**Chlamydia trachomatis** growth inhibition and restoration of LDL-receptor level in HepG2 cells treated with mevastatin

Yuriy K Bashmakov†, Nailya A Zigangirova1,2†, Yulia P Pashko2, Lidia N Kapotina2, Ivan M Petyaev1

**Abstract**

**Background:** Perihepatitis is rare but consistently occurring extragenital manifestation of untreated *Chlamydia trachomatis* infection. Despite of possible liver involvement in generalized *C. trachomatis* infection, the ability of the pathogen to propagate in the hepatic cells and its impact on liver functions is not thoroughly investigated. The effect of mevastatin, an inhibitor of 3-hydroxy-3-methylglutaryl CoA reductase, on *C. trachomatis* growth in human hepatoma cell line HepG2 has been studied. Bacterial growth was assessed by immunostaining with FITC-labeled monoclonal antibody against chlamydial lipopolysaccharide and by RT-PCR for two chlamydial genetic markers (16S rRNA and euo).

**Results:** Chlamydial inclusion bodies were seen in approximately 50% of hepatocytes at 48 hours in the post infection period. Lysates obtained from infected hepatocytes were positive in the infective progeny test at 48 and especially in 72 hours after infection initiation. It has been shown that chlamydial infection in hepatocytes also leads to the decline of LDL-receptor mRNA which reflects infection multiplicity rate. Additions of mevastatin (1, 20 and 40 μM) 1 hour before inoculation restored and upregulated LDL-receptor mRNA level in a dose-dependent manner. Mevastatin treatment had no effect on internalization of chlamydial particles. However it reduced drastically the number of chlamydial 16S rRNA and euo transcripts as well as overall infection rate in HepG-2 cells. Complete eradication of infection has been seen by immunofluorescent staining at 40 μM mevastatin concentration, when expression level of chlamydial 16S rRNA and euo was undetectable. Lower concentration of mevastatin (20 μM) promoted euo expression level and the appearance of atypically small chlamydial inclusions, while there was a noticeable reduction in the number of infected cells and 16S rRNA transcripts.

**Conclusions:** *C. trachomatis* can efficiently propagate in hepatocytes affecting transcription rate of some liver-specific genes. Ongoing cholesterol synthesis is essential for chlamydial growth in hepatocytes. Inhibitors of cholesterol biosynthesis can supplement conventional strategy in the management of *C. trachomatis* infection.

**Background**

*Chlamydia trachomatis* is a prevalent bacterial pathogen causing most of the cases of urogenital infections and preventable blindness in the world. Epididymitis and urethritis in men, cervical as well as the urethral inflammation in woman may lead to acute pelvic inflammatory disease and variety of other extragenital manifestations in both sexes. Among most frequent extragenital manifestations of *C. trachomatis* are sexually acquired reactive arthritis (SARA), conjunctivitis and perihepatitis [1]. In most of the cases of ophthalmological manifestations *C. trachomatis* can be detected and/or isolated in the eye swabs [2]. It is believed that immunological and hormonal phenotype as well as some genotype characteristics, particularly expression of human leucocyte antigen B27, predetermine the severity of extragenital manifestations caused by *C. trachomatis* [3]. Delayed cell-mediated immunological response is also known to play an important role in the systemic generalization of chlamydial disease [4].

However there is a growing body of evidence that *C. trachomatis* can be present and isolated from...
extragenital tissues and organs. Bacterial antigens, DNA and/or RNA can be detected in whole blood [5,6] since C. trachomatis can efficiently propagate in mononuclear cells [7] as well as in astrocytes [8], muscle cells [9] and myocardioctyes [10]. Virulent forms of C. trachomatis can be isolated from synovial exude [11], ascitic fluid [12,13], liver biopsy material [14], and respiratory secretion fluids [15]. Similar pattern of extragenital manifestations has been reported in animal experiments.

Lesions containing virulent C. trachomatis have been reported in lungs, liver and spleen of BALB/c mice in the post-infection period [16]. With the exception of a single report [14] there are no confirmed cases of C. trachomatis isolation from the human liver or any well articulated insights on the potential role of chlamydial infection in hepatobiliary pathology. However, recently shown ability of C. trachomatis to propagate in hepatocytes [17,18] leads to many questions about possible involvement of liver in systemic chlamydial disease.

In the present paper we have investigated the infectibility of C. trachomatis toward immortalized human hepatoma cells (HepG2 cell line) and some metabolic consequences of chlamydia propagation in the hepatic cell line. In particular, of mRNA regulation of major lipogenic genes in the host cells and effect of mevastatin, an inhibitor of 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase), in cases of chlamydial infection. The results have been summarized in the subsequent text.

HepG2 plates were infected with C. trachomatis at multiplicities of infection (MOI) of 1 or 2 in DMEM with 0.4% glucose without FBS and cycloheximide and centrifuged for 0.5 hour at 1500 g. The cells were harvested for RNA analysis in 24 hours (expression of chlamydial genes) and in 48 hours (expression of eukaryotic genes and immunofluorescence analysis) after infection after the inoculation of C. trachomatis. Accl viability assay was conducted routinely for each group of the experiment using 2% trypan blue exclusion test. The cells monolayers with viability > 85% were used for RNA extraction and/or immunostaining. There was a significant decrease in number of viable hepatocytes during the late stage of chlamydial infection in HepG2 cells (72 hours).

**Immunofluorescence staining**

Infected HepG2 monolayers grown 48 hours on coverslips in 24 well plates, which were fixed with methanol. Permeabilized cells were stained by direct immunofluorescence using FITC - conjugated monoclonal antibody against chlamydial lipopolysaccharide (NearMedic Plus, RF). Inclusion-containing cells were visualized using Nikon Eclipse 50 i microscope fluorescence microscope at X1350 magnification.

**Internalization assay**

Internalization assay has been performed as described [20]. Briefly, to visualize attachment of C. trachomatis to HepG2 cells, elementary bodies (EB) of C. trachomatis were added at MOI 50 to the 24 well plates with coverslips containing hepatocytes monolayers. The EB were allowed to attach in presence or absence of 40 μM mevastatin for 60 min at 4°C after which the inoculum was removed, cell were washed 3 times with ice-cold PBS. To visualize attached particles, the cell monolayers were fixed in 4% paraformaldehyde for 15 min on ice. This regimen of fixation is believed to maintain the integrity of the plasma membrane in the host cells [20]. After fixation the cells were washed with PBS and incubated for 30 min with monoclonal chlamydial LPS-specific antibody labeled with FITC (1 μg/ml, NearMedic Plus, RF) for visualization of attached particles. Internalization has been studied in separate set of experiments. To allow attachment, HepG2 cells were incubated with EB of C. trachomatis in presence or absence of 40 μM mevastatin for 1 hour at 4°C after which the inoculum was removed and the cells were washed 3 times with ice-cold PBS. The cells were transferred to 37°C for 1 hour to permit internalization. After fixation with 4% paraformaldehyde (15 min, room temperature) the cells were incubated for 30 min with the polyclonal antibody raised against EB of C. trachomatis (Gamaleya Institute of Microbiology and Epidemiology, Moscow, RF). This
step was performed in order to block attachment sites of non-internalized EB. After fixation with methanol (15 min, room temperature), which allows penetration of antibody inside of the cells [20], cell monolayers were incubated for 30 min with 1 μg/ml of monoclonal FITC-conjugated antibody against \textit{C. trachomatis} major outer membrane protein (MOMP) (NearMedic Plus, RF). The cells were washed thoroughly with PBS and analyzed by immunofluorescent microscope.

**Assessment of infective progeny**

In order to assess the infective progeny accumulation in HepG2 cells after 48 hour cultivation period, HepG2 cells were harvested, frozen and thawed, as described elsewhere. Serial dilutions of lysates were inoculated onto Hep-2 cells and centrifuged for 0.5 hour at 1500 g. The infected cells were visualized with \textit{C. trachomatis} LPS-specific antibody in 48 hours of the post-infection period.

**RNA extraction and reverse transcription**

RNA was isolated from HepG2 monolayers grown on 6-well plates using TRIZol (Invitrogen). Total mRNA pre-treated with DNase I (DNA-free™, Ambion) and quantified on the spectrophotometer NanoDrop ND-100 (ThermoFisher Scientific, Wilmington, USA) was converted into cDNA using random hexamer primers and a SuperScript III First-Strand Synthesis Kit (Invitrogen, Karlsruhe, Germany).

**Quantitative real-time PCR**

The mRNA levels for two different developmental genes of \textit{C. trachomatis} were analyzed in HepG2 cells by quantitative RT-PCR using thermocycler ANK 32 (Syntol, RF). The 16S rRNA and gene encoding DNA-binding protein Euo were studied as constitutive markers of the early stage of chlamydial developmental cycle. Primers for \textit{C. trachomatis} 16S rRNA (sense - 5′-GGCGATATTGCGGATTCGAGT AACC-3′, antisense - 5′-TCAAATCCAGGGGTATTA ACCGCCT-3′) and \textit{C. trachomatis} Euo (sense - 5′-TC CCCGACGTCTCTCTTTCA-3′, antisense - 5′-CTCG TCAGGCTATATCTGTTG-3′) were verified and used under thermal cycling conditions - 95°C for 10 min and 50 cycles of 95°C for 15 seconds, 60°C for 1 min and 72°C for 20 seconds. Serial dilutions of \textit{C. trachomatis} RNA, extracted from chlamydia-infected Hep-2 cells, were used as a standard for quantification of chlamydial gene expression. The results of PCR analysis for chlamydia-specific genes were normalized to mRNA values of human beta actin (β-actin, primers: sense - 5′-GCACCCGACAAATGAAAGAT-3′, antisense - 5′-GCC CGATCCACCGGAGTAC-3′). Among other human-specific genes studied were major lipogenic enzymes: 3-hydroxy-3-methylglutaryl CoA reductase (HMG CoA reductase, primers: sense-5′-CAAGGAGCATGCAAAGT AATACCC-3′, antisense - 5′-GCCATTACCGTCC CACACA-3′); 3-hydroxy-3-methylglutaryl CoA synthase (HMG CoA Syn, primers: sense - 5′-GACCTGTGC ATTCAAACATAGCAA-3′, antisense - 5′-GCTGTAGGC GGGAGTCCTGTGACT-3′); squalene synthase (SS, primers: sense - 5′-ATGACCATCAGTGTGGAAAG AAG-3′, antisense - 5′-CCGACGTCGTTGGTGTA-3′); and fatty acid synthase (FAS, primers: sense-5′-TC GTGGGCTACAGCATGTT-3′, antisense - 5′-GCC CTCTGAAATCGAAGAAAG-3′).

The mRNA levels for lipogenic enzymes as well as mRNAs for LDL-receptor (LDL-R, primers: sense - 5′-GCCCTGCGTTAATGTGACACTCT-3′, antisense - 5′-CTCTAGCCTGTGGTGCAGACTTTGT-3′) and LDL-receptor related protein (LRP, primers: sense - 5′-CCT CCTGACGCCTGA TTITG-3′, antisense - 5′-GCC CCCCCATGAGTGGT-3′) in the host cells were normalized to human β-actin expression level. The mRNA expression levels in the host cells were referenced to the CT values in uninfected HepG2 cells grown at the same conditions. That reference value was taken as 1.00. Each cDNA sample was tested by PCR at least three times. All experiments were repeated at least twice. Representative sets of results are shown below.

**Results**

\textit{C. trachomatis} growth in HepG2 cells

Immunofluorescent images of HepG2 infected cells reveal that \textit{C. trachomatis} can efficiently grow in immortalized hepatocytes cells line. Positive immunofluorescence was first apparent within 24 hours of post-infection period and did not differ in intensity at MOIs of 1 and 2. Inclusion bodies were seen in about 50% of cells at 48 hours in the post-infection period at MOI of 1. Up to 70% of the infected cells were seen at multiplicity rate of 2. Most of the immunostaining was localized throughout whole cytoplasm. However some cells had perinuclear pattern of immunofluorescence with no intranuclear inclusions seen. At 48 and especially 72 hours of the post-infection period, immunostaining was stronger with numerous inclusion bodies. Some of them were released from the ruptured cells. To determine if \textit{C. trachomatis} can be cultured from HepG2 monolayers, we harvested 24 and 48 hour cultures of hepatocytes. Replication was not observed when 24 hour lysates of hepatocytes were inoculated to Hep2 cells. However the lysates obtained in 48 and especially 72 hour were positive in the infective progeny test.

**LDL-receptor mRNA and multiplicity of infection**

As can be seen from Table 1, 48 hour propagation of \textit{C. trachomatis} in HepG2 cells did not affect mRNA for a major housekeeping gene - 36B4, nor mRNAs for
Table 1 Folds and mRNA changes in HepG2 cells infected with C. trachomatis at different infectivity rates.

| Parameter       | Non-infected cells | Infected cells |
|-----------------|--------------------|----------------|
|                 |                    | MOI 1          | MOI 2          |
| 36B4           | 18.37              | 18.26 18.01    |
| HMG-CoA Red    | 1                  | 1.31 0.98      |
| HMG-CoA Synth   | 1                  | 1.06 0.87      |
| SS             | 1                  | 1.21 0.89      |
| LDL-R          | 1                  | 0.76 0.56      |
| LRP            | 1                  | 0.87 0.99      |
| FAS            | 1                  | 0.88 0.89      |

Mevastatin inhibits chlamydial growth in HepG2 cells

Figure 1 shows representative immunofluorescent images of HepG2 cells infected with C. trachomatis in presence of increasing concentrations of mevastatin. As can be seen, the effect of mevastatin was marginal at the concentration of 1 μM, though some decline in the number of infected cells has been noticed. However, 20 μM mevastatin reduced both the number of inclusion bodies in the infected cells, promoting a perinuclear pattern of staining. Mevastatin-treated cells (20 μM) appeared to contain smaller inclusion bodies similar to those that occur during persistent chlamydial infection [23]. The highest concentration of mevastatin tested (40 μM) abolished the number of infected cells almost completely. Analysis of bacterial transcripts showed a similar tendency. As can be seen from Figure 2, 16S rRNA and euo mRNA were undetectable at highest concentration of mevastatin used, whereas at 20 μM and 1 μM mevastatin reduced the expression level for 16S rRNA by 8 and 3 fold respectively. There is significant induction of euo mRNA at 20 μM mevastatin concentration.

Mevastatin reverses LDL-receptor mRNA decline

Inhibitors of HMG-CoA reductase are the most powerful activators of LDL receptor, whose activity on the LDL-receptor is mediated by SREBP pathway [21]. The addition of mevastatin to HepG2 cells infected with C. trachomatis at MOI of 1 did not affect cell viability nor mRNA levels of 36B4 (Table 2). However, LDL-receptor mRNA level was dose-dependently upregulated with the increasing concentrations of mevastatin, reaching 2 fold induction at 40 μM level. This effect was even more pronounced at 72 hours of the post-infection period though cell viability was declining (results not shown). There is also dose-dependent upregulation of choleseterologenic enzymes (HMG-CoA reductase, HMG-CoA synthase, SS) which is well known effect of statins in the cultures cells [22]. Notably, LDL-receptor related protein mRNA was not impacted under all conditions studied.

Discussion

Although there is a small but growing body of evidence that C. trachomatis can be disseminated widely throughout the human body, the physiological consequences and overall medical relevance of extragenital propagation of C. trachomatis remains poorly understood. First of all, our results confirm initial observations [25] showing the ability of C. trachomatis to propagate in HepG2 hepatoma cell line. More importantly, we have demonstrated that propagation of C. trachomatis in hepatocytes follows full infectious cycle leading to the formation of infectious progeny in 48 and 72 hours of post-infection period. Propagation of the pathogen
Figure 1 Immunofluorescent images of HepG2 cells infected with *C. trachomatis* in presence of mevastatin. HepG2 cells were set up, grown and infected with *C. trachomatis* in presence or absence of mevastatin as described in Methods. Immunofluorescence analysis was performed 48 hours after inoculation of the pathogen. **A** - non-infected cells; **B** – infected cells with no mevastatin; **C** – infected cells in presence of 1 μM mevastatin; **D** – infected cells in presence of 20 μM mevastatin; **E** – infected cells in presence of 40 μM mevastatin. Scale bar = 10 μm.
distinctively affects some specific functions of the liver cells. In particular, *C. trachomatis* ameliorates transcription of LDL-receptor in hepatocytes, which may have various consequences for lipid homeostasis.

Chlamydial organisms are strict intracellular parasites, whose requirements in the metabolites are covered by the host cells. Enhanced uptake of the substrates and metabolites by the infected host cells is a well known “signature” strategy of chlamydial infection mandatory for successful accomplishment of its infectious cycle [25]. However, in the case of the chlamydial growth in HepG2 cells we have seen significant decline in LDL-receptor mRNA, which may potentially result in the reduction of lipid uptake. The biological significance of this finding remains unclear. However it is possible to assume, that decline in the LDL-receptor mRNA might represent a mechanism of metabolic adaption of the host cell to chlamydial infection targeted on limitation of lipid supply and chlamydial growth in the cells. Unfortunately we were not able to document corresponding changes in LDL-receptor protein level due to decline in number of viable HepG2 cells that occurs at 72 hour time point of post-infection period. Models of persistent chlamydial infection might be required for evaluating hepatic LDL-receptor turnover in the infected liver cells.

**Figure 2** Expression of chlamydial 16S RNA and euo in infected hepatocytes grown at different concentration of mevastatin. HepG2 cells were set up, grown and infected with *C. trachomatis* in presence or absence of mevastatin as described in Methods. RNA was extracted in 24 hours after inoculation of the bacteria. Expression of chlamydial genes was normalized to copy number of eukaryotic β-actin.

**Figure 3** Attachment of chlamydial particles to plasma membrane of hepatocytes in presence or absence of mevastatin. HepG2 cells were set up, grown and incubated with chlamydial particles (EB) in presence or absence of mevastatin as described in Methods. Attached particles were visualized with FITC-labeled antibody against chlamydial LPS. A – attachment of chlamydial particles in absence of 40 μM mevastatin; B – attachment of chlamydial particles in presence of 40 μM mevastatin. Scale bar = 10 μm.
Secondly, we have clearly shown that mevastatin, an inhibitor of cholesterol biosynthesis, restores LDL-receptor mRNA and has a significant anti-chlamydial activity reducing chlamydial growth in infected hepatocytes. Genome of C. trachomatis does not contain genes responsible for lipid biosynthesis. Chlamydial species are known to acquire cholesterol, fatty acids and triglycerides from the host cells [26]. Therefore, it was reasonable to believe that targeting the cholesterol biosynthetic pathway in the host cells might affect chlamydial infection rate. This prediction was confirmed by RT PCR analysis. It is well acknowledged, that C. trachomatis 16S rRNA gene expression is an informative criterion of chlamydial developmental cycle expressed in both early and late stages of C. trachomatis infection [27]. Detection of 16S rRNA transcript as a marker of viable and metabolically active Chlamydia allows to evaluate the effectiveness of different antibacterial agents [28]. Maximum inhibition of 16S rRNA as well as drastic reduction in the number of infected immunofluorescence-positive cell has been seen at 40 μM mevastatin level. Less pronounced decline in 16S rRNA transcript level has been observed at 20 μM mevastatin concentration. Even though addition of 20 μM mevastatin did not result in complete inhibition of chlamydial growth in HepG2 cells, there was formation of smaller chlamydial inclusions. Those are often observed in antibiotic- and/or cytokine-treated cells when concentration of the agent is not enough to induce complete eradication of the pathogen [23]. “Aberrant” chlamydial cells are known to have some metabolic activity but fail to induce new rounds of chlamydial infection [23,28]. Therefore inhibition of chlamydial growth in mevastatin-treated HepG2 cells takes place in clearly dose-dependent manner. Step-wise decline in 16S rRNA level was accompanied by reduction in the number of infected cells (1 and 20 μM mevastatin), as well as the appearance of “aberrant” chlamydial forms (20 μM mevastatin) until complete eradication of chlamydial growth takes place (40 μM mevastatin). Euo mRNA level has been changing in a similar manner, except inconsistent increase seen at 20 μM concentration of mevastatin. However, it is known that euo mRNA can be highly induced when the developmental cycle of C. trachomatis in cultured cells is compromised by addition of cytokines and other substances affecting chlamydial growth [28]. It has been proposed, that increased expression of euo may inhibit transcription of the genes specific for “late phase” of chlamydial developmental cycle [28,29]. Thus, enhanced transcription rate of euo may represent self-sufficient mechanism predetermining anti-chlamydial activity of mevastatin.

It is also important to conclude, that according to our results mevastatin has no effect on initial interaction of chlamydial particles with host cell, allowing the entry of the pathogen into hepatocytes. Therefore we assume that later stages of chlamydial developmental cycle are affected by mevastatin treatment. The effect of different metabolites and inhibitors of mevalonate pathway needs to be tested in hepatocytes infected with C. trachomatis in presence of mevastatin. It is possible, that anti-chlamydial activity of mevastatin takes place due to reduced geranylgeranylation of host cell proteins as it happens in case of lovastatin-treated hepatocytes infected with hepatitis C virus [30].

Conclusions
We have demonstrated that ongoing cholesterol synthesis is essential for chlamydial growth in hepatocytes. Although the precise mechanism of anti-chlamydial activity of mevastatin remains to be elucidated, targeting the cholesterol biosynthetic pathway may represent an effective strategy in management of chlamydial infection.

Acknowledgements
Ms Agni Roce is appreciated for invaluable help during experimental work and manuscript preparation.

Author details
1 Cambridge Theranostics Ltd, Babraham Research Campus, Babraham, Cambridge, CB2 4AT, UK. 2 Department of Medical Microbiology, Institute of Epidemiology and Microbiology RAMS, 18 Gamaley Str, Moscow 123098, Russia.

Authors’ contributions
YKB and NAZ contributed equally into design, acquisition of data, analysis and interpretation of the results. YPP and LNK performed immunostaining and RNA protocols. IMP contributed into primary concept, drafting the manuscript, and final approval for publishing the results. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Received: 1 July 2009 Accepted: 28 January 2010 Published: 28 January 2010

References
1. Baguley S, Greenhouse P. Non-genital manifestations of Chlamydia trachomatis. Clinical Medicine 2003, 3:206-208.
2. Yang JL, Hong KC, Schachter J, Moncada J, Lekew T, House J, Zhou Z, Neuwelt MD, Rutar T, Halfpenny C, Shah N, Whitcher JP, Lietman TM. Detection of Chlamydia trachomatis ocular infection in trachoma-endemic communities by rRNA amplification. Invest Ophthalmol Vis Sci 2009, 50:90-94.
3. Kobayashi S, Kida I. Reactive arthritis: recent advances and clinical manifestations. Intern Med 2005, 44:408-412.
4. Bilenki L, Wang S, Yang J, Fan Y, Joyce AG, Yang X. Chlamydia trachomatis NK T cell activation promotes infection in vivo. J Immunol 2005, 175:3197-3206.
5. Roubalova K. A simultaneous detection of Chlamydia pneumoniae and Chlamydia trachomatis DNA by real-time PCR. Epidemiol Mikrobiol Imunol 2007, 56:166-173.
6. Nasion TA, Cheong SF, Lim CT, Leong EW, Ngnew YF. Multiplex PCR for the detection of urogenital pathogens in mothers and newborns. Malays J Pathol 2007, 29:19-24.
7. Schrader S, Klox A, Hess S, Zeidler H, Kuipers JG, Rihl M. Expression of inflammatory host genes in Chlamydia trachomatis-infected human monocytes. Arthritis Res Ther 2007, 9:R54.
8. Dreses-Werringloer U, Gérard HC, Whittum-Hudson JA, Hudson AP. Chlamydiophila (Chlamydia) pneumoniae infection of human astrocytes and microglia in culture displays an active, rather than a persistent, phenotype. *Am J Med Sci* 2006, 332:168-174.

9. Yang X, Coriolan D, Schultz K, Golnik DD, Beasley D. Toll-like receptor 2 mediates persistent chemokine release by *Chlamydia pneumoniae*-infected vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 2005, 25:2308-2314.

10. Wang G, Buczynski F, Harinoff B, Zhong G. Infection of myocytes with *Chlamydiae*. *Microbiology* 2002, 148:3955-3969.

11. Rihl M, Köhler L, Kos A, Zedler H. Persistent infection of *Chlamydia* in reactive arthritis. *Ann Rheum Dis* 2006, 65:281-284.

12. Shabot JM, Roak GD, Truant AL. *Chlamydia trachomatis* in the ascitic fluids of patients with chronic liver disease. *Am J Gastroenterol* 1983, 78:291-294.

13. Shabot AM. *Chlamydia trachomatis* and ascites: Going with the flow?. *Hepatology* 2005, 9:505-506.

14. Dan M, Terrell LDJ, Goldsand G. Isolation of *Chlamydia trachomatis* from the liver of patients with prolonged fever. *Gut* 1987, 28:1514-1516.

15. Chen CJ, Wu KG, Tang RB, Yuan HC, Soong WI, Hwang BT. Characteristics of *Chlamydia trachomatis* infection in hospitalized infants with lower respiratory tract infection. *J Microbiol Immunol Infect* 2007, 40:255-259.

16. Artenanova N, Theodor J, Peterson EM, de la Maza LM. Role of neutrophils in controlling early stages of a *Chlamydia trachomatis* infection. *Infect Immun* 1996, 64:4830-4833.

17. Hatch GM, McClarty G. *Chlamydia*-infection accelerates metabolism of phosphatidylcholine in endoplasmic reticulum from low density lipoproteins but does not affect phosphatidylcholine secretion from hepatocytes. *BMC Microbiology* 2004, 4:8.

18. Wang G, Buczynski F, Anderson J, Zhong G. Effect of host fatty acid-binding protein and fatty acid uptake on growth of *Chlamydia trachomatis* L2. *Microbiology* 2007, 153:1935-1939.

19. Caldwell KD, Kornhout J, Schachter J. Purification and partial characterization of the major outer membrane protein of *Chlamydia trachomatis*. *Infect Immun* 1981, 31:1161-1176.

20. Carabeo RA, Greshaber SS, Fisher E, Hackstadt T. *Chlamydia trachomatis* induces remodeling of the actin cytoskeleton during attachment and entry into HeLa cells. *Infect Immun* 2002, 70:3793-3803.

21. Goldstein JL, Brown MS. The LDL receptor. *Arterioscler Thromb Vasc Biol* 2009, 29:431-8.

22. Medina M, Cho F, Ruan W, Rotter J, Krauss RM. Alternative splicing of 3-hydroxy-3-methylglutaryl coenzyme A reductase is associated with plasma low-density lipoprotein cholesterol response to simvastatin. *Circulation* 2008, 118:355-362.

23. Hammerschlag MR. The intracellular life of chlamydiae. *Semin Pediatr Infect Dis* 2002, 13:239-248.

24. Stuart ES, Webley WC, Norkin LC. Lipid rafts, caveolae, caveolin-1, and entry by *Chlamydiae* into host cells. *Exp Cell Res* 2003, 287:67-78.

25. Wang G, Buczynski F, Anderson J, Zhong G. Effect of host fatty acid-binding protein and fatty acid uptake on growth of *Chlamydia trachomatis* L2. *Microbiology* 2007, 153:1935-1939.

26. Kumar Y, Cocciairo J, Valdivia RH. The obligate intracellular pathogen *Chlamydia trachomatis* targets host lipid droplets. *Curr Biol* 2006, 16:1646-1651.

27. Bellard RJ, Zhong G, Crane DD, Hogan D, Studenwalt D, Sharma J, Beatty WL, Caldwell HD. Genomic transcriptional profiling of the developmental cycle of *Chlamydia trachomatis*. *Proc Natl Acad Sci USA* 2003, 100:8478-8483.

28. Perry LL, Su H, Feilzer K, Messor R, Hughes S, Whittine W, Caldwell HD. Differential sensitivity of distinct *Chlamydia trachomatis* isolates to IFN-gamma-mediated inhibition. *J Immunol* 1999, 162:3541-3548.

29. Zhang L, Douglas AL, Hatch TP. Characterization of a *Chlamydia psittaci* DNA binding protein (EUO) synthesized during the early and middle phases of the developmental cycle. *Infect Immun* 1998, 66:1167-1173.

30. Ye J, Wang G, Sumpter R Jr, Brown MS, Goldstein JL, Galle M Jr. Disruption of hepatic C virus RNA replication through inhibition of host protein geranylgeranylation. *Proc Natl Acad Sci USA* 2003, 100:15865-15870.