-3' ds
Ct237 / fabG
5'-tattcgctgagcaaggg-3' us
5'-gttctgaacttgtgcggaa-3' ds
Ct393 / proS
5'-tcgtggctcagtggctaa-3' us
5'-aactctttggcacgcataa-3' ds
Ct701 / secA
5'-ctgtctgacgacgagcttc-3' us
5'-cacccagaatctgcacatc-3' ds
Ct762 / murC
5'-gttctgctcgatcgaggat-3' us
5'-aattcggctcgatgaactac-3' ds
Ct624 / mviN
5'-cattgctgccgtagtgaa-3' us
5'-taccatagcatcgctaactgac-3' ds
Ct052 / hemN
5'-ctctcccaatccaacacatc-3' us
5'-ccttgattctctagtagctctt-3' ds
Ct868 / Conserved Hypothetical Protein
5'-agatccctttacagacgctt-3' us
5'- tcatggcgttctacagaatc -3'ds
Ct437 / fusA
5'-tgcgcaacgctgagaa-3' us
5'-gcacttccgcaaagaactg-3' ds
Ct727 / cadA
5'-tctccgcaactttttttcc-3' us
5'-ttccgccatccctgat-3' ds
Ct820 / ftsY
5'-gaaacaaactccgctctctt-3' us
5'-gcccgttcatcaggatt-3' ds
Ct414 / pmpC
5'-gcactctcctccgttactga-3' us
5'-agcagctcgatactctgtgtt-3' ds
Ct394 / hrcA
5'-caactgcgagcatcgaa-3' us
5'-aagtttcgagcctactggtt-3' ds

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Results

C. trachomatis genes orthologous to M. tuberculosis persistence-related genes.

To determine whether any of the 194 genes from *M. tuberculosis* identified as being required for growth and persistence *in vivo* in mice [22] have orthologs in *C. trachomatis*, we performed a BLAST evaluation of each identified mycobacterial coding sequence against the full chlamydial genome [www.stdgen.lanl.gov]. Of the 194 genes, we identified 67 orthologs with Expect values of 10^{-1} or better, representing 35% of the total number of genes assessed. Table 3 provides a complete list of the chlamydial coding sequences identified, their related genes in *M. tuberculosis*, and the Expect values. The chlamydial orthologs of mycobacterial persistence-related genes identified fall into general categories similar to those in the *M. tuberculosis* study, *i.e.*, genes encoding products involved in cell envelope synthesis or modification, synthesis of cofactors, transport, translation, and so on [22]. Interestingly, nine genes specifying proteins of unknown function were identified on the *C. trachomatis* genome, all but one of which correspond to similarly unknown genes in *M. tuberculosis*. No genes specifying components of the chlamydial Type III secretion system were identified, but *secA*, a component of an apparent Type II secretion system, was identified (but see below). Three secretion-related mycobacterial genes were shown to be up-regulated in the earlier study, including *secA2*. A few potentially important differences emerged in the panel of chlamydial orthologs identified. For example, we showed earlier that expression of genes encoding products for the glycolytic and pentose phosphate pathways is severely attenuated in persistent *C. trachomatis* cells [9], while transcription of several genes involved in energy production and conversion were shown to be up-regulated in the mycobacterial study. Importantly, 107 of the 194 genes identified in the mycobacterial study as required for *in vivo* growth and persistence were coding sequences of unknown function; the majority of these 107 genes had no orthologous coding sequence in *C. trachomatis* (see Discussion).

Expression of selected C. trachomatis orthologs during persistent infection in vitro.

To confirm that the *Chlamydia* genes identified are not only expressed during persistent infection but also up-regulated over their level of expression during active infection, we selected 16 chlamydial orthologs from various functional categories and assessed their relative expression level by real time RT-PCR during both infection states; the genes subjected to this analysis are indicated in bold in Table 3. In previous studies of chlamydial persistence, we have employed an *in vitro* model system using normal human monocytic cells infected with *C. trachomatis* serovar K; in this system, chlamydiae transit from a more or less normal infection state to the persistent state by 3 d p.i. [*e.g.*, 7-10]. We analyzed RNA from infected monocytes at d 5 p.i. and compared transcript levels from each of the 16 targeted genes to the level of their expression at 12 hr p.i. during active infection of HEp-2 cells. Previous microarray-based studies have shown that all coding sequences on the *C. trachomatis* chromosome and plasmid are expressed at some time during the normal developmental cycle [24,25]. Consistent with those data, each of the 16 genes assessed here was being transcribed at 12 hr p.i. in HEp-2 cells (data not shown). Fig. 1 provides a summary of the transcript level at d 5 p.i. in the *in vitro* monocyte model system for each of the 16 chlamydial genes targeted relative to their expression level during active HEp-2 cell infection. Interestingly, all but 4 of the chlamydial genes assayed showed relatively strong up-regulation during persistence in comparison to expression during active infection. The 4 genes showing no/minimal transcriptional up-regulation

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Table 3. *C. trachomatis* genes identified in the BLAST search using *M. tuberculosis* persistence-related genes as query.

Mtb Gene ID / Name[&]	Ct Gene ID / Name*	Gene Function	E value
Metabolism :			
Rv3114 / UNK	Ct844 / *yfhC*	cytosine deaminase	6.00E-04
Rv2976c / *ung*	Ct067 / *ytgA*	N-uracyl glycosylase	1.00E-34
Rv3868 / UNK	Ct040 / *ruvB*	Holliday Junction DNA helicase	2.00E-05
Rv2241 / *aceE*	Ct750 / *tktB*	Transketolase	9.00E-05
Rv1028c / *kdpD*	Ct205 / *pfpA*	pyrophosphate-fructose 6-phosphate 1-phosphotransferase	5.00E-03
Rv2857c / UNK	**Ct237 / *fabG***	3-oxyacyl [acyl-carrier protein] reductase	8.00E-37
Rv1238 / *sugC*	Ct202 / *oppF*	oligopeptide ABC transporter protein	3.00E-25
Rv2048c / *pks12*	Ct770 / *fabF*	beta-ketoacyl-ACP synthase	2.00E-21
Rv3502c / UNK	Ct237 / *fabG*	3-oxyacyl [acyl-carrier protein] reductase	8.00E-21
Rv0687 / UNK	Ct237 / *fabG*	3-oxyacyl [acyl-carrier protein] reductase	9.00E-19
Rv1144 / UNK	Ct237 / *fabG*	3-oxyacyl [acyl-carrier protein] reductase	8.00E-18
Rv1013 / *pks16*	**Ct776 / *aas***	2-acylglycerophosphoethanolamine acyltransferase	5.00E-14
Rv1185c / *fad21*	Ct776 / *aas*	2-acylglycerophosphoethanolamine acyltransferase	9.00E-06
Rv2502c / *accD1*	Ct265 / *accA*	acetyl-CoA carboxylase transferase subunit alpha	6.00E-03
Rv099 / *fad10*	Ct776 / *aas*	2-acylglycerophosphoethanolamine acyltransferase	8.00E-02
Rv2692 / *trkB*	Ct714 / *gpsA*	glycerol-3-phosphate dehydrogenase	9.00E-02
Rv1111c / UNK	Ct451 / *cdsA*	phosphatidate cytidylyltransferase (CDP diglyceride)	3.00E-01
Rv3556c / *fadA6*	Ct770 / *fabF*	beta-ketoacyl-ACP synthase	7.00E-01
Cell envelope related :			
Rv1109c / UNK	**Ct414 / *pmpC***	outer membrane protein C (Chlamydia-specific)	3.00E-01
Rv3484 / *cpsA*	**Ct414 / *pmpC***	pmpC : outer membrane protein C (Chlamydia-specific)	6.00E-01
Rv2335 / *cysE*	Ct243 / *firA*	UDP-3-O-(3-hydoxymyristoyl)-glucosamine-N-acyltransferase	1.00E-02
Rv2335 / *cysE*	Ct531 / *lpxA*	UDP-N-acetylglucosamine acyltransferase	1.00E-02
Rv2981 / *ddlA*	**Ct762 / *murC***	MUR-NAc-L-alanine and D-alanine-D-alanine ligase	2.00E-36
Rv3717 / UNK	Ct268 / *amiA*	probable N-acetylmuramoyl-l-alanine amidase	1.00E-07
Cellular processes :			
Rv2921c / *ftsY*	**Ct820 / *ftsY***	signal recognition particle	6.00E-46
Rv3870 / UNK	Ct739 / *ftsK*	cell division protein (ATPase)	1.00E-13
Rv3871 / UNK	Ct739 / *ftsK*	cell division protein (ATPase)	8.00E-02
Rv2912c / UNK	Ct570 / *gspF*	type II secretion pathway protein (protein F)	6.80E-01
Rv1821 / *secA2*	**Ct701 / *secA***	preprotein translocase subunit A protein	1.00E-62
Transport and binding :			
Rv1469 / *ctpD*	**Ct727 / *zntA***	cation-transporting ATPase	1.00E-78
Rv3270 / *ctpC*	Ct727 / *zntA*	cation-transporting ATPase	2.00E-65
Rv1272c / UNK	Ct264 / *msbA*	ABC transporter protein, MSD-NBD fusion protein	2.00E-56
Rv3663c / *dppD*	Ct689/690/201/202	oligopeptide ABC transporter protein	7.00E-46

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Rv2936 / *drrA*	Ct653 / *yhbG*	Probable ABC transporter atp binding protein	2.00E-27
Rv0655 / UNK	Ct130 / *glnQ*	glutamine ABC transporter	1.00E-23
Rv3758c / *proV*	**Ct130/180 *glnQ/ tauB***	ABC transporter, ATP-binding protein	6.00E-23
Rv2038c / UNK	Ct202/689	ABC transporter protein	2.00E-20
Rv3781 / UNK	**Ct653 / *yhbG***	Probable ABC transporter ATP-binding	1.00E-11
Rv3501c / UNK	Ct194 / *mgtE*	Mg2+ transport protein	4.00E-01
Rv2813 / UNK	Ct130 / *glnQ*	glutamine ABC transporter	1.00E+00
Translation :			
Rv2845c / *proS*	**Ct393 / *proS***	prolyl-tRNA synthetase	4.00E-88
Rv1640c / *lysX*	Ct781 / *lysS*	lysyl tRNA synthetase	9.00E-69
Rv3375 / *amiD*	Ct003 / *gluA*	Glu-tRNA Gln amidotransferase subunit	1.00E-52
Rv3419c / *gcp*	Ct197 / *gcp2*	O-sialoglycoprotein endopeptidase	6.00E-49
Rv3560c / *fadE30*	**Ct437 / *fusA***	elongation factor G protein	6.00E-01
Rv3563 / *fadE32*	Ct437 / *fusA*	elongation factor G protein	6.00E-01
Rv3210c / UNK	Ct459 / *prfB*	peptide chain release factor RF-2	8.60E-01
Rv0101 / *nrp*	Ct766 / *miaA*	2-acylglycerophosphoethanolamine acyltransferase	4.00E-04
Regulatory functions :			
Rv2374c / *hrcA*	**Ct394 / *hrcA***	heat inducible transcription regulator HrcA	1.00E-14
Rv3246c / *mtrA*	**Ct468 / *pilR***	two-component sensor system regulatory subunit	3.00E-08
Rv0490 / *senX3*	Ct467 / *pilS*	two-component sensor histidine kinase (ATOS-related)	2.00E-07
Rv2483c / UNK	Ct743 / *hctA*	Hc1 histone analog	2.00E-04
Biosynthesis of cofactors :			
Rv2388c / *hemN*	**Ct052 / *hemN***	oxygen-independent coproporphyrinogen III oxidase	2.00E-34
Rv1569 / *bioF*	Ct777 / *bioF*	8-amino-7-oxononanoate synthase	2.00E-20
Rv1568 / *bioA*	Ct210 / *hemL*	glutamate-1-semialdehyde 2,1-aminomutase	9.00E-16
Rv1405c / UNK	Ct428 / *ubiE*	probable ubiquiUNK/menaquiUNK methyltransferase	5.00E-04
Chlamydia Other and Uncategorized :			
Rv3877 / UNK	Ct115 / UNK	hypothetical protein (basic)	2.00E-01
Rv3910 / UNK	**Ct624 / *mviN***	MviN virulence factor	8.00E-09
Rv0670 / *end*	Ct625 / *end4*	endonuclease IV	2.00E-16
Rv3876 / UNK	Ct868 / UNK	conserved hypothetical protein	2.00E-08
Rv3103c / UNK	**Ct868 / UNK**	conserved hypothetical protein	9.00E-06
Rv3683 / UNK	Ct461 / UNK	conserved hypothetical protein	2.00E-05
Rv1021 / UNK	Ct255 / UNK	conserved hypothetical protein (acidic)	9.00E-05
Rv3400 / UNK	Ct464 / UNK	conserved hypothetical protein	1.00E-04
Rv0171 / UNK	**Ct868 / UNK**	conserved hypothetical protein	2.00E-03
Rv3723 / UNK	Ct664 / UNK	conserved hypothetical protein	4.00E-03
Rv3810 / *pirG*	Ct456 / UNK	conserved hypothetical protein	5.00E-01

[&]UNK, gene of unknown function

***C. trachomatis* genes indicated in bold were chosen for real time RT-PCR transcript analyses, as indicated in the text.

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during persistence were Ct701 (*secA*, preprotein translocase subunit A), Ct762 (*murC/murF*, an apparent fusion protein with MUR-NAc-L-alanine and D-alanine-D-alanine ligase activity), Ct624 (*mviN*, a putative virulence factor), and Ct868 (hypothetical protein, possibly a membrane thiol protease). Four other chlamydial ortholog genes showed powerful up-regulation of expression during persistence, consistent with data from the mycobacterial study. These were Ct393 (*proS*, prolyl tRNA synthetase), Ct052 (*hemN*, oxygen-independent coproporphyrinogen III oxidase), Ct727 (*zntA/cadA*, cation-transporting ATPase), and Ct820 (*ftsY*, component of signal recognition particle). All other chlamydial genes assessed showed moderate to fairly strong transcriptional up-regulation in persistent *C. trachomatis* compared to that shown in actively infecting organisms.

Expression of selected C. trachomatis orthologs during persistent infection in vivo.

With one interesting exception, the human monocyte model of *C. trachomatis* persistence has proved to be extremely accurate in reflecting the pattern of chlamydial gene expression *in vivo* in synovial tissues [*e.g.*, 8,9]. That exception was the identification of a different transcript pattern from the chlamydial *groEL* gene (Ct110, encodes the authentic hsp60 protein) between the monocyte model of persistence and synovial tissue samples from patients with *Chlamydia*-associated arthritis [10]; we understood this observation to indicate that the cellular context in the synovium differs in important ways from that of pure monocytic cells in culture. To determine whether *in vivo* and *in vitro* expression differed for any of the 16 tested chlamydial persistence ortholog genes, we repeated the real time RT-PCR assays using RNA/cDNA prepared from synovial biopsies of three arthritis patients known to be PCR-positive for *C. trachomatis* in that tissue. The results of those assays are presented in Fig. 2. The Ct393, Ct052, Ct727, and Ct820 genes all showed expression in these patient samples, as they did in the monocyte model of chlamydial persistence. However, the genes that displayed essentially no up-regulation of expression in the monocyte system (Ct701, Ct762, Ct624, Ct868) all showed some level of increased expression in the patient samples, consistent with data from the mycobacterial study. Ct468 (*pilR/atoC*, sensor subunit of a two-component system), Ct414 (*pmpC*, polymorphic membrane protein), and Ct394 (*hrcA*, transcriptional repressor) all showed similar levels of expression in one or more of the patient samples relative to that found in the monocyte model of persistence. Ct437 (*fusA*, elongation factor G) and Ct776 (*aas*, 2-acylglycerophospho ethanolamine acyltransferase) also were expressed at generally the same levels in patient samples as in the *in vitro* monocyte system. We assume that differences in transcript levels among the three patients for the genes assessed (*e.g.*, Ct414, Ct820) were a function of disease duration and genetic and other characteristics specific to each individual; these characteristics determine the details of host-pathogen interaction for each patient, in turn influencing the course of pathogenesis for each individual.

Discussion

Molecular genetic and other observations have demonstrated that *C. trachomatis* infecting synovial tissues in patients with *Chlamydia*-associated arthritis are in the persistent, rather than the actively-growing, state [2,3 for reviews]. Although we have some knowledge of the transcriptional and morphologic characteristics of persistent

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Chlamydia, we have essentially no understanding of which specific aspects of host-pathogen interaction are responsible for eliciting chlamydial persistence in either the monocyte model system or in synovial materials. This lack of understanding results primarily from the absence of a system for genetic manipulation of any chlamydial species, and from the large number of genes specifying proteins of unknown function encoded by all chlamydial genomes so far sequenced. In the present report, we extrapolate to *C. trachomatis* molecular genetic data from a persistence-related study of *M. tuberculosis*, an organism amenable to genetic manipulation and which undergoes persistent infection [17,22]. We show that 35% of the 194 mycobacterial genes identified in the parent study as showing significant transcriptional up-regulation in support of persistent infection have orthologous coding sequences on the *C. trachomatis* genome. We further show that most of a selected set of those chlamydial orthologs do indeed show increased expression in an *in vitro* model of chlamydial persistence and in relevant patient samples, compared to their normal expression levels during active infection. Thus, the results presented here indicate that some molecular genetic details underlying chlamydial persistence can be gleaned or extrapolated from experimental work in other organisms. Most importantly, however, the data given add new details to the emerging picture of the genetic basis underlying persistent infection by *C. trachomatis*, in both a relevant *in vitro* system and *in vivo* in arthritis patient samples.

The genome of *M. tuberculosis* is about 4.4 mbp in length and encodes about 4000 genes [26]. Most of the 194 genes identified in the persistence study of this organism are expressed at only low level or not at all during axenic growth in culture [22], suggesting that persistence for *M. tuberculosis* may depend on expression of a particular set of genes devoted largely or exclusively to establishment of that state. In contrast, the *C. trachomatis* genome specifies just over 900 coding sequences, and all of them are expressed at one time or another during active growth of the organism [15,16,24,25]. This suggests that, unlike *M. tuberculosis*, *C. trachomatis* does not possess a gene or gene set whose sole function is the genesis and/or maintenance of the persistent infection state. Thus, while *M. tuberculosis* persistence may derive largely from expression of a specific set of genes, in *C. trachomatis* development of that state must be a function of readjustment of transcript levels from genes already being expressed. The challenge will be to understand whether, and if so by what means, that readjustment results from input from the host monocytic cell in the joint, and how that readjustment of gene expression alters chlamydial biochemistry and physiology.

It is difficult to deduce from the results presented here, and from those in the mycobacterial study, whether and if so how metabolic processes are similarly modified overall between the actively-growing and persistent infection states of these two bacterial pathogens. While many of the *M. tuberculosis* genes up-regulated during *in vivo* growth and persistence have known functions, the majority of genes (107/194, 55%) so identified encode proteins of unknown function [22]. Nine of the *C. trachomatis* orthologs of mycobacterial genes found in our BLAST search also were coding sequences specifying proteins of unknown function, but importantly, most of the unknown *M. tuberculosis* genes in the persistence study were shown to be unique to that organism. As pointed out in the Sassetti and Rubin study, these observations indicate that mycobacteriae appear to have evolved mechanisms for *in vivo* survival and persistence that are unique, *i.e.*, not shared by other obligate or facultative intracellular pathogens. Similarly, most coding sequences specifying products of unknown function in the *C. trachomatis* genome are either *Chlamydia*- (Genus-) specific or specific to that organism, probably indicating that the genetic underpinnings of chlamydial persistence are essentially unique to this group as well. Indeed, we suspect that many critical molecular genetic and other details differ between these two organisms with regard to metabolic and other characteristics of

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persistence. Nonetheless, while caution must be exercised in extrapolating the genetic mechanisms underlying persistence in one organism to those performing a similar function in another, circumspect exercise of such extrapolations can be useful.

The panel of *M. tuberculosis* genes underlying *in vivo* growth and persistence, and the group of *C. trachomatis* genes identified as orthologous to them, fall into the same general functional categories, with the exception that the former panel included more genes encoding lipid metabolism-related products than did the latter. As mentioned, given the limited panel of chlamydial genes identified as being orthologous to mycobacterial persistence-related genes, it is difficult to form a detailed picture of the overall transcript pattern underlying chlamydial persistence in monocytic cells, and the overall metabolic characteristics of persistence for the organism that result from that gene expression pattern. One recent study provided a transcriptome analysis of chlamydial gene expression in an *in vitro* model of persistence different than the monocyte system used here [27]. Collaborative studies between this laboratory and another have shown, however, that the profiles of gene expression differ importantly among the several currently employed *in vitro* model systems of persistence studied in various laboratories (A. Klos, H.C.G., A.P.H., unpublished observations). In the case of the monocyte model of chlamydial persistence, which we consider to be the most relevant for studies of pathogenesis in *Chlamydia*-associated arthritis, understanding the transcriptional and metabolic modifications underlying persistence must derive from full transcriptome analyses of that system. We are now performing those analyses.

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Competing Interest Statement

None of the authors has any competing interest to declare.

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FIGURE LEGENDS

Figure 1. Real time RT-PCR analysis of transcript levels from 16 *C. trachomatis* genes identified as orthologous to *M. tuberculosis* genes required for *in vivo* growth and persistence, using cDNA prepared from an *in vitro* model of chlamydial persistence. Preparation of RNA/cDNA and details of the analyses are given in Patients and Methods. Bars represent standard error.

Figure 2. Real time RT-PCR analysis of transcript levels from 16 *C. trachomatis* genes identified as orthologous to *M. tuberculosis* genes required for *in vivo* growth and persistence, using cDNA prepared from *Chlamydia*-infected synovial tissue samples. Preparation of RNA/cDNA and details of the analyses are given in Patients and Methods. Bars represent standard error.

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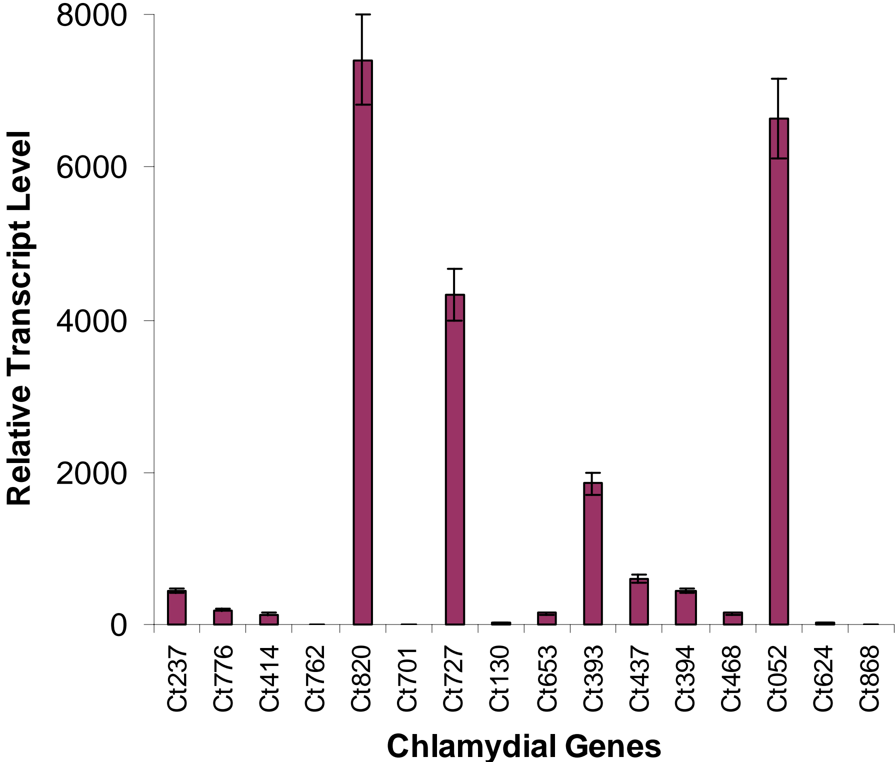
(FIGURE 1)



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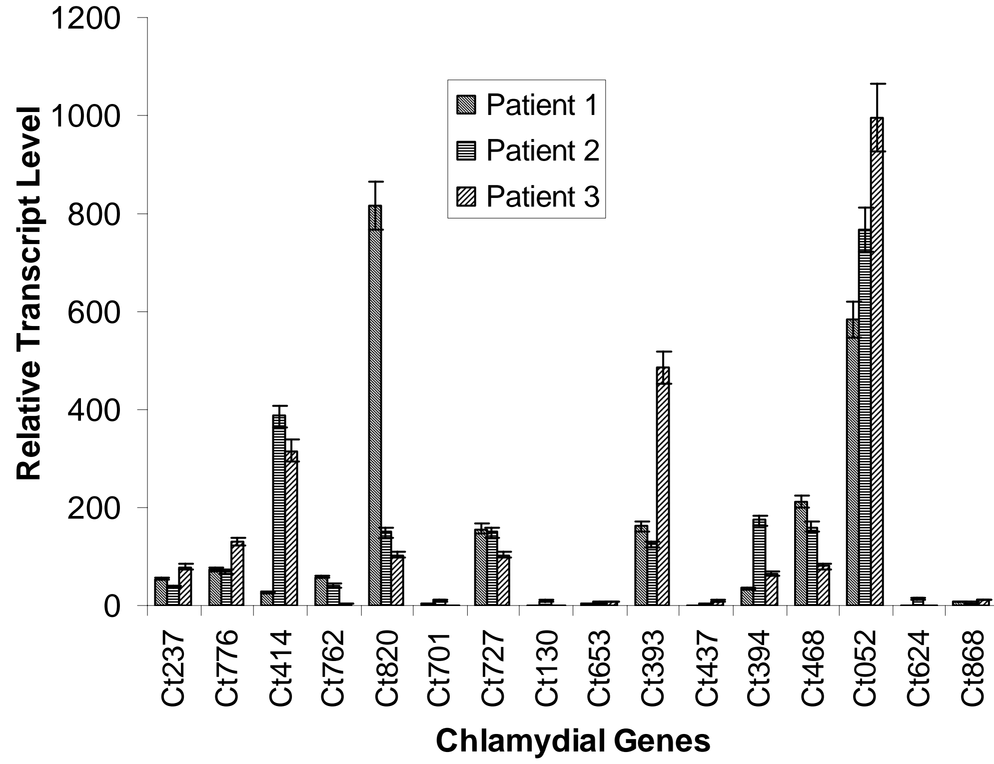
(FIGURE 2)



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