ELSEVIER

Institut Pasteur

Microbes and Infection xx (2017) 1−8

www.elsevier.com/locate/micinf

Chlamydiales, *Anaplasma* and *Bartonella*: persistence and immune escape of intracellular bacteria

Aurélie Scherler, Nicolas Jacquier, Gilbert Greub*

Centre for Research on Intracellular Bacteria, Institute of Microbiology, University Hospital Centre and University of Lausanne, Lausanne, Switzerland

Received 19 September 2017; accepted 7 November 2017
Available online ■ ■ ■

Abstract

Intracellular bacteria, such as *Chlamydiales*, *Anaplasma* or *Bartonella*, need to persist inside their host in order to complete their developmental cycle and to infect new hosts. In order to escape from the host immune system, intracellular bacteria have developed diverse mechanisms of persistence, which can directly impact the health of their host.

Keywords: Persistence; Intracellular bacteria; Aberrant bodies; *Anaplasma*; *Bartonella*; *Chlamydiales*

1. Introduction

Historically, persistence phenomenon was first described in 1944 when J. W. Bigger discovered that in presence of penicillin, a small proportion of bacteria named “persisters”, were able to survive without acquiring antibiotic resistance [1]. Indeed, subcultures obtained from persisters and treated with antibiotic were as sensitive as the pure culture grown in absence of antibiotics. Bacterial persistence is commonly defined as the ability of a specific subpopulation of bacteria to grow in presence of antibiotic treatment or to escape from innate and adaptive immunity by becoming “invisible”. Additionally, persistence can also describe a viable but not cultivable stage resulting in the prolonged colonization of the host. Because of these diverse definitions, the term persistence should be used carefully since it can be interpreted in different ways.

Due to their obligate or facultative intracellular lifestyle, bacteria such as *Chlamydiales*, *Anaplasma* and *Bartonella* need to survive in the inhospitable environment of the host cell. They have to avoid detection and degradation by the host immunity. Furthermore, obligate intracellular bacteria need host resources for their own development as well as to ensure their transmission to a new host. Therefore, intracellular bacteria have developed diverse strategies in order to escape host immunity and finally proliferate inside their host. Interestingly, in adverse conditions in which proliferation is not possible, some obligate intracellular bacteria have developed mechanisms of persistence, allowing their survival, waiting for more favourable conditions.

This review aimed to highlight the diversity of persistence mechanisms used by different obligate intracellular bacteria such as *Chlamydiales*, *Anaplasma* or *Bartonella*. Members of the *Chlamydiales* order are known to form a persistent stage, named aberrant bodies (ABs), or to interfere with the nuclear factor-kappa B (NFκB), a protein complex known to play a key role in regulating the immune response to infection. In contrast, *Anaplasma* persistence can occur by antigenic variation of the major surface proteins (MSPs) or by the lack of peptidoglycan (PG) layer or lipopolysaccharide (LPS). Finally, *Bartonella* spp. have developed diverse mechanisms such as harbouring a low-potency LPS, stimulation of interleukine-10 (IL-10) secretion or antigenic variation of their adhesins.

* Corresponding author. Institute of Microbiology, University of Lausanne, Bugnon 48, 1011 Lausanne, Switzerland. Fax: +41 21 314 40 60.
E-mail address: gilbert.greub@chuv.ch (G. Greub).

https://doi.org/10.1016/j.micinf.2017.11.002

2. Chlamydiales

2.1. Pathogenicity

The *Chlamydiales* order is composed of obligate intracellular Gram-negative bacteria that can cause widespread infections in human and in animals. The order is divided between the *Chlamydiaceae*, including two well-studied human pathogens, *Chlamydia trachomatis* and *Chlamydia pneumoniae*, and the *Chlamydia*-related bacteria grouping nine described families up to date [2].

C. trachomatis represents the most common bacterial sexually transmitted infection and leads to fallopian tubes injuries and infertility in women. Nearly 130 million new incident cases are reported each year [3]. Furthermore, this bacterium is responsible for trachoma which remains the world's leading infectious cause of blindness despite efforts to eradicate the disease. *C. pneumoniae* is responsible for acute respiratory diseases in human, including pneumonia and bronchitis. An inflammatory disease, the reactive arthritis, is also associated with *C. trachomatis* and to a lesser extent with *C. pneumoniae* [4]. *Chlamydia abortus* causes abortion in mammals and represents a zoonotic risk to pregnant women resulting in adverse pregnancy outcomes [5]. Furthermore, birds such as parrots or parakeets represent the main reservoir of *Chlamydia psittaci*, the agent responsible for human and avian psittacosis [6]. *Chlamydia suis*, an animal pathogen, causes arthritis, conjunctivitis, pneumonia, reproductive disorders and rhinitis in pigs [7]. Finally, *Waddlia chondrophila*, a *Chlamydia*-related bacterium, is probably associated with abortion in ruminants [8] and human miscarriages [9].

2.2. Biology

All chlamydial members share a typical biphasic life cycle including two developmental stages: the infectious non-dividing elementary bodies (EBs) [10] and the non-infectious dividing reticulate bodies (RBs) [11]. The life cycle starts with the entry of EBs into the host cells by endocytosis or phagocytosis. Once inside the cells, EBs reside in a vacuole called inclusion and are finally converted into RBs. Ultimately, RBs redifferentiate into EBs which are released by exocytosis or cell lysis in order to start a new life cycle. In some stress conditions, the chlamydial bacteria exhibit an alternative and persistent stage called ABs. The stress stimuli removal allows the redifferentiation of ABs into RBs, and subsequently into infectious EBs [12].

2.3. Aberrant bodies

Diverse stimuli can induce ABs such as the addition of β-lactam antibiotics [13], including penicillin (Fig. 1), starvation of iron or nutrients [14], treatment with interferon-gamma (IFN-γ) [15] or phosphomycin [16], as well as coinfection of the host with herpes or other viruses [17,18]. ABs are characterized by an abnormal enlargement of the bacteria that seems to be due to a continuous DNA replication without division [19]. Indeed, when *C. trachomatis* is treated with penicillin, each AB accumulates more than 16 chromosomal copies [20]. As mentioned earlier, the removal of stress stimuli allows the bacteria to re-enter its normal life cycle. As an example, *C. trachomatis* needs between 10 and 20 h to recover its normal developmental cycle after penicillin removal. Furthermore, ABs seem to re-differentiate by budding process but do not produce initially normal sized RBs [21]. It was also shown that *C. psittaci* infecting HeLa cells could be maintained in a viable and persistent infection for 6–9 months with the presence of penicillin and antiserum. When the antibiotics and the antibodies were removed, an acute infectious resumed, characterized by recoverable infectious EBs [22]. From these observations, ABs are considered as a persistent stage and this feature might be conserved among *Chlamydiales* as they have been described in *W. chondrophila*, a *Chlamydia*-related bacterium [23]. In addition, ABs can be induced in absence of exogenous stress stimuli as reported in untreated human endometrial cells infected with *W. chondrophila*. Indeed, ABs were observed at 72 h post infection (hpi), 96 hpi and 120 hpi, probably in response to nutrient starvation [23].

Furthermore, numerous studies have investigated how stress-inducing treatments impact the expression of diverse functional subsets of chlamydial genes compared to untreated infected cells. The expression of some highly antigenic proteins such as major outer membrane protein (MOMP) was reduced in persistence induced by herpes simplex virus co-infection [18] or by IFN-γ treatment [24]. However, another study has also reported an up-regulation of the *ompA* gene encoding MOMP in *C. pneumoniae*-infected cells and treated with IFN-γ [25]. Real-Time RT-PCR on *C. trachomatis* infected Hep-2 cells has identified increased expression of heat-shock response genes *ct604* and *ct755* at 96 hpi in response to IFN-γ [26]. Interestingly, *ct604* and *ct755* were not described as up-regulated genes in persistent *C. trachomatis* infection in a study published earlier [27]. In this study, genes implying tryptophan utilization, DNA repair and recombination, phospholipid biosynthesis, protein translation, and general stress genes were shown to be up-regulated and genes involved in proteolysis, peptide transport, and cell division were down-regulated during persistence [27]. From these results, no subset of genes can be clearly defined as a marker of persistence. The differences in up/down regulated chlamydial gene sets may represent the key event to understand the occurrence of ABs but the mechanism behind their formation remains unclear [19]. These in vitro models provide evidence for abnormal chlamydial growth under stress conditions but they do not bring any clue regarding the occurrence of ABs as an in vivo persistence mechanism in infected patients.

2.4. Clinical importance

Although chlamydial persistence in vitro has been well documented, only few studies have demonstrated in vivo persistence. By using transmission electron microscopy, Pospischil et al. have detected ABs in the intestinal enterocytes of

Please cite this article in press as: Scherler A, et al., *Chlamydiales*, *Anaplasma* and *Bartonella*: persistence and immune escape of intracellular bacteria, Microbes and Infection (2017), https://doi.org/10.1016/j.micinf.2017.11.002

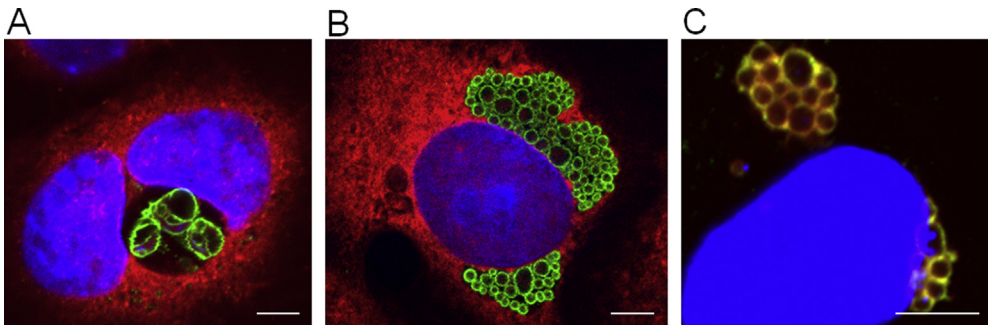

Fig. 1. Penicillin treatment induces aberrant bodies in different cell lines infected with distinct *Chlamydiales* members at 24 h post infection (hpi): (A). *C. trachomatis* infected McCoy cells treated with 0.6 μg/ml penicillin and stained with anti-LPS antibodies (green) (B). Vero cells infected with *W. chondrophila*, treated with penicillin at 1,000 μg/ml and labelled with anti-*Waddlia* antibodies (green) (C). Penicillin treatment at 500 μg/ml in Vero cells infected with *Estrella lausannensis* labelled with anti-OMP antibodies (green). In all conditions, drugs were added at 2 hpi and infected cells were fixed with methanol at 24 hpi. Fixed cells were labelled with DAPI (blue), concanavalin A (red, panels A and B) or specific antibodies against *E. lausannensis* (panel C). Scale bars represent 5 μm.

pigs infected both naturally and experimentally with *C. suis* [28]. Furthermore, aberrant bacterial forms were observed by electron microscopy in the female genital tract from non-treated *C. trachomatis* infected patients [29]. More recently, whole-genome sequencing was performed on strains obtained from patients with long-term persistent *C. trachomatis* infection and data showed remarkable genome stability over a long period of time. According to these results, persistence is thus apparently not related to pathogen mutational event [30].

From these in vivo observations, persistent stage is thought to play an important role in the initiation of persistent infections by *Chlamydiales*, which leads to serious complications, including pelvic pain and ectopic pregnancy. Nevertheless, only few in vivo cases were reported and therefore their consequences and occurrence rate remain undefined. Repeated infections are also difficult to differentiate from long-term persistence. Therefore, the link between persistent forms observed in vitro and the in vivo survival mechanisms should be clarified in the future.

2.5. Other mechanisms of persistence

The formation of ABs might be one possible cause of chlamydial persistence but it might also be caused by others mechanisms. For example, *C. trachomatis* infections can persist asymptomatically for months before being spontaneously cleared by the host, suggesting a likely escape from the host immunity. A possible strategy might involve the bacterial interference with the inflammatory transcription factor, NFκB, a regulator of immune responses to infection. Indeed, *Chlamydia pecorum*, an animal pathogen, was shown to induce significant NFκB activation at 24 hpi in human cells, suggesting its role in early inflammatory response. In contrast, *C. trachomatis* and *C. suis* exhibited less NFκB activation [31]. To escape from host-innate immunity, *Chlamydia* has also developed a mechanism to reduce the recognition of PAMPs (pathogen-associated molecular patterns) by guarantying the stability of the pathogen-containing vacuole during the infectious intracellular stage. Therefore, inclusions are stabilized by a network of F-actin and intermediate filaments avoiding thus the efflux of bacterial content into the host and its subsequent detection [32]. Furthermore, a truncated LPS with low endotoxic activity is localized on the chlamydial outer membrane and helps to reduce the recognition of *Chlamydiae* [33]. Finally, cytokines production elicited during productive infection and persistent infection varies according to the infected cells, as well as the immune status of the individual [34].

3. Anaplasma

3.1. Pathogenicity

Another obligate intracellular genus, *Anaplasma*, from the order Rickettsiales, is composed of ixodid tick-transmitted Gram-negative bacteria including two notable species: *Anaplasma marginale* and *Anaplasma phagocytophilum*. *Anaplasma* spp. can be transmitted by biting flies or by blood-contaminated fomites including needles, tattooing instruments or nose tongs [35].

Present worldwide and exclusively in ruminants' infection, *A. marginale* replicates in erythrocytes leading to bovine anaplasmosis principally in cattle and to a less extent in sheep, goats, buffalo, or in some wild ruminants [35]. Following transmission, an acute infection occurs with a peak between 3 and 7 weeks post-infection due to the ascending bacteraemia resulting in symptoms such as anaemia, fever, weight loss, dehydration, lower milk production, constipation, abortion in pregnant animals and finally death [36].

Contrary to *A. marginale*, *A. phagocytophilum* can infect human as well as animals including carnivores, ruminants, rodents, birds and reptiles [37]. Furthermore, *A. phagocytophilum* does not replicate in erythrocytes but within the vacuoles of neutrophils and leads to human granulocytic anaplasmosis. In infected humans, clinical manifestations vary from asymptomatic to severe disease with fever, malaise, headache, myalgia, anaemia, nausea, thrombocytopenia or leukopenia. In a small number of cases (<3%), patients experiment acute anaplasmosis due to toxic shock-like syndrome or acute respiratory distress syndrome that can lead to

Please cite this article in press as: Scherler A, et al., *Chlamydiales*, *Anaplasma* and *Bartonella*: persistence and immune escape of intracellular bacteria, Microbes and Infection (2017), https://doi.org/10.1016/j.micinf.2017.11.002

death in 1% of all cases [38]. In addition, *A. phagocytophilum* is the causative agent of tick borne fever, which affects principally small ruminants such as sheep and to a less extent, goats, cattle, horses, dogs and cats (reviewed in Ref. [39]). The main symptoms of tick borne fever in sheep include a high fever and a leukopenia implicating both granulocytes and lymphocytes [40].

Finally, four other species are pathogenic towards animals: *Anaplasma bovis*, *Anaplasma centrale*, *Anaplasma ovis* and *Anaplasma platys* [39].

3.2. Biology

As mentioned above, *Anaplasma* spp. are transmitted by ixodid ticks which feed on a wide variety of vertebrate hosts in which bacteria can potentially establish persistent infections. During blood meal on an infected host, *Anaplasma* spp. enter the tick midgut epithelium. After initial replication in tick gut cells, bacteria reach the tick salivary glands where a second replication occurs, and then migrate to the saliva allowing *Anaplasma* transmission to the next vertebrate host [41]. Interestingly, the *Anaplasma* developmental cycle exhibits two distinct forms. The first named "reticulate cell" represents the non-infectious replicative form and then it changes to the infectious "dense-cored cell" containing a dense nucleoid and surviving outside the host cells. Both morphological forms replicate by binary fission [42].

3.3. In vitro strategies developed by Anaplasma spp.

Anaplasma spp. have developed numerous strategies to persistently infect ticks and hosts which subsequently become reservoirs of infection allowing the transmission of the pathogen. The antigenic variation through the major surface proteins (MSPs) appears to be one of the major mechanisms to establish persistent infections. MSPs, such as MSP1, MSP2 (P44), MSP3 or MSP4, are immunodominant outer membrane proteins present in the *Anaplasmataceae* family and have been broadly studied especially in *A. marginale* and *A. phagocytophilum*. In both bacteria, a recombinant mechanism is used to generate new antigenic variants of their surface proteins in order to evade host immune response. For example, *A. marginale* possesses pseudogenes for two gene families, *msp2* and *msp3*, which can be recombined into the functional expression site to produce new antigenic variants during bacterial proliferation [43]. Furthermore, a second level of variation occurs when short segments of pseudogenes are recombined into the expression site by gene conversion allowing the generation of a large collection of outer membrane protein variants [44]. Concerning *A. phagocytophilum*, comparable combinatorial mechanisms happen with MSP2(P44), a MSP2 homologue of *A. marginale*, in order to generate a broad diversity of variants [45]. To summarize, immunodominant surface proteins MSP2 and MSP3 in *A. marginale* and MSP2(P44) in *A. phagocytophilum* are undergoing antigenic variations to allow bacterial escape from host immune responses, which leads to persistent infection.

Additionally, members of *Anaplasma* genus show no evidence of peptidoglycan (PG) layer or lipopolysaccharide (LPS) biosynthesis [46]. LPS or PG are normally recognized as PAMPs by pattern recognition receptors such as Toll-like and NOD receptors, expressed by macrophages or neutrophils, which lead to innate immune responses in order to eliminate the pathogens. Genome sequencing of *A. phagocytophilum* confirmed the absence of genes coding for the biosynthesis of lipid A and nearly all genes required for PG biosynthesis [47]. Consequently, the absence of PG and LPS allows *Anaplasma* to infect its host cell without activating the innate immune response and thus enables the bacterial persistence in the host.

3.4. Persistent Anaplasma infections

Various studies have established in vivo persistence confirming the key role of the different mechanisms previously described in vitro. For example, in *A. marginale* infected cattle, the acute infection is characterized by high bacteraemia reaching between 10^8 and 10^9 bacteria/ml of blood. However, the acute infection is not completely cleared in surviving animals and cycles of bacteraemia varying between 10^2 and 10^7 bacteria/ml of blood continue to occur every 6–8 weeks throughout lifelong persistent infection [48]. Additionally, numerous studies have reported persistent infections with *A. phagocytophilum* in various vertebrate hosts such as sheep, dogs, lambs, cattle or horses. For example, in sheep infected experimentally with *A. phagocytophilum*, bacterial DNA was still detectable within peripheral blood almost one year after the beginning of the infection, indicating a long-term DNA persistence. However, no reported case of persistent infection with *A. phagocytophilum* has been reported in humans up to now and no clinical evidences have confirmed the potential impact of persistent infection on the onset of chronic illness [38]. In contrast to *A. phagocytophilum*, *A. platys*, which is known to be implicated in persistent infections in dogs, was repetitively detected by PCR testing of blood samples taken from two family members and their dog confirming its intravascular persistence in human [49].

To conclude, numerous studies have confirmed the persistence of *Anaplasma* spp. inside mammalian hosts which are therefore considered as reservoirs of infection. Indeed, persistently infected species act as a source of infections for the tick vectors and thus impact directly the epidemiology and the transmission of anaplasmosis. Nevertheless, persistent infections are not always identified as such due to the low concentrations of bacteria present in blood samples and to the inaccuracy of techniques previously used for the detection.

4. Bartonella

4.1. Pathogenicity

The *Bartonella* genus is composed of Gram-negative facultative intracellular bacteria which have developed different strategies to persist in intraerythrocytic niche.

Please cite this article in press as: Scherler A, et al., *Chlamydiales*, *Anaplasma* and *Bartonella*: persistence and immune escape of intracellular bacteria, Microbes and Infection (2017), https://doi.org/10.1016/j.micinf.2017.11.002

Confined to the Andes region, *Bartonella bacilliformis* is a human-restricted pathogen causing Carrion's disease which can reach up to 88% of mortality in untreated patients during the acute infection [50]. The disease is characterized by two distinct phases beginning with the Oroya fever, which symptoms are fever, haemolytic anaemia, headache or muscle pains. A subsequent and chronic stage, called verruga peruana, occurs, causing distinctive red-to-purple vascular lesions of the skin [51]. Another member of this genus, *Bartonella quintana*, infects humans and causes trench fever characterized by peaks of fever, rash, bone pain and headache with only limited morbidity. Furthermore, *B. quintana* infection can induce a persistent bacteraemia [52]. Finally, *Bartonella henselae* causes cat scratch disease with mild symptoms such as fever, chills, malaise or papule at the inoculation site [51]. Infection by *B. henselae* is usually associated with low morbidity but, in some patients, more serious manifestations can occur such as neuroretinitis, encephalitis or endocarditis [53].

To summarize, human bartonellosis are mainly caused by the three species described above but the *Bartonella* genus contains as many as 45 species, among which 13 have been implicated in human diseases. These include *Bartonella clarridgeiae*, *Bartonella elizabethae* and *Bartonella rochalimae*, which were isolated from various vertebrate reservoirs such as cats, rats or foxes [54].

4.2. Biology

Bartonellosis vectors are sandfly species of the genera *Lutzomyia* for *B. bacilliformis* [55] and human body lice for *B. quintana*. For both bacteria, humans are considered to represent the main reservoir since no animal reservoir has been clearly established. However, natural or experimental inoculation of *B. quintana* in rhesus macaques lead to infection suggesting that *Bartonella* spp. may also use few other primates as reservoirs [56,57]. In contrast, the main host of *B. henselae* is domestic cats, which then transmit the bacterium to humans via cat fleas, animal scratches or bites.

Following *Bartonella* transmission, the pathogen resides in a still debating primary niche which seems to be required for the completion of the developmental life cycle. The bacteria are then transported to the vascular endothelium where bacterial persistence can occur. Later on, the bacteria are disseminated into the bloodstream where they invade erythrocytes, potentially allowing the reinfection of the primary niche. Interestingly, they can persist in the intraerythrocytic niche leading to specific asymptomatic bacteraemia [58].

4.3. In vitro persistence

Various strategies have been developed by the *Bartonella* spp. to establish persistent infection inside the host by evading innate and adaptive immune responses. One of these strategies consists in harbouring a low-potency LPS. Indeed, *B. henselae* was shown to create a deep-rough-type LPS depleted of O-chain. In addition, the pathogen harbours some unusual structural characteristics such as a long-chain fatty acid, which is also present in other bacteria known to cause chronic intracellular infections like *Chlamydia*. Interestingly, Toll-like receptor 4 signalling was activated at least 1,000-fold less by the *B. henselae* LPS compared with that of *Salmonella* [59]. According to these results, the modified form of LPS found in *Bartonella* spp. seems to contribute largely to the bacterial establishment and persistence.

Furthermore, members of the *Bartonella* genus can stimulate the secretion of IL-10, a key cytokine regulating immunity. Indeed, IL-10 suppresses the activity of various immune cells such as dendritic cells, macrophages/monocytes and T helper cells, which leads to an interference with the host immune responses. In IL-10 knockout mice, *Bartonella birtlesii*, a bacterium isolated from field mouse, could not establish a stable infection [60]. These results confirm the important role of IL-10 to promote persistence establishment of *Bartonella* infection. In addition, the Trw type IV secretion system (T4SS) may be necessary to establish persistent bacteraemia in erythrocytes as it displays extremely effective source of antigenic variability, which represents a crucial factor of erythrocyte adhesion [61]. Interestingly, *B. bacilliformis*, which does not likely cause intraerythrocytic persistent bacteraemia, lacks a T4SS in contrary to *B. quintana* and *B. henselae* which are able to persist in erythrocytes and possess two T4SS, Trw system and VirB/VirD4 [62].

Numerous surface proteins can undergo phase or antigenic variation as demonstrated for two adhesins, the variably expressed OMP (Vomp) [56] and the *Bartonella* adhesin A (BadA) [63]. Using a macaque model of *B. quintana* infection, phase variation of Vomp was shown to be mediated by deletion of one or more *vomp* genes during persistent bloodstream infection [56]. This antigenic variation allows the bacteria to subvert the host immune response and then persist in the erythrocytes.

To summarize, intraerythrocytic persistent bacteraemia might occur in almost all *Bartonella* spp. as confirmed for *B. quintana* and *B. henselae* in host infection model such as rhesus macaque or cats. Indeed, the persistence in erythrocytes allows the escape from host's immune system as well as the continuous transmission by arthropod vectors. Nevertheless, intraerythrocytic persistence has never been reported in *B. bacilliformis* which is probably the only exception among *Bartonella* genus that can trigger massive haemolysis of the infected human erythrocytes [64].

4.4. In vivo persistence

Some in vivo persistence cases were reported in host reservoirs such as human, cats or dogs. During a three-year period, *B. henselae* was isolated from the blood culture of a single cat on four occasions. *B. clarridgeiae*, another cat scratch disease agent, could be isolated from another cat on two distinct occasions. These observations suggest that a persistent infection is present inside the reservoir. Furthermore, asymptomatic persistent infection with *Bartonella vinsonii* subsp. *berkhoffii*, an emerging pathogen, was also described in a healthy dog along a period of 16 months [65]. In

Please cite this article in press as: Scherler A, et al., *Chlamydiales*, *Anaplasma* and *Bartonella*: persistence and immune escape of intracellular bacteria, Microbes and Infection (2017), https://doi.org/10.1016/j.micinf.2017.11.002

Table 1
Summary of persistence mechanisms present in *Chlamydiales*, *Anaplasma* and *Bartonella* spp.

	Chlamydiales	*Anaplasma*	*Bartonella*
Abnormal morphology	Enlarged aberrant bodies	Absent	Absent
Stimuli	β-lactam antibiotics [13] Iron or nutrients deprivation [14] Interferon-γ [15] Phosphomycin [16]	/	/
In vitro evidences	Presence of aberrant bodies induced by different stimuli in various cell lines	/	/
In vivo evidences	Aberrant bodies in intestinal enterocytes of pig with *C. suis* [28] Aberrant bodies in female genital tract [29]	/	/
Modulation of host immunity	Interference with NF-κB [31]	/	Stimulation of IL-10 secretion [60] Trw type IV secretion system [61,62]
Strutural modification	Stabilization of pathogen-containing vacuoles [32] Truncated LPS [33]	Antigenic variation via major surface proteins [43–45] Absence of PG and of LPS [46]	Antigenic variation via adhesins [56,63] Low-potency LPS [59]
Implications of persistence	Considered to be involved in serious complications; pelvic pain and ectopic pregnancy	Transmission to tick vectors	Transmission to insect vectors

another study, cats experimentally infected with *B. henselae* and/or *B. clarridgeiae* showed persistent bacteraemia for almost 450 days. Furthermore, clinical symptoms were minimal or even absent although *Bartonella* DNA was amplified from different organs such as the liver, the brain or the kidney [66]. *Bartonella* persistence has been also documented in 5 homeless patients infected with *B. quintana* for at least several weeks as demonstrated by positive blood cultures [67]. Furthermore, clinical manifestations of persistent *B. henselae* infections can occur in the form of bacillary angiomatosis and bacillary peliosis in immunocompromised patients [61].

Taken all together, these results confirm that persistent bacteraemia can be detected in the blood of cats, dogs and human or as a vascular and proliferative form on the skin. However, the maximal duration of persistence in reservoirs or hosts is still unknown due to the difficulty to isolate *Bartonella* species and to measure low bacteraemia.

5. Conclusions

As summarized in the Table 1, obligate or facultative intracellular bacteria have developed a variety of mechanisms in order to persist inside their hosts allowing thus access to their resources. Among the groups of bacteria reviewed above, interference with the immune responses is shared by *Chlamydiales* and *Bartonella* spp. through NFκB modulation or the stimulation of IL-10 expression, respectively. One common feature shared between *Anaplasma* and *Bartonella* is the absence of LPS or the presence of low-potency LPS allowing the evasion from host immunity detection. Furthermore, no evidence of PG layer is found in *Anaplasma* genus. Interestingly, *Chlamydiaceae* are known to have PG only at the division septum, which lowers immunity responses from the host [68]. In order to subvert the host immune responses, antigenic variation occurs through MSPs in *Anaplasma* or via surface proteins such as Vomp and BadA adhesins in *Bartonella*. Furthermore, the stabilization of chlamydial inclusions and the presence of T4SS in *Bartonella* can be mentioned as mechanisms of persistence. Finally, typical forms of persistence, such as aberrant bodies, are not found in *Anaplasma* or *Bartonella* spp. which indicates that ABs are presumably specific to *Chlamydia*. However, they also result in the maintenance of low bacteraemia and escape from host immune system.

To conclude, different essential mechanisms of persistence have been developed by intracellular bacteria in order to persist and then to be efficiently transmitted to new hosts. Indeed, in case of over-stimulation of immune system by bacteria, host immune responses will kill the pathogen and cause disease with noticeable symptoms such as fever or headache. On the contrary, if the host does not present any symptoms, his behaviour will remain unchanged and the bacterial transmission will continue. For example, if the infection with *C. trachomatis* caused severe symptoms, the host would not have sexual relations and then the bacterial transmission would decrease significantly. Similarly, persistent bacteraemia with low or absent symptoms in human and animals directly impacts the transmission of pathogens to other accidental host reservoirs like human.

It is important to note that the diversity of persistence strategies as well as their precise mechanisms remain unclear and should be investigated in the future. Due to the low number of pathogens and the asymptomatic manifestations, in vivo bacterial persistence is not always detected in human or in animals. Thus, future improvements of the diagnostic techniques will allow the detection of persistent infection despite the very low quantity of bacterial DNA, and thus lead to an improvement of our knowledge on these persistence mechanisms. Additionally, a better comprehension of bacterial persistence will improve the treatments of infected human or

Please cite this article in press as: Scherler A, et al., *Chlamydiales*, *Anaplasma* and *Bartonella*: persistence and immune escape of intracellular bacteria, Microbes and Infection (2017), https://doi.org/10.1016/j.micinf.2017.11.002

animals. Furthermore, systematic screening for pathogens known to persist should be encouraged in order to detect and treat asymptomatic infected patients and thus lower the prevalence of these pathogens.

Conflict of interest

The authors have no conflict of interest.

Acknowledgments

This work was supported by a grant from the Swiss National Science Foundation no. 310030-162603.

References

[1] Bigger JW. Treatment of staphylococcal infections with penicillin by intermittent sterilisation. Lancet 1944;244:497–500.
[2] Taylor-Brown A, Vaughan L, Greub G, Timms P, Polkinghorne A. Twenty years of research into *Chlamydia*-like organisms: a revolution in our understanding of the biology and pathogenicity of members of the phylum *Chlamydiae*. Pathog Dis 2015;73:1–15.
[3] Newman L, Rowley J, Vander Hoorn S, Wijesooriya NS, Unemo M, Low N, et al. Global estimates of the prevalence and incidence of four curable sexually transmitted infections in 2012 based on systematic review and global reporting. PLoS One 2015;10, e0143304.
[4] Carter JD, Hudson AP. Recent advances and future directions in understanding and treating *Chlamydia*-induced reactive arthritis. Expert Rev Clin Immunol 2017;13:197–206.
[5] Essig A, Longbottom D. *Chlamydia abortus*: new aspects of infectious abortion in sheep and potential risk for pregnant women. Curr Clin Microbiol Rep 2015;2:22–34.
[6] Knittler MR, Sachse K. *Chlamydia psittaci*: update on an underestimated zoonotic agent. Pathog Dis 2015;73:1–15.
[7] Schautteet K, Vanrompay D. *Chlamydiaceae* infections in pig. Vet Res 2011;42:29.
[8] Barkallah M, Gharbi Y, Hassena AB, Slima AB, Mallek Z, Gautier M, et al. Survey of infectious etiologies of bovine abortion during mid- to late gestation in dairy herds. PLoS One 2014;9, e91549.
[9] Baud D, Goy G, Osterheld MC, Croxatto A, Borel N, Vial Y, et al. Role of *Waddlia chondrophila* placental infection in miscarriage. Emerg Infect Dis 2014;20:460–4.
[10] Matsumoto A. Fine structures of cell envelopes of *Chlamydia* organisms as revealed by freeze-etching and negative staining techniques. J Bacteriol 1973;116:1355–63.
[11] Friis RR. Interaction of L cells and *Chlamydia psittaci*: entry of the parasite and host responses to its development. J Bacteriol 1972;110:706–21.
[12] Matsumoto A, Manire GP. Electron microscopic observations on the effects of penicillin on the morphology of *Chlamydia psittaci*. J Bacteriol 1970;101:278–85.
[13] Kintner J, Lajoie D, Hall J, Whittimore J, Schoborg RV. Commonly prescribed beta-lactam antibiotics induce *C. trachomatis* persistence/stress in culture at physiologically relevant concentrations. Front Cell Infect Microbiol 2014;4:44.
[14] Raulston JE. Response of *Chlamydia trachomatis* serovar E to iron restriction in vitro and evidence for iron-regulated chlamydial proteins. Infect Immun 1997;65:4539–47.
[15] Pantoja LG, Miller RD, Ramirez JA, Molestina RE, Summersgill JT. Characterization of *Chlamydia pneumoniae* persistence in HEp-2 cells treated with gamma interferon. Infect Immun 2001;69:7927–32.
[16] Jacquier N, Frandi A, Pillonel T, Viollier PH, Greub G. Cell wall precursors are required to organize the chlamydial division septum. Nat Commun 2014;5:3578.
[17] Borel N, Dumrese C, Ziegler U, Schifferli A, Kaiser C, Pospischil A. Mixed infections with *Chlamydia* and porcine epidemic diarrhea virus – a new in vitro model of chlamydial persistence. BMC Microbiol 2010;10:201.
[18] Deka S, Vanover J, Dessus-Babus S, Whittimore J, Howett MK, Wyrick PB, et al. *Chlamydia trachomatis* enters a viable but non-cultivable (persistent) state within herpes simplex virus type 2 (HSV-2) co-infected host cells. Cell Microbiol 2006;8:149–62.
[19] Wyrick PB. *Chlamydia trachomatis* persistence in vitro: an overview. J Infect Dis 2010;201(Suppl 2):S88–95.
[20] Lambden PR, Pickett MA, Clarke IN. The effect of penicillin on *Chlamydia trachomatis* DNA replication. Microbiology 2006;152:2573–8.
[21] Skilton RJ, Cutcliffen LT, Barlow D, Wang Y, Salim O, Lambden PR, et al. Penicillin induced persistence in *Chlamydia trachomatis*: high quality time lapse video analysis of the developmental cycle. PLoS One 2009;4:e7723.
[22] Galasso GJ, Manire GP. Effect of antiserum and antibiotics on persistent infection of HeLa cells with meningopneumonitis virus. J Immunol 1961;86:382–5.
[23] Kebbi-Beghdadi C, Cisse O, Greub G. Permissivity of Vero cells, human pneumocytes and human endometrial cells to *Waddlia chondrophila*. Microbes Infect 2011;13:566–74.
[24] Beatty WL, Byrne GI, Morrison RP. Morphologic and antigenic characterization of interferon gamma-mediated persistent *Chlamydia trachomatis* infection in vitro. Proc Natl Acad Sci USA 1993;90:3998–4002.
[25] Mathews S, George C, Flegg C, Stenzel D, Timms P. Differential expression of ompA, ompB, pyk, nlpD and Cpn0585 genes between normal and interferon-gamma treated cultures of *Chlamydia pneumoniae*. Microb Pathog 2001;30:337–45.
[26] Kokab A, Jennings R, Eley A, Pacey AA, Cross NA. Analysis of modulated gene expression in a model of Interferon-gamma-induced persistence of *Chlamydia trachomatis* in HEp-2 cells. Microb Pathog 2010;49:217–25.
[27] Belland RJ, Nelson DE, Virok D, Crane DD, Hogan D, Sturdevant D, et al. Transcriptome analysis of chlamydial growth during IFN-gamma-mediated persistence and reactivation. Proc Natl Acad Sci USA 2003;100:15971–6.
[28] Pospischil A, Borel N, Chowdhury EH, Guscetti F. Aberrant chlamydial developmental forms in the gastrointestinal tract of pigs spontaneously and experimentally infected with *Chlamydia suis*. Vet Microbiol 2009;135:147–56.
[29] Lewis ME, Belland RJ, AbdelRahman YM, Beatty WL, Aiyar AA, Zea AH, et al. Morphologic and molecular evaluation of *Chlamydia trachomatis* growth in human endocervix reveals distinct growth patterns. Front Cell Infect Microbiol 2014;4:71.
[30] Suchland RJ, Dimond ZE, Putman TE, Rockey DD. Demonstration of persistent infections and genome stability by whole-genome sequencing of repeat-positive, same-serovar *Chlamydia trachomatis* collected from the female genital tract. J Infect Dis 2017;215:1657–65.
[31] Leonard CA, Schoborg RV, Borel N. Productive and penicillin-stressed *Chlamydia pecorum* infection induces nuclear factor kappa B activation and interleukin-6 secretion in vitro. Front Cell Infect Microbiol 2017;7:180.
[32] Kumar Y, Valdivia RH. Actin and intermediate filaments stabilize the *Chlamydia trachomatis* vacuole by forming dynamic structural scaffolds. Cell Host Microbe 2008;4:159–69.
[33] Kosma P. Chlamydial lipopolysaccharide. Biochim Biophys Acta 1999;1455:387–402.
[34] Rusconi B, Greub G. *Chlamydiales* and the innate immune response: friend or foe? FEMS Immunol Med Microbiol 2011;61:231–44.
[35] Kocan KM, de la Fuente J, Blouin EF, Coetzee JF, Ewing SA. The natural history of *Anaplasma marginale*. Vet Parasitol 2010;167:95–107.
[36] Aubry P, Geale DW. A review of bovine anaplasmosis. Transbound Emerg Dis 2011;58:1–30.
[37] Stuen S, Granquist EG, Silaghi C. *Anaplasma phagocytophilum* – a widespread multi-host pathogen with highly adaptive strategies. Front Cell Infect Microbiol 2013;3:31.

Please cite this article in press as: Scherler A, et al., *Chlamydiales*, *Anaplasma* and *Bartonella*: persistence and immune escape of intracellular bacteria, Microbes and Infection (2017), https://doi.org/10.1016/j.micinf.2017.11.002

[38] Bakken JS, Dumler JS. Human granulocytic anaplasmosis. Infect Dis Clin North Am 2015;29:341−55.
[39] Battilani M, De Arcangeli S, Balboni A, Dondi F. Genetic diversity and molecular epidemiology of *Anaplasma*. Infect Genet Evol 2017;49: 195−211.
[40] Woldehiwet Z. *Anaplasma phagocytophilum* in ruminants in Europe. Ann N Y Acad Sci 2006;1078:446−60.
[41] Ueti MW, Knowles DP, Davitt CM, Scoles GA, Baszler TV, Palmer GH. Quantitative differences in salivary pathogen load during tick transmission underlie strain-specific variation in transmission efficiency of *Anaplasma marginale*. Infect Immun 2009;77:70−5.
[42] Troese MJ, Kahlon A, Ragland SA, Ottens AK, Ojogun N, Nelson KT, et al. Proteomic analysis of *Anaplasma phagocytophilum* during infection of human myeloid cells identifies a protein that is pronouncedly upregulated on the infectious dense-cored cell. Infect Immun 2011;79: 4696−707.
[43] Brayton KA, Knowles DP, McGuire TC, Palmer GH. Efficient use of a small genome to generate antigenic diversity in tick-borne ehrlichial pathogens. Proc Natl Acad Sci USA 2001;98:4130−5.
[44] Brayton KA, Palmer GH, Lundgren A, Yi J, Barbet AF. Antigenic variation of *Anaplasma marginale* msp2 occurs by combinatorial gene conversion. Mol Microbiol 2002;43:1151−9.
[45] Barbet AF, Meeus PF, Belanger M, Bowie MV, Yi J, Lundgren AM, et al. Expression of multiple outer membrane protein sequence variants from a single genomic locus of *Anaplasma phagocytophilum*. Infect Immun 2003;71:1706−18.
[46] Rikihisa Y. The tribe *Ehrlichieae* and ehrlichial diseases. Clin Microbiol Rev 1991;4:286−308.
[47] Lin M, Rikihisa Y. *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* lack genes for lipid A biosynthesis and incorporate cholesterol for their survival. Infect Immun 2003;71:5324−31.
[48] Palmer GH, Rurangirwa FR, Kocan KM, Brown WC. Molecular basis for vaccine development against the ehrlichial pathogen *Anaplasma marginale*. Parasitol Today 1999;15:281−6.
[49] Breitschwerdt EB, Hegarty BC, Qurollo BA, Saito TB, Maggi RG, Blanton LS, et al. Intravascular persistence of *Anaplasma platys, Ehrlichia chaffeensis*, and *Ehrlichia ewingii* DNA in the blood of a dog and two family members. Parasit Vectors 2014;7:298.
[50] Gray GC, Johnson AA, Thornton SA, Smith WA, Knobloch J, Kelley PW, et al. An epidemic of Oroya fever in the Peruvian Andes. Am J Trop Med Hyg 1990;42:215−21.
[51] Greub G, Raoult D. *Bartonella* infections resurgence in the new Century. In: Reemergence of established pathogens in the 21st century. Springer; 2004. p. 35−68.
[52] Ohl ME, Spach DH. *Bartonella quintana* and urban trench fever. Clin Infect Dis 2000;31:131−5.
[53] Florin TA, Zaoutis TE, Zaoutis LB. Beyond cat scratch disease: widening spectrum of *Bartonella henselae* infection. Pediatrics 2008;121: e1413−25.
[54] Okaro U, Addisu A, Casanas B, Anderson B. *Bartonella* species, an emerging cause of blood-culture-negative endocarditis. Clin Microbiol Rev 2017;30:709−46.
[55] Sanchez Clemente N, Ugarte-Gil CA, Solorzano N, Maguina C, Pachas P, Blazes D, et al. *Bartonella bacilliformis*: a systematic review of the literature to guide the research agenda for elimination. PLoS Negl Trop Dis 2012;6:e1819.
[56] Zhang P, Chomel BB, Schau MK, Goo JS, Droz S, Kelminson KL, et al. A family of variably expressed outer-membrane proteins (Vomp) mediates adhesion and autoaggregation in *Bartonella quintana*. Proc Natl Acad Sci USA 2004;101:13630−5.
[57] Huang R, Liu Q, Li G, Li D, Song X, Birtles RJ, et al. *Bartonella quintana* infections in captive monkeys, China. Emerg Infect Dis 2011; 17:1707−9.
[58] Harms A, Dehio C. Intruders below the radar: molecular pathogenesis of *Bartonella* spp. Clin Microbiol Rev 2012;25:42−78.
[59] Zahringer U, Lindner B, Knirel YA, van den Akker WM, Hiestand R, Heine H, et al. Structure and biological activity of the short-chain lipopolysaccharide from *Bartonella henselae* ATCC 49882T. J Biol Chem 2004;279:21046−54.
[60] Marignac G, Barrat F, Chomel B, Vayssier-Taussat M, Gandoin C, Bouillin C, et al. Murine model for *Bartonella birtlesii* infection: new aspects. Comp Immunol Microbiol Infect Dis 2010;33:95−107.
[61] Pulliainen AT, Dehio C. Persistence of *Bartonella* spp. stealth pathogens: from subclinical infections to vasoproliferative tumor formation. FEMS Microbiol Rev 2012;36:563−99.
[62] Saenz HL, Engel P, Stoeckli MC, Lanz C, Raddatz G, Vayssier-Taussat M, et al. Genomic analysis of *Bartonella* identifies type IV secretion systems as host adaptability factors. Nat Genet 2007;39: 1469−76.
[63] Riess T, Raddatz G, Linke D, Schafer A, Kempf VA. Analysis of *Bartonella* adhesin A expression reveals differences between various B. henselae strains. Infect Immun 2007;75:35−43.
[64] Chomel BB, Boulouis HJ, Breitschwerdt EB, Kasten RW, Vayssier-Taussat M, Birtles RJ, et al. Ecological fitness and strategies of adaptation of *Bartonella* species to their hosts and vectors. Vet Res 2009;40:29.
[65] Kordick DL, Breitschwerdt EB. Persistent infection of pets within a household with three *Bartonella* species. Emerg Infect Dis 1998;4: 325−8.
[66] Kordick DL, Brown TT, Shin K, Breitschwerdt EB. Clinical and pathologic evaluation of chronic *Bartonella henselae* or *Bartonella clarridgeiae* infection in cats. J Clin Microbiol 1999;37:1536−47.
[67] Brouqui P, Lascola B, Roux V, Raoult D. Chronic *Bartonella quintana* bacteremia in homeless patients. N Engl J Med 1999;340:184−9.
[68] Packiam M, Weinrick B, Jacobs Jr WR, Maurelli AT. Structural characterization of muropeptides from *Chlamydia trachomatis* peptidoglycan by mass spectrometry resolves “chlamydial anomaly”. Proc Natl Acad Sci USA 2015;112:11660−5.

Please cite this article in press as: Scherler A, et al., *Chlamydiales*, *Anaplasma* and *Bartonella*: persistence and immune escape of intracellular bacteria, Microbes and Infection (2017), https://doi.org/10.1016/j.micinf.2017.11.002