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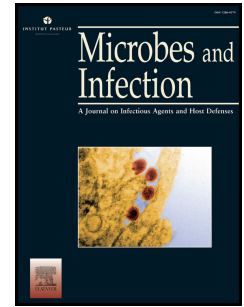
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1 ***Immunobiology of monocytes and macrophages during***
2 ***Chlamydia trachomatis infection***

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Abstract

16 Infections caused by the intracellular bacterium *Chlamydia trachomatis* are a global health
17 burden affecting more than 100 million people annually causing damaging long-lasting
18 infections. In this review, we will present and discuss important aspects of the interaction
19 between *C. trachomatis* and monocytes/macrophages.

20
21 **Keywords:** Monocytes; macrophages; *Chlamydia trachomatis*

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34 **1 Introduction**

35 *Chlamydia trachomatis* (*C. trachomatis*) is a small intracellular Gram-negative human
36 pathogenic bacterium, which comprises a range of serovars based on variations in the major
37 outer membrane protein (MOMP). These serovars are genetically similar, but cause different
38 pathological manifestations. Serovar A-C cause the blinding eye condition, trachoma; D-K
39 cause sexually transmitted genital infection, which can lead to pelvic inflammatory disease,
40 ectopic pregnancy, and infertility. Finally, serovar L1-L3 can spread from the genital tract to
41 the lymphatic system causing more disseminated infections.

42 *Chlamydiae* are obligate intracellular bacteria with a unique biphasic developmental cycle.
43 Initially, the small (0.3 μm) infectious but metabolic inactive elementary body (EB) infects
44 the epithelial host cell. Intracellularly, the EB transforms to a larger (1 μm) and metabolic
45 active reticular body (RB) and the RB starts to replicate.

46 *C. trachomatis* serovars preferably infect mucosal epithelium, but can also infect a range of
47 other cells including fibroblasts and cells of the immune system [1].

48 Monocytes and macrophages are recruited to the genital tract during experimental genital
49 *Chlamydia* infection and the initial engagement between macrophages and *C. trachomatis*
50 may determine the overall outcome of the infection [2,3]. Efficient phagocytosis and
51 intracellular killing can limit ascension of the infection and provide antigenic material for
52 activating CD4⁺ T-cells towards a Th1-mediated immune response - the most critical immune
53 response to eradicate *C. trachomatis* infections [4]. Different murine infection models have
54 demonstrated the importance of these mechanisms in controlling *Chlamydia* infections.[5,6].
55 However, if intracellular elimination in macrophages fails, macrophages may be used as
56 Trojan horses for bacterial dissemination to the lymphatic system with bacterial replication in
57 the draining lymph nodes. Especially the L-biovars have been linked to intracellular survival
58 and dissemination [7]. Lastly, monocytes and macrophages also play important roles in the

59 immunopathology of *C. trachomatis* infections by secreting proinflammatory cytokines
60 causing collateral tissue damage [3]. Thus, understanding the interaction between
61 macrophages and *C. trachomatis* is critical to understand how protective immunity develops
62 and how the immunological response causes pathology.

63 A proposed role for *C. trachomatis*-infected monocytes in the pathogenesis of reactive
64 arthritis prompted a number of studies in the late 1980's trying to understand the interaction
65 between monocytes/macrophages and *C. trachomatis*. Since these initial studies, several
66 efforts have been made to understand monocyte/macrophage functions in *Chlamydia*-induced
67 inflammation and to understand why *C. trachomatis* infections tend to be chronic.
68 Clearly, the intracellular fate of *C. trachomatis* in macrophages is completely distinct from
69 the normal developmental cycle observed in epithelial cells. Thus, before discussing the
70 immunobiology of macrophages during chlamydial infection, we will begin with a concise
71 presentation of current knowledge about the developmental cycle in epithelial cells to set the
72 scene for discussions.

73 **2 The developmental cycle of *C. trachomatis* in epithelial cells**

74 The developmental cycle of *C. trachomatis* in epithelial cells has been studied in decades and
75 is now rather well characterized. Depending on the serovar, *C. trachomatis* EBs engage
76 epithelial cells in the eye or in the genital mucosa where they attach to host cell surface
77 components namely heparan sulfate proteoglycans. Upon attachment, *C. trachomatis* induces
78 its own uptake by secreting pre-formed effector proteins into the host cell cytosol through a
79 type III secretion system. One of these effectors is translocated actin-recruiting
80 phosphoprotein (TarP), which is tyrosin phosphorylated by host cell kinases when
81 translocated [8,9]. TarP is an actin modifying protein inducing rearrangement of the actin
82 cytoskeleton and uptake of *C. trachomatis* into a membrane-enclosed vesicle [10]. Each
83 chlamydial EB is taken up in an independent vesicle, which is transported to the microtubule-

84 organizing center in the perinuclear area of the cell. This process is facilitated by interaction
85 with microtubules and the motor protein dynein [11]. At the microtubule organizing center,
86 the independent *Chlamydia*-containing vesicles undergo homotypic fusion thereby
87 establishing a single large membrane enclosed vacuole called an inclusion [12].
88 The stability and unique physiology of this replicative niche is established by inserting
89 translocated secreted inclusion membrane proteins (Incs) into the inclusion membrane. Inc
90 proteins face the cytoplasmic site of the inclusion membrane and interact with different
91 membrane-sorting proteins including numerous Ras-related protein Rab (Rab) GTPases.
92 These interactions inhibit fusion with destructive vesicular compartments, e.g. lysosomes
93 while promoting fusion with nutrient-rich compartments such as lipid-rich Golgi-derived
94 vesicles [13].
95 During inclusion formation, the infectious EBs differentiate into metabolically active RBs
96 that start replicating by binary fission or polarized cell division leading to growth of the
97 inclusion [14]. After 48-72 hours, the end of the developmental cycle is reached when RBs
98 have transformed back to EBs. Burst of the cell or membrane extrusion liberates infectious
99 EBs ready for new rounds of infection. Generally, the underlying mechanisms mediating host
100 cell exit remain poorly described. However, it was recently shown that chlamydial membrane
101 extrusion is mediated by interaction with inclusion membrane proteins and host Ca^{2+} -channels
102 reducing myosin motor activity necessary for extrusion formation [15].

103 **3 Macrophage encounter of *C. trachomatis***

104 The first encounter between *Chlamydia* and mononuclear phagocytes takes place in the
105 genital tract mucosa. The genital mucosa contains tissue-resident macrophages and
106 monocytes which engage *Chlamydia* EBs once liberated from lysed epithelial cells after
107 completion of the developmental cycle [16]. In early infectious stages, epithelial cells secrete

108 several chemokines and proinflammatory cytokines leading to local inflammation and
109 leukocyte recruitment [17,18]. Using mouse models of genital *C. trachomatis* infection it was
110 demonstrated that CD11b-positive cells (monocytes/macrophages) infiltrate the mucosa
111 during infection [2]. This recruitment is likely induced by secretion of chemokines including
112 CCL2 and macrophage inflammatory protein-1 α known to attract monocytes to the site of
113 infection [19,20]. Thus, both resident macrophages and monocyte-derived macrophages
114 recruited from the bloodstream engage invading *C. trachomatis* in the genital mucosa. The
115 encountered *Chlamydia* organisms, liberated from the epithelial cells, consist of both EBs and
116 RBs. Both forms can trigger the inflammatory response and provide antigenic material as
117 discussed in the following sections.

118 **4 Macrophage sensing of *C. trachomatis***

119 At the site of infection, macrophages recognize the bacteria directly through different innate
120 immune receptors. Abundant evidence shows that *C. trachomatis* recognition activates
121 MyD88- and P38/ERK-dependent signaling pathways, suggesting a role for pattern
122 recognition receptors (PRRs) in chlamydial sensing [21–23].

123 Monocytes and macrophages are equipped with numerous PRRs, which detect a variety of
124 conserved structural motifs known as pathogen associated molecular patterns (PAMPs). *C.*
125 *trachomatis* contains several PAMPs; the most well-studied being LPS and Heat Shock
126 Protein (HSP) 60. Furthermore, HSP70, pORF5, lipoproteins, and macrophage infectivity
127 potentiator (MIP) have been confirmed to activate host macrophages through PRRs
128 [21,23,24].

129 Using photo-chemically inactivated *C. trachomatis* EBs, Bas et al. show a prominent cell
130 activation of monocytes and macrophages [24]. In addition, macrophages stimulated with
131 viable or inactivated *C. trachomatis* display different cytokine profiles [25–27]. Collectively,

132 these observations suggest that both surface and intracellular receptors detect and respond to
133 chlamydial infection presumably activating different downstream signaling pathways.
134 Particularly, members of the toll-like receptor (TLR) family and the nucleotide-binding
135 oligomerization domain (NOD) like receptor family have been implicated in chlamydial
136 recognition.
137 The macrophage receptors involved in *C. trachomatis* recognition and the subsequent
138 intracellular events are illustrated in Fig. 1.

139 **4.1 Toll-like receptors in *C. trachomatis* recognition**

140 Like other Gram-negative bacteria *C. trachomatis* contains LPS in the outer membrane, a
141 potent ligand for TLR4 and the co-receptor CD14. Therefore, it is rational to expect an
142 important role of TLR4 in *C. trachomatis* recognition. Using CD14 and TLR4 transfected cell
143 lines, early studies did indeed discover a role for these receptors in recognition of chlamydial
144 LPS [28,29]. In support, Heine et al. showed that preincubating human peripheral blood
145 mononuclear cells with a CD14-blocking antibody completely abrogated cellular activation
146 by chlamydial LPS confirming the *Chlamydia*-sensing role of CD14 [29]. More recent
147 studies, however, suggest that the contribution of TLR4 in chlamydial recognition by
148 monocytes may be limited [23,24,30]. Instead, several reports suggest that *C. trachomatis*
149 induced activation of monocytes is TLR2 dependent. These observations originate from
150 studies using different strategies including cell lines transfected with different TLRs, primary
151 cells treated with receptor-blocking antibodies, and primary cells from TLR-deficient mice
152 [21,23,24,30–32]. Collectively, these studies suggest that TLR2 recognizes live *C.*
153 *trachomatis* EBs together with several PAMPs such as LPS, pORF, lipoproteins, and MIP.
154 Interestingly, Agrawal et al. found that both TLR2 and TLR4 are involved in *C. trachomatis*
155 recognition in human cervical monocytes with a time-dependent contribution of each
156 receptor[16]. Thus, early detection was TLR4-dependent, but switched to TLR2-dependent

157 recognition at later time points. In addition, activation through TLR4, but not TLR2, induced
158 interleukin(IL)-12 production [16]. These observations outline the necessity of careful
159 interpretation of studies investigating chlamydial activation of host cell receptors when
160 considering experimental design.

161 An interesting study by Nagarajan et al. found that neither TLR2 nor TLR4 are involved in *C.*
162 *trachomatis* induced interferon (IFN)- β production. Instead they showed the induction of
163 IFN- β was dependent on endosome acidification and the adaptor molecule MyD88 [26]. The
164 authors did not identify the involved receptors, but suggested that the recognition could be
165 mediated by intracellular TLRs, including TLR7, -8, and -9 [26]. However, using
166 macrophages from TLR7- and TLR9 KO mice, the same authors demonstrated that these
167 receptors are dispensable for IFN- β production [32]. Applying macrophages generated from
168 human induced pluripotent stem cells, Yeung and colleagues demonstrated an important role
169 for interferon regulatory factor 5 (IRF5) in intracellular survival of *C. trachomatis* in
170 macrophages [33]. IRF5 is activated downstream of TLR7 and TLR8, suggesting a possible
171 role for these receptors in chlamydia recognition by human macrophages.

172 Lastly, also TLR1 and TLR6 have been shown to participate in chlamydial recognition by
173 inducing cell activation in response to chlamydial MIP and the lipopeptide PamCSK4 [24].
174 Yet, blocking these receptors does not have the same effect as blocking TLR2. Thus, TLR2
175 seems to be the predominating TLR used for macrophage recognition of *C. trachomatis* while
176 *Chlamydia*-induced type I interferon response is TLR-independent highlighting the
177 importance of other PRRs outside the TLR family.

178 **4.2 NOD-like receptors**

179 TLR-deficiency or TLR-blockage does not abrogate cellular activation completely, proposing
180 a redundancy in TLR-based *C. trachomatis* recognition. NOD-like receptors are cytosolic
181 receptors playing an important role in microbial sensing and innate defense. The NOD-like

182 receptor family consists of 23 members of which two have been reported in *C. trachomatis*
183 sensing: NOD1 and nucleotide-binding domain, leucine-rich repeat family, pyrin domain
184 containing 3 (NLRP3). The involvement of NOD1 in *Chlamydia* recognition was established
185 using expression and gene knockdown studies in HeLa cells [32,34,35]. At present, no direct
186 evidence for NOD-based recognition in macrophages exists, although NOD contribution has
187 been confirmed for other intracellular bacteria and may also be involved in macrophage
188 recognition of *C. trachomatis* [36]. Nonetheless, the contribution of NOD1 has been obscure
189 since these receptors recognize and ligate peptidoglycan fragments from the bacterial cell wall
190 [37]. Until recently, peptidoglycan has not been directly detected in *C. trachomatis*, even
191 though the *C. trachomatis* genome contains all necessary genes for peptidoglycan assembly
192 and is sensitive to beta-lactam antibiotics [38]. In 2014, the Maurelli group, however, directly
193 detected peptidoglycan in *C. trachomatis* using a novel metabolic cell wall labeling approach
194 [39] and later confirmed the presence of muropeptides using mass spectrometry [40]. Finally,
195 it has been demonstrated that NOD2 expression is upregulated in *C. trachomatis*-infected
196 macrophages, suggesting that NOD2 may also participate in macrophage recognition of *C.*
197 *trachomatis* [41].

198 NLRP3 is another NOD-like receptor which senses molecules associated with cell damage
199 including adenosine triphosphate (ATP) and uric acid [37]. It constitutes the pattern
200 recognition moiety of a large multiprotein complex known as the inflammasome. PAMP
201 mediated inflammasome activation leads to caspase-1 activation and subsequently cleavage
202 and secretion of IL-1 β and IL-18. Chlamydial infection of monocytes activates the
203 inflammasome in a NLRP3, AIM2 and MyD88-dependent manner [27,42,43]. Whether
204 NLRP3 directly recognizes chlamydial PAMPs or if the activation results from endogenous
205 danger-associated molecular patterns (DAMPs) induced by *C. trachomatis* is not fully

206 understood, but a role for reactive oxygen species (ROS) [43] and autocrine cytokine
207 signaling (please see the section below) [27] have been proposed.

208 **4.3 Cytosolic DNA receptors**

209 Finally, the cytosolic DNA sensors stimulator of interferon genes (STING) and the absent in
210 melanoma 2 (AIM2) might also participate in *C. trachomatis* recognition by sensing
211 chlamydial nucleic acids (Fig. 1). STING detects cytosolic double-stranded DNA and plays
212 an important role during both bacterial and viral infections. It was previously demonstrated
213 that STING mediates IFN- β induction in *Chlamydia* infected HeLa cells and that *C.*
214 *muridarum* induced IFN- β production in J774 macrophages was cyclic GMA-AMP synthase
215 (cGAS)-dependent. cGAS is a cytosolic DNA-sensing enzyme that detects foreign DNA
216 converting it to cyclic nucleic acids which is recognized by STING [32,44]. Direct STING-
217 mediated recognition of *Chlamydia* by macrophages was shown recently by Webster and
218 colleagues [27]. They demonstrated that STING recognizes cyclic di-AMP from metabolic
219 active *C. trachomatis* in murine macrophages leading to IFN- β secretion and autocrine IFN- β
220 dependent inflammasome activation and IL-1 β secretion [27]. However, this observation
221 awaits confirmation in human primary macrophages. Translating this conclusion directly to
222 human conditions is controversial due to the debatable metabolic state of *C. trachomatis* in
223 human primary macrophages.

224 AIM2 is another cytosolic receptor sensing double-stranded DNA and like NLRP3 involved
225 in inflammasome activation. A recent study showed that *C. trachomatis*-induced
226 inflammasome activation in murine macrophages was AIM2 dependent implying that AIM2
227 might detect chlamydial DNA [27,42].

228 **4.4 Cellular activation and cytokine production**

229 Although the exact mechanisms mediating macrophage recognition of *C. trachomatis* are not
230 fully comprehended, macrophage engagement with *C. trachomatis* elicits a potent cell
231 activation inducing the expression of several cytokines, chemokines, and growth factors that
232 are summarized in Table 1.

233 **5 *C. trachomatis* entry into macrophages**

234 Several *C. trachomatis* serovars are internalized into both murine and human primary
235 macrophages and into different cell lines. However, the involved receptors and molecular
236 mechanisms mediating chlamydial entry into host immune cells have not been determined yet
237 [1,43,45,46]. The entry mechanisms are supposedly carried out by phagocytosis or by
238 receptor-mediated endocytosis [46–48] and the involved receptors might be located to lipid
239 rafts in the plasma membrane [49].

240 Comparing chlamydial infection rates in cell types with different surface receptor profiles
241 could highlight the involvement of receptors and receptor families. Since *C. trachomatis*
242 infects many different cell types the receptors involved may be ubiquitously expressed or
243 involve multiple entry mechanisms working with essentially equal efficiency [1,50,51]. This
244 theory is supported by the findings by Sun et al. who observed a similar infection rate
245 between HeLa cells and murine RAW macrophages [52]. In contrast, others find that *C.*
246 *trachomatis* entry occurs much less efficiently in monocytes compared to epithelial cells
247 indicating involvement of cell-specific receptors [53]. However, this study, among others,
248 evaluated the entry efficiency by enumerating inclusions two days post infection. Thus, the
249 data presented in this study may not reflect the actual entry efficiency, since inclusion
250 numbers after two days also depend on bacterial survival and replication.

251 Glycosylated chlamydial surface proteins may provide a moiety for host cell attachment and
252 entry. Kuo et al demonstrated that *C. trachomatis* entry into macrophages was significantly
253 reduced in macrophages deficient in the mannose receptor [54]. The chlamydial ligand
254 attaching to the mannose receptor has not been identified, but it has been suggested that
255 chlamydial MOMP is glycosylated by mannose [55] and might therefore serve as ligand for
256 the mannose receptor facilitating chlamydial entry. The mannose receptor is used by
257 *Mycobacterium tuberculosis* to enter macrophages and entry through this receptor is
258 beneficial for intracellular survival [56].

259 Another receptor involved in *Mycobacterium tuberculosis* entry is the complement receptor
260 CR3 [57]. Complement receptors are also likely involved in chlamydial entry because *C.*
261 *trachomatis* is opsonized by the complement C3 fragment iC3b which is recognized by CR3
262 expressed on monocytes and macrophages [58,59]. We recently demonstrated that
263 complement C3 facilitates rapid uptake of *C. trachomatis* in human monocytes supporting the
264 role for CR3 in chlamydial uptake, [59].

265 Lastly, chlamydial recognition and uptake may be dependent on how *Chlamydia* are liberated
266 from infected epithelial cells after completing the development cycle. *C. trachomatis* liberated
267 by membrane extrusion is engulfed by murine macrophages through an actin-dependent
268 mechanism involving extrusion membrane phosphatidylserine (PS) [60]. PS is normally
269 exposed in the membrane of apoptotic cells and is recognized by apoptotic receptors on
270 phagocytes. However, blocking PS-receptor interaction by annexin V only partially inhibit
271 macrophage uptake of *Chlamydia* containing extrusions, indicating involvement of other
272 receptor-ligand interactions [60].

273 **6 The intracellular fate of *C. trachomatis* in macrophages**

274 Studies exploring the intracellular fate of *C. trachomatis* in macrophages have been carried
275 out since the 80's, but despite more than 30 years of research there is still no clear
276 understanding of the intracellular trafficking and fate of *C. trachomatis* in macrophages. Early
277 studies indicated that *C. trachomatis* can persist in monocytes for more than 7 days [61–63],
278 while others, more recent studies, show that *C. trachomatis* is rapidly degraded in
279 macrophages [52]. One thing is however certain; the intracellular fate of *C. trachomatis* in
280 monocytes and macrophages differs drastically from the normal developmental cycle seen in
281 epithelial cells as demonstrated in Fig. 2.

282 After macrophage entry *C. trachomatis* can induce a state of persistency, where the bacterium
283 is viable and metabolic active, but does not replicate [1,22,63]. This phenomenon has been
284 demonstrated for several serovars including Ba, D, K, and L2. Although viable and metabolic
285 active, the different serovars cannot maintain the developmental cycle, except for serovar L2
286 [22,64,65]. It appears that serovar L2 can maintain its infectious potency during monocyte
287 infection, because lysates from L2-infected monocytes induce inclusion formation in HeLa
288 cells [53,65]. Nonetheless, we recently demonstrated that *C. trachomatis* L2 were unable to
289 maintain its infectious and growth potential after 24 hours of incubation within monocytes
290 [59]. Different infection/incubation protocols are likely to cause these discrepancies. Table II
291 provides an overview of studies investigating the intracellular fate of *C. trachomatis* in
292 monocytes and macrophages as well as the main findings. Collectively, these findings
293 indicate that monocytes may respond differently to different serovars; that serovar-specific
294 survival mechanisms exist; that infection protocols may affect the chlamydial outcome and/or
295 different macrophage cell types respond differently to *C. trachomatis* infection.

296

297 **6.1 Macrophage strategies to restrict *C. trachomatis* growth**

298 Why is the development of *C. trachomatis* infection successful in epithelial cells but not in
299 macrophages? Following entry into the epithelial cell, *C. trachomatis* forms a membrane-
300 bound vacuole; the inclusion, as previously described in section 1. Yet, *C. trachomatis* fails to
301 form a mature inclusion in macrophages and this failure is likely due to several mechanisms
302 involving phagosome-lysosome fusion, autophagy, and nutrient starvation.

303 **6.1.1 Targeting *C. trachomatis* for lysosomal degradation**

304 Lysosomal degradation of engulfed bacteria is an important mechanism for bacteria
305 elimination. Usually, a coordinated procedure involving sequential trafficking to vesicles of
306 increased acidity target endocytosed or phagocytosed bacteria to lysosomes. . Recruitment of
307 the proton pump vacuolar H⁺ ATPase (V-ATPase) mediates the acidification and the
308 sequential trafficking is coordinated by a set of GTP-binding proteins including the Rab
309 GTPases. Of these, Rab5 and Rab7 target vesicles for early endosomes and late endosomes,
310 respectively [66].

311 Several studies propose that *C. trachomatis* fails to inhibit phagosome-lysosome fusion in
312 macrophages. Shortly after entry into murine macrophages, chlamydial EBs locate to Rab7-
313 positive compartments, a late endosome marker, and subsequently associate with the
314 lysosome marker lysosomal-associated membrane protein 1 (Lamp1) [52,67]. Reducing
315 lysosome acidification by inhibiting V-ATPase supports chlamydial growth in macrophages
316 and suggests that *C. trachomatis* EBs are trafficked through the conventional
317 phagosome/lysosome pathway in macrophages [52,67,68]. This is completely different from
318 epithelial cells where Rab GTPases, different from Rab5 and Rab7, are recruited and target
319 the *Chlamydia*-containing vesicles to non-destructive vesicular compartments.

320 **6.1.2 Anti-chlamydial defense by autophagy**

321 Autophagy is another means of targeting bacteria to lysosomes. Autophagy induction by *C.*
322 *trachomatis* was first described by Pachikara et al. in HeLa cells [69] and accumulating
323 evidence suggests that autophagy also plays a substantial role in macrophage clearance of *C.*
324 *trachomatis* [52,67].

325 Autophagy is a ubiquitous mechanism used to degrade and sequester cytosolic protein and
326 organelles to maintain cell homeostasis [70]. During autophagy, a double membrane structure
327 assembles which surrounds the protein/organelle/pathogen thereby creating a vesicular
328 structure called an autophagosome. The autophagosome is directed to lysosomes and after
329 fusion, the autophagosomal content is degraded [70]. The autophagic pathway is illustrated in
330 Fig. 3.

331 Upon entry into macrophages, *C. trachomatis* associates with the autophagosomal marker
332 LC3 and is observed in large doubled membrane structures resembling autophagosomes
333 [52,68]. In accordance, functional experiments show that autophagic activity is elicited in
334 infected macrophages, but not in infected epithelial cells [52]. Knockdown of autophagy
335 protein 5 (ATG5), a key regulator of autophagy, increases *C. trachomatis* progeny numbers in
336 THP-1 cells [67]. The autophagic potency of macrophages can be enhanced by IFN- γ
337 stimulation mediated by IFN-inducible proteins called guanylate-binding proteins. During
338 IFN- γ cell activation, these proteins co-localize with chlamydial EBs and direct them for
339 lysosomal fusion through an autophagy-dependent pathway [67]. External ATP stimulation
340 can induce chlamydial vacuole fusion with lysosomes in addition to IFN- γ activation, but
341 whether this process occurs through autophagy has not been determined [71]. The entry and
342 intracellular trafficking of *C. trachomatis* into macrophages is illustrated in Fig. 3.

343

344 **6.1.3 Direct interaction by perforin-2**

345 Perforin-2 is a phylogenetic conserved pore-forming protein containing a domain, which is
346 also found in other vital immunological proteins such as complement C9 and perforin-1 [72].
347 Varying expression of perforin-2 during *C. trachomatis* infection may account for the
348 different infection outcome between macrophages and epithelial cells [73]. Monocytes and
349 macrophages constitutively express perforin-2, and IFN- γ stimulation induce expression in
350 epithelial cells. Unfortunately, this induction is inhibited by chlamydial proteins [73].
351 Perforin-2 expression increases in macrophages, but not in epithelial cells, during *C.*
352 *trachomatis* infection indicating that perforin-2 expression may be regulated by gene
353 regulatory factors acting downstream of immune receptors. The local cytokine milieu
354 generated by *C. trachomatis* infected epithelial cells increases perforin-2 expression in either
355 resident macrophages or invading monocytes, potentially boosting perforin-2 expression
356 before direct contact with the bacterium [74].
357 Inducing perforin-2 knock down by small interfering RNA in macrophages leads to
358 maturation of *C. trachomatis* inclusions and the growth pattern resembles that of epithelial
359 cells. In addition, chlamydial growth is restricted in perforin-2 expressing epithelial cells. The
360 anti-chlamydial defense mechanism responsible for these observations is mediated through
361 direct contact with the bacterium [73]. Thus, macrophages synthesize perforin-2 in response
362 to *C. trachomatis* and prevent chlamydial-induced perforin-2 degradation by limiting
363 chlamydial de novo protein synthesis. This provides an efficient chlamydial killing
364 mechanism involving direct contact with the bacterium.

365

366 **6.1.4 Induction of reactive oxygen and nitrogen species**

367 Production of reactive oxygen species and reactive nitrogen species (ROS and RNS) are
368 important microbicidal mechanisms against various pathogens [75]. Inducible nitric oxide

369 synthase (iNOS) is produced during *C. trachomatis* infection in macrophages and leads to
370 nitric oxide production [16,64], which is strongly correlated with chlamydial clearance [76].
371 The mechanisms leading to iNOS induction involve a ROS- and cathepsin-dependent
372 mechanism acting downstream of TLR2 activation [77]. In addition, *C. trachomatis*, but not
373 *C. pneumoniae*, induces ROS production in macrophages. The differential induction of ROS
374 could explain why *C. trachomatis* is killed earlier than *C. pneumoniae* in macrophages [64].
375 Indeed, macrophages deficient in NADPH oxidase, a ROS generating enzyme, support
376 intracellular survival and replication of *C. trachomatis* [27]. Finally, ROS has also been
377 implicated in inflammasome activation since adding an antioxidant to *C. trachomatis* infected
378 macrophages reduces caspase-1 activation [43].

379

380 **6.1.5 Limiting access to host cell nutrients**

381 *C. trachomatis* exploits a parasitic nature relying on host cell components for maintaining
382 metabolism and survival. Hence, restricting chlamydial access to host cell nutrients inhibits
383 bacterial growth.

384 Tryptophan is an essential amino acid required for chlamydial growth and survival. An
385 essential anti-chlamydial defense mechanism is IFN- γ induced expression of indoleamine 2,3-
386 dioxygenase (IDO). IDO catabolizes tryptophan to L-kynurenine leading to depletion of
387 cytosolic tryptophan and chlamydial growth restriction [78]. Macrophages induce IDO
388 expression in response to *C. trachomatis* infection by different serovars, which may
389 contribute to the growth restriction observed in macrophages [22,79].

390 Acquisition of host cell lipids to the inclusion membrane is regarded an essential step in
391 chlamydial inclusion maturation and reproduction [80]. This process involves Golgi-
392 disruption and acquisition of lipid-containing Golgi-vesicles. By preventing cleavage of
393 golgin84, macrophages prevent Golgi-disruption during infection thereby preventing

394 inclusion maturation [52]. In epithelial cells however, golgin84 is cleaved leading to Golgi
395 disruption and acquisition of lipid-rich Golgi-vesicles to the growing inclusion [52].
396 Another approach of restricting chlamydial growth by nutrient starvation is by reducing
397 intracellular iron levels [81]. Increasing intracellular iron levels by reducing surface-
398 expressed ferroportin in macrophages increases the fraction of large *C. trachomatis* inclusions
399 [82]. Thus, chlamydial growth is dependent on host-cell iron metabolism. Modulation of these
400 pathways could provide a defense mechanism against *C. trachomatis*. Expression of ferritin
401 heavy chain is increased during *C. trachomatis* infection of monocytes [79]. Ferritin could be
402 anti-chlamydial by binding intracellular iron thereby decreasing the concentration of free iron
403 available for *C. trachomatis* in the infected cell.

404 **7 Antigen-presentation of *C. trachomatis* infected macrophages**

405 The primary role for monocytes and macrophages in anti-bacterial immunity is mediated by
406 phagocytosis and secretion of proinflammatory cytokines. However, monocytes and
407 especially macrophages contain major histocompatibility complex (MHC) class I and MHC
408 class II molecules making them competent inducers of adaptive immunity. Possible antigen-
409 presentation pathways in *C. trachomatis* infected macrophages are illustrated in Fig. 4.

410

411 **7.1 Macrophages and CD4+ T-cells in *C. trachomatis* infection**

412 Th1 responses are the predominant adaptive immunological response to control and eliminate
413 *C. trachomatis* infection like most other intracellular bacteria [83]. Activated Th1 cells secrete
414 IFN- γ and TNF- α , which potentiate microbicidal mechanisms in macrophages and inhibit
415 chlamydial growth in infected epithelial cells as previously described.

416 How do monocytes and macrophages contribute to Th1 immunity during chlamydial
417 infection? Activation of naïve CD4+ T-cells requires T-cell recognition of chlamydial

418 antigens presented in MHC class II molecules together with co-receptor ligation and an
419 appropriate cytokine signal. Several *C. trachomatis* proteins have been shown to contain
420 MHC class II epitopes including HSP60, MOMP and PMP [84]. During infection with *C.*
421 *trachomatis* monocytes upregulate the expression of MHC class II molecules and the co-
422 stimulatory receptors CD40, CD80 and CD86 [16,41,74,79]. IFN- γ and IL-12 drive T-cell
423 polarization in the Th1 direction. Several studies have shown that *C. trachomatis* leads to
424 IFN- γ and IL-12 expression and secretion from infected macrophages (Table I) [16,41,85,86].
425 Hence, macrophages infected with *C. trachomatis* seem to direct the adaptive response
426 towards Th1 immunity.

427 Although Th1 mediated immunity is pivotal for infection control and resolution, the
428 macrophage induced T-cell response is not directed solely against Th1 activation. Some
429 investigations suggest that *C. trachomatis* infected monocytes might also drive a Th2
430 mediated response or modulate the effector functions of activated T-cells [87–89]. Lu et al.
431 showed that murine macrophages pulsed *ex vivo* with UV-inactivated *C. muridarum* failed to
432 induce a Th1 dominant response when adoptively transferred. Instead, mice immunized with
433 *ex vivo* pulsed macrophages had high titers of IgG1 *Chlamydia*-specific antibodies suggesting
434 an IL-4 mediated Th2 response [88]. The authors did not evaluate whether macrophages in
435 fact induced IL-4 secretion in response to *C. trachomatis* pulsing. In fact, macrophage
436 secretion of IL-4 have not yet been established, but micro array analysis have shown that IL-4
437 mRNA is upregulated in human monocytes early after infection [90].

438 **7.2 Macrophages and CD8+ T-cells in *C. trachomatis* infection**

439 Besides the Th1- response, cell-mediated immunity against *Chlamydia* may also involve
440 CD8+ T-cells. When activated, these cells differentiate into cytotoxic T-cells, which possess
441 efficient killing mechanisms targeted against host cells infected with intracellular pathogens.
442 The relevance and importance of CD8+ mediated immunity during chlamydial infections has

443 not yet been fully established. Different studies have shown that *Chlamydia*-specific CD8+ T-
444 cells are generated during *C. trachomatis* infection and that they participate actively in anti-
445 chlamydial immunity [91].

446 CD8+ T-cells recognize small peptides loaded on MHC class I molecules. Therefore,
447 pathogen-derived antigens need to be proteolytically processed before loading onto MHC
448 class I happens. Enzymatic processing of MHC class I antigens is mediated by the
449 ubiquitin/proteasome system located in the cytosol. Thus, only pathogens/antigens accessing
450 the cytosol are targets for MHC class I antigen presentation and CD8+ T-cell activation. The
451 process of presenting exogenously acquired antigens on MHC class I is known as antigen
452 cross-presentation and this immunological mechanism is restricted to professional antigen-
453 presenting cells, such as dendritic cells and macrophages [92]. Accordingly, *C. trachomatis* is
454 only a potential target for antigen cross-presentation if chlamydial antigens enter the cytosol.

455 In epithelial cells, *C. trachomatis* secretes different proteins into the host cell cytosol. If these
456 proteins are secreted in macrophages too, entering MHC class I processing is possible[9,93–
457 95]. However, these proteins are important for inclusion formation and may not be secreted in
458 macrophages since *C. trachomatis* fail to induce inclusion maturation in macrophages.

459 Interestingly though, Prantner et al. demonstrated that the translocon protein sec61 locates to
460 the chlamydial inclusion in macrophages [32]. Sec61 has recently been demonstrated to
461 facilitate antigen translocation from an endosomal compartment into the cytosol [96]. Thus,
462 when *C. trachomatis* EBs or RBs are degraded in macrophages, chlamydial proteins may
463 escape the vesicular compartment entering the cytosol and may be tagged for MHC class I
464 presentation. This process is potentially facilitated by increased expression of MHC class I
465 and transporter associated with antigen processing (TAP1) in macrophages activated by
466 conditioned medium from *C. trachomatis* infected epithelial cells [74]. TAP is a

467 transmembrane protein that facilitates transport of antigenic peptides from the cytosol to the
468 MHC class I loading compartment in the ER.

469 **7.3 Modulation of T-cell responses**

470 Although chlamydial infection initiates both CD4+ and CD8+ cell-mediated immune
471 responses, eradication of the infection does not occur. The insufficiency of chlamydial
472 clearance mechanisms may be due to chlamydial-induced attenuation of T-cell immunity.
473 Jendro and colleagues demonstrated that culture supernatants from *C. trachomatis* infected
474 monocytes induced apoptosis of T-cells by a TNF- α dependent mechanism [97,98]. Another
475 way of regulating T-cell immunity is by attenuating T-cell effector functions. It has been
476 demonstrated that chlamydial-infected macrophages reduce IFN- γ release from co-cultured T-
477 cells [99].

478 **8 Summary**

479 Chlamydial growth in monocytes and macrophages is limited and differs drastically from the
480 classical growth pattern seen in epithelial cells. The restricted growth pattern is mediated by
481 several mechanisms including lysosome trafficking, perforin-2 interaction, production of
482 reactive species, and nutrient starvation. The receptors and mechanisms mediating chlamydial
483 recognition and entry are poorly understood and need further investigation. Additionally,
484 there is still dissension on the intracellular trafficking of *C. trachomatis* in macrophages.
485 Confirmation of current observations in human primary cells remains.

486

487 **Conflict of interest**

488 The authors declare no conflicts of interest.

489

490

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813 **Figure legends**

814

815 **Figure 1. Macrophage receptors involved in recognition of *C. trachomatis*.** TLRs

816 expressed at the cell surface recognize several chlamydial PAMPs such as LPS, HSP60,

817 lipoproteins, MIP, pORF5, and probably many others. Ligation of the different TLRs initiate

818 a signaling cascade that ultimately leads to nuclear translocation of transcription factors and
819 expression of genes encoding proinflammatory cytokines. After entry, *C. trachomatis* is
820 engaged by another set of receptors in the intracellular compartment. Induction of IFN- β is
821 dependent on endosomal acidification and MyD88, suggesting that intracellular TLRs may
822 participate in IFN- β induction. The cytosolic NOD-like receptor NOD1 recognizes
823 peptidoglycan and ligation leads to activation of IKKs (I κ B kinases) and NF- κ B. NLRP3,
824 another NOD-like receptor, recruits the adaptor protein ASC (apoptosis-associated speck-like
825 protein containing a CARD) and P-Casp1 (pro-caspase 1) during infection forming a
826 multiprotein complex known as the inflammasome. Inflammasome assembly leads to
827 caspase-1 activation and caspase-1-dependent cleavage and secretion of IL-1 β . The NLRP3
828 activating compound has not been identified yet, but several endogenous molecules including
829 ATP and ROS (reactive oxygen species) can activate NLRP3. Also the DNA-sensing receptor
830 AIM2 is involved in *Chlamydia*-induced inflammsome activation. Finally, chlamydial DNA
831 can be recognized by the ER-associated receptor STING (stimulator of interferon genes).
832 STING ligation leads to translocation of IRF3 (Interferon Regulatory Factor 3) and
833 transcription of type I interferons.

834

835 **Figure 2. *C. trachomatis* infection in epithelial cell and monocyte.**

836 Both cell types have been cultured with *C. trachomatis* L2 for 24 hours. In HeLa cells (left),
837 *C. trachomatis* replicate and form a large inclusion at 24 hours, but it fails to do so in
838 monocytes (right).

839

840 **Figure 3. Entry and intracellular trafficking of *C. trachomatis* in macrophages.**

841 *C. trachomatis* entry into macrophages is facilitated by both ubiquitous and cell type-specific
842 surface receptors. The entry mechanisms are supposedly carried out by receptor-mediated

843 endocytosis and phagocytosis, involving the mannose receptor, complement receptors and
844 possibly receptors recognizing phosphatidylserine (PS) in *Chlamydia*-containing extrusions.
845 Upon entry, *C. trachomatis* EBs are localized to Rab7-positive compartments indicative of
846 late endosomes. Rab7 traffics *Chlamydia*-containing vesicles to lysosomes, where the bacteria
847 are killed by the acidic pH and lysozymes.

848 Autophagy is another mechanism that targets *C. trachomatis* to lysosomes. Here, several
849 ATG proteins facilitate the formation of a double-membrane structure that surrounds the
850 bacteria creating an autophagosome. Interferon-inducible GBPs (guanylate binding proteins)
851 modifies the autophagosomal membrane and facilitates fusion with lysosomes creating
852 autolysosomes that leads to chlamydial killing. Finally, perforin-2 leads to *C. trachomatis*
853 growth restriction by targeting EB directly or by modulating the compartment in which the
854 bacteria reside.

855

856 **Figure 4. Antigen-presentation in *Chlamydia*-infected macrophages.** Both CD4⁺ T-cells
857 and CD8⁺ T-cells are activated during *C. trachomatis* infection. Both MHC class II and the
858 co-stimulatory molecules CD80/CD86 are upregulated in macrophages during chlamydial
859 infection. In addition, both IL-12 and IFN- γ are secreted from activated macrophages
860 directing the CD4⁺ T-cell differentiation in a Th1 direction. Activated Th1 cells secrete TNF-
861 α and IFN- γ , which potentiate the microbicidal potency of macrophages. In addition, also Th2
862 immunity is elicited and the Th2-differentiation is mediated by IL-4, which is
863 transcriptionally upregulated in response to infection.

864 The mechanisms involved in *Chlamydia*-induced CD8⁺ T-cell activation have not been
865 elucidated, but it may occur through antigen cross-presentation. In this pathway, chlamydial
866 proteins may escape the endosomal compartment leading to enzymatically processing by the
867 proteasome. *Chlamydia*-derived peptides are trafficked to the ER or endosomal structures via

868 TAP (Transporter associated with antigen processing) where they are loaded onto MHC class
869 I molecules.

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Table 1. Cytokines and chemokines induced by *Chlamydia* in macrophages

Study	Cytokine/chemokine	Species	Cells	<i>Chlamydia</i> spp.	Reference
Abdul-Sater et al.	IL-1 β	Human	THP-1	<i>C. trachomatis</i> L2	[37]
Agrawal et al.	IL-1 β , IL-6, IL-12, IFN- γ	Human	Cervical monocytes	<i>C. trachomatis</i> *	[9]
Bas et al.	IL-1 β , IL-6, IL-8, TNF- α	Human	Monocytes	<i>C. trachomatis</i> L2	[18]
Darville et al.	IL-6, TNF- α	Mouse	Peritoneal macrophages	<i>C. muridarum</i>	[39]
Datta et al.	IL-1 β , IL-10, TNF- α	Human	Monocytes	<i>C. trachomatis</i> Ba, D, and L2.	[16]
Hui et al.	IL-1 β , IL-8, TNF- α	Human	THP-1	pORF5 from <i>C. trachomatis</i>	[15]
Jendro et al.	TNF- α	Human	Monocytes	<i>C. trachomatis</i> K	[40]
Kol et al.	IL-6	Human	Monocytes	HSP60 from <i>C. trachomatis</i>	[41]
Krausse-Opatz et al.	IL-8	Human	Monocytes	<i>C. trachomatis</i> K	[42]
Lausen et al.	IL-6, IL8	Human	Monocytes	<i>C. trachomatis</i> L2	[43]
Manor et al.	TNF- α	Human	Monocyte-derived macrophages	<i>C. trachomatis</i> K and L2	[44]
Marangoni et al.	IFN- γ , TNF- α	Human	Monocytes	<i>C. trachomatis</i> D	[45]
Mpiga et al.	IL-1 β , IL-6, IL-8, IL-12	Human	THP-1 (human)	<i>C. trachomatis</i> L2	[46]
Nagajaran et al.	IFN α , IFN β , IP10, TNF- α	Mouse	Peritoneal macrophages	<i>C. trachomatis</i> Nigg.	[20]
Rothermel et al.	IL-1 α , IL-1 β	Human	Monocytes	<i>C. trachomatis</i> L2	[47]
Schrader et al.	IL-1 α , IL-1 β , IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-11, IL-15, IL-16, IL-17, IL-18, IFN- γ , TGF- β 1, TGF- β 2, TNF- α	Human	Monocytes	<i>C. trachomatis</i> K	[48]
Yilma et al.	IL-6, IL-8, TNF- α	Mouse	J774 macrophages	<i>C. muridarum</i>	[19]
Yilma et al.	IL-1 α , IL-1 β , IL-6, IL-9, IL-12, IL-15, GM-CSF, G-CSF, CCL2, CXCL1, CXCL5, CXCL10.	Mouse	J774 macrophages	<i>C. muridarum</i>	[35]
Wang et al.	MIP-2	Mouse	Monocyte-derived macrophages	Recombinant predicted lipoproteins from <i>C. trachomatis</i> D.	[17]

*Serovar not specified

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Table 2. Intracellular survival of *C. trachomatis* in monocytes/macrophages

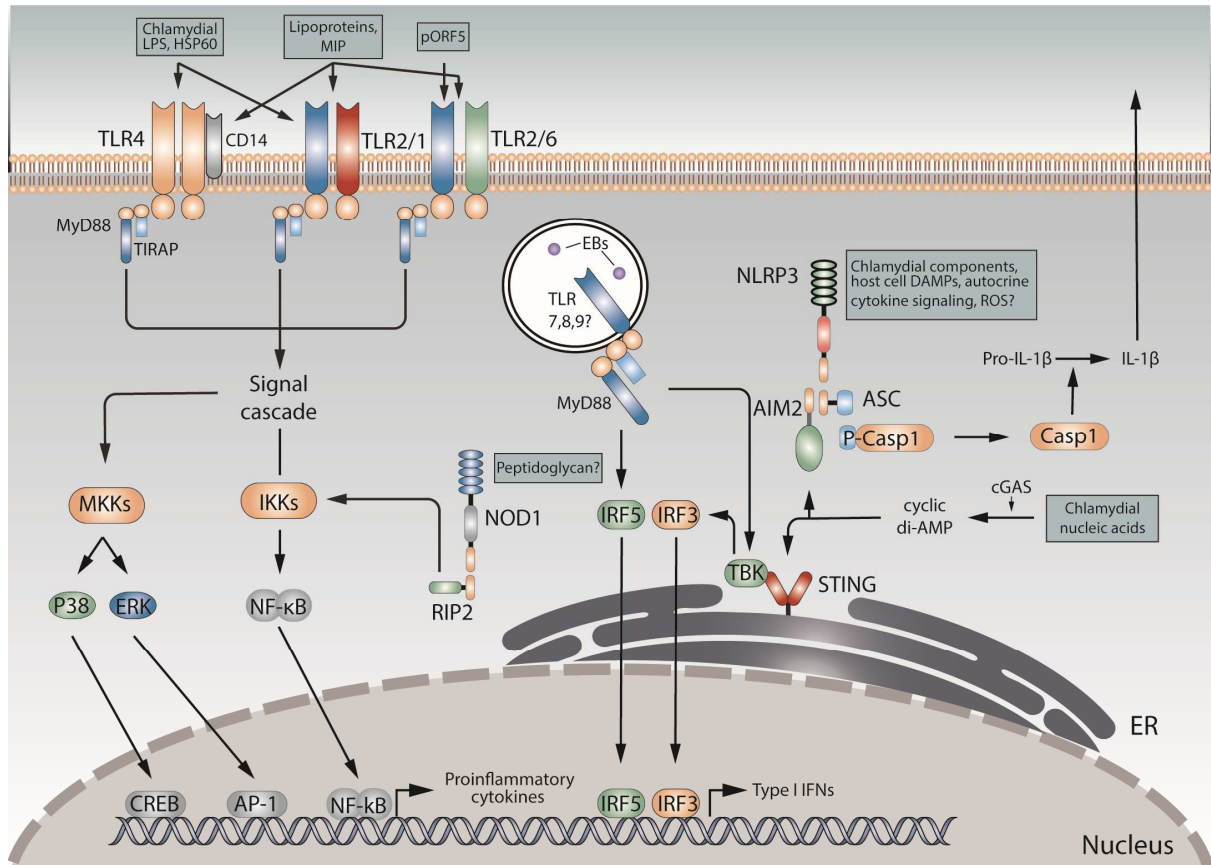
Study	<i>Chlamydia</i> spp.	Species	Cells	Method	Results	Ref
Lausen et al. 2018	<i>C. trachomatis</i> L2	Human	Peripheral blood monocytes	IFU ¹ on McCoy cells	No IFUs 24h p.i. ²	[59]
Nagarajan et al. 2018	<i>C. muridarum</i>	Mouse	Peritoneal macrophages	IFU on L929 cells	200% of initial IFUs are recovered 24h p.i.	[100]
Webster et al. 2017	<i>C. trachomatis</i> ?*	Mouse	BMDM ³	Quantification of LPS and qPCR on 16S RNA	LPS can be detected 24h p.i.	[27]
Yeung et al. 2017	<i>C. trachomatis</i> F	Human	iPSdM ⁴ and monocyte-derived macrophages	Quantification of GFP and IFU assay on McCoy cells	Bacteria replicates in iPSdM for 48h p.i.	[33]
Zuck et al. 2017	<i>C. trachomatis</i> L2	Mouse	BMDM	IFU assay on HeLa cells	No IFUs 4h and 8h p.i.	[60]
Zuck et al. 2016	<i>C. trachomatis</i> B, D, L2 and <i>C. muridarum</i>	Mouse	BMDM	Direct detection by fluorescence	All serovars except serovar B are detected 6h p.i.	[101]
Finethy et al. 2015	<i>C. muridarum</i>	Mouse	BMDM	qPCR on 16S RNA	Survives 24h p.i.	[42]
Rajaram et al. 2015	<i>C. muridarum</i>	Mouse	RAW264.7 cells	IFU assay on McCoy cells	Reproductive infection is observed 24h p.i. and is MOI ⁵ dependent	[77]
Datta et al. 2014	<i>C. trachomatis</i> Ba, D and L2	Human	Peripheral blood monocytes	IFU assay on HeLa cells	Serovar L2, but not Ba and D survives for 2 days p.i.	[53]
Marangoni et al. 2014	<i>C. trachomatis</i> D	Human	Peripheral blood monocytes	IFU assay on LLC-MK2 cells	No detectable IFUs 24h p.i.	[64]
Chen et al. 2013	<i>C. trachomatis</i> G	Human	Peripheral blood monocytes	IFU assay on Hep-2 cells	IFUs are detected 48h p.i.	[102]
Fields et al. 2013	<i>C. trachomatis</i> B, D, L2 and <i>C. muridarum</i>	Mouse	BV2 macrophages + RAW 264.7 cells	IFU assay on HeLa cells	L2 IFUs are detected 24h p.i	[73]
Sun et al. 2012	<i>C. trachomatis</i> L2	Mouse	RAW 264.7 cells	IFU assay on HeLa cells	IFUs are detected 24h p.i.	[52]
Azenabor et al. 2011	<i>C. trachomatis</i> ?*	Human	THP-1 cells	IFU assay on Hep-2 cells	Few detectable IFUs 72h	[99]
Yasir et al. 2011	<i>C. trachomatis</i> L2 and <i>C. muridarum</i>	Mouse	RAW 264.7 cells	IFU assay on HeLa cells	Four times as many IFUs are recovered from muridarum compared to L2 24h p.i	[68]

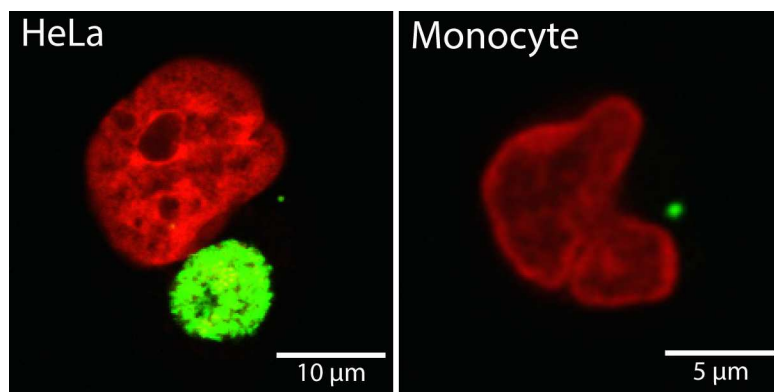
Paradkar et al. 2008	<i>C. trachomatis</i> ?*	Mouse	BMDM	Direct inclusion visualization	10% cells contain large inclusions 24h p.i.	[82]
Schnitger et al. 2006	<i>C. trachomatis</i> K	Human	Peripheral blood monocytes	qPCR on ompA, euo and groEL1	Expression of all genes are observed after 7 days in monocytes	[103]
Gerard et al. 2002	<i>C. trachomatis</i> K	Human	Peripheral blood monocytes	qPCR on chlamydial rRNA and metabolic enzymes	Most mRNAs are detected after 2 days and rRNA after 5 days p.i.	[104]
Gerard et al. 1998	<i>C. trachomatis</i> K	Human	Peripheral blood monocytes	qPCR on chlamydial mRNA	Transcripts of glyQs, gseA, hsp60 and omp2 are observed 10 days p.i.	[105]
Nettelbreker et al. 1998	<i>C. trachomatis</i> K	Human	U937 cells	Direct inclusion visualization and IFU assay on Hep-2 cells	Chlamydial replication is observed from day 1 to 10 p.i.	[106]
Chen et al. 1996	<i>C. trachomatis</i> D	Mouse	RAW 264.7 cells	Direct inclusion visualization and IFU assay on McCoy cells	IFU recovery increases from 24h to 48h and IFUs are recovered 6 days p.i.	[76]
Koehler et al. 1996	<i>C. trachomatis</i> K	Human	Peripheral blood monocytes	TEM ⁶ visualization of inclusions	Atypical inclusions are observed for up to 10 days p.i.	[63]
Numazaki et al. 1995	<i>C. trachomatis</i> L2	Human	U937 cells	PCR on chlamydial DNA	DNA is detected 90 days p.i.	[62]
Schmitz et al. 1993	<i>C. trachomatis</i> K	Human	Peripheral blood monocytes	IFU assay on Hep-2 cells	3-5% of original inoculum is detected after 2h and few inclusions after 24h. MOMP is detected up until 14 days p.i.	[61]
Zhong et al. 1988	<i>C. trachomatis</i> L1	Mouse	Peritoneal macrophages	In vivo infection followed by IFU assay on HeLa cells	IFUs are recovered 60h p.i.	[107]
Bard et al. 1987	<i>C. trachomatis</i> L2	Human	HL-60 cells	Direct inclusion visualization and IFU assay on McCoy cells	IFUs are recovered 72h p.i.	[108]
Yong et al. 1987	<i>C. trachomatis</i> B, C, I, L1 and L2	Human	Peripheral blood monocytes	Direct inclusion visualization and IFU assay on HeLa cells	LGV biovars survive and replicate for 48h, but only in cells incubated for 8-9 days. Trachoma biovars do not survive.	[109]
Bard et al. 1986	<i>C. trachomatis</i> L2	Human	Peripheral blood monocytes	Direct inclusion visualization and IFU assay on McCoy cells	<0,5% IFU yield compared to initial inoculum 32h p.i.	[110]
Manor et al. 1986	<i>C. trachomatis</i> L2	Human	Peripheral blood monocytes and monocyte-derived macrophages	TEM visualization of inclusions + IFU assay on MA-104 cells	Recovery of IFUs from monocytes at all tested time points up until 120h p.i. IFUs from macrophages decrease at 24h and increase at 72h.	[111]

[Kuo et al. 1978	<i>C. trachomatis</i> B and L2	Mouse	Peritoneal macrophages	IFU assay on HeLa cells	Macrophages contain typical inclusions.	
					Both serovars form inclusions at 48h.	[112]
					4% of serovar B is recovered after 48h while 50% of serovar L2 is recovered. B is detected for 4 days and L2 for 9 days p.i..	

¹IFU: Inclusion forming units; ²p.i.: post inoculation; ³BMDM: Bone marrow-derived macrophages; ⁴iPSdM: Induced pluripotent stem cell-derived macrophages; ⁵MOI: Multiplicity of infection, ⁶TEM: Transmission electron microscopy.

*Serovar not specified





ACCEPTED MANUSCRIPT

